Doctoral theses at NTNU, 2016:225

Siri Marte Hollekim-Strand

Effects of exercise training on cardiac function and cardiometabolic risk factors in type 2 diabetes

Doctoral thesis

Siri Marte Hollekim-Strand

ISBN 978-82-326-1788-3 (printed ver.)
ISBN 978-82-326-1789-0 (electronic ver.)
ISSN 1503-8181

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine
Department of Circulation and Medical Imaging

Doctoral theses at NTNU, 2016:225

NTNU
Norwegian University of
Science and Technology

Siri Marte Hollekim-Strand

Effects of exercise training on cardiac function and cardiometabolic risk factors in type 2 diabetes

Thesis for the Degree of Philosophiae Doctor

Trondheim, august 2016

Norwegian University of Science and Technology
Faculty of Medicine
Department of Circulation and Medical Imaging

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Medicine
Department of Circulation and Medical Imaging

ISBN 978-82-326-1788-3 (printed ver.)
ISBN 978-82-326-1789-0 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2016:225

Printed by NTNU Grafisk senter

EFFEKTEN AV UTHOLDENHETSTRENING PÅ HJERTEFUNKSJON OG RISIKOFAKTORER FOR HJERTE- OG KARSYKDOM HOS PERSONER MED TYPE 2 DIABETES.

I dag antas det at mellom 300 000 og 400 000 nordmenn har sukkersyke (type 2 diabetes). Omtrent 200 000 vet at de har diagnosen, resten er udiagnostisert. Antallet mennesker med diabetes vokser raskt verden over; fra 108 millioner i 1980 til 422 millioner mennesker i 2014. Det er i lav- og mellominntektsland (spesielt i Sør-Øst Asia og vest i stillehavsregionen) type 2 diabetes vokser raskest og om tjue år forventes forekomsten av sykdommen å være mer enn doblet på verdensbasis, sammenlignet med i dag.

Blant annet er inaktivitet, overvekt og fedme viktige årsaker til utvikling av type 2 diabetes. Risikofaktorene for å utvikle denne sykdommen er ofte de samme som for å utvikle hjerte- og karsykdom. Nesten 80 % av de som har type 2 diabetes dør av en form for hjerte- og karsykdom eller slag. Risikoen for å dø av hjerte- og karsykdom er doblet og risikoen for å få hjertesvikt er opptil fem ganger større sammenlignet med personer som ikke har sykdommen.

Forekomsten av hjertesvikt vokser sammen med økende diabetesforekomst. Omtrent annenhver person med type 2 diabetes uten symptomer på hjertesykdom har redusert hjertefunksjon i den fasen hvor hjertet fylles med blod (diastolisk dysfunksjon). Dette kan føre til hjertesvikt i det lange løp.

Regelmessig fysisk trening har vist seg å kunne forbedre risikofaktorer forbundet med type 2 diabetes. Forskning foreslår at utholdenhetstrening på høy intensitet kan være mer effektiv enn moderat intensitet når det kommer til å redusere risiko for hjertesykdom. Det er likevel enda usikkert hvilken type treningsintensitet som er best for å oppnå hensiktsmessige effekter på den diastoliske hjertefunksjonen spesielt, samt på risikofaktorene for hjerte- og karsykdom generelt, hos personer med type 2 diabetes.

Den diastoliske hjertefunksjonen bestemmes av mange faktorer; blant annet vridningen som skjer i hjertet når det pumper blod. Hjertet vris som en klut når det pumper blod ut i kroppen (systole) og vris tilbake når det skal fylles med blod (diastole). Denne «tilbakevridningen» er viktig for at blodet effektivt skal suges inn i hjertet i tidlig diastole. Det er usikkert om personer med type 2 diabetes og diastolisk dysfunksjon har dårligere vridningsegenskaper enn friske og om trening (høy- og/eller moderat intensitet) kan påvirke disse egenskapene.

Usunn mat, rik på energi, raske karbohydrater og mettet fett (hurtigmat) kan akutt føre til stor økning i blodsukker og/eller fettinnhold i blodet, noe som er vanlig å ha med type 2 diabetes. Høyt blodsukker og/eller høye fettnivåer i blodet generelt sett, og/eller store svingninger i blodsukkernivåer gjennom dagen representerer en økt risiko for hjerte- og karsykdom i det lange løp. Det er dog usikkert hvordan ett enkelt måltid hurtigmat påvirker hjertefunksjonen hos personer med type 2 diabetes. Det er videre usikkert om en enkel treningsøkt kan redusere en eventuelt økt arbeidsbelastning på hjertet i fasen etter et måltid; slik som akutt trening tidligere har vist å kunne forbedre blodårefunksjonen etter et måltid hurtigmat hos friske.

Hensikten med denne doktorgradsavhandlingen var å finne ut om utholdenhetstrening, og i så fall hvilken treningsintensitet, som kan forbedre diastolisk funksjon hos personer med type 2 diabetes og diastolisk dysfunksjon. Videre var hensikten å undersøke om vridningsegenskapene i hjertet hos denne gruppen pasienter er forringet, og hvis så; om utholdenhetstrening (høy- eller moderat intensitet) kan forbedre disse egenskapene. I tillegg ønsket vi å undersøke om hurtigmat akutt påvirker arbeidsbelastningen på hjertet til personer med type 2 diabetes sammenlignet med friske overvektige; samt om trening i forkant av måltidet kan bidra til å endre de eventuelle effektene usunn mat har på hjertets arbeid rett etter måltidet.

Resultatene fra studiene gjennomført i denne doktorgradsavhandlingen viser at trening kan forbedre redusert diastolisk funksjon hos personer med type 2 diabetes og at relativ høy treningsintensitet er viktig for å effektivt oppnå dette. Doktorgradsavhandlingen viser også at hjertet til personer med type 2 diabetes og diastolisk dysfunksjon kan ha en forsinket tid til maksimal tilbakevridningshastighet sammenlignet med friske. Trening, uansett intensitet, bidro til å normalisere dette. Videre viser denne doktorgradsavhandlingen at hurtigmat bidrar til akutt økt arbeidsbelastning for hjertet hos personer med type 2 diabetes og friske overvektige i tiden etter et måltid; og at hurtigmat kan påvirke diastolisk funksjon, systolisk blodtrykk og hjertefrekvens i større grad hos personer med type 2 diabetes sammenlignet med friske overvektige. Trening før et hurtigmat-måltid påvirket ikke de observasjoner vi gjorde på hjertefunksjonen eller andre variabler etter måltidet.

Siri Marte Hollekim-Strand

Institutt for Sirkulasjon og Bildediagnostikk, Det Medisinske Fakultet, NTNU. Hovedveileder: Dr. Med, PhD Charlotte B. Ingul. Biveileder: professor Ulrik Wisløff.

Finansieringskilder: Helse Midt-Norge, NTNU, UNIKARD, Stiftelsen K.G. Jebsen og Diabetesforbundet.

EFFECTS OF ENDURANCE TRAINING ON CARDIAC FUNCTION AND CARDIOMETABOLIC RISK FACTORS IN TYPE 2 DIABETES

In 2014, 422 million people around the world had diabetes (global prevalence: 8.5%). Approximately 90–95% of the population with diabetes have type 2 diabetes (T2D). The prevalence is expected to increase by 55% worldwide in the next 20 years. The countries with the most rapid diabetes increase are middle- and low-income countries (i.e., South-East Asia and Western Pacific Regions). In Norway, 300,000 to 400,000 people have T2D, including up to 50% who are undiagnosed.

Inactivity, being overweight, and obesity are important contributing factors to the development of T2D. Many T2D risk factors are the same as those that increase the risk of cardiovascular disease. Nearly 80% of the T2D population die from cardiovascular disease; the risk of dying from cardiovascular disease is at least doubled and the risk of developing heart failure is up to five times greater than in non-diabetes individuals.

The prevalence of heart failure is increasing in concordance with the increasing diabetes prevalence. About every second person with T2D without traditional symptoms of cardiovascular disease has reduced cardiac function in the phase of the cardiac cycle when the heart is filled with blood (diastolic dysfunction). Diastolic dysfunction can ultimately lead to heart failure.

Exercise training improves risk factors related to T2D. Research suggests that high-intensity exercise training is more effective than moderate-intensity exercise training when it comes to reducing risk for cardiovascular disease. However, it is not established which exercise training intensity is the most effective in reducing risk factors for cardiovascular disease in general and in improving diastolic cardiac function in particular in T2D.

Diastolic function is determined by a number of factors; among others, the wringing and unwringing motion that happens when the heart ejects blood (systole) and fills with blood (diastole), respectively. The unwringing of the heart is important for diastolic function as it contributes to the suction of blood into the heart in early diastole. However, even though diastolic dysfunction is common in T2D, it is uncertain whether people with T2D and diastolic dysfunction have reduced wringing and/or unwringing properties and whether exercise (high and/or moderate-intensity exercise) can influence these properties.

Fast food, high in energy, refined carbohydrates and saturated fat can contribute to excessive increase in circulating glucose and lipids, which is common in people with T2D. High blood sugar and/or lipid levels in general and/or great fluctuations in blood sugar throughout the day represents an increased risk for cardiovascular disease in the long run. However, it is uncertain how fast food affects cardiac workload in the acute phase after the meal in people with T2D. Furthermore, we do not know whether a single exercise bout prior to food ingestion can reduce putative acute negative effects to the heart after a meal, similar to what is previously observed in arterial function in healthy individuals.

The aim of this PhD-thesis was to investigate whether endurance training (and if so, at which exercise intensity) could improve cardiac function and cardiovascular risk factors in T2D individuals, without signs of cardiovascular disease, but with diastolic dysfunction when assessed by echocardiography. Furthermore, the aim was to investigate whether the wringing properties of the heart are different in people with T2D compared to healthy individuals, and if so whether endurance exercise (at high or moderate-intensity) can modify the difference. In addition, we wanted to investigate whether fast food affects cardiac workload in individuals with T2D versus overweight healthy counterparts, and if so, whether acute exercise can contribute to modulating these effects on cardiac function.

Results from the studies carried out in this thesis show that endurance exercise training can improve cardiac function in T2D individuals with diastolic dysfunction and indicate that intensity is important to achieve improvements. Furthermore, this thesis shows that patients with T2D have similar wringing velocities as their healthy counterparts, but that they have delayed time to peak unwringing velocity. Endurance exercise, independent of intensity, normalized the timing of peak unwringing velocity. Moreover, we found that fast food induces increased cardiac workload in both T2D and healthy overweight individuals. Our findings also indicate that fast food induces compensations in diastolic function, blood pressure, and heart rate to a larger extent in T2D individuals compared to healthy overweight controls. A single prior exercise bout did not modulate the observed alterations in cardiac function or other variables measured after the meal.

Siri Marte Hollekim-Strand

TABLE OF CONTENTS

Acknowledgements

The work presented in this thesis was carried out from 2010-2016 at the Department of Circulation and Medical Imaging, The Faculty of Medicine, Norwegian University of Science and Technology (NTNU). The financial support was granted by the Liaison Committee between the Central Norway Regional Health Authority and NTNU as well as UNIKARD, the K.G. Jebsen Foundation and the Norwegian Diabetes Association, and is gratefully acknowledged.

First of all, I would like to thank the participants of the two clinical trials conducted in this project. Your time and extraordinary efforts are highly appreciated.

I would like to express my deepest gratitude to my main supervisor, Charlotte Björk Ingul. Thank you for believing in me and for inviting me into this journey. You certainly made it unique: The number of hours you have from dusk 'til dawn, your passion for research, cardiology and echocardiography, the diversity of interests in which you commit so strongly to, your broad knowledge, your clinical competence as well as your engagement as a supervisor; it cannot be explained, it has to be experienced. You have really invested your time and heart in this project and to my development as a researcher—and as a person—for the past six years. I am so grateful for that. My gratefulness also goes to my co-supervisor, Ulrik Wisløff. Thank you for making me feel welcomed into your excellent Cardiac Exercise Research Group (CERG). Your feedback, which is always spot on, and sometimes painfully honest, has immensely contributed to a valuable learning experience for me and reflects your profound competence and great experience in the field of exercise in medicine. I am so grateful for your guidance and the time you have invested in this project as well as in my development as a researcher.

Furthermore, I would like to extend my warmest thanks to the researchers and clinicians contributing to the projects of this thesis. A special thanks goes to the remaining co-authors of the papers (Marit Bjørgaas, Grethe Albrektsen, Arnt Erik Tjønna, Vegard Malmo, Turid Follestad, Sigve Fredrik Høydahl and Håvard Dalen), the clinical staff at the research unit (Nina Backlund and Gøril Rønning) for their tremendous help with "the fast food study," Ellen Gjerløw at the Department of Endocrinology for being so flexible (DXA measurements), as well as the clinicians at the Department of Cardiology at the St. Olavs University Hospital, who kindly stepped in and performed echocardiography when logistics went wild during "the fast food study" (Torfinn Eriksen, Ola Kleveland, Thomas Renhult Skaug, Bjørn Olav Haugen, Erik Madsen and Asbjørn Støylen). I would also like to thank Bjarne Nes, Tor Arne Grindal, Ingrid Aarbo, Ragnhild Røsbjørgen, Atefe Tari, Mansoureh Hosseini and Anita Timilsina for your goodwill when I needed your advice or help. I would also like to grab the opportunity to honor my other great colleagues at CERG and in particular my office-mates over the past several years. Thank you for being forthcoming and caring. Even though the meetings between us have only been brief at times, I have felt that I have sincere friends in you.

A special thank you goes to my dear parents, brother, sister as well as brother- and mother-in-law (Grethe-, Jon Arve-, Ole Jakob- and Solveig Hollekim, Jørgen Brun-Pedersen and Dagny Strand) and of course, tante Grete Estenstad. You have been supportive every step of the way, not only towards me but most importantly towards our beloved children when work-hours have

been massive. A humongous thank you also goes to my dear friends for listening to me talk about the joys and challenges of this project for what feels like forever and ever! Elin Kvernberg, you have been priceless.

Finally, I would like to address my beautiful four daughters. First, my precious little ones, Hannah and Sanne Hollekim-Strand: Thank you for your patience, which has been inexhaustible to an extent that is unbelievable. Words cannot explain how proud I am to be your mother. So, to my cherished "grown-up-girls," Vibeke and Nina Bjerkaker Strand. I feel so privileged to have you in my life. Now, I look forward to more time with you, my bouquet of daughters, heart-melting grandchildren (Sandra and Elise) and my beautiful nieces (Synnøve and Ane). Remember, girls and young women: "You is kind, you is smart, you is important" (quote: Eibileen Clark).

And most of all, I would like to express my deep, deep gratitude towards my dearest friend and husband, Roar Strand. There is no doubt; you are the best team-mate! You have been absolutely amazing every step of the way and I am forever grateful to you. The kids and you are my home and my heart; and what we have means the world to me!

«A sad and a happy heart» *by Sanne Hollekim-Strand, 4 years.*

List of papers

The following studies were carried out at the Norwegian University of Science and Technology, Faculty of Medicine, Department of Circulation and Medical Imaging from 2010 to 2013. The numbers listed below refer to the original papers included in this thesis.

Paper I

High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: A randomized controlled trial

Hollekim-Strand, S.M.; Bjørgaas, M.R.; Albrektsen, G.; Tjønna, A.E.; Wisløff, U.; Ingul, C.B.

J Am Coll Cardiol, 2014. 64(16): p. 1758–60.

Paper II

Exercise training normalizes timing of left ventricular untwist rate, but not peak untwist rate, in individuals with type 2 diabetes and diastolic dysfunction: A pilot study

Hollekim-Strand S.M.; Høydahl, S.F.; Follestad, T.; Dalen, H.; Bjørgaas, M.R.; Wisløff, U; Ingul, C.B.

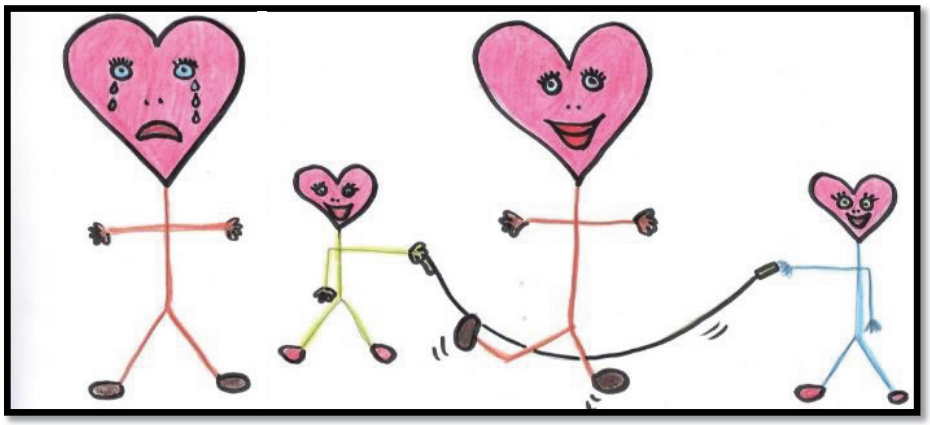
J Am Soc Echocardiogr.2016. 29(5): p. 421–430

Paper III

Fast food increases postprandial cardiac workload in type 2 diabetes independent of pre-exercise: A pilot study

Hollekim-Strand, S.M.; Malmo, V.; Follestad, T.; Wisløff, U.; Ingul, C.B.

Nutr J, 2015. 14(1): p. 79–90.



«Three hearts exercising, and one that's not» *by Hannah Hollekim-Strand, 11 years.*

Abbreviations

A	Peak late diastolic mitral inflow velocity
a'	Peak late diastolic tissue Doppler velocity
Ar	Atrial reversal
BMI	Body mass index (kg/m^2)
CAN	Cardiac autonomic neuropathy
CO	Cardiac output
CVD	Cardiovascular disease
DT	Deceleration time
E	Peak early diastolic mitral inflow velocity
e'	Peak early diastolic tissue Doppler velocity
ECG	Electrocardiogram
EF	Ejection fraction
FMD	Flow-mediated dilatation
HbA_{1c}	Glycosylated hemoglobin
HF	Heart failure
HIIE	High-intensity interval exercise training
HR_{max}	Maximum heart rate
IVRT	Isovolumic relaxation time
LA	Left atrium
LV	Left ventricular
MIE	Moderate-intensity exercise training
pw	Pulsed wave
S'	Peak systolic tissue Doppler velocity
SV	Stroke volume
T2D	Type 2 diabetes
TDI	Tissue Doppler imaging
UTR	Untwist rate
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake

Definitions and explanations

Aerobic capacity is physiologically determined by maximal oxygen uptake (VO_{2max}), work economy, and lactate threshold (Pate and Kriska, 1984).

Afterload is defined as the stress/tension developed in the left ventricle during blood ejection opposing resistance towards ejection. Afterload is dependent on LaPlace law (tension upon the myocytes in the cardiac wall multiplied by volume within the ventricle divided by wall thickness) and the aortic pressure/systemic vascular resistance (Støylen).

β-cells is a cell type in the islet of Langerhans of the pancreas that produces and secretes insulin (Holt et al., 2010).

Cardiorespiratory fitness is defined as the ability to perform dynamic whole body (large) muscle work at moderate- to high intensities over a (longer) period of time (Pate and Kriska, 1984). In the present thesis, cardiorespiratory fitness refers to $VO_{2max/peak}$.

Cardiovascular disease (CVD) is any disease to the heart and circulatory system, including coronary heart disease, valvular disease, cardiomyopathy, heart failure, arrhythmia, peripheral artery disease and cerebrovascular disease (stroke) (American Heart Association; World Health Organization).

Cardiometabolic risk (CMR) factors are factors that both increase the risk of developing cardiovascular and metabolic disease (i.e., T2D and metabolic syndrome) (Brunzell et al., 2008).

C-peptide (or connecting-peptide) is produced by the pancreatic cells during insulin synthesis. The level of C-peptide reflects insulin production (Holt et al., 2010).

Diastole is a phase of the cardiac cycle in which the heart fills with blood. Diastolic blood pressure is the minimum arterial pressure during relaxation and filling of the ventricles (Widmaier, 2004).

Diabetic cardiomyopathy is a structural heart disease in diabetes without symptoms of heart failure (Lam, 2015), which affects cardiac structure and function of the ventricle in the absence of a recognized cause, such as hypertension or coronary artery disease (Boudina and Abel, 2007; Nagueh et al., 2009). Diabetic cardiomyopathy is commonly recognized through diastolic dysfunction and left ventricular hypertrophy (Isfort et al., 2014).

Diastolic dysfunction refers to an abnormal ventricular relaxation and filling due to stiffening of the ventricle (Nagueh, 2003; Nagueh et al., 2009). Diastolic dysfunction in this thesis was defined as reduced peak early tissue Doppler velocity (e' < 8 cm/s).

Dyslipidemia reflects elevated lipid concentrations above normal blood values in the fasting and/or postprandial state (Solano and Goldberg, 2006).

Exercise training refers to planned, repetitive, structured, and purposeful physical activity with the objective of improving or maintaining aerobic capacity (World Health Organization, 2015).

Glucose is produced from carbohydrates, fat, and protein and is the major source of energy for living cells in the body. Without the help of insulin, cells cannot use glucose (Holt et al., 2010).

Glycosylated hemoglobin (HbA_{1c}) is glucose bound to hemoglobin. It reflects average blood glucose levels during a two- to three-month period and is a standard biomarker for glycemic control (American Diabetes Association, 2013).

Glucose transporter 4 (GLUT4) is a protein facilitating glucose transport across the plasma membrane of the myocytes (Holt et al., 2010).

Heart failure (HF) is defined as an abnormality of the heart's structure or function, which leads to an inability of the heart to pump enough blood to satisfy other organs' need for oxygen and substrate (McMurray et al., 2012). As defined by the American College of Cardiology (ACC)/American Heart Association (AHA) and the New York Heart Association (NYHA), HF includes patients within which clinical symptoms of HF are present; this includes dyspnea during less than ordinary physical activity and rest (Stage C-D and level II-IV, respectively; Table 1.2) (Lam, 2015; Yancy et al., 2013).

Hyperglycemia reflects elevated blood glucose levels above normal values, in the fasting (>5.7 mmol/L) and/or in the postprandial state (two-hour post-glucose load measured by oral glucose tolerance test ≥7.8 mmol/L) (Holt et al., 2010).

Impaired fasting glucose (IFG) or prediabetes refers to when fasting blood glucose is above normal range, but lower than the diagnostic threshold for type 2 diabetes: 5.6 to 6.9 mmol/L) (Holt et al., 2010).

Impaired glucose tolerance (IGT) refers to blood glucose values above normal range, but under the diagnostic threshold for diabetes after ingesting a standard amount of glucose in an oral glucose tolerance test (Holt et al., 2010).

Insulin is an anabolic hormone essential for life. The main action of insulin is to enable the cells to absorb glucose from the blood for energy production and to regulate utilization and storage of glucose and fat (Holt et al., 2010).

Insulin resistance is a condition in which insulin, in normal circulating concentrations, no longer produces the usual biological action that it does in healthy individuals (Holt et al., 2010).

Left ventricular compliance (ΔVolume/ΔPressure) or inversely LV stiffness (ΔPressure/ΔVolume); refers to the left ventricle's ability to stretch (dispensability of the ventricle) in end-diastole (Nagueh et al., 2009).

Left ventricular contractility is the ability to generate force/the intrinsic strength of the ventricle (Støylen).

Left ventricular elastance refers to the left ventricle's ability to return to its original form/recoil after pressure removal (Støylen). **Elastic recoil** is generated due to the restoring forces, which are built up from the preceding systole; i.e., cardiac untwist in early diastole creates recoil and thus assists filling through the generation of suction (Nagueh et al., 2009).

Left ventricular relaxation is "the process whereby the myocardium returns after contraction to its unstressed length and force. (…) Contraction and relaxation belong to the same molecular process of transient activation of the myocyte and are closely intertwined" (Nagueh et al., 2009).

Left ventricular twist, untwist is defined as the difference between the basal and apical rotation, it increases with the distance from the base to the apex, and it is reported in degrees (Buchalter et al., 1990). The temporal derivative of twist is referred to the twisting and untwisting rate and is reported in degrees/s. The rate of untwisting is often referred to as the recoil rate in the literature. **Left ventricular torsion** is twist normalized to the distance between the image planes (degrees/cm) (cannot be measured by 2D echocardiography).

Maximal oxygen uptake (VO_{2max}) and peak oxygen uptake (VO_{2peak}) refer to the highest rate at which an individual can transport and utilize oxygen during severe dynamic work with

large muscle mass (Bassett and Howley, 2000). VO_{2max} is commonly expressed in litres (volume) per minute or relative to body mass or in millilitres of oxygen per kilogram of body mass per minute (ml/kg/min), but it can also be expressed through allometric scaling ($ml/kg^{0.75}$/min) to adjust for the effect of body dimensions (Åstrand, 2003). VO_{2peak} can be used as term instead of VO_{2max} because maximal effort does not necessarily always give a levelling-off in VO_2 (which defines VO_{2max}), in spite of increased workload (Balady et al., 2010).

Metabolic syndrome is a condition in which at least three out of the following five cardiometabolic risk factors are present: hyperglycemia (>5.6 mmol/L); obesity (in particular central obesity: in Europe defined as waist circumference of >94 and 80cm for men and women, respectively, or else defined by population and country specifications); hypertension (defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg); increased triglycerides (>1.7 mmol/L); and low high-density lipoprotein cholesterol (< 1.0 mmol/L and <1.3 mmol/L in men and women, respectively) (Alberti et al., 2009).

Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α is a key regulator of energy metabolism and an important transcriptional coactivator in mitochondrial biogenesis (Lin et al., 2005).

Physical activity is any bodily movement produced by the skeletal muscles resulting in an increase of energy expenditure from resting levels (World Health Organization, 2015).

Postprandial means “after a meal”.

Preload is the pressure that stretches the left ventricular wall (end-diastolic volume) in end-diastole before the start of contraction (Nagueh et al., 2009; Støylen).

Systole is the phase of the cardiac cycle in which the heart ejects blood. Systolic blood pressure refers to peak pressure in the arteries during systole (Widmaier, 2004).

Total left ventricular load (or load) refers to the force the left ventricle has to overcome to contract (preload + afterload) (Støylen).

References (Definitions and explanations):

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . International Association for the Study of, O. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation, 120*(16), 1640-1645.

American Diabetes Association. (2013). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 36 Suppl 1*, S67-74.

American Heart Association. (2015.04.28). What is cardiovascular disease? Retrieved from http://www.heart.org/HEARTORG/Caregiver/Resources/WhatisCardiovascularDisease/What-is-Cardiovascular-Disease_UCM_301852_Article.jsp Accessed: May 2015

Balady, G. J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G. F., . . . Outcomes, R. (2010). Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation, 122*(2), 191-225.

Bassett, D. R., Jr., & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med. Sci. Sports Exerc., 32*(1), 70-84.

Boudina, S., & Abel, E. D. (2007). Diabetic cardiomyopathy revisited. *Circulation, 115*(25), 3213-3223.

Brunzell, J. D., Davidson, M., Furberg, C. D., Goldberg, R. B., Howard, B. V., Stein, J. H., . . . American College of Cardiology, F. (2008). Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care, 31*(4), 811-822.

Buchalter, M. B., Weiss, J. L., Rogers, W. J., Zerhouni, E. A., Weisfeldt, M. L., Beyar, R., & Shapiro, E. P. (1990). Noninvasive quantification of left ventricular rotational deformation in normal humans using magnetic resonance imaging myocardial tagging. *Circulation, 81*(4), 1236-1244.

Holt, R. I. G., Cockram, C. S., Flyvbjerg, A., & Goldstein, B. J. (2010). *Textbook of Diabetes. -4th ed.* (4 ed.): Blackwell Publishing Ltd.

Isfort, M., Stevens, S. C., Schaffer, S., Jong, C. J., & Wold, L. E. (2014). Metabolic dysfunction in diabetic cardiomyopathy. *Heart Fail. Rev., 19*(1), 35-48.

Lam, C. S. (2015). Diabetic cardiomyopathy: An expression of stage B heart failure with preserved ejection fraction. *Diab. Vasc. Dis. Res.*

Lin, J., Handschin, C., & Spiegelman, B. M. (2005). Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab., 1*(6), 361-370.

McMurray, J. J., Adamopoulos, S., Anker, S. D., Auricchio, A., Bohm, M., Dickstein, K., . . . Guidelines, E. S. C. C. f. P. (2012). ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment

of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur. Heart J., 33*(14), 1787-1847.

Nagueh, S. F. (2003). Search for non-invasive load-independent indices of left ventricular relaxation. *Clin. Sci. (Lond.), 105*(4), 395-397.

Nagueh, S. F., Appleton, C. P., Gillebert, T. C., Marino, P. N., Oh, J. K., Smiseth, O. A., . . . Evangelisa, A. (2009). Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur. J. Echocardiogr., 10*(2), 165-193.

Pate, R. R., & Kriska, A. (1984). Physiological basis of the sex difference in cardiorespiratory endurance. *Sports Med., 1*(2), 87-98.

Solano, M. P., & Goldberg, R. B. (2006). Management of dyslipidemia in diabetes. *Cardiol. Rev., 14*(3), 125-135.

Støylen, A. Strain rate imaging. Retrieved from http://folk.ntnu.no/stoylen/strainrate/ Accessed: May, 2015

Widmaier, E. P. R., H,;Strang, K.T. (2004). *Human Physiology* (9th ed.): McGraw-Hill Companies, Inc.

World Health Organization. Definition of cardiovascular diseases. Retrieved from http://www.euro.who.int/en/health-topics/noncommunicable-diseases/cardiovascular-diseases/cardiovascular-diseases2/definition-of-cardiovascular-diseases Accessed: May, 2015

World Health Organization. (2015). Physical activity. *Global Strategy on Diet, Physical Activity and Health.* Retrieved from http://www.who.int/dietphysicalactivity/pa/en/ Accessed: May, 2015

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E., Jr., Drazner, M. H., . . . American Heart Association Task Force on Practice, G. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol., 62*(16), e147-239.

Åstrand, P. O. R., K. (2003). *Textbook of work physiology - physiological bases of exercise* (Fourth Edition ed.): Human Kinetics.

CHAPTER 1

Background and Introduction

TABLE OF CONTENTS

CHAPTER 1

1.1 BACKGROUND

1.1.1 The global burden of diabetes

In 2014, 422 million people worldwide had diabetes and the prevalence is expected to rise to 592 million people by year 2035 (International Diabetes Federation, 2014; World Health Organization, 2016) (Figure 1.1). Furthermore, it is predicted to become the world's seventh-leading cause of death by the year 2030 (World Health Organization, 2016). Annually, approximately five million people die from diabetic complications (International Diabetes Federation, 2013, 2014).

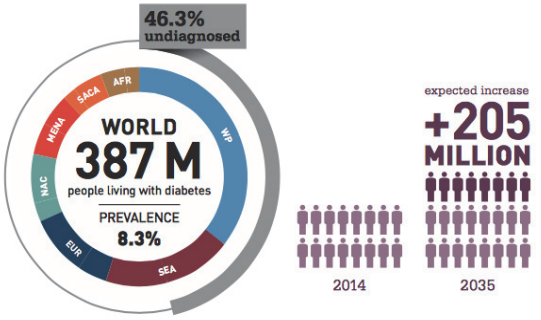


Figure 1.1 Diabetes prevalence (2014) and its expected rise by 2035. With permission from the International Diabetes Federation (IDF): idf.org/diabetesatlas

The increasing diabetes epidemic is due to the increased prevalence of type 2 diabetes (T2D) in particular (International Diabetes Federation, 2013). Currently, 90–95% of individuals with diabetes have T2D (Mozaffarian et al., 2015).

This disease develops gradually, and many are diagnosed several years after onset, when diabetic micro- and macro-vascular complications are already present (Holt et al., 2010). Cardiovascular disease (CVD) stands out as a major complication in T2D (Buse et al., 2007; Fox et al., 2007; Ryden et al., 2013) and is the leading cause of disease and death in this population (International Diabetes Federation): Compared to people without diabetes, individuals with T2D have two times the greater risk of cardiovascular mortality and a two- to five times higher risk of developing heart failure (HF) (Go et al., 2014; Kannel et al., 1974; Nichols et al., 2004). Management methods to reduce CVD risk factors should thus be targeted in T2D (Go et al., 2014; Ryden et al., 2013).

1.1.2 Type 2 diabetes

1.1.2.1 The characteristics and classification of type 2 diabetes

T2D is characterized by hyperglycemia due to the failure of the β-cells with increasing insulin resistance in insulin-sensitive tissues (in particular, skeletal, muscle, and liver tissues) (Holt et

al., 2010; Kahn et al., 2014). Insulin resistance in the context of glucose metabolism leads to reduced muscle glucose uptake and increased release of glucose from the liver, which, in turn, results in hyperglycemia (Figure 1.2), both in the fasting and postprandial states.

Therefore, T2D is diagnosed by measuring either fasting blood glucose, glycosylated hemoglobin (HbA_{1c}), and/or a 75g oral glucose load in a glucose tolerance test; one of the criteria shown in Table 1.1 must be fulfilled in order to make a diagnosis of T2D.

The molecular mechanisms leading to insulin resistance are not entirely understood, and whether this entity leads to β-cell dysfunction or vice versa is a subject of debate. Nevertheless, insulin resistance is associated with lipid accumulation in the muscles and liver, altered function, and number of insulin receptors and glucose transporters (i.e. GLUT4) in skeletal muscle, impaired glycogen synthesis, impaired ability to suppress liver glucose production, very low-density lipoprotein production (hypertriglyceridemia and low levels of high-density lipoprotein), an impaired ability to suppress lipolysis, adiponectin deficiency, inflammation in the adipose tissue, and oxidative stress (due to persistent hyperglycemia), as well as chronic hyperglycemia per se (Holt et al., 2010; Patel et al., 2015).

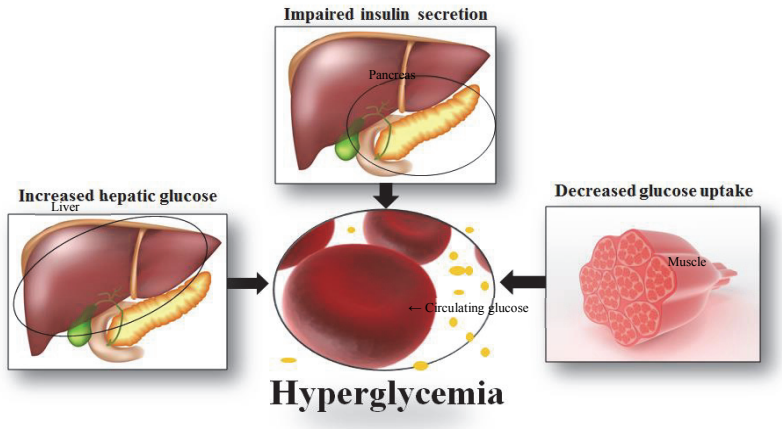


Figure 1.2. Core deficits in type 2 diabetes. Insulin resistance, especially in muscle and hepatic tissue, contributes to hyperglycemia in addition to impaired insulin secretion. Illustrations of muscle, liver, blood cells and pancreas are purchased from http://www.dreamstime.com. Figure is composed by SMH.

Table 1.1 The clinical definition of type 2 diabetes.

Measurement	Cut point	Comment
HbA_{1c}	≥6.5%	(Impaired fasting glucose, IFG: 5.7–6.4%)
Fasting plasma glucose (FPG)	≥7.0 mmol/L ≥126 mg/dL	Fasting defined as no energy-intake <8 hours. (IFG: 5.6–6.9 mmol/L or 100–125 mg/dl.)
2-h plasma glucose	≥11.1 mmol/L ≥200 mg/dL	During oral glucose tolerance test. (Impaired glucose tolerance, IGT: 7.8–11.0 mmol/L or 140–199 mg/dl.)
Random plasma glucose	≥11.1 mmol/L ≥200 mg/dL	When classic symptoms of hyperglycemia or hyperglycemic crisis. (Confirmed by repeated testing if absence of unequivocal hyperglycemia.)

HbA_{1c}, glycosylated hemoglobin. One criterion must be fulfilled. Test procedures are to be conducted according to WHO criteria. Modified after Executive Summary: Standards of Medical Care in Diabetes- 2014 (American Diabetes Association, 2014; Holt et al., 2010).

1.1.2.2 Type 2 diabetes management

The precise causes of T2D are not fully known, but they seem to be a complex interplay of genetic, environmental and behavioral factors (such as physical inactivity, high-fat diets and excess body weight) (Holt et al., 2010), which are also risk factors for CVD, commonly referred to as cardiometabolic risk (CMR) factors (Figure 1.3) (Brunzell et al., 2008; Mozaffarian et al., 2015).

To reduce CMR and thus microvascular and macrovascular complications, the primary goal in T2D management is to improve glycemic control (goal $HbA_{1c} \leq 7\%$) (American Diabetes Association, 2014). For most T2D individuals, the disease requires continuous medical care, initially by oral antidiabetic agents (i.e. metformin) and eventually by insulin administration. Indeed, in the early stages of the disease, some T2D patients manage to achieve glycemic control through lifestyle interventions only (i.e. physical activity, nutrition therapy, weight loss) and it is established that lifestyle intervention can protect and postpone the development of T2D in high-risk individuals (Knowler et al., 2002; Pan et al., 1997; Tuomilehto et al., 2001). Nonetheless, T2D management requires multifactorial risk reduction strategies, also beyond glycemic control. In addition to pharmaceutical interventions, physical activity, reduced energy intake, improved diet composition and weight loss are considered cornerstones in T2D management (American Diabetes Association, 2014; Leiter, 2011).

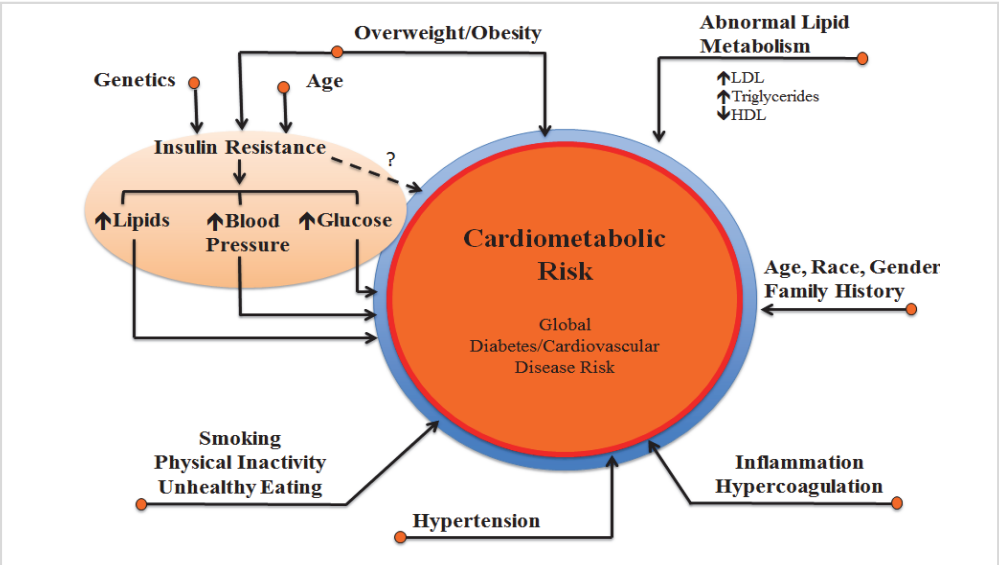


Figure 1.3. Factors contributing to cardiometabolic risk.
Modified after Brunzell et al., Diabetes Care®, Vol. 31, 2008; 811–822. Copyright 2008 American Diabetes Association (ADA). Reprinted with permission from ADA.

Exercise as therapy

In general, physical inactivity has emerged as a major risk factor for many metabolic disorders and is associated with CMR factor clustering, including T2D, insulin resistance, obesity, hypertension, hyperglycemia, and dyslipidemia (Aspenes et al., 2011; Byrkjeland et al., 2014; Earnest et al., 2013; Hawley and Lessard, 2008; Lakka et al., 2003; LaMonte et al., 2005; Laukkanen et al., 2001; Paffenbarger et al., 1993; Sassen et al., 2009; Sawada et al., 2010; Snowling and Hopkins, 2006).

An extensive amount of evidence indicates the correlation between physical activity levels and cardiovascular mortality in individuals with T2D (Hu et al., 2005; Kokkinos et al., 2009; Reddigan et al., 2012; Sluik et al., 2012; Tanasescu et al., 2003; Zethelius et al., 2014). For instance, a recent Swedish study (Zethelius et al., 2014) showed that self-reported inactive individuals with T2D (mean age of 60 years) who were followed for five years had a 25% to 70% increased risk of CVD and premature mortality compared to active individuals with T2D. In line with these observations, increased physical activity levels have been shown to be associated with reduced all-cause mortality and reduced cardiovascular risk in this patient group (Kodama et al., 2013; Moe et al., 2015), and any physical activity seems to be better than none at all (Kodama et al., 2013).

Overall, it is widely accepted that physical activity (i.e. exercise training) has positive effects in terms of prevention and treatment in T2D (Reddigan et al., 2012; Sluik et al., 2012). Exercise has the potential to improve a number of factors contributing to CMR, such as (but not limited to): HbA_{1c} (Boule et al., 2001; Boule et al., 2003; Sigal et al., 2007; Snowling and Hopkins, 2006); insulin sensitivity (Braun et al., 1995; Hawley and Lessard, 2008; Winnick et al., 2008); β-cell function (Dela et al., 2004; Lee et al., 2015); body weight (Ross et al., 2000; Ross et al., 2004); blood pressure (Green et al., 2008; Pi-Sunyer et al., 2007; Wing et al., 2013); dyslipidemia (Halverstadt et al., 2007; Kelley and Kelley, 2007); oxidative stress (Hafstad et al., 2013; Vinetti et al., 2015); and chronic inflammation (Hayashino et al., 2014).

Of direct clinical importance in regard to physical activity in T2D is its potential to improve glycemic control (i.e. to reduce HbA_{1c}) to an extent similar to pharmaceutical therapy (Snowling and Hopkins, 2006). Indeed, exercise training interventions (≥ 8 weeks) may improve HbA_{1c} by an average of 0.66 percentage points in individuals with T2D, independent of changes in BMI (Boule et al., 2001) (Further discussed in section 1.1.6.2). However, the effects of physical activity on overall mortality and cardiovascular risk exceed those that can be explained by reduced glucose (Church et al., 2004; Reddigan et al., 2012; Sluik et al., 2012; Wei et al., 2000). In line with this, exercise training seems as beneficial in reducing CMR as statin treatment (Ridker et al., 2008; Wilt et al., 2004), even though exercise appears to yield only modest improvements in circulating lipids (Green et al., 2008; Halverstadt et al., 2007). Similarly, exercise has been shown to exert only modest effects on blood pressure when compared to medications (Blood Pressure Lowering Treatment Trialists et al., 2008; Green et al., 2008). Furthermore, intervention trials have shown that moderate- to high-intensity exercise (~150 min per week) substantially decreases the number of at-risk individuals who actually develop T2D over a 5-to-10-year follow-up period (Lindstrom et al., 2006). Moreover, exercise seems to be better than medication for the prevention of the development of T2D in high-risk individuals (Knowler et al., 2002; Tuomilehto et al., 2001). Indeed, less than half of the 40% risk reduction, due to physical activity observed in the Nurse's Health Study (Mora et al., 2007), could be attributed to improvements in traditional risk factors, such as HbA_{1c}/T2D, BMI, hypertension, and lipids. In summary, this indicates that there are also other important mechanisms at play, which contribute to the observed CVD risk reduction induced by physical activity (i.e. exercise training). Among these are cardiorespiratory fitness (further discussed in section 1.1.5)

1.1.3 Diabetic cardiomyopathy

1.1.3.1 Risk of heart failure in type 2 diabetes

T2D is an independent risk factor for the development of HF (de Simone et al., 2010; Iribarren et al., 2001; Kannel et al., 1974; Kannel and McGee, 1979; Nichols et al., 2004; Nichols et al., 2001; Shindler et al., 1996), which is increasing in prevalence (Holt et al., 2010; Horwich and Fonarow, 2010; Ryden et al., 2013). Furthermore, the incidence of HF in individuals with T2D is high (Boonman-de Winter et al., 2012; He et al., 2001; Yancy et al., 2013). In the general population, 1% to 4% has HF, and 0.3% to 0.5% has both HF and T2D (Catapano et al., 2011). Moreover, while the global prevalence of diabetes is 8.5% (International Diabetes Federation, 2014), the prevalence of diabetes in individuals with HF is 12% to 13%, with increased prevalence up to 40% in patients who are hospitalized (MacDonald et al., 2008; Whiting et al., 2011). The association between T2D and HF is due to diabetic cardiomyopathy (Ernande et al., 2011; Ichikawa et al., 2013; Nichols et al., 2004; Redfield et al., 2003; Ryden et al., 2013; Stefanidis et al., 2009).

1.1.3.2 Diabetic cardiomyopathy

As a general term, cardiomyopathy describes a myocardial dysfunction caused by structural and functional alterations of the myocardium. Cardiomyopathy can be caused by many conditions, such as coronary heart disease and hypertension, in particular, but metabolic disease is also listed as a risk factor (Fang et al., 2004; Horwich and Fonarow, 2010). As defined by the American College of Cardiology (ACC), the AHA, and the New York Heart Association (NYHA), cardiomyopathy is seen in individuals who have structural and functional heart disease but no current or prior symptoms of HF (stage A-B and level I, respectively; Table 1.2). Thus, cardiomyopathy is classified as a risk factor for HF (Yancy et al., 2013).

Even though the increased risk of CVD and mortality in T2D is partly accelerated due to atherosclerosis, substantial epidemiological and clinical research indicate that diabetes increases the risk for cardiac dysfunction and HF independent of other risk factors, such as coronary artery disease and hypertension. The term “diabetic cardiomyopathy” thus describes a specific myocardial dysfunction characterized by structural (increased left ventricular [LV] mass and wall thickness) and functional myocardial alterations, which are not related to coronary artery disease, hypertension, or other heart disease (Boudina and Abel, 2007, 2010; Devereux et al., 2000; Fang et al., 2004; Marwick, 2008). It was first described in 1972 when four T2D cases with HF and no coronary artery disease were present (Rubler et al., 1972).

Table 1.2: Presentation of ACC/AHA stages of HF and the NYHA functional classification.

ACC/AHA stages of HF (Hunt et al., 2009)		NYHA functional classification	
A	At high risk for HF but without structural heart disease or symptoms of HF (i.e. T2D (Lam, 2015))	None	
B	Structural heart disease but without signs or symptoms of HF (i.e. asymptomatic diastolic dysfunction (Lam, 2015))	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF.
C	Structural heart disease without prior or current symptoms of HF	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF.
		II	Slight limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in symptoms of HF.
		III	Marked limitation of physical activity. But less than ordinary physical activity results in symptoms of HF.
		IV	Unable to carry out physical activity without symptoms of HF or symptoms of HF at rest.
D	Refractory HF requiring specialized interventions	IV	Unable to carry out physical activity without symptoms of HF or symptoms of HF at rest.

Modified from Table in Yancy et al. (2013). Abbreviations: ACC, American College of Cardiology; AHA, American Heart Association; HF, Heart failure; NYHA, New York Heart Association

1.1.3.3 Diastolic dysfunction

Although diabetic cardiomyopathy describes a progression of heart disease, the earliest signs of this entity are subclinical structural and functional abnormalities. In apparently healthy, normotensive, asymptomatic (absence of traditional symptoms of CVD), well-controlled (i.e. glucose control) individuals with T2D, signs of diabetic cardiomyopathy are commonly present as subclinical LV diastolic dysfunction (Fang et al., 2005; Hordern et al., 2009; Zabalgoitia et al., 2001), which may precede the development of systolic dysfunction (Bell, 2003; Liu et al., 2001). Even though other studies have found that systolic alterations precede diastolic impairment (Ernande et al., 2011; Faden et al., 2013), LV diastolic dysfunction is nevertheless considered a hallmark feature of diabetic cardiomyopathy (Fang et al., 2004).

Typically, diastolic dysfunction is evidenced by impaired LV relaxation and pseudonormal filling pressure is measured by conventional and tissue Doppler echocardiography (Boudina

and Abel, 2010; Nagueh et al., 2009). The diagnostic procedure to identify diastolic dysfunction is complex and many considerations are taken into account before the diagnosis is established. In the present thesis, however, diastolic dysfunction was defined as mean peak early diastolic tissue Doppler velocity (e') (i.e. impaired relaxation) < 8 cm/s. This cut-off point was based on the findings of Von Bibra et al. who reported that T2D individuals (n=43; 60.6±7 years; both with and without evidence of coronary artery disease) had compromised relaxation measured as mean e' (in septal, anteroseptal, anterior, lateral, posterior and inferior walls) compared to controls at rest (8.5+1.7 vs. 9.6+1.9 cm/sec, respectively; p<0.02) (Von Bibra et al., 2005).

In general, studies performing Doppler echocardiography on individuals with T2D without overt coronary artery disease, have revealed a prevalence of diastolic dysfunction (i.e. impaired relaxation and pseudonormal filling) of 20% to 60% (Di Bonito et al., 2005; Fang et al., 2005; Hare et al., 2011; Poirier et al., 2001; Redfield et al., 2003; Von Bibra et al., 2005; Zabalgoitia et al., 2001). Indeed, a study performing tissue Doppler imaging reported LV diastolic dysfunction, measured as e' < 8cm/s (in septal or lateral walls), in up to 75% of individuals with T2D (n=57; mean 49 years, range 31 to 59 years) (Boyer et al., 2004). However, the latter might be an over-estimation as e' decreases with age (Dalen et al., 2010; Nikitin et al., 2003). Nonetheless, Fang et al. demonstrated reduced diastolic function (e') in 27% of T2D individuals (n=120; 57+/-10 years) after adjusting for age and gender (Fang et al., 2005), which overall indicated that it was reasonable to expect diastolic dysfunction to be present in at least 20%–30% of patients with T2D.

In addition to age, diastolic function is dependent on several clinical parameters, such as gender, glycaemic control, blood pressure, diabetes medication, adiposity, and insulin resistance. An association between reduced diastolic function (measured as e') and increased age, presence of hypertension, absence of insulin, and presence metformin treatment has been reported (Fang et al., 2005). The latter indicates adverse effects of oral anti-hyperglycemic agents. This is in contrast with the Strong Heart Study finding that individuals with diabetes and diastolic dysfunction (i.e. abnormal relaxation) had elevated fasting glucose and HbA_{1c} compared to those with normal diastolic function (Liu et al., 2001). Furthermore, the association between diastolic dysfunction and insulin resistance in overweight individuals with essential hypertension indicates a role in the development of diabetic cardiomyopathy (Galderisi et al., 1997; Guida et al., 2001). The duration of T2D does not seem significant to the development of diastolic dysfunction as in the development in microvascular disease. In fact, diastolic dysfunction has been observed in individuals with recent onset T2D (Boyer et al., 2004).

The clinical significance of diastolic dysfunction is related to its association with increased risk of CVD. The presence of subclinical LV dysfunction in T2D is associated with increased mortality and risk of HF (From et al., 2010). Even mild diastolic dysfunction in T2D is associated with a fivefold higher mortality compared to individuals with diastolic dysfunction and no T2D (Redfield et al., 2003).

1.1.3.4 Pathogenesis of diabetic cardiomyopathy

The development of diabetic cardiomyopathy is complex and most likely multifactorial and induced by a range of systemic changes. Several hypotheses have been proposed as mechanisms responsible for the functional and structural derangements leading to diabetic cardiomyopathy, such as (but not limited to) the following (Figure 1.4):

Metabolic disturbances

The metabolic disturbances characterized by insulin resistance may be an important contributor to the development of diabetic cardiomyopathy.

Shift in cardiac substrate utilization

In experimental trials, the shift in substrate metabolism in T2D has been associated with systolic and diastolic dysfunction (Hafstad et al., 2013; Lopaschuk and Russell, 1991). Several mechanisms are suggested to cause the shift in cardiac substrate utilization in the diabetic heart (Figure 1.4).

As for the whole body, insulin resistance can affect cardiac metabolism with alterations in insulin-stimulated glucose uptake, glycolytic rate, glucose oxidation, and glycogen synthesis as well as free fatty-acid metabolism (Abel, 2005; Boudina and Abel, 2010; Hafstad et al., 2015; Holt et al., 2010; Patel et al., 2015). The alterations in glucose utilization is proposed in particular to be due to reduced myocardial GLUT4 content and altered GLUT 4 translocation (Berger et al., 1989; Buchanan et al., 2005; Camps et al., 1992; Eckel and Reinauer, 1990; Garvey et al., 1993; Russell et al., 1998; Sivitz et al., 1989). Indeed, patients with T2D and HF have shown to have greater down regulation of GLUT4 than patients without diabetes and HF (Razeghi et al., 2002). Increased circulating glucose may contribute to changes in nonoxidative glucose pathways, resulting in, for example, increased levels of glycation end-products (AGEs), which may lead to myocardial stiffness (Candido et al., 2003). Increased AGE production may subsequently contribute to increased production of cytokines, reactive oxygen species (ROS) and reduced nitric oxide (NO) bioavailability (Goldin et al., 2006). Thus, as oxidative stress

seems to be important in the development of vascular dysfunction in T2D, it appears as if this also has a role in the development of diabetic cardiomyopathy (Candido et al., 2003).

Furthermore, the high levels of circulating fatty acids and the subsequent increased fatty acid oxidation suppress glucose oxidation (Liedtke et al., 1988; Wright et al., 2009). The reduced glucose utilization in the diabetic heart (10% in diabetes versus 30% in normal myocardial energy production) results in a concordant increase in free fatty-acid oxidation, which subsequently increases myocardial oxygen consumption, compared to the normal heart (Abel et al., 2008; Boardman et al., 2009; Boudina and Abel, 2007, 2010; Bugger and Abel, 2008; Hafstad et al., 2015; Holt et al., 2010).

The increased myocardial oxygen consumption due to increased fatty-acid oxidation has been shown to reduce cardiac efficiency (ratio of energy output [cardiac work] to energy input [myocardial oxygen consumption]) in T2D (Boardman et al., 2009; How et al., 2006; Mazumder et al., 2004; Peterson et al., 2008; Peterson et al., 2004). However, the mechanisms leading to cardiac inefficiency in cardiac dysfunction are not clear and can be a result of several factors in addition to oxygen wasting, due to elevations in circulating fatty acids, such as mechanisms related to Ca^{2+} handling and oxidative stress in cardiac tissue (Hafstad et al., 2015). Nonetheless, it is yet to be determined whether reduced cardiac efficiency promotes diabetic cardiomyopathy.

Alterations in calcium (Ca^{2+}) *homeostasis*

Alterations in intracellular Ca^{2+} homeostasis are also believed to contribute to diabetic cardiomyopathy in T2D (Figure 1.4). The abnormal metabolism characteristic for T2D may contribute to cardiac contractile dysfunction as well as impaired myocardial relaxation through impaired sarcoplasmatic reticulum (SR) function with altered intracellular Ca^{2+} handling and sensitivity (Malhotra and Sanghi, 1997). Impaired Ca^{2+} homeostasis has been associated with both diastolic and systolic dysfunction (Stolen et al., 2009; Van den Bergh et al., 2008). For example, Stølen et al. showed increased SR Ca^{2+} leak and reduced synchrony of Ca^{2+} release, transverse-tubule density, peak systolic and diastolic Ca^{2+} release as well as reduced SR Ca^{2+} ATPase–mediated Ca^{2+} uptake during diastole in mice with diabetic cardiomyopathy (Stolen et al., 2009).

Also, reduced adenosine triphosphate (ATP) availability in cytosol is associated with impaired Ca^{2+} handling and relaxation of cardiomyocytes (Diamant et al., 2003).

Mitochondrial dysfunction

Mitochondrial dysfunction has been suggested to be an important contributor to the development of diabetic cardiomyopathy (Figure 1.4). Overall, the metabolic abnormalities in T2D can result in reduced mitochondrial capacity and subsequently a net reduction in ATP availability in the myocardium. This is proposed to result in "an energetically compromised heart with reduced working capacity" (Hafstad et al., 2015).

Changes in cardiac mitochondrial form, structure, respiratory capacity, and reduced number of mitochondria has been observed in rodent models with T2D (Boudina et al., 2005; Boudina et al., 2007). Furthermore, reduced mitochondrial oxygen consumption has been found in individuals with diabetes having coronary artery bypass surgery (Anderson et al., 2009). Insulin resistance appears to contribute to reduced levels of insulin-signaling proteins in the cardiomyocytes, such as peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α (see Definitions and explanations) (Huo and Scarpulla, 2001) and thus regulate the production of ATP in the mitochondria (Mootha, 2003; Patti et al., 2003; Russell et al., 2004). Indeed, insulin has shown to acutely regulate mitochondrial metabolism by increasing ATP-levels and oxygen consumption in cardiomyocytes (Parra et al., 2014).

Moreover, reduced cardiac energetics (low creatine phosphate/ATP-ratio) because of cardiac mechanical inefficiency is suggested to contribute to diabetic cardiomyopathy. An imbalance between substrate availability and demand can, as noted above, lead to reduced cardiac work capacity. A decrease in cardiac energetics in individuals with diabetes and those who are obese without overt coronary artery disease has been observed (Diamant et al., 2003; Scheuermann-Freestone et al., 2003), although not in all studies (Rijzewijk et al., 2009). Reduced myocardial creatine phosphate to ATP ratio can, together with the factors listed under Metabolic disturbances (Shift in cardiac substrate utilization) in the present section, contribute to reduced ATP availability in the sarcomeres that mitochondrial ATP production cannot compensate for (Lamb et al., 1999).

Structural changes

Myocardial fibrosis

Myocardial fibrosis is one of the most commonly proposed mechanisms of diabetic cardiomyopathy (Figure 1.4). In the past decades, autopsy studies have identified increased presence of myocardial fibrosis in individuals with T2D (Campbell et al., 2011; Factor et al., 1980; Rubler et al., 1972; Shimizu et al., 1993; Sunni et al., 1986; van Hoeven and Factor,

1990). However, the studies in humans are few, small, and performed on individuals with end-stage disease, which does not allow for a separation of the effect of T2D versus comorbidities such as coronary artery disease and/or HF. Nevertheless, diastolic dysfunction in T2D individuals without overt CVD has been associated with serum markers of collagen biosynthesis, which suggest that myocardial fibrosis has a role in diastolic dysfunction in this patient group (Gonzalez-Vilchez et al., 2005; Ihm et al., 2007). Furthermore, studies on animal models have also reported findings of increased myocardial fibrosis in pre-diabetes as well as T2D (Mizushige et al., 2000). A recent experimental study observed myocardial fibrosis in both T2D and type 1 diabetes, but the fibrosis in the type 1 diabetes models were significantly more pronounced compared to the T2D models (Radovits et al., 2015). However, myocardial fibrosis has not been observed in all experimental models (Van den Bergh et al., 2008).

The mechanisms contributing to increased myocardial fibrosis in T2D are not completely understood. Hyperglycemia, resulting in oxidative stress and cell death has been proposed as one mechanism that may explain the development of myocardial fibrosis (Frustaci et al., 2000). Furthermore, lack of insulin receptors in the cardiomyocytes (McQueen et al., 2005), decreased levels of insulin-like growth factor (IGF) (Kajstura et al., 2001), and increased expression of connective tissue growth factor and transforming growth factor (Ban and Twigg, 2008; Lee et al., 1995; Way et al., 2002) have been suggested to contribute to myocardial fibrosis and thus myocardial stiffness.

Cell death

Cell death due to apoptosis and/or necrosis is suggested to contribute to diabetic cardiomyopathy (Figure 1.4). In myocardial biopsies from individuals with T2D and HF, necrotic and apoptotic cell death in the cardiac cell has been found in cell populations that were not affected by myocardial ischemia (Frustaci et al., 2000). In subjects with T2D and hypertension, necrotic cell death, but not apoptosis, was further increased (Frustaci et al., 2000). Experimental studies have also reported increased apoptosis in cardiomyocytes with obesity and T2D (Barouch et al., 2006) as well as increased collapse of myofibers and myocardial degeneration in T2D (Radovits et al., 2015).

Although the mechanisms for cell death are not entirely understood, several mechanisms have been suggested to cause this, and thus the pathogenesis of diabetic cardiomyopathy: For example, oxidative stress due to hyperglycemia (or impaired antioxidant defense), oxidative stress or Lipotoxicity due to lipid accumulation (increased fatty acid oxidation) (Boudina and

Abel, 2010; Wende and Abel, 2010) as well as increased oxidative stress, apoptosis and necrosis due to activation of the renin-angiotensin system (RAS) (Dhalla et al., 1998; Frustaci et al., 2000) have been suggested to cause myocardial fibrosis and thus myocardial stiffness.

Intra-myocardial lipids

Lipid accumulation in the myocardium has also been proposed as a mechanism that partly explains diabetic cardiomyopathy (Figure 1.4). Indeed, T2D has been associated with lipid accumulation in the myocardium (McGavock et al., 2007; Rijzewijk et al., 2008). Furthermore, intramyocardial triglyceride has been associated with reduced diastolic function, but not systolic dysfunction (McGavock et al., 2007; Rijzewijk et al., 2008), which has been confirmed in mice (Christoffersen et al., 2003; Sharma et al., 2004). Although lipid accumulation in the myocardium is not considered toxic per se, it can contribute to accumulation of intermediate metabolites and thus lipotoxicity, which may increase fibrosis due to a number of mechanisms, such as apoptosis and activation of growth factors (Hafstad et al., 2015). However, whether lipid accumulation per se causes changes in cardiac function or whether these observations are simply a marker of the metabolic disturbances is unknown.

Pathological cardiomyocyte hypertrophy

Pathological LV hypertrophy can contribute to reduced cardiac compliance in subjects with T2D (Figure 1.4), independent of hypertension (Aneja et al., 2008). It has been found that individuals with T2D have a substantial (75^{th} percentile) greater risk of having increased LV mass compared to normal subjects, also after adjusting for hypertension and other covariates (Eguchi et al., 2008). The Strong Heart study observed greater LV mass and wall thickness in both men and women with diabetes (Devereux et al., 2000), whereas the Framingham study reported this in women with diabetes only (Galderisi et al., 1991). It appears as if changes in LV mass are a result of long standing T2D (Rerkpattanapipat et al., 2009). Several experimental studies confirm the findings of myocardial hypertrophy in humans. For example, Radovits et al. recently reported increased mean cardiomyocyte width in T2D rats compared to controls (Radovits et al., 2015).

Insulin signaling appears to act as a growth factor in the heart (Belke et al., 2002; Riehle et al., 2014) and insulin resistance and hyperinsulinemia have been correlated with increased LV mass (Karason et al., 2003). Evidence also suggests the role of cytokines, produced by adipose tissue, as a mechanism behind the development of LV hypertrophy; for example, leptin and resistin (adipose-derived hormones, similar to a cytokines, which are often associated with insulin

resistance) have been linked to cardiomyocyte hypertrophy, although the mechanisms behind this are still not fully known (Barouch et al., 2003; Kim et al., 2008; Xu et al., 2004).

Other proposed mechanisms

Small vessel disease

Small vessel disease (microangiopathy) (Figure 1.4) of the myocardium in subjects with T2D may cause reduced blood flow in the myocardium, cardiac hypertrophy, and cardiac dilatation (Adameova and Dhalla, 2014). Low myocardial blood flow is closely related to fasting glucose and HbA_{1c} levels (Meyer and Schwaiger, 1997; Yokoyama et al., 1998). Indeed, as for the whole body, chronic hyperglycemia or insulin resistance can induce a series of vascular changes, including reduced vascular sensitivity, depressed autonomic function, and reduced vascular wall compliance in the heart (Nitenberg et al., 1993; Strauer et al., 1997).

The characteristic of metabolic changes for T2D, which promotes oxidative stress and reduces NO bioavailability, in combination with alterations in the neurohormonal systems, contributes to the development of microangiopathy, resulting in endothelial dysfunction (Adameova and Dhalla, 2014; Bucala et al., 1991; Klein, 2008). Under normal conditions, insulin enhances NO production by the endothelium, which leads to vasodilation, increased blood flow, and improved glucose disposal in the skeletal muscle (Vakkilainen et al., 2000; Vehkavaara and Yki-Jarvinen, 2004; Westerbacka et al., 2004; Yki-Jarvinen and Utriainen, 1998). Endothelial function in both small and large vessels seems to be impaired in T2D individuals, even when the presence of atherosclerosis is considered low (Johnstone et al., 1993; Nitenberg et al., 1993). However, it is yet to be known to what extent small vessel disease plays a role in diabetic cardiomyopathy.

Cardiac autonomic neuropathy

Cardiac autonomic neuropathy (CAN) is also proposed to contribute to the development of diabetic cardiomyopathy (Boudina and Abel, 2007, 2010; Campbell et al., 2011; Fang et al., 2004; Hafstad et al., 2015) (Figure 1.4). It describes the impairment of the autonomic nervous system's ability to regulate factors such as heart rate (HR), cardiac performance (cardiac output and contractility), catecholamines, blood pressure and blood vessel dynamics. It furthermore causes several cardiac disorders, including resting tachycardia and silent myocardial ischemia. Insulin plays a role in, for example, muscle sympathetic nervous activity, level of norepinephrine release from sympathetic nerve endings in the tissues, and HR (insulin decreases vagal tone and increases sympathetic drive) (Berne et al., 1992; Patel et al., 2015)

and CAN has been associated with diastolic dysfunction in T2D (Poirier et al., 2003; Vanninen et al., 1992), independent of age, LV wall thickness, HbA1c, BMI, and present cardiovascular disease (Vanninen et al., 1992). However, the evidence supporting a relationship between CAN and the development of diabetic cardiomyopathy in T2D is scarce as most studies investigating CAN has been performed in T1D.

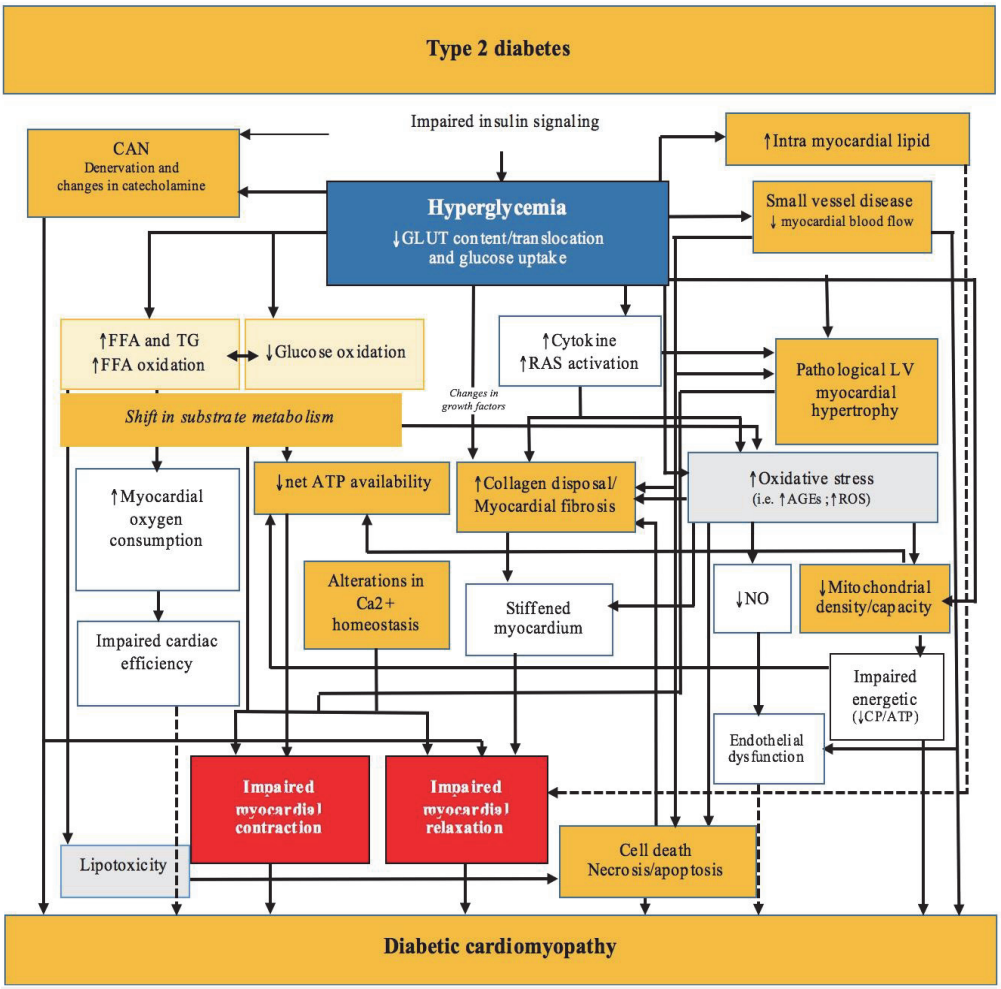


Figure 1.4. Proposed mechanisms for the development of diabetic cardiomyopathy in type 2 diabetes. Abbreviations: AGEs, Advanced glycemic end-products; ATP, adenosine triphosphate; Ca^{2+}, Calcium; CAN, Cardiac autonomic neuropathy; CP, creatine phosphate; FFA, free fatty acids; glucose transporter protein, GLUT; LA, left ventricular; NO, nitric oxide; RAS, renin angiotensin system; ROS, reactive oxygen species; TG, triglycerides. Modified after Falco-Pires and Leite-Moreira 2012 (Falcao-Pires and Leite-Moreira, 2012)

1.1.3.5 Controversies

Although there is evidence for the existence of diabetic cardiomyopathy (Fang et al., 2004; Horwich and Fonarow, 2010; Kannel et al., 1974; Lewis et al., 2009; Poornima et al., 2006) (and this condition has been confirmed by epidemiological, clinical, and experimental trials that have shown associations between T2D and HF, and diastolic dysfunction and structural and functional changes in the myocardium), the mechanisms listed in section 1.1.3.4 as being possibly responsible for this entity are controversial.

Most of the evidence for the possible mechanisms of diabetic cardiomyopathy outlined in the previous section (1.1.3.4) are found in diabetic animal models (Boardman et al., 2009; Candido et al., 2003; Connelly et al., 2009; Gross et al., 2004; Hayashi et al., 2003; Hsueh et al., 2007; Stolen et al., 2009). Large-scale studies would be ideal to confirm these findings in humans, however, this may not be possible as it would require biopsies of the human heart with subclinical heart disease. As the few biopsy studies in humans are performed on patients with, for example, coronary heart disease or heart failure, there is significant controversy with regard to whether diabetic cardiomyopathy is initiated by myocardial fibrosis or other alterations, such as cardiomyocyte hypertrophy and altered cardiac microvasculature function/structure, or whether these factors are simply a consequence of cardiac dysfunction (Campbell et al., 2011). In addition, it is established that untreated hypertension and/or ischemic heart disease may accelerate the development of a mild, subclinical diabetic cardiomyopathy and it is difficult to distinguish the additive roles of these common comorbidities in the development of diabetic cardiomyopathy. Moreover, silent ischemia complicates the diagnosis further. Finally, it is still unknown to what degree diabetic cardiomyopathy represents a cardiac pathology, as opposed to a pathology that predominantly affects the peripheral vasculature.

1.1.3.6 Prevention

Early diabetic cardiomyopathy appears to consist primarily of physiological adaptations to metabolic alterations and may at this stage be partly or completely reversible (Fang et al., 2004; Klein, 2008). However, as the dysfunction progresses, alterations in diastolic function seem to be more permanently compromised due to structural changes, with only limited capacity for repair (Fang et al., 2004; Klein, 2008). Therefore, as diabetic cardiomyopathy often evolves silently, early identification and intervention is critical in order to potentially delay or prevent the progression of a more permanent condition and ultimately the development of HF.

Exercise intervention approaches, which potentially can delay or prevent the development of diabetic cardiomyopathy, and reduce cardiac metabolic disturbances and CMR are discussed in the following sections of this chapter.

1.1.4 Cardiac adaptations to aerobic exercise training

A large number of molecular and signaling mechanisms underpin cardiac adaptations to exercise. In normal hearts, exercise is known to induce a range of cardioprotective molecular mechanisms (Golbidi and Laher, 2011; Powers et al., 2008). There are indications that endurance training has the potential to improve cardiac function in individuals with T2D (Friis Schmidt et al., 2013; Hordern et al., 2009; Sacre et al., 2014; Schrauwen-Hinderling et al., 2011) and beneficial effects after exercise training have been reported in experimental animal studies with diabetes (Bidasee et al., 2008; Epp et al., 2013; Hafstad et al., 2013; Korte et al., 2005; Stolen et al., 2009). However, at the time that this PhD project began, no randomized controlled trial had investigated the effects of aerobic exercise in a group with T2D and established diastolic dysfunction.

Although the myocardial metabolic response to endurance training is not fully known, a number of studies have reported changes in gene and protein expression associated with myocardial metabolism in normal hearts (Hafstad et al., 2011; Liu et al., 2009; Strom et al., 2005) as well as diabetic hearts (Hafstad et al., 2013; Shearer et al., 2011) after exercise training. However, few studies have directly addressed myocardial metabolic response to exercise; most studies are experimental, and results are conflicting. For example, Broderick et al. (Broderick et al., 2005) failed to find changes in myocardial glucose oxidation and glycolysis after treadmill running, whereas Burelle et al. (Burelle et al., 2004) found increased glucose and fatty acid oxidation and decreased glycolysis. The discrepancy between these two findings may be due to differences in exercise training intensity. Hafstad et al. reported that high-intensity exercise training, but not moderate-intensity exercise training (MIE), induces alterations in myocardial substrate utilization associated with improved cardiac function in mice (Hafstad et al., 2013).

The potential oxygen sparing effect of exercise as a result of changes in substrate utilization can improve cardiac efficiency (Hafstad et al., 2015). In mice, cardiac mechanical efficiency was shown to improve through reduced cardiac oxygen consumption (Hafstad et al., 2013), which may be related to improvements in Ca^{2+} homeostasis (Stolen et al., 2009). It has been suggested that exercise induced increases in cardiac oxygen availability, due to improved

cardiac efficiency, contribute to reduced ischemic susceptibility in hearts of T2D subjects (Hafstad et al., 2015).

Furthermore, it has been shown that endurance exercise can improve or preserve cardiac function associated with improvements in mitochondrial biogenesis in diabetic cardiomyopathy in rodents (Hafstad et al., 2013; Stolen et al., 2009; Wang et al., 2015). For example, Hafstad et al. reported exercise-induced improvements in cardiac function associated with mitochondrial respiratory capacity (increased mitochondrial density), improved mitochondrial efficiency (increased respiratory coupling) (Hafstad et al., 2013). For example, it has been demonstrated that exercise induces mitochondrial biogenesis in the skeletal muscle, but not in the heart of mice (Li et al., 2011). However, exercise intensity seem to be important to increase cardiac mitochondrial content both in lean (Hafstad et al., 2011) and obese (Hafstad et al., 2013) animal models. (Exercise intensity will be further discussed in section 1.1.6).

There appears to be a link between improvements in mitochondrial capacity after exercise and enhanced mitochondrial Ca^{2+} handling (Hafstad et al., 2015). In addition, aerobic exercise-induced improvements in cardiomyocyte contractility have been associated with improvements Ca^{2+} homeostasis (in rodents with diabetic cardiomyopathy) (Epp et al., 2013; Stolen et al., 2009). Nonetheless, the effects of exercise on cardiac mitochondrial biogenesis in humans are unclear.

As for the effect of exercise on whole body glycemic control (discussed in section 1.1.2.2), it is suggested that exercise has the potential to improve the adverse effects of hyperglycemia (i.e. oxidative stress) in the myocardium of T2D individuals (Hafstad et al., 2015). In line with this, exercise training also appears to have an anti-inflammatory effect in the cardiac tissue (Botta et al., 2013). Cardiac stress induced by exercise may lead to a transient increase in oxidative stress (Hafstad et al., 2015; Radak et al., 2001), which appears to be important for the cardiac exercise adaptations, as antioxidant therapy has been shown to have altered exercise adaptations in healthy individuals (Ristow et al., 2009). However, as for the whole body, exercise training most likely decreases oxidative stress in the cardiomyocytes due to systemic changes, such as improved insulin signaling, plasma lipid profile, and reduced RAS activity as well reduced inflammation (Hafstad et al., 2015).

Furthermore, an exercise-induced reduction in intramyocardial triglyceride content has been reported in mice (Hafstad et al., 2013), suggesting that exercise can reduce the susceptibility for cardiac lipotoxicity. However, this has not been confirmed in T2D individuals (Jonker et

al., 2013; Schrauwen-Hinderling et al., 2011) and the role of these exercise-induced metabolic changes in diabetic cardiomyopathy is unknown.

Physical activity (i.e. exercise training) can increase stroke volume (SV) and reduce resting- and submaximal HRs. For example, previously untrained individuals who perform regular vigorous activity over a ≥ 6-month period can be expected to lower resting and submaximal exercise HRs by 5-20 beats per minute with ≥20% increase in SV as well as improvement in contractility (Wilson et al., 2016). In addition, exercise training can induce cardiac adaptations with increased internal RV and LV volumes and slight increases in wall thickness (cardiomyocyte hypertrophy), and thus an increase in total muscle mass (Scharhag et al., 2010; Wilson et al., 2011).

A previous meta-analysis showed that male elite athletes had greater LV cardiac structures than sedentary controls and that endurance trained athletes hearts had larger volumes (Utomi et al., 2013). Exercise induces normal, physiological, cardiac growth associated with normal cardiac structure due to factors such as cell hypertrophy (Kemi et al., 2002; Waring et al., 2014), reduced cell death and fibrosis (Jin et al., 2000; Kwak et al., 2006; Siu et al., 2004), and normal or improved cardiac function (Jin et al., 2000; Waring et al., 2014). In addition, exercise may protect against obesity-induced LV remodeling and myocardial fibrosis (Hafstad et al., 2013; Sacre et al., 2014), and thus contribute to reduced wall stress and improved cardiac efficiency.

In general, with exercise, the production of key growth factors, such as IGF-1 and NO and their associated signaling pathways, increase (Czarkowska-Paczek et al., 2006; Ellison et al., 2011; Neri Serneri et al., 2001; Rastaldo et al., 2007). For example, exercise has been shown to improve insulin-signaling cascades in the myocardium of obese rats (Medford et al., 2012; Pieri et al., 2014). Improved endothelial function is considered a whole-body response to exercise (Hawley et al., 2014) and endothelial function has been shown to improve after exercise training in individuals with T2D (Montero et al., 2013; Sixt et al., 2010). As for the whole body, there are also indications that exercise training has the potential to improve endothelial function in coronary vasculature as well as to increase capillary formation (Bowles et al., 2000). However, the molecular responses underpinning adaptations to exercise training in the cardiac muscle are not fully established (Zanesco and Antunes, 2007).

1.1.5 Cardiorespiratory fitness and aerobic exercise

Cardiorespiratory fitness per se stands out as an important cardiovascular risk factor in addition to traditional risk factors such as age, dyslipidemia, smoking, and hypertension. Indeed, cardiorespiratory fitness is a strong predictor of future cardiovascular health and is inversely related to premature mortality. Reduced cardiorespiratory fitness appears to be an even stronger predictor of cardiovascular morbidity and mortality than physical inactivity in T2D and metabolic syndrome (see Definitions and explanations) as well as in healthy patients – even after controlling for traditional risk factors (Blair et al., 1996; Carnethon et al., 2003; Chase et al., 2009; Keteyian et al., 2008; Kodama et al., 2009; Kohl et al., 1992; Lakka et al., 2003; Lee et al., 2011; Myers et al., 2002; Nes et al., 2014; Sassen et al., 2009; Talbot et al., 2002; Wei et al., 2000). For instance, Nes et al. (2014) found that cardiorespiratory fitness was inversely associated with all-cause and cardiovascular mortality (men and women < 60 years of age): for every ~3.5 mL/kg/min (1 MET) higher VO_2, the risk of all-cause mortality was 15% and 8% lower in men and women, respectively, as well as 21% lower for cardiovascular mortality in both genders (Nes et al., 2014). Combined, these observations indicate that exercise training strategies that effectively improve cardiorespiratory fitness are more beneficial to reduce cardiovascular risk factors than physical activity in general.

Reduced cardiorespiratory fitness is a common finding in individuals with T2D (Gurdal et al., 2015; Hordern et al., 2009; Wei et al., 1999), and is associated with physical inactivity in this patient group (Bjorgaas et al., 2005). Moreover, diastolic dysfunction is associated with reduced cardiorespiratory fitness (Edelmann et al., 2011; Kosmala et al., 2015; Poirier et al., 2000). Indeed, Poirier et al. showed that the more severe grade of diastolic dysfunction, the lower the exercise performance was on the maximal treadmill test (Poirier et al., 2000). However, it is uncertain whether this association is in fact due to the relative sedate nature of this patient group (Egan et al., 2013; Nelson et al., 2002; Thomas et al., 2004) or whether the reduced cardiorespiratory fitness is due to diastolic dysfunction per se. Furthermore, one might also speculate whether the observed sedate behavior in the T2D population is due to diastolic dysfunction.

1.1.5.1 Maximal oxygen uptake

Maximal oxygen uptake (VO_{2max}) is considered the best measure of cardiorespiratory fitness (Balady et al., 2010; Fletcher et al., 2013). It is the product of oxygen delivery and the arteriovenous oxygen difference, and it measures the energy turnover in the body by reflecting

the capacity to take up, transport, and utilize oxygen (Fletcher et al., 2013).

VO_{2max} is thus determined by the capacity of the central nervous system to recruit motor units, the functional capacity of the lungs and cardiovascular system to supply oxygen to the working skeletal muscles, and the ability of these muscles to utilize oxygen in the oxidative and metabolic pathways (i.e. oxidative capacity of the mitochondria) (Hawley et al., 2014; Saltin and Strange, 1992). Even though each step in this oxygen supply chain determines VO_{2max}, healthy individuals appear to be limited by the ability of the cardiovascular system to supply oxygen to the working muscles (Gonzalez-Alonso and Calbet, 2003) rather than by limitations in the oxidative capacity of muscle mitochondria (Boushel et al., 2011). However, compared to healthy individuals, those with T2D appear to have reduced mitochondrial respiration due to reduced mitochondrial density (Boushel et al., 2007). For instance, low VO_{2max} in T2D appears to be strongly associated with the coordinated down-regulation of PGC-1α responsive genes (Mootha, 2003). Whether it is T2D or inactivity that is responsible for these mechanisms is uncertain. Nevertheless, endurance exercise training may induce a shift from demand to supply limitation of VO_{2max} (Wagner, 2000).

It is the acute cardiovascular adaptations to aerobic exercise that, over time, result in the cardiac remodeling and adaptations that increase VO_{2max} (Egan and Zierath, 2013; Hawley et al., 2014). Cardiorespiratory fitness can be improved by aerobic-exercise-induced changes in both the oxygen supply and oxygen demand chain: maximal oxygen supply can increase due to several factors, such as increased SV, blood volume, and myocardial contractility (Amundsen et al., 2008; Warburton et al., 2004; Wisloff et al., 2007). For instance, increased venous return increases preload (see "Definitions and explanations") and thus stretch to the ventricle, which triggers volume hypertrophy.

In addition, the vascular system is remodeled because endurance exercises induce increases in vessel diameter, arteriole number, and capillary density in skeletal muscles (Green et al., 2012). This structural remodeling is primarily specific to the working muscle, except for the endothelial function, which is considered a whole-body response to exercise (Hawley et al., 2014).

At the oxygen demand end of the chain, the major contributor to changes in metabolism induced by exercise training is the skeletal muscle. This is because muscle tissue has a high mitochondrial density (relative to other tissues) and relies on oxidative phosphorylation to produce energy (Egan and Zierath, 2013). Endurance exercise training improves the metabolic

properties of the myofibers by increasing mitochondrial density as well as by changing the pattern of substrate oxidation (from carbohydrate to fat utilization at submaximal exercise intensities) (Calvo et al., 2008; Holloszy, 1967), all of which promote increases in arteriovenous oxygen difference. These changes are determined by a number of signals and transcriptional factors, including Ca^{2+}-signaling pathways and PGC-1α (Hawley et al., 2014). There are also a number of other factors, such as cytokines and peptides, which are responsible for the adaptive responses to exercise training in the whole body, such as glucose metabolism (Hawley et al., 2014; Karstoft and Pedersen, 2015; Zierath and Wallberg-Henriksson, 2015).

Indeed, mounting evidence is showing improvements in cardiorespiratory fitness following exercise training in individuals with T2D (Belli et al., 2011; Bjorgaas et al., 2005; Cuff et al., 2003; Kadoglou et al., 2007; Little, Gillen, et al., 2011), metabolic syndrome (Tjonna et al., 2008), obesity (Schjerve et al., 2008), and CVD (Kavanagh et al., 2003; Rognmo et al., 2004; Warburton et al., 2005; Wisløff et al., 2007), as well as in healthy individuals (Burgomaster et al., 2008; Gibala et al., 2006; Helgerud et al., 2001; Helgerud, Hoydal, et al., 2007; Kemi and Wisløff, 2010; Ogawa et al., 1992).

1.1.6 Exercise intensities

It has been established that exercise adaptations are specific to exercise mode, determined by the volume, frequency, and intensity of contractile stimuli (Egan and Zierath, 2013; Hawley, 2002; Hawley et al., 2014; Zierath and Wallberg-Henriksson, 2015). Importantly, increased exercise intensity appears to magnify training stimulus and associated adaptations, such as SV and other adaptations to the oxygen supply-and-demand chain (discussed in section 1.1.4) (Gibala et al., 2006; Laursen et al., 2005; Swain and Franklin, 2006; Weston et al., 2014).

Previous research on risk classification and exercise as a treatment in general has traditionally investigated endurance exercise models involving continuous, relatively high-volume exercise at low-to-moderate intensities (Fletcher et al., 2013; Levinger et al., 2015). An increasing body of evidence, however, suggests that although moderate intensity aerobic exercise (MIE) is highly beneficial, high-intensity exercise appears to induce equal or even greater health benefits—at least relative to the time spent in physical activity (Gebel et al., 2015; Lee et al., 2003; Nes et al., 2012; Wen et al., 2011; Wisloff et al., 2006).

Up to two-thirds of patients with T2D may not participate in any regular physical activity at all (Thomas et al., 2004), and few achieve the recommended amount of weekly exercise (Egan et

al., 2013; Nelson et al., 2002; Thomas et al., 2004). In general, lack of time seems to be the most frequently reported reason for not exercising (Booth et al., 1997; Kimm et al., 2006; Stutts, 2002; Trost et al., 2002). In this context, high-intensity exercise training may represent an effective alternative to MIE training for individuals with T2D.

1.1.6.1 High-intensity interval exercise

High-intensity interval exercise (HIIE) is considered a tolerable and effective exercise model of high-intensity exercise (at least in the short term) in patients with cardiometabolic disease (Weston et al., 2014).

In general, HIIE involves intermittent work bouts of high-intensity exercise interspersed with recovery periods of rest or low-intensity exercise (Gibala et al., 2012; Weston et al., 2014). The terminology that describes HIIE varies, and so do intensity, volume, frequency, and duration of the exercise interventions studied, making them somewhat difficult to compare. Overall, the term HIIE commonly includes models of low-volume to high-volume HIIE.

Low-volume HIIE is characterized by short exercise sessions (approximately 20–30 minutes, including warm up and cool down), and the most common protocols are four-to-six 60-second "all-out" work bouts with four-minute recovery periods or 10 one-minute (~90% of HR_{max}) with one-minute recovery periods (Baekkerud et al., 2015; Burgomaster et al., 2005; Freyssin et al., 2012; Gibala et al., 2006; Jacobs et al., 2013; Little, Gillen, et al., 2011; Little, Safdar, et al., 2011; Little et al., 2010; Roditis et al., 2007). The high-volume HIIE models typically consist of longer exercise sessions (approximately 40 minutes) and longer work bouts; one of the most common is a four-times-four work bout interspersed with three-minute active recovery periods (Helgerud, Hoydal, et al., 2007; Weston et al., 2014). The latter method is commonly applied in studies investigating the effects of HIIE on risk classification and treatment in individuals with cardiometabolic disease (Fu et al., 2013; Iellamo et al., 2013; Moholdt et al., 2009; Molmen-Hansen et al., 2012; Rognmo et al., 2004; Schjerve et al., 2008; Tjonna et al., 2008; Wisloff et al., 2007).

The rationale behind the low-volume HIIE protocols has been mainly to induce adaptations in the demand side of the chain, such as muscle oxidative potential and insulin sensitivity (i.e. GLUT4; see section 1.1.6.2) (Burgomaster et al., 2005; Jacobs et al., 2013; Little, Gillen, et al., 2011; Little et al., 2010; Roditis et al., 2007), whereas the rationale behind the high-volume HIIE approach has primarily been to increase the central parts of the oxygen supply chain (Fu

et al., 2013; Helgerud, Høydal, et al., 2007; Molmen-Hansen et al., 2012; Wisloff et al., 2009; Wisloff et al., 2007).

1.1.6.2 Effects of high-intensity interval exercise versus moderate-intensity exercise

Effect of exercise intensity on cardiac function

Few studies have investigated the effects of HIIE (> 90% HR_{max}) on cardiac function in individuals with T2D. Thus, the impact of exercise intensity on cardiac structure and function in this population is uncertain (Balducci et al., 2012; Hordern et al., 2009). However, there are studies that indicate that exercise intensity is crucial to improving diastolic function in persons with T2D (Hordern et al., 2009) and to achieving optimal cardiac remodeling in HF patients (Wisloff et al., 2007). It has also been demonstrated that HIIE is superior to MIE in decreasing work-independent myocardial oxygen consumption, increasing cardiac maximal mitochondrial respiratory capacity, and improving cardiac substrate utilization (by increased glucose utilization) in mice (Hafstad et al., 2011; Stolen et al., 2009). Furthermore, it has been demonstrated that these factors may contribute to the observed superior effects of HIIE versus MIE (when isocaloric) for improving cardiorespiratory fitness in mice (Hafstad et al., 2013).

MIE in accordance with current exercise recommendations (International Diabetes Federation) seems to be insufficient to improve myocardial function in T2D (Hordern et al., 2009). Moreover, it appears that work bouts at high intensities that are longer than those typical for low-volume HIIE are required to induce cardiac remodeling (Gibala et al., 2012; Weston et al., 2014). This may be due to MIE not allowing for intensities ≥ 90 % of HR_{max} and low-volume HIIE affecting mostly peripheral components of the oxygen supply chain; whereas higher-volume HIIE appears to trigger a greater stimulus to the central components of the oxygen supply chain.

Effect of exercise intensity on cardiorespiratory fitness

A substantial amount of evidence, however, suggests superior effects of HIIE on the oxygen supply and/or demand chain—relative to the time spent exercising in particular (Gibala et al., 2012; Weston et al., 2014). In general, high-volume HIIE appears to be more effective than MIE (Helgerud, Hoydal, et al., 2007; Rognmo et al., 2004), and low-volume HIIE appears to be equal to MIE in terms of improving VO_{2max} (Baekkerud et al., 2015; Burgomaster et al., 2008).

Several studies have shown superior improvements in cardiorespiratory fitness after high-volume HIIE compared to MIE in patients with T2D (Balducci et al., 2012; Boule et al., 2003;

Gormley et al., 2008; Hordern et al., 2009), metabolic syndrome (Tjonna et al., 2008), HF (Freyssin et al., 2012; Fu et al., 2013; Haykowsky et al., 2013; Wisloff et al., 2007), coronary artery disease (Amundsen et al., 2008; Moholdt et al., 2009; Rognmo et al., 2004), hypertension (Molmen-Hansen et al., 2012), and obesity (Schjerve et al., 2008), as well as in the general population (Swain and Franklin, 2006). Indeed, a recent meta-analysis performed by Weston et al. identified that HIIE has greater physiological benefits than MIE in lifestyle-induced cardiometabolic disease when it comes to improving VO_{2max} (19.4% vs. 10.3% improvement, respectively) (Weston et al., 2014). Animal studies using HIIE to reduce risk factors also support the clinical findings listed above (Wisloff et al., 2009). However, a recent large-scale trial investigating HIIE versus MIE failed to find a difference between these exercise intensities in a coronary artery disease population (Conraads et al., 2015) and thus the role of intensity in CVD is not yet established.

A greater potential to increase SV during exercise after high-volume HIIE may explain the superior improvements in VO_{2max} compared to that induced by MIE and low-volume HIIE (Baekkerud et al., 2015; Helgerud, Hoydal, et al., 2007). SV seems to progressively increase toward VO_{2max} (Gledhill et al., 1994; Zhou et al., 2001), and high-volume HIIE allows for longer periods at a steady workload $\geq$ 90% HR_{max} compared to MIE and low-volume HIIE, which may explain differences in cardiorespiratory adaptations. One might speculate whether blood volume, oxygen-carrying capacity, or peripheral adaptations could contribute to the superior cardiorespiratory effects seen following high-volume HIIE. However, an increase in SV after HIIE training (in the short term) does not seem to be particularly influenced by changes in preload due to increased blood volume (Baekkerud et al., 2015; Helgerud, Høydal, et al., 2007; Wisloff et al., 2009). Furthermore, it is uncertain to what extent changes in oxygen-carrying capacity (i.e. hemoglobin mass) differ between HIIE and MIE (Baekkerud et al., 2015; Helgerud, Høydal, et al., 2007; Warburton et al., 2004). Moreover, both HIIE models and MIE seem to improve muscle mitochondrial potential (Baekkerud et al., 2015; Duscha et al., 2012; Little et al., 2010) and changes in peripheral adaptations are therefore not likely to explain higher increase in VO_{2max} with high-volume HIIE. Thus, the superior improvements in SV after high-volume HIIE may be explained by improved LV suction as a result of increased contractility and/or redistribution of blood (Fletcher et al., 2013; Opdahl et al., 2009; Sengupta et al., 2008; Stoylen et al., 2003). Although exercise volume is suggested as crucial to improve VO_{2max} (Duscha et al., 2012), intensity appears to be an even more important factor. Tjønna et al. found that only one four-minute work bout at 90%–95% of HR_{max} three times a week for 10

weeks improved VO_{2max} to the same extent as four work bouts of four-minutes each of HIIE in untrained, overweight individuals (Tjonna et al., 2013). Thus, it might not be the total duration at high intensity that determines changes in VO_{2max} but rather the difference in the duration of the work bout. The fact that less time is spent at a steady workload ≥ 90% HR_{max} during low-volume compared to high-volume HIIE and that the majority of time during low-volume HIIE is spent with a fluctuating HR may play a role in this regard. The latter supports the assumption that time spent at a steady-state HR ≥ 90% HR_{max} might be a key factor to induce increased SV and thus VO_{2max} (at workloads of which the MIE model does not allow for). Overall, the greater increase in cardiorespiratory fitness after high-volume HIIE compared to MIE indicates different adaptations in the oxygen supply chain due to difference in intensities, whereas differences between high- and low-volume HIIE indicate different adaptations to the oxygen supply chain due to difference in work bout duration at high intensity.

The choice of HIIE model in this thesis is thus high-volume HIIE (four, four-minute work bouts) based on the increasing amount of empirical data indicating that high-volume HIIE is superior to MIE and low-volume HIIE for improving central parts of the oxygen supply chain (VO_{2max}).

Effect of exercise intensity on glycemic control and other CMR factors and markers

Exercise intensity also appears to be important to glycemic control in T2D (Boule et al., 2003; Little et al., 2014; Marwick et al., 2009) and to insulin sensitivity in overweight/obese subjects at risk of developing T2D (in the short term) (Bajpeyi et al., 2009; Earnest et al., 2013; Hood et al., 2011).

Although the mechanisms that cause exercise-induced improvements in glycemic control are not entirely understood, the improvements in glycemic control and insulin sensitivity can be due to increased muscle glucose transport capacity mediated in part by increased glucose transporter proteins (GLUT4) (Holloszy, 2003; Hood et al., 2011; Little, Gillen, et al., 2011). At rest, glucose uptake is primarily insulin dependent as glucose entrance into the cells cytoplasm is facilitated by GLUT 4 being translocated to the cell membrane. Exercise increases the concentration of GLUT 4 in the cell membrane. GLUT4 are thus activated by insulin acting upon insulin receptors as well as by muscle contractions, all of which increase GLUT4 translocation and total GLUT4 quantity (Colberg et al., 2010). It is the insulin-dependent glucose uptake needed for glucose transport at rest and in the postprandial state that is considered impaired in T2D. Glucose uptake due to muscle contraction is, however, not

considered dysfunctional in this patient group (Colberg et al., 2010). This mechanism may also partly explain the observed exercise-induced improvements in cardiac glucose utilization (Broderick et al., 2005; Hafstad et al., 2013). Aerobic exercise (and resistance exercise, albeit outside the scope of this thesis) has shown to promote improvements in circulating glucose concentrations and insulin sensitivity within 2–72 hours post exercise, apparently dependent on exercise intensity as well as duration of exercise (Boule et al., 2003; Colberg et al., 2010). This emphasizes the importance of frequent exercise for individuals with T2D to achieve acute blood glucose-lowering effects of exercise.

In general, fluctuating glucose levels throughout the day is highly prevalent in T2D (van Dijk et al., 2011), which is not entirely reflected by HbA_{1c} and also represents an increased CVD risk in this patient group in the long term (Cavalot et al., 2011). Even a single bout of endurance exercise can reduce postprandial glucose elevations in patients with T2D as well as improve triglycerides and/or oxidative stress and/or endothelial function in healthy individuals (Gillen et al., 2012; Karstoft et al., 2014; Tyldum et al., 2009). One exercise bout can also affect fluctuating excessive elevations in circulating glucose throughout the day in insulin resistant and T2D individuals (Francois et al., 2014; Van Dijk et al., 2013). Although MIE has been shown to be effective in improving 24 hours glycemic control in T2D (Van Dijk et al., 2013), it appears as if HIIE can be a time efficient and effective way to achieve this compared to individuals with insulin resistance and T2D (Francois et al., 2014; Gillen et al., 2012). Furthermore, exercise intensity also appears to matter in reducing postprandial triglyceride levels (Burns et al., 2015). Moreover, HIIE has previously shown to be more effective than MIE in reducing the negative effect of fast food on endothelial function and oxidative stress in healthy individuals in the postprandial phase (Tyldum et al., 2009).

Furthermore, improvements in markers of metabolic control and arterial compliance have been shown to be similar to what is observed with traditional, continuous MIE training after low-volume HIIE training in healthy individuals (Burgomaster et al., 2008; Rakobowchuk et al., 2008). Moreover, high-volume HIIE has been shown to be superior to MIE in improving endothelial function (assessed using flow-mediated dilatation of the brachial artery) in metabolic syndrome (Tjonna et al., 2008), as well as endothelial function and antioxidant status in HF patients (Wisloff et al., 2007).

Overall, Weston et al. (2014) report that the adaptations that appear to occur more often with HIIE compared to MIE in lifestyle-induced cardiometabolic disease include (in addition to

improved cardiorespiratory fitness and cardiac function): reduction in systolic and diastolic blood pressure; fasting glucose and triglyceride levels; and oxidative stress and inflammation; as well as increased PGC-1α activation in skeletal muscle and maximal rate of Ca^{2+} reuptake, HDL, adiponectin, insulin sensitivity, β-cell function, availability of NO, and enjoyment of exercise and improved quality of life (Weston et al., 2014). However, the studies reviewed in the meta-analysis (Weston et al., 2014) had, in general, small sample sizes and a great variety of diseases under the term "cardiometabolic diseases"; in addition, it consisted of relatively few studies due to limited research in this area.

1.2 THESIS INTRODUCTION

Cardiovascular disease (CVD) stands out as a major complication in T2D (International Diabetes Federation) and epidemiological evidence supports heart failure (HF) as being responsible for a substantial proportion of this disease's burden. Compared to people without diabetes, individuals with T2D have a two to five times higher risk of developing HF and a two times higher risk of cardiovascular mortality (Go et al., 2014; Kannel et al., 1974; Nichols et al., 2004). Diabetic cardiomyopathy substantially increases the risk of developing HF, and 20% to 60% of individuals with T2D (without traditional evidence of CVD) can have diastolic dysfunction in the early stages of T2D.

Exercise is an important part of the management plan for T2D and has been shown to reduce CVD risk factors. In addition, evidence suggests that aerobic exercise may prevent or contribute to delaying the development of diabetic cardiomyopathy.

Today, numerous public health institutions worldwide, such as the AHA, the International Diabetes Federation (IDF), the American College of Sports Medicine (ACSM) and the American Diabetes Association (ADA) in a joint position statement, recommend that individuals with T2D perform at least 150 minutes of moderate (50% to 70% of HR_{max}) to vigorous intensity aerobic activity distributed over at least three days a week, with no inactivity on more than two consecutive days (Alberti et al., 2009; American Diabetes Association, 2014; Buse et al., 2007; Colberg et al., 2010; International Diabetes Federation; Mendes et al., 2015). In 2009, the AHA (Marwick et al., 2009) stated that although very high intensities (i.e. $\geq$ 80% of HR_{max}) may be less well tolerated in people with T2D, vigorous activity should be targeted if it is tolerated in this patient group. Resistance training is also recommended at least twice a week (American Diabetes Association, 2014; Colberg et al., 2010; Mendes et al., 2015), but this will not be further discussed as it is outside the scope of this thesis.

HIIE training (see section 1.1.6) has recently gained increasing interest due to its success in improving various health outcomes in cardiometabolic diseases (Bird and Hawley, 2012; Weston et al., 2014). However, research regarding the effect of high-intensity versus moderate-intensity exercise on cardiac function in T2D individuals is limited. Therefore, this thesis includes two studies (Studies 1 and 2, Chapters 3–5) comparing the effects of HIIE versus MIE in individuals with T2D.

The primary study (Study 1, Chapter 3) of this thesis investigated the effect of 12 weeks of supervised HIIE training (90–95% HR_{max}) versus home-based MIE training, according to exercise guidelines, on cardiac function in individuals with T2D and established diastolic dysfunction (measured as e' < 8 cm/s). Furthermore, the effects of HIIE versus MIE on cardiorespiratory fitness, endothelial function and various other CMR factors and markers were investigated. In addition to testing the magnitude of exercise effects after 12 weeks of HIIE or MIE, the objective of this one-year randomized controlled study intervention was to investigate whether the initial 12-week intervention could sustain or yield additional health benefits after one year. The goal of this study was to provide further evidence about the proposed most effective exercise prescription for improving cardiac function, cardiorespiratory fitness, and other cardiometabolic risk factors in T2D individuals with diastolic dysfunction, which may influence future exercise guidelines for the T2D population.

Furthermore, a sub study of Study 1 (Chapter 4) is encompassed in this thesis, investigating left ventricular (LV) twist parameters in T2D individuals with diastolic dysfunction as well as the effect of exercise on these. Untwisting is considered an important component in early diastolic filling, and LV untwist rate (UTR) has recently been introduced as a clinical marker for diastolic function (Opdahl et al., 2012; Sengupta et al., 2008). Furthermore, the timing of untwist appears to be important for optimal early diastolic function (Sengupta et al., 2008; Wang et al., 2007) and overall cardiac function, as improved cardiac contractility improves diastolic suction (Fletcher et al., 2013; Opdahl et al., 2009; Sengupta et al., 2008; Stoylen et al., 2003). However, there is limited information regarding the role of LV untwist in individuals with T2D and diastolic dysfunction. Thus, the overall aim of this combined prospective (T2D individuals participating in Study 1) and retrospective (healthy controls participating in HUNT3) study was to test whether T2D individuals with diastolic dysfunction have altered cardiac twist parameters compared to healthy individuals and whether exercise at HIIE and/or MIE has the potential to improve twist parameters in this population. The goal of this study was also to provide further evidence about the role of LV twist in T2D individuals with diastolic dysfunction and the effect of exercise, which may influence the direction of further research seeking early clinical echocardiographic markers of diastolic dysfunction.

Finally, we included an acute exercise study (Study 2, Chapter 5) in this thesis. This study investigated the effects of acute prior HIIE and MIE on cardiac function in the postprandial phase; after an energy dense meal rich in refined carbohydrates and saturated fat ("fast food"), which may acutely alter cardiac efficiency through increased circulating glucose and/or cardiac

fatty acid oxidation. The overall aim of this two-group cross-over trial was to investigate whether fast food alters cardiac workload in T2D individuals versus age- and BMI-matched healthy individuals in the postprandial phase; as well as to investigate whether pre-exercise could modify potential alterations in cardiac work load after fast food in T2D individuals and age- and BMI-matched healthy individuals. However, identifying an optimal time, model, intensity, volume, and frequency for exercise prescription to reduce fluctuating cardiac metabolic disturbances in individuals with T2D warrants an extended amount of research. Thus, the goal of this study was to develop pilot data for a larger study initiative to search for the optimal timing and intensity of exercise to reduce overall cardiac metabolic disturbances in patients with T2D.

REFERENCES

Abel, E. D. (2005). Myocardial insulin resistance and cardiac complications of diabetes. *Curr Drug Targets Immune Endocr Metabol Disord, 5*(2), 219-226.

Abel, E. D., Litwin, S. E., & Sweeney, G. (2008). Cardiac remodeling in obesity. *Physiol. Rev., 88*(2), 389-419.

Adameova, A., & Dhalla, N. S. (2014). Role of microangiopathy in diabetic cardiomyopathy. *Heart Fail. Rev., 19*(1), 25-33.

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . International Association for the Study of, O. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation, 120*(16), 1640-1645.

American Diabetes Association. (2014). Standards of medical care in diabetes-2014. *Diabetes Care, 37 Suppl 1*, S14-80.

Amundsen, B. H., Rognmo, O., Hatlen-Rebhan, G., & Slordahl, S. A. (2008). High-intensity aerobic exercise improves diastolic function in coronary artery disease. *Scand. Cardiovasc. J., 42*(2), 110-117.

Anderson, E. J., Kypson, A. P., Rodriguez, E., Anderson, C. A., Lehr, E. J., & Neufer, P. D. (2009). Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J. Am. Coll. Cardiol., 54*(20), 1891-1898.

Aneja, A., Tang, W. H., Bansilal, S., Garcia, M. J., & Farkouh, M. E. (2008). Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. *Am. J. Med., 121*(9), 748-757.

Aspenes, S. T., Nilsen, T. I., Skaug, E. A., Bertheussen, G. F., Ellingsen, O., Vatten, L., & Wisloff, U. (2011). Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med. Sci. Sports Exerc., 43*(8), 1465-1473.

Baekkerud, F. H., Solberg, F., Leinan, I. M., Wisloff, U., Karlsen, T., & Rognmo, O. (2015). Comparison of Three Popular Exercise Modalities on VO2max in Overweight and Obese. *Med. Sci. Sports Exerc.*

Bajpeyi, S., Tanner, C. J., Slentz, C. A., Duscha, B. D., McCartney, J. S., Hickner, R. C., . . . Houmard, J. A. (2009). Effect of exercise intensity and volume on persistence of insulin sensitivity during training cessation. *J Appl Physiol. , 106*(4), 1079-1085.

Balady, G. J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G. F., . . . Outcomes, R. (2010). Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation, 122*(2), 191-225.

Balducci, S., Zanuso, S., Cardelli, P., Salvi, L., Bazuro, A., Pugliese, L., . . . Italian Diabetes Exercise Study, I. (2012). Effect of high- versus low-intensity supervised aerobic and

resistance training on modifiable cardiovascular risk factors in type 2 diabetes; the Italian Diabetes and Exercise Study (IDES). *PLoS One, 7*(11), e49297.

Ban, C. R., & Twigg, S. M. (2008). Fibrosis in diabetes complications: pathogenic mechanisms and circulating and urinary markers. *Vasc Health Risk Manag, 4*(3), 575-596.

Barouch, L. A., Berkowitz, D. E., Harrison, R. W., O'Donnell, C. P., & Hare, J. M. (2003). Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation, 108*(6), 754-759.

Barouch, L. A., Gao, D., Chen, L., Miller, K. L., Xu, W., Phan, A. C., . . . Hare, J. M. (2006). Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. *Circ. Res., 98*(1), 119-124.

Belke, D. D., Betuing, S., Tuttle, M. J., Graveleau, C., Young, M. E., Pham, M., . . . Abel, E. D. (2002). Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. *J. Clin. Invest., 109*(5), 629-639.

Bell, D. S. (2003). Diabetic cardiomyopathy. *Diabetes Care, 26*(10), 2949-2951.

Belli, T., Ribeiro, L. F., Ackermann, M. A., Baldissera, V., Gobatto, C. A., & Galdino da Silva, R. (2011). Effects of 12-week overground walking training at ventilatory threshold velocity in type 2 diabetic women. *Diabetes Res. Clin. Pract., 93*(3), 337-343.

Berger, J., Biswas, C., Vicario, P. P., Strout, H. V., Saperstein, R., & Pilch, P. F. (1989). Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting. *Nature, 340*(6228), 70-72.

Berne, C., Fagius, J., Pollare, T., & Hjemdahl, P. (1992). The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia, 35*(9), 873-879.

Bidasee, K. R., Zheng, H., Shao, C. H., Parbhu, S. K., Rozanski, G. J., & Patel, K. P. (2008). Exercise training initiated after the onset of diabetes preserves myocardial function: effects on expression of beta-adrenoceptors. *J Appl Physiol (1985), 105*(3), 907-914.

Bird, S. R., & Hawley, J. A. (2012). Exercise and type 2 diabetes: new prescription for an old problem. *Maturitas, 72*(4), 311-316.

Bjorgaas, M., Vik, J. T., Saeterhaug, A., Langlo, L., Sakshaug, T., Mohus, R. M., & Grill, V. (2005). Relationship between pedometer-registered activity, aerobic capacity and self-reported activity and fitness in patients with type 2 diabetes. *Diabetes Obes. Metab., 7*(6), 737-744.

Blair, S. N., Kampert, J. B., Kohl, H. W., 3rd, Barlow, C. E., Macera, C. A., Paffenbarger, R. S., Jr., & Gibbons, L. W. (1996). Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *JAMA, 276*(3), 205-210.

Blood Pressure Lowering Treatment Trialists, C., Turnbull, F., Neal, B., Ninomiya, T., Algert, C., Arima, H., . . . MacMahon, S. (2008). Effects of different regimens to lower blood

pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. *BMJ, 336*(7653), 1121-1123.

Boardman, N., Hafstad, A. D., Larsen, T. S., Severson, D. L., & Aasum, E. (2009). Increased O2 cost of basal metabolism and excitation-contraction coupling in hearts from type 2 diabetic mice. *Am. J. Physiol. Heart Circ. Physiol., 296*(5), H1373-1379.

Boonman-de Winter, L. J., Rutten, F. H., Cramer, M. J., Landman, M. J., Liem, A. H., Rutten, G. E., & Hoes, A. W. (2012). High prevalence of previously unknown heart failure and left ventricular dysfunction in patients with type 2 diabetes. *Diabetologia, 55*(8), 2154-2162.

Booth, M. L., Bauman, A., Owen, N., & Gore, C. J. (1997). Physical activity preferences, preferred sources of assistance, and perceived barriers to increased activity among physically inactive Australians. *Prev. Med., 26*(1), 131-137.

Botta, A., Laher, I., Beam, J., Decoffe, D., Brown, K., Halder, S., . . . Ghosh, S. (2013). Short term exercise induces PGC-1alpha, ameliorates inflammation and increases mitochondrial membrane proteins but fails to increase respiratory enzymes in aging diabetic hearts. *PLoS One, 8*(8), e70248.

Boudina, S., & Abel, E. D. (2007). Diabetic cardiomyopathy revisited. *Circulation, 115*(25), 3213-3223.

Boudina, S., & Abel, E. D. (2010). Diabetic cardiomyopathy, causes and effects. *Rev. Endocr. Metab. Disord., 11*(1), 31-39.

Boudina, S., Sena, S., O'Neill, B. T., Tathireddy, P., Young, M. E., & Abel, E. D. (2005). Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial energetics in obesity. *Circulation, 112*(17), 2686-2695.

Boudina, S., Sena, S., Theobald, H., Sheng, X., Wright, J. J., Hu, X. X., . . . Abel, E. D. (2007). Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes, 56*(10), 2457-2466.

Boule, N. G., Haddad, E., Kenny, G. P., Wells, G. A., & Sigal, R. J. (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA, 286*(10), 1218-1227.

Boule, N. G., Kenny, G. P., Haddad, E., Wells, G. A., & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia, 46*(8), 1071-1081.

Boushel, R., Gnaiger, E., Calbet, J. A., Gonzalez-Alonso, J., Wright-Paradis, C., Sondergaard, H., . . . Saltin, B. (2011). Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion, 11*(2), 303-307.

Boushel, R., Gnaiger, E., Schjerling, P., Skovbro, M., Kraunsoe, R., & Dela, F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia, 50*(4), 790-796.

Bowles, D. K., Woodman, C. R., & Laughlin, M. H. (2000). Coronary smooth muscle and endothelial adaptations to exercise training. *Exerc. Sport Sci. Rev., 28*(2), 57-62.

Boyer, J. K., Thanigaraj, S., Schechtman, K. B., & Perez, J. E. (2004). Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am. J. Cardiol., 93*(7), 870-875.

Braun, B., Zimmermann, M. B., & Kretchmer, N. (1995). Effects of exercise intensity on insulin sensitivity in women with non-insulin-dependent diabetes mellitus. *J Appl Physiol (1985), 78*(1), 300-306.

Broderick, T. L., Poirier, P., & Gillis, M. (2005). Exercise training restores abnormal myocardial glucose utilization and cardiac function in diabetes. *Diabetes Metab. Res. Rev., 21*(1), 44-50.

Brunzell, J. D., Davidson, M., Furberg, C. D., Goldberg, R. B., Howard, B. V., Stein, J. H., . . . American College of Cardiology, F. (2008). Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care, 31*(4), 811-822.

Bucala, R., Tracey, K. J., & Cerami, A. (1991). Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J. Clin. Invest., 87*(2), 432-438.

Buchanan, J., Mazumder, P. K., Hu, P., Chakrabarti, G., Roberts, M. W., Yun, U. J., . . . Abel, E. D. (2005). Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. *Endocrinology, 146*(12), 5341-5349.

Bugger, H., & Abel, E. D. (2008). Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. *Clin. Sci. (Lond.), 114*(3), 195-210.

Burelle, Y., Wambolt, R. B., Grist, M., Parsons, H. L., Chow, J. C., Antler, C., . . . Allard, M. F. (2004). Regular exercise is associated with a protective metabolic phenotype in the rat heart. *Am. J. Physiol. Heart Circ. Physiol., 287*(3), H1055-1063.

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J. Physiol., 586*(1), 151-160.

Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J., Bradwell, S. N., & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol (1985), 98*(6), 1985-1990.

Burns, S. F., Miyashita, M., & Stensel, D. J. (2015). High-Intensity Interval Exercise and Postprandial Triacylglycerol. *Sports Med., 45*(7), 957-968.

Buse, J. B., Ginsberg, H. N., Bakris, G. L., Clark, N. G., Costa, F., Eckel, R., . . . American Diabetes, A. (2007). Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation, 115*(1), 114-126.

Byrkjeland, R., Edvardsen, E., Njerve, I. U., Arnesen, H., Seljeflot, I., & Solheim, S. (2014). Insulin levels and HOMA index are associated with exercise capacity in patients with type 2 diabetes and coronary artery disease. *Diabetol. Metab. Syndr., 6*(1), 36.

Calvo, J. A., Daniels, T. G., Wang, X., Paul, A., Lin, J., Spiegelman, B. M., . . . Rangwala, S. M. (2008). Muscle-specific expression of PPARgamma coactivator-1alpha improves exercise performance and increases peak oxygen uptake. *J Appl Physiol (1985), 104*(5), 1304-1312.

Campbell, D. J., Somaratne, J. B., Jenkins, A. J., Prior, D. L., Yii, M., Kenny, J. F., . . . Kelly, D. J. (2011). Impact of type 2 diabetes and the metabolic syndrome on myocardial structure and microvasculature of men with coronary artery disease. *Cardiovasc. Diabetol., 10*, 80.

Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M., & Zorzano, A. (1992). Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. *Biochem. J., 282 (Pt 3)*, 765-772.

Candido, R., Forbes, J. M., Thomas, M. C., Thallas, V., Dean, R. G., Burns, W. C., . . . Burrell, L. M. (2003). A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. *Circ. Res., 92*(7), 785-792.

Carnethon, M. R., Gidding, S. S., Nehgme, R., Sidney, S., Jacobs, D. R., Jr., & Liu, K. (2003). Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. *JAMA, 290*(23), 3092-3100.

Catapano, A. L., Reiner, Z., De Backer, G., Graham, I., Taskinen, M. R., Wiklund, O., . . . Committees. (2011). ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis, 217 Suppl 1*, S1-44.

Cavalot, F., Pagliarino, A., Valle, M., Di Martino, L., Bonomo, K., Massucco, P., . . . Trovati, M. (2011). Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. *Diabetes Care, 34*(10), 2237-2243.

Chase, N. L., Sui, X., Lee, D. C., & Blair, S. N. (2009). The association of cardiorespiratory fitness and physical activity with incidence of hypertension in men. *Am. J. Hypertens., 22*(4), 417-424.

Christoffersen, C., Bollano, E., Lindegaard, M. L., Bartels, E. D., Goetze, J. P., Andersen, C. B., & Nielsen, L. B. (2003). Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology, 144*(8), 3483-3490.

Church, T. S., Cheng, Y. J., Earnest, C. P., Barlow, C. E., Gibbons, L. W., Priest, E. L., & Blair, S. N. (2004). Exercise capacity and body composition as predictors of mortality among men with diabetes. *Diabetes Care, 27*(1), 83-88.

Colberg, S. R., Albright, A. L., Blissmer, B. J., Braun, B., Chasan-Taber, L., Fernhall, B., . . . American Diabetes, A. (2010). Exercise and type 2 diabetes: American College of

Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Med. Sci. Sports Exerc., 42*(12), 2282-2303.

Connelly, K. A., Kelly, D. J., Zhang, Y., Prior, D. L., Advani, A., Cox, A. J., . . . Gilbert, R. E. (2009). Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. *Circ. Heart Fail., 2*(2), 129-137.

Conraads, V. M., Pattyn, N., De Maeyer, C., Beckers, P. J., Coeckelberghs, E., Cornelissen, V. A., . . . Vanhees, L. (2015). Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study. *Int. J. Cardiol., 179*, 203-210.

Cuff, D. J., Meneilly, G. S., Martin, A., Ignaszewski, A., Tildesley, H. D., & Frohlich, J. J. (2003). Effective exercise modality to reduce insulin resistance in women with type 2 diabetes. *Diabetes Care, 26*(11), 2977-2982.

Czarkowska-Paczek, B., Bartlomiejczyk, I., & Przybylski, J. (2006). The serum levels of growth factors: PDGF, TGF-beta and VEGF are increased after strenuous physical exercise. *J. Physiol. Pharmacol., 57*(2), 189-197.

Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A., & Stoylen, A. (2010). Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ. Cardiovasc. Imaging, 3*(5), 614-622.

de Simone, G., Devereux, R. B., Chinali, M., Lee, E. T., Galloway, J. M., Barac, A., . . . Howard, B. V. (2010). Diabetes and incident heart failure in hypertensive and normotensive participants of the Strong Heart Study. *J. Hypertens., 28*(2), 353-360.

Dela, F., von Linstow, M. E., Mikines, K. J., & Galbo, H. (2004). Physical training may enhance beta-cell function in type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab., 287*(5), E1024-1031.

Devereux, R. B., Roman, M. J., Paranicas, M., O'Grady, M. J., Lee, E. T., Welty, T. K., . . . Howard, B. V. (2000). Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation, 101*(19), 2271-2276.

Dhalla, N. S., Liu, X., Panagia, V., & Takeda, N. (1998). Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc. Res., 40*(2), 239-247.

Di Bonito, P., Moio, N., Cavuto, L., Covino, G., Murena, E., Scilla, C., . . . Sibilio, G. (2005). Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging. *Diabet. Med., 22*(12), 1720-1725.

Diamant, M., Lamb, H. J., Groeneveld, Y., Endert, E. L., Smit, J. W., Bax, J. J., . . . Radder, J. K. (2003). Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J. Am. Coll. Cardiol., 42*(2), 328-335.

Duscha, B. D., Annex, B. H., Johnson, J. L., Huffman, K., Houmard, J., & Kraus, W. E. (2012). Exercise dose response in muscle. *Int. J. Sports Med., 33*(3), 218-223.

Earnest, C. P., Lupo, M., Thibodaux, J., Hollier, C., Butitta, B., Lejeune, E., . . . Church, T. S. (2013). Interval training in men at risk for insulin resistance. *Int. J. Sports Med., 34*(4), 355-363.

Eckel, J., & Reinauer, H. (1990). Insulin action on glucose transport in isolated cardiac myocytes: signalling pathways and diabetes-induced alterations. *Biochem. Soc. Trans., 18*(6), 1125-1127.

Edelmann, F., Gelbrich, G., Dungen, H. D., Frohling, S., Wachter, R., Stahrenberg, R., . . . Pieske, B. (2011). Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J. Am. Coll. Cardiol., 58*(17), 1780-1791.

Egan, A. M., Mahmood, W. A., Fenton, R., Redziniak, N., Kyaw Tun, T., Sreenan, S., & McDermott, J. H. (2013). Barriers to exercise in obese patients with type 2 diabetes. *QJM, 106*(7), 635-638.

Egan, B., & Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab., 17*(2), 162-184.

Eguchi, K., Boden-Albala, B., Jin, Z., Rundek, T., Sacco, R. L., Homma, S., & Di Tullio, M. R. (2008). Association between diabetes mellitus and left ventricular hypertrophy in a multiethnic population. *Am. J. Cardiol., 101*(12), 1787-1791.

Ellison, G. M., Torella, D., Dellegrottaglie, S., Perez-Martinez, C., Perez de Prado, A., Vicinanza, C., . . . Nadal-Ginard, B. (2011). Endogenous cardiac stem cell activation by insulin-like growth factor-1/hepatocyte growth factor intracoronary injection fosters survival and regeneration of the infarcted pig heart. *J. Am. Coll. Cardiol., 58*(9), 977-986.

Epp, R. A., Susser, S. E., Morissette, M. P., Kehler, D. S., Jassal, D. S., & Duhamel, T. A. (2013). Exercise training prevents the development of cardiac dysfunction in the low-dose streptozotocin diabetic rats fed a high-fat diet. *Can. J. Physiol. Pharmacol., 91*(1), 80-89.

Ernande, L., Bergerot, C., Rietzschel, E. R., De Buyzere, M. L., Thibault, H., Pignonblanc, P. G., . . . Derumeaux, G. (2011). Diastolic dysfunction in patients with type 2 diabetes mellitus: is it really the first marker of diabetic cardiomyopathy? *J. Am. Soc. Echocardiogr., 24*(11), 1268-1275 e1261.

Factor, S. M., Minase, T., & Sonnenblick, E. H. (1980). Clinical and morphological features of human hypertensive-diabetic cardiomyopathy. *Am. Heart J., 99*(4), 446-458.

Faden, G., Faganello, G., De Feo, S., Berlinghieri, N., Tarantini, L., Di Lenarda, A., . . . Cioffi, G. (2013). The increasing detection of asymptomatic left ventricular dysfunction in patients with type 2 diabetes mellitus without overt cardiac disease: Data from the SHORTWAVE study. *Diabetes Res. Clin. Pract., 101*(3), 309-316.

Falcao-Pires, I., & Leite-Moreira, A. F. (2012). Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. *Heart Fail. Rev., 17*(3), 325-344.

Fang, Z. Y., Prins, J. B., & Marwick, T. H. (2004). Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocr. Rev., 25*(4), 543-567.

Fang, Z. Y., Schull-Meade, R., Downey, M., Prins, J., & Marwick, T. H. (2005). Determinants of subclinical diabetic heart disease. *Diabetologia, 48*(2), 394-402.

Fletcher, G. F., Ades, P. A., Kligfield, P., Arena, R., Balady, G. J., Bittner, V. A., . . . Prevention. (2013). Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation, 128*(8), 873-934.

Fox, C. S., Coady, S., Sorlie, P. D., D'Agostino, R. B., Sr., Pencina, M. J., Vasan, R. S., . . . Savage, P. J. (2007). Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. *Circulation, 115*(12), 1544-1550.

Francois, M. E., Baldi, J. C., Manning, P. J., Lucas, S. J., Hawley, J. A., Williams, M. J., & Cotter, J. D. (2014). 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia, 57*(7), 1437-1445.

Freyssin, C., Verkindt, C., Prieur, F., Benaich, P., Maunier, S., & Blanc, P. (2012). Cardiac rehabilitation in chronic heart failure: effect of an 8-week, high-intensity interval training versus continuous training. *Arch. Phys. Med. Rehabil., 93*(8), 1359-1364.

Friis Schmidt, J., Rostgaard Andersen, T., Horton, J., Brix, J., Tarnow, L., Krustrup, P., . . . Riis Hansen, P. (2013). Soccer Training Improves Cardiac Function in Men with Type 2 Diabetes. *Med. Sci. Sports Exerc.*

From, A. M., Scott, C. G., & Chen, H. H. (2010). The development of heart failure in patients with diabetes mellitus and pre-clinical diastolic dysfunction a population-based study. *J. Am. Coll. Cardiol., 55*(4), 300-305.

Frustaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., . . . Anversa, P. (2000). Myocardial cell death in human diabetes. *Circ. Res., 87*(12), 1123-1132.

Fu, T. C., Wang, C. H., Lin, P. S., Hsu, C. C., Cherng, W. J., Huang, S. C., . . . Wang, J. S. (2013). Aerobic interval training improves oxygen uptake efficiency by enhancing cerebral and muscular hemodynamics in patients with heart failure. *Int. J. Cardiol., 167*(1), 41-50.

Galderisi, M., Anderson, K. M., Wilson, P. W., & Levy, D. (1991). Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am. J. Cardiol., 68*(1), 85-89.

Galderisi, M., Paolisso, G., Tagliamonte, M. R., Alfieri, A., Petrocelli, A., de Divitiis, M., . . . de Divitiis, O. (1997). Is insulin action a determinant of left ventricular relaxation in uncomplicated essential hypertension? *J. Hypertens., 15*(7), 745-750.

Garvey, W. T., Hardin, D., Juhaszova, M., & Dominguez, J. H. (1993). Effects of diabetes on myocardial glucose transport system in rats: implications for diabetic cardiomyopathy. *Am. J. Physiol., 264*(3 Pt 2), H837-844.

Gebel, K., Ding, D., Chey, T., Stamatakis, E., Brown, W. J., & Bauman, A. E. (2015). Effect of Moderate to Vigorous Physical Activity on All-Cause Mortality in Middle-aged and Older Australians. *JAMA Intern Med, 175*(6), 970-977.

Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol., 590*(Pt 5), 1077-1084.

Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., . . . Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J. Physiol., 575*(Pt 3), 901-911.

Gillen, J. B., Little, J. P., Punthakee, Z., Tarnopolsky, M. A., Riddell, M. C., & Gibala, M. J. (2012). Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes Obes. Metab., 14*(6), 575-577.

Gledhill, N., Cox, D., & Jamnik, R. (1994). Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. *Med. Sci. Sports Exerc., 26*(9), 1116-1121.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Blaha, M. J., . . . Stroke Statistics, S. (2014). Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation, 129*(3), e28-e292.

Golbidi, S., & Laher, I. (2011). Molecular mechanisms in exercise-induced cardioprotection. *Cardiol. Res. Pract., 2011*, 972807.

Goldin, A., Beckman, J. A., Schmidt, A. M., & Creager, M. A. (2006). Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation, 114*(6), 597-605.

Gonzalez-Alonso, J., & Calbet, J. A. (2003). Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation, 107*(6), 824-830.

Gonzalez-Vilchez, F., Ayuela, J., Ares, M., Pi, J., Castillo, L., & Martin-Duran, R. (2005). Oxidative stress and fibrosis in incipient myocardial dysfunction in type 2 diabetic patients. *Int. J. Cardiol., 101*(1), 53-58.

Gormley, S. E., Swain, D. P., High, R., Spina, R. J., Dowling, E. A., Kotipalli, U. S., & Gandrakota, R. (2008). Effect of intensity of aerobic training on VO2max. *Med. Sci. Sports Exerc., 40*(7), 1336-1343.

Green, D. J., O'Driscoll, G., Joyner, M. J., & Cable, N. T. (2008). Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol (1985), 105*(2), 766-768.

Green, D. J., Spence, A., Rowley, N., Thijssen, D. H., & Naylor, L. H. (2012). Vascular adaptation in athletes: is there an 'athlete's artery'? *Exp. Physiol., 97*(3), 295-304.

Gross, M. L., Heiss, N., Weckbach, M., Hansen, A., El-Shakmak, A., Szabo, A., . . . Amann, K. (2004). ACE-inhibition is superior to endothelin A receptor blockade in preventing abnormal capillary supply and fibrosis of the heart in experimental diabetes. *Diabetologia, 47*(2), 316-324.

Guida, L., Celentano, A., Iannuzzi, R., & Ferrara, L. A. (2001). Insulin resistance, ventricular mass and function in normoglycaemic hypertensives. *Nutr. Metab. Cardiovasc. Dis., 11*(5), 306-311.

Gurdal, A., Kasikcioglu, E., Yakal, S., & Bugra, Z. (2015). Impact of diabetes and diastolic dysfunction on exercise capacity in normotensive patients without coronary artery disease. *Diab. Vasc. Dis. Res.*

Hafstad, A. D., Boardman, N., & Aasum, E. (2015). How Exercise May Amend Metabolic Disturbances in Diabetic Cardiomyopathy. *Antioxid Redox Signal*.

Hafstad, A. D., Boardman, N. T., Lund, J., Hagve, M., Khalid, A. M., Wisloff, U., . . . Aasum, E. (2011). High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. *J Appl Physiol (1985), 111*(5), 1235-1241.

Hafstad, A. D., Lund, J., Hadler-Olsen, E., Hoper, A. C., Larsen, T. S., & Aasum, E. (2013). High- and moderate-intensity training normalizes ventricular function and mechanoenergetics in mice with diet-induced obesity. *Diabetes, 62*(7), 2287-2294.

Halverstadt, A., Phares, D. A., Wilund, K. R., Goldberg, A. P., & Hagberg, J. M. (2007). Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. *Metabolism, 56*(4), 444-450.

Hare, J. L., Hordern, M. D., Leano, R., Stanton, T., Prins, J. B., & Marwick, T. H. (2011). Application of an exercise intervention on the evolution of diastolic dysfunction in patients with diabetes mellitus: efficacy and effectiveness. *Circ. Heart Fail., 4*(4), 441-449.

Hawley, J. A. (2002). Adaptations of skeletal muscle to prolonged, intense endurance training. *Clin. Exp. Pharmacol. Physiol., 29*(3), 218-222.

Hawley, J. A., Hargreaves, M., Joyner, M. J., & Zierath, J. R. (2014). Integrative biology of exercise. *Cell, 159*(4), 738-749.

Hawley, J. A., & Lessard, S. J. (2008). Exercise training-induced improvements in insulin action. *Acta Physiol. (Oxf.), 192*(1), 127-135.

Hayashi, T., Sohmiya, K., Ukimura, A., Endoh, S., Mori, T., Shimomura, H., . . . Kitaura, Y. (2003). Angiotensin II receptor blockade prevents microangiopathy and preserves diastolic function in the diabetic rat heart. *Heart, 89*(10), 1236-1242.

Hayashino, Y., Jackson, J. L., Hirata, T., Fukumori, N., Nakamura, F., Fukuhara, S., . . . Ishii, H. (2014). Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Metabolism, 63*(3), 431-440.

Haykowsky, M. J., Timmons, M. P., Kruger, C., McNeely, M., Taylor, D. A., & Clark, A. M. (2013). Meta-analysis of aerobic interval training on exercise capacity and systolic function in patients with heart failure and reduced ejection fractions. *Am. J. Cardiol., 111*(10), 1466-1469.

He, J., Ogden, L. G., Bazzano, L. A., Vupputuri, S., Loria, C., & Whelton, P. K. (2001). Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Arch. Intern. Med., 161*(7), 996-1002.

Helgerud, J., Engen, L. C., Wisløff, U., & Hoff, J. (2001). Aerobic endurance training improves soccer performance. *Med. Sci. Sports Exerc., 33*(11), 1925-1931.

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., . . . Hoff, J. (2007). Aerobic high-intensity intervals improve VO2max more than moderate training. *Med. Sci. Sports Exerc., 39*(4), 665-671.

Helgerud, J., Høydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., . . . Hoff, J. (2007). Aerobic High-Intensity Intervals Improve VO2max More Than Moderate Training. *Med. Sci. Sports Exerc., 39*(4), 665-671.

Holloszy, J. O. (1967). Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem., 242*(9), 2278-2282.

Holloszy, J. O. (2003). A forty-year memoir of research on the regulation of glucose transport into muscle. *Am. J. Physiol. Endocrinol. Metab., 284*(3), E453-467.

Holt, R. I. G., Cockram, C. S., Flyvbjerg, A., & Goldstein, B. J. (2010). *Textbook of Diabetes. -4th ed.* (4 ed.): Blackwell Publishing Ltd.

Hood, M. S., Little, J. P., Tarnopolsky, M. A., Myslik, F., & Gibala, M. J. (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Med. Sci. Sports Exerc., 43*(10), 1849-1856.

Hordern, M. D., Coombes, J. S., Cooney, L. M., Jeffriess, L., Prins, J. B., & Marwick, T. H. (2009). Effects of exercise intervention on myocardial function in type 2 diabetes. *Heart, 95*(16), 1343-1349.

Horwich, T. B., & Fonarow, G. C. (2010). Glucose, obesity, metabolic syndrome, and diabetes relevance to incidence of heart failure. *J. Am. Coll. Cardiol., 55*(4), 283-293.

How, O. J., Aasum, E., Severson, D. L., Chan, W. Y., Essop, M. F., & Larsen, T. S. (2006). Increased myocardial oxygen consumption reduces cardiac efficiency in diabetic mice. *Diabetes, 55*(2), 466-473.

Hsueh, W., Abel, E. D., Breslow, J. L., Maeda, N., Davis, R. C., Fisher, E. A., . . . Goldberg, I. J. (2007). Recipes for creating animal models of diabetic cardiovascular disease. *Circ. Res., 100*(10), 1415-1427.

Hu, G., Jousilahti, P., Barengo, N. C., Qiao, Q., Lakka, T. A., & Tuomilehto, J. (2005). Physical activity, cardiovascular risk factors, and mortality among Finnish adults with diabetes. *Diabetes Care, 28*(4), 799-805.

Hunt, S. A., Abraham, W. T., Chin, M. H., Feldman, A. M., Francis, G. S., Ganiats, T. G., . . . American Heart, A. (2009). 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *J. Am. Coll. Cardiol., 53*(15), e1-e90.

Huo, L., & Scarpulla, R. C. (2001). Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. *Mol. Cell. Biol., 21*(2), 644-654.

Ichikawa, R., Daimon, M., Miyazaki, T., Kawata, T., Miyazaki, S., Maruyama, M., . . . Daida, H. (2013). Influencing factors on cardiac structure and function beyond glycemic control in patients with type 2 diabetes mellitus. *Cardiovasc. Diabetol., 12*, 38.

Iellamo, F., Manzi, V., Caminiti, G., Vitale, C., Castagna, C., Massaro, M., . . . Volterrani, M. (2013). Matched dose interval and continuous exercise training induce similar cardiorespiratory and metabolic adaptations in patients with heart failure. *Int. J. Cardiol., 167*(6), 2561-2565.

Ihm, S. H., Youn, H. J., Shin, D. I., Jang, S. W., Park, C. S., Kim, P. J., . . . Choi, K. B. (2007). Serum carboxy-terminal propeptide of type I procollagen (PIP) is a marker of diastolic dysfunction in patients with early type 2 diabetes mellitus. *Int. J. Cardiol., 122*(3), e36-38.

International Diabetes Federation. (2012). *Global Guideline for Type 2 Diabetes* Available at: http://www.idf.org/sites/default/files/IDF%20T2DM%20Guideline.pdf Accessed: October, 2015.

International Diabetes Federation. (2013). *IDF Diabetes Atlas, 6th edn.* Retrieved from https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf Accessed: October, 2015:

International Diabetes Federation. (2014). *Key findings 2014; Diabetes Atlas* International Diabetes Federation Retrieved from http://www.idf.org/sites/default/files/Atlas-poster-2014_EN.pdf Accessed: October, 2015.

Iribarren, C., Karter, A. J., Go, A. S., Ferrara, A., Liu, J. Y., Sidney, S., & Selby, J. V. (2001). Glycemic control and heart failure among adult patients with diabetes. *Circulation, 103*(22), 2668-2673.

Jacobs, R. A., Fluck, D., Bonne, T. C., Burgi, S., Christensen, P. M., Toigo, M., & Lundby, C. (2013). Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. *J Appl Physiol (1985), 115*(6), 785-793.

Jin, H., Yang, R., Li, W., Lu, H., Ryan, A. M., Ogasawara, A. K., . . . Paoni, N. F. (2000). Effects of exercise training on cardiac function, gene expression, and apoptosis in rats. *Am. J. Physiol. Heart Circ. Physiol., 279*(6), H2994-3002.

Johnstone, M. T., Creager, S. J., Scales, K. M., Cusco, J. A., Lee, B. K., & Creager, M. A. (1993). Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation, 88*(6), 2510-2516.

Jonker, J. T., de Mol, P., de Vries, S. T., Widya, R. L., Hammer, S., van Schinkel, L. D., . . . Lamb, H. J. (2013). Exercise and type 2 diabetes mellitus: changes in tissue-specific fat distribution and cardiac function. *Radiology, 269*(2), 434-442.

Kadoglou, N. P., Iliadis, F., Angelopoulou, N., Perrea, D., Ampatzidis, G., Liapis, C. D., & Alevizos, M. (2007). The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology, 14*(6), 837-843.

Kahn, S. E., Cooper, M. E., & Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet, 383*(9922), 1068-1083.

Kajstura, J., Fiordaliso, F., Andreoli, A. M., Li, B., Chimenti, S., Medow, M. S., . . . Anversa, P. (2001). IGF-1 overexpression inhibits the development of diabetic cardiomyopathy and angiotensin II-mediated oxidative stress. *Diabetes, 50*(6), 1414-1424.

Kannel, W. B., Hjortland, M., & Castelli, W. P. (1974). Role of diabetes in congestive heart failure: the Framingham study. *Am. J. Cardiol., 34*(1), 29-34.

Kannel, W. B., & McGee, D. L. (1979). Diabetes and cardiovascular disease. The Framingham study. *JAMA, 241*(19), 2035-2038.

Karason, K., Sjostrom, L., Wallentin, I., & Peltonen, M. (2003). Impact of blood pressure and insulin on the relationship between body fat and left ventricular structure. *Eur. Heart J., 24*(16), 1500-1505.

Karstoft, K., Christensen, C. S., Pedersen, B. K., & Solomon, T. P. (2014). The acute effects of interval- Vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a crossover, controlled study. *J. Clin. Endocrinol. Metab., 99*(9), 3334-3342.

Karstoft, K., & Pedersen, B. K. (2015). Exercise and type 2 diabetes: focus on metabolism and inflammation. *Immunol. Cell Biol.*

Kavanagh, T., Mertens, D. J., Shephard, R. J., Beyene, J., Kennedy, J., Campbell, R., . . . Yacoub, M. (2003). Long-term cardiorespiratory results of exercise training following cardiac transplantation. *Am. J. Cardiol., 91*(2), 190-194.

Kelley, G. A., & Kelley, K. S. (2007). Effects of aerobic exercise on lipids and lipoproteins in adults with type 2 diabetes: a meta-analysis of randomized-controlled trials. *Public Health, 121*(9), 643-655.

Kemi, O. J., Loennechen, J. P., Wisloff, U., & Ellingsen, O. (2002). Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. *J Appl Physiol (1985), 93*(4), 1301-1309.

Kemi, O. J., & Wisløff, U. (2010). High-intensity aerobic exercise training improves the heart in health and disease. *J. Cardiopulm. Rehabil. Prev., 30*(1), 2-11.

Keteyian, S. J., Brawner, C. A., Savage, P. D., Ehrman, J. K., Schairer, J., Divine, G., . . . Ades, P. A. (2008). Peak aerobic capacity predicts prognosis in patients with coronary heart disease. *Am. Heart J., 156*(2), 292-300.

Kim, M., Oh, J. K., Sakata, S., Liang, I., Park, W., Hajjar, R. J., & Lebeche, D. (2008). Role of resistin in cardiac contractility and hypertrophy. *J. Mol. Cell. Cardiol., 45*(2), 270-280.

Kimm, S. Y., Glynn, N. W., McMahon, R. P., Voorhees, C. C., Striegel-Moore, R. H., & Daniels, S. R. (2006). Self-perceived barriers to activity participation among sedentary adolescent girls. *Med. Sci. Sports Exerc., 38*(3), 534-540.

Klein, K. L. G., M.J. (2008). *Diastology: Clinical Approach to Diastolic Heart Failure* (K. L. G. Klein, M.J. Ed. 1st ed.). Elsevier Inc.: Natasha Andjelkovic.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., . . . Diabetes Prevention Program Research, G. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med., 346*(6), 393-403.

Kodama, S., Saito, K., Tanaka, S., Maki, M., Yachi, Y., Asumi, M., . . . Sone, H. (2009). Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA, 301*(19), 2024-2035.

Kodama, S., Tanaka, S., Heianza, Y., Fujihara, K., Horikawa, C., Shimano, H., . . . Sone, H. (2013). Association between physical activity and risk of all-cause mortality and cardiovascular disease in patients with diabetes: a meta-analysis. *Diabetes Care, 36*(2), 471-479.

Kohl, H. W., Gordon, N. F., Villegas, J. A., & Blair, S. N. (1992). Cardiorespiratory fitness, glycemic status, and mortality risk in men. *Diabetes Care, 15*(2), 184-192.

Kokkinos, P., Myers, J., Nylen, E., Panagiotakos, D. B., Manolis, A., Pittaras, A., . . . Singh, S. (2009). Exercise capacity and all-cause mortality in African American and Caucasian men with type 2 diabetes. *Diabetes Care, 32*(4), 623-628.

Korte, F. S., Mokelke, E. A., Sturek, M., & McDonald, K. S. (2005). Exercise improves impaired ventricular function and alterations of cardiac myofibrillar proteins in diabetic dyslipidemic pigs. *J Appl Physiol (1985), 98*(2), 461-467.

Kosmala, W., Jellis, C. L., & Marwick, T. H. (2015). Exercise limitation associated with asymptomatic left ventricular impairment: analogy with stage B heart failure. *J. Am. Coll. Cardiol., 65*(3), 257-266.

Kwak, H. B., Song, W., & Lawler, J. M. (2006). Exercise training attenuates age-induced elevation in Bax/Bcl-2 ratio, apoptosis, and remodeling in the rat heart. *FASEB J., 20*(6), 791-793.

Lakka, T. A., Laaksonen, D. E., Lakka, H. M., Mannikko, N., Niskanen, L. K., Rauramaa, R., & Salonen, J. T. (2003). Sedentary lifestyle, poor cardiorespiratory fitness, and the metabolic syndrome. *Med. Sci. Sports Exerc., 35*(8), 1279-1286.

Lam, C. S. (2015). Diabetic cardiomyopathy: An expression of stage B heart failure with preserved ejection fraction. *Diab. Vasc. Dis. Res.*

Lamb, H. J., Beyerbacht, H. P., van der Laarse, A., Stoel, B. C., Doornbos, J., van der Wall, E. E., & de Roos, A. (1999). Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation, 99*(17), 2261-2267.

LaMonte, M. J., Barlow, C. E., Jurca, R., Kampert, J. B., Church, T. S., & Blair, S. N. (2005). Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. *Circulation, 112*(4), 505-512.

Laukkanen, J. A., Lakka, T. A., Rauramaa, R., Kuhanen, R., Venalainen, J. M., Salonen, R., & Salonen, J. T. (2001). Cardiovascular fitness as a predictor of mortality in men. *Arch. Intern. Med., 161*(6), 825-831.

Laursen, P. B., Shing, C. M., Peake, J. M., Coombes, J. S., & Jenkins, D. G. (2005). Influence of high-intensity interval training on adaptations in well-trained cyclists. *J. Strength Cond. Res., 19*(3), 527-533.

Lee, A. A., Dillmann, W. H., McCulloch, A. D., & Villarreal, F. J. (1995). Angiotensin II stimulates the autocrine production of transforming growth factor-beta 1 in adult rat cardiac fibroblasts. *J. Mol. Cell. Cardiol., 27*(10), 2347-2357.

Lee, D. C., Sui, X., Ortega, F. B., Kim, Y. S., Church, T. S., Winett, R. A., . . . Blair, S. N. (2011). Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. *Br. J. Sports Med., 45*(6), 504-510.

Lee, I. M., Sesso, H. D., Oguma, Y., & Paffenbarger, R. S., Jr. (2003). Relative intensity of physical activity and risk of coronary heart disease. *Circulation, 107*(8), 1110-1116.

Lee, S. F., Pei, D., Chi, M. J., & Jeng, C. (2015). An investigation and comparison of the effectiveness of different exercise programmes in improving glucose metabolism and pancreatic beta cell function of type 2 diabetes patients. *Int. J. Clin. Pract., 69*(10), 1159-1170.

Leiter, L. A. F., D. H.; Gilbert, R. E.; Gupta, M.; Mancini, G. B.; McFarlane, P. A.; Ross, R.; Teoh, H.; Verma, S.; Anand, S.; Camelon, K.; Chow, C. M.; Cox, J. L.; Despres, J. P.; Genest, J.; Harris, S. B.; Lau, D. C.; Lewanczuk, R.; Liu, P. P.; Lonn, E. M.; McPherson, R.; Poirier, P.; Qaadri, S.; Rabasa-Lhoret, R.; Rabkin, S. W.; Sharma, A. M.; Steele, A. W.; Stone, J. A.; Tardif, J. C.; Tobe, S.; Ur, E. (2011). Identification and management of cardiometabolic risk in Canada: a position paper by the cardiometabolic risk working group (executive summary). *Can. J. Cardiol., 27*(2), 124-131.

Levinger, I., Shaw, C. S., Stepto, N. K., Cassar, S., McAinch, A. J., Cheetham, C., & Maiorana, A. J. (2015). What Doesn't Kill You Makes You Fitter: A Systematic Review of High-Intensity Interval Exercise for Patients with Cardiovascular and Metabolic Diseases. *Clin. Med. Insights Cardiol., 9*, 53-63.

Lewis, E. F., Solomon, S. D., Jablonski, K. A., Rice, M. M., Clemenza, F., Hsia, J., . . . Investigators, P. (2009). Predictors of heart failure in patients with stable coronary artery disease: a PEACE study. *Circ. Heart Fail., 2*(3), 209-216.

Li, L., Muhlfeld, C., Niemann, B., Pan, R., Li, R., Hilfiker-Kleiner, D., . . . Rohrbach, S. (2011). Mitochondrial biogenesis and PGC-1alpha deacetylation by chronic treadmill exercise: differential response in cardiac and skeletal muscle. *Basic Res. Cardiol., 106*(6), 1221-1234.

Liedtke, A. J., DeMaison, L., Eggleston, A. M., Cohen, L. M., & Nellis, S. H. (1988). Changes in substrate metabolism and effects of excess fatty acids in reperfused myocardium. *Circ. Res., 62*(3), 535-542.

Lindstrom, J., Ilanne-Parikka, P., Peltonen, M., Aunola, S., Eriksson, J. G., Hemio, K., . . . Finnish Diabetes Prevention Study, G. (2006). Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet, 368*(9548), 1673-1679.

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol (1985), 111*(6), 1554-1560.

Little, J. P., Jung, M. E., Wright, A. E., Wright, W., & Manders, R. J. (2014). Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. *Appl. Physiol. Nutr. Metab., 39*(7), 835-841.

Little, J. P., Safdar, A., Bishop, D., Tarnopolsky, M. A., & Gibala, M. J. (2011). An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1alpha and activates mitochondrial biogenesis in human skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 300*(6), R1303-1310.

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A., & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J. Physiol., 588*(Pt 6), 1011-1022.

Liu, J. E., Palmieri, V., Roman, M. J., Bella, J. N., Fabsitz, R., Howard, B. V., . . . Devereux, R. B. (2001). The impact of diabetes on left ventricular filling pattern in normotensive and hypertensive adults: the Strong Heart Study. *J. Am. Coll. Cardiol., 37*(7), 1943-1949.

Liu, L., Shi, X., Bharadwaj, K. G., Ikeda, S., Yamashita, H., Yagyu, H., . . . Goldberg, I. J. (2009). DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. *J. Biol. Chem., 284*(52), 36312-36323.

Lopaschuk, G. D., & Russell, J. C. (1991). Myocardial function and energy substrate metabolism in the insulin-resistant JCR:LA corpulent rat. *J Appl Physiol (1985), 71*(4), 1302-1308.

MacDonald, M. R., Petrie, M. C., Hawkins, N. M., Petrie, J. R., Fisher, M., McKelvie, R., . . . McMurray, J. J. (2008). Diabetes, left ventricular systolic dysfunction, and chronic heart failure. *Eur. Heart J., 29*(10), 1224-1240.

Malhotra, A., & Sanghi, V. (1997). Regulation of contractile proteins in diabetic heart. *Cardiovasc. Res., 34*(1), 34-40.

Marwick, T. H. (2008). Diabetic heart disease. *Postgrad. Med. J., 84*(990), 188-192.

Marwick, T. H., Hordern, M. D., Miller, T., Chyun, D. A., Bertoni, A. G., Blumenthal, R. S., . . . Rocchini, A. (2009). Exercise training for type 2 diabetes mellitus: impact on cardiovascular risk: a scientific statement from the American Heart Association. *Circulation, 119*(25), 3244-3262.

Mazumder, P. K., O'Neill, B. T., Roberts, M. W., Buchanan, J., Yun, U. J., Cooksey, R. C., . . . Abel, E. D. (2004). Impaired cardiac efficiency and increased fatty acid oxidation in insulin-resistant ob/ob mouse hearts. *Diabetes, 53*(9), 2366-2374.

McGavock, J. M., Lingvay, I., Zib, I., Tillery, T., Salas, N., Unger, R., . . . Szczepaniak, L. S. (2007). Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation, 116*(10), 1170-1175.

McQueen, A. P., Zhang, D., Hu, P., Swenson, L., Yang, Y., Zaha, V. G., . . . Litwin, S. E. (2005). Contractile dysfunction in hypertrophied hearts with deficient insulin receptor signaling: possible role of reduced capillary density. *J. Mol. Cell. Cardiol., 39*(6), 882-892.

Medford, H. M., Chatham, J. C., & Marsh, S. A. (2012). Chronic ingestion of a Western diet increases O-linked-beta-N-acetylglucosamine (O-GlcNAc) protein modification in the rat heart. *Life Sci., 90*(23-24), 883-888.

Mendes, R., Sousa, N., Almeida, A., Subtil, P., Guedes-Marques, F., Reis, V. M., & Themudo-Barata, J. L. (2015). Exercise prescription for patients with type 2 diabetes-a synthesis of international recommendations: narrative review. *Br. J. Sports Med.*

Meyer, C., & Schwaiger, M. (1997). Myocardial blood flow and glucose metabolism in diabetes mellitus. *Am. J. Cardiol., 80*(3A), 94A-101A.

Mizushige, K., Yao, L., Noma, T., Kiyomoto, H., Yu, Y., Hosomi, N., . . . Matsuo, H. (2000). Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation, 101*(8), 899-907.

Moe, B., Augestad, L. B., Flanders, W. D., Dalen, H., & Nilsen, T. I. (2015). The adverse association of diabetes with risk of first acute myocardial infarction is modified by physical activity and body mass index: prospective data from the HUNT Study, Norway. *Diabetologia, 58*(1), 59-66.

Moholdt, T. T., Amundsen, B. H., Rustad, L. A., Wahba, A., Lovo, K. T., Gullikstad, L. R., . . . Slordahl, S. A. (2009). Aerobic interval training versus continuous moderate exercise after coronary artery bypass surgery: a randomized study of cardiovascular effects and quality of life. *Am. Heart J., 158*(6), 1031-1037.

Molmen-Hansen, H. E., Stolen, T., Tjonna, A. E., Aamot, I. L., Ekeberg, I. S., Tyldum, G. A., . . . Stoylen, A. (2012). Aerobic interval training reduces blood pressure and improves myocardial function in hypertensive patients. *Eur J Prev Cardiol, 19*(2), 151-160.

Montero, D., Walther, G., Benamo, E., Perez-Martin, A., & Vinet, A. (2013). Effects of Exercise Training on Arterial Function in Type 2 Diabetes Mellitus : A Systematic Review and Meta-Analysis. *Sports Med.*

Mootha, V. K. L., C.M.; Eriksson, K.F.;Subramanian, A.;Sihag, S.;Lehar, J.;Puigserver, P.;Carlsson, E.;Ridderstråle, M.;Laurila, E.;Houstis, N.;Daly M.J.;Patterson, N.;Mesirov, J.P.;Golub, T.R.;Tamayo, P.;Spiegelman, B.;Lander, E.S.;Hirschhorn, J.N.;Altshuler, D.;Groop, L.C. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet., 34*, 267-273.

Mora, S., Cook, N., Buring, J. E., Ridker, P. M., & Lee, I. M. (2007). Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation, 116*(19), 2110-2118.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., . . . Stroke Statistics, S. (2015). Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation, 131*(4), e29-322.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002). Exercise capacity and mortality among men referred for exercise testing. *N. Engl. J. Med., 346*(11), 793-801.

Nagueh, S. F., Appleton, C. P., Gillebert, T. C., Marino, P. N., Oh, J. K., Smiseth, O. A., . . . Evangelisa, A. (2009). Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur. J. Echocardiogr., 10*(2), 165-193.

Nelson, K. M., Reiber, G., Boyko, E. J., & Nhanes, III. (2002). Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III). *Diabetes Care, 25*(10), 1722-1728.

Neri Serneri, G. G., Boddi, M., Modesti, P. A., Cecioni, I., Coppo, M., Padeletti, L., . . . Galanti, G. (2001). Increased cardiac sympathetic activity and insulin-like growth factor-I formation are associated with physiological hypertrophy in athletes. *Circ. Res., 89*(11), 977-982.

Nes, B. M., Janszky, I., Aspenes, S. T., Bertheussen, G. F., Vatten, L. J., & Wisloff, U. (2012). Exercise patterns and peak oxygen uptake in a healthy population: the HUNT study. *Med. Sci. Sports Exerc., 44*(10), 1881-1889.

Nes, B. M., Vatten, L. J., Nauman, J., Janszky, I., & Wisloff, U. (2014). A simple nonexercise model of cardiorespiratory fitness predicts long-term mortality. *Med. Sci. Sports Exerc., 46*(6), 1159-1165.

Nichols, G. A., Gullion, C. M., Koro, C. E., Ephross, S. A., & Brown, J. B. (2004). The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes Care, 27*(8), 1879-1884.

Nichols, G. A., Hillier, T. A., Erbey, J. R., & Brown, J. B. (2001). Congestive heart failure in type 2 diabetes: prevalence, incidence, and risk factors. *Diabetes Care, 24*(9), 1614-1619.

Nikitin, N. P., Witte, K. K., Thackray, S. D., de Silva, R., Clark, A. L., & Cleland, J. G. (2003). Longitudinal ventricular function: normal values of atrioventricular annular and myocardial velocities measured with quantitative two-dimensional color Doppler tissue imaging. *J. Am. Soc. Echocardiogr., 16*(9), 906-921.

Nitenberg, A., Valensi, P., Sachs, R., Dali, M., Aptecar, E., & Attali, J. R. (1993). Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes, 42*(7), 1017-1025.

Ogawa, T., Spina, R. J., Martin, W. H., 3rd, Kohrt, W. M., Schechtman, K. B., Holloszy, J. O., & Ehsani, A. A. (1992). Effects of aging, sex, and physical training on cardiovascular responses to exercise. *Circulation, 86*(2), 494-503.

Opdahl, A., Remme, E. W., Helle-Valle, T., Edvardsen, T., & Smiseth, O. A. (2012). Myocardial relaxation, restoring forces, and early-diastolic load are independent determinants of left ventricular untwisting rate. *Circulation, 126*(12), 1441-1451.

Opdahl, A., Remme, E. W., Helle-Valle, T., Lyseggen, E., Vartdal, T., Pettersen, E., . . . Smiseth, O. A. (2009). Determinants of left ventricular early-diastolic lengthening velocity: independent contributions from left ventricular relaxation, restoring forces, and lengthening load. *Circulation, 119*(19), 2578-2586.

Paffenbarger, R. S., Jr., Hyde, R. T., Wing, A. L., Lee, I. M., Jung, D. L., & Kampert, J. B. (1993). The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. *N. Engl. J. Med., 328*(8), 538-545.

Pan, X. R., Li, G. W., Hu, Y. H., Wang, J. X., Yang, W. Y., An, Z. X., . . . Howard, B. V. (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care, 20*(4), 537-544.

Parra, V., Verdejo, H. E., Iglewski, M., Del Campo, A., Troncoso, R., Jones, D., . . . Lavandero, S. (2014). Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFkappaB-Opa-1 signaling pathway. *Diabetes, 63*(1), 75-88.

Patel, T. P., Rawal, K., Bagchi, A. K., Akolkar, G., Bernardes, N., Dias, D. D., . . . Singal, P. K. (2015). Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. *Heart Fail. Rev.*

Patti, M. E., Butte, A. J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., . . . Mandarino, L. J. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc. Natl. Acad. Sci. U. S. A., 100*(14), 8466-8471.

Peterson, L. R., Herrero, P., McGill, J., Schechtman, K. B., Kisrieva-Ware, Z., Lesniak, D., & Gropler, R. J. (2008). Fatty acids and insulin modulate myocardial substrate metabolism in humans with type 1 diabetes. *Diabetes, 57*(1), 32-40.

Peterson, L. R., Herrero, P., Schechtman, K. B., Racette, S. B., Waggoner, A. D., Kisrieva-Ware, Z., . . . Gropler, R. J. (2004). Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation, 109*(18), 2191-2196.

Pi-Sunyer, X., Blackburn, G., Brancati, F. L., Bray, G. A., Bright, R., Clark, J. M., . . . Yanovski, S. Z. (2007). Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care, 30*(6), 1374-1383.

Pieri, B. L., Souza, D. R., Luciano, T. F., Marques, S. O., Pauli, J. R., Silva, A. S., . . . De Souza, C. T. (2014). Effects of physical exercise on the P38MAPK/REDD1/14-3-3 pathways in the myocardium of diet-induced obesity rats. *Horm. Metab. Res., 46*(9), 621-627.

Poirier, P., Bogaty, P., Garneau, C., Marois, L., & Dumesnil, J. G. (2001). Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. *Diabetes Care, 24*(1), 5-10.

Poirier, P., Bogaty, P., Philippon, F., Garneau, C., Fortin, C., & Dumesnil, J. G. (2003). Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic neuropathy in men with uncomplicated well-controlled type 2 diabetes. *Metabolism, 52*(8), 1056-1061.

Poirier, P., Garneau, C., Bogaty, P., Nadeau, A., Marois, L., Brochu, C., . . . Dumesnil, J. G. (2000). Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol., 85*(4), 473-477.

Poornima, I. G., Parikh, P., & Shannon, R. P. (2006). Diabetic cardiomyopathy: the search for a unifying hypothesis. *Circ. Res., 98*(5), 596-605.

Powers, S. K., Quindry, J. C., & Kavazis, A. N. (2008). Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury. *Free Radic. Biol. Med., 44*(2), 193-201.

Radak, Z., Taylor, A. W., Ohno, H., & Goto, S. (2001). Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exerc. Immunol. Rev., 7*, 90-107.

Radovits, T., Korkmaz, S., Matyas, C., Olah, A., Nemeth, B. T., Pali, S., . . . Szabo, G. (2015). An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of type-1 and type-2 diabetic cardiac dysfunction. *J Diabetes Res, 2015*, 728741.

Rakobowchuk, M., Tanguay, S., Burgomaster, K. A., Howarth, K. R., Gibala, M. J., & MacDonald, M. J. (2008). Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 295*(1), R236-242.

Rastaldo, R., Pagliaro, P., Cappello, S., Penna, C., Mancardi, D., Westerhof, N., & Losano, G. (2007). Nitric oxide and cardiac function. *Life Sci., 81*(10), 779-793.

Razeghi, P., Young, M. E., Cockrill, T. C., Frazier, O. H., & Taegtmeyer, H. (2002). Downregulation of myocardial myocyte enhancer factor 2C and myocyte enhancer factor 2C-regulated gene expression in diabetic patients with nonischemic heart failure. *Circulation, 106*(4), 407-411.

Reddigan, J. I., Riddell, M. C., & Kuk, J. L. (2012). The joint association of physical activity and glycaemic control in predicting cardiovascular death and all-cause mortality in the US population. *Diabetologia, 55*(3), 632-635.

Redfield, M. M., Jacobsen, S. J., Burnett, J. C., Jr., Mahoney, D. W., Bailey, K. R., & Rodeheffer, R. J. (2003). Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA, 289*(2), 194-202.

Rerkpattanapipat, P., D'Agostino, R. B., Jr., Link, K. M., Shahar, E., Lima, J. A., Bluemke, D. A., . . . Hundley, W. G. (2009). Location of arterial stiffening differs in those with impaired fasting glucose versus diabetes: implications for left ventricular hypertrophy from the Multi-Ethnic Study of Atherosclerosis. *Diabetes, 58*(4), 946-953.

Ridker, P. M., Danielson, E., Fonseca, F. A., Genest, J., Gotto, A. M., Jr., Kastelein, J. J., . . . Group, J. S. (2008). Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N. Engl. J. Med., 359*(21), 2195-2207.

Riehle, C., Wende, A. R., Zhu, Y., Oliveira, K. J., Pereira, R. O., Jaishy, B. P., . . . Abel, E. D. (2014). Insulin receptor substrates are essential for the bioenergetic and hypertrophic response of the heart to exercise training. *Mol. Cell. Biol., 34*(18), 3450-3460.

Rijzewijk, L. J., van der Meer, R. W., Lamb, H. J., de Jong, H. W., Lubberink, M., Romijn, J. A., . . . Diamant, M. (2009). Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies with cardiac positron emission tomography and magnetic resonance imaging. *J. Am. Coll. Cardiol., 54*(16), 1524-1532.

Rijzewijk, L. J., van der Meer, R. W., Smit, J. W., Diamant, M., Bax, J. J., Hammer, S., . . . Lamb, H. J. (2008). Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J. Am. Coll. Cardiol., 52*(22), 1793-1799.

Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., . . . Bluher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl. Acad. Sci. U. S. A., 106*(21), 8665-8670.

Roditis, P., Dimopoulos, S., Sakellariou, D., Sarafoglou, S., Kaldara, E., Venetsanakos, J., . . . Nanas, S. (2007). The effects of exercise training on the kinetics of oxygen uptake in patients with chronic heart failure. *Eur. J. Cardiovasc. Prev. Rehabil., 14*(2), 304-311.

Rognmo, O., Hetland, E., Helgerud, J., Hoff, J., & Slordahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur. J. Cardiovasc. Prev. Rehabil., 11*(3), 216-222.

Ross, R., Dagnone, D., Jones, P. J., Smith, H., Paddags, A., Hudson, R., & Janssen, I. (2000). Reduction in obesity and related comorbid conditions after diet-induced weight loss or

exercise-induced weight loss in men. A randomized, controlled trial. *Ann. Intern. Med., 133*(2), 92-103.

Ross, R., Janssen, I., Dawson, J., Kungl, A. M., Kuk, J. L., Wong, S. L., . . . Hudson, R. (2004). Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obes. Res., 12*(5), 789-798.

Rubler, S., Dlugash, J., Yuceoglu, Y. Z., Kumral, T., Branwood, A. W., & Grishman, A. (1972). New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am. J. Cardiol., 30*(6), 595-602.

Russell, L. K., Mansfield, C. M., Lehman, J. J., Kovacs, A., Courtois, M., Saffitz, J. E., . . . Kelly, D. P. (2004). Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1alpha promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. *Circ. Res., 94*(4), 525-533.

Russell, R. R., 3rd, Yin, R., Caplan, M. J., Hu, X., Ren, J., Shulman, G. I., . . . Young, L. H. (1998). Additive effects of hyperinsulinemia and ischemia on myocardial GLUT1 and GLUT4 translocation in vivo. *Circulation, 98*(20), 2180-2186.

Ryden, L., Grant, P. J., Anker, S. D., Berne, C., Cosentino, F., Danchin, N., . . . Xuereb, R. G. (2013). ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur. Heart J., 34*(39), 3035-3087.

Sacre, J. W., Jellis, C. L., Jenkins, C., Haluska, B. A., Baumert, M., Coombes, J. S., & Marwick, T. H. (2014). A six-month exercise intervention in subclinical diabetic heart disease: effects on exercise capacity, autonomic and myocardial function. *Metabolism, 63*(9), 1104-1114.

Saltin, B., & Strange, S. (1992). Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med. Sci. Sports Exerc., 24*(1), 30-37.

Sassen, B., Cornelissen, V. A., Kiers, H., Wittink, H., Kok, G., & Vanhees, L. (2009). Physical fitness matters more than physical activity in controlling cardiovascular disease risk factors. *Eur. J. Cardiovasc. Prev. Rehabil., 16*(6), 677-683.

Sawada, S. S., Lee, I. M., Naito, H., Noguchi, J., Tsukamoto, K., Muto, T., . . . Blair, S. N. (2010). Long-term trends in cardiorespiratory fitness and the incidence of type 2 diabetes. *Diabetes Care, 33*(6), 1353-1357.

Scharhag, J., Thunenkotter, T., Urhausen, A., Schneider, G., & Kindermann, W. (2010). Echocardiography of the right ventricle in athlete's heart and hearts of normal size compared to magnetic resonance imaging: which measurements should be applied in athletes? *Int. J. Sports Med., 31*(1), 58-64.

Scheuermann-Freestone, M., Madsen, P. L., Manners, D., Blamire, A. M., Buckingham, R. E., Styles, P., . . . Clarke, K. (2003). Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation, 107*(24), 3040-3046.

Schjerve, I. E., Tyldum, G. A., Tjonna, A. E., Stolen, T., Loennechen, J. P., Hansen, H. E., . . . Wisloff, U. (2008). Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. *Clin. Sci. (Lond.), 115*(9), 283-293.

Schrauwen-Hinderling, V. B., Meex, R. C., Hesselink, M. K., van de Weijer, T., Leiner, T., Schar, M., . . . Kooi, M. E. (2011). Cardiac lipid content is unresponsive to a physical activity training intervention in type 2 diabetic patients, despite improved ejection fraction. *Cardiovasc. Diabetol., 10*, 47.

Sengupta, P. P., Tajik, A. J., Chandrasekaran, K., & Khandheria, B. K. (2008). Twist mechanics of the left ventricle: principles and application. *JACC Cardiovasc. Imaging, 1*(3), 366-376.

Sharma, S., Adrogue, J. V., Golfman, L., Uray, I., Lemm, J., Youker, K., . . . Taegtmeyer, H. (2004). Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J., 18*(14), 1692-1700.

Shearer, J., Ross, K. D., Hughey, C. C., Johnsen, V. L., Hittel, D. S., & Severson, D. L. (2011). Exercise training does not correct abnormal cardiac glycogen accumulation in the db/db mouse model of type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab., 301*(1), E31-39.

Shimizu, M., Umeda, K., Sugihara, N., Yoshio, H., Ino, H., Takeda, R., . . . Nakanishi, I. (1993). Collagen remodelling in myocardia of patients with diabetes. *J. Clin. Pathol., 46*(1), 32-36.

Shindler, D. M., Kostis, J. B., Yusuf, S., Quinones, M. A., Pitt, B., Stewart, D., . . . Wilson, A. C. (1996). Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) Trials and Registry. *Am. J. Cardiol., 77*(11), 1017-1020.

Sigal, R. J., Kenny, G. P., Boule, N. G., Wells, G. A., Prud'homme, D., Fortier, M., . . . Jaffey, J. (2007). Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Ann. Intern. Med., 147*(6), 357-369.

Siu, P. M., Bryner, R. W., Martyn, J. K., & Alway, S. E. (2004). Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J., 18*(10), 1150-1152.

Sivitz, W. I., DeSautel, S. L., Kayano, T., Bell, G. I., & Pessin, J. E. (1989). Regulation of glucose transporter messenger RNA in insulin-deficient states. *Nature, 340*(6228), 72-74.

Sixt, S., Beer, S., Bluher, M., Korff, N., Peschel, T., Sonnabend, M., . . . Niebauer, J. (2010). Long- but not short-term multifactorial intervention with focus on exercise training improves coronary endothelial dysfunction in diabetes mellitus type 2 and coronary artery disease. *Eur. Heart J., 31*(1), 112-119.

Sluik, D., Buijsse, B., Muckelbauer, R., Kaaks, R., Teucher, B., Johnsen, N. F., . . . Nothlings, U. (2012). Physical Activity and Mortality in Individuals With Diabetes Mellitus: A Prospective Study and Meta-analysis. *Arch. Intern. Med., 172*(17), 1285-1295.

Snowling, N. J., & Hopkins, W. G. (2006). Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: a meta-analysis. *Diabetes Care, 29*(11), 2518-2527.

Stefanidis, A., Bousboulas, S., Kalafatis, J., Baroutsi, K., Margos, P., Komninos, K., . . . Papasteriadis, E. (2009). Left ventricular anatomical and functional changes with ageing in type 2 diabetic adults. *Eur. J. Echocardiogr., 10*(5), 647-653.

Stolen, T. O., Hoydal, M. A., Kemi, O. J., Catalucci, D., Ceci, M., Aasum, E., . . . Wisloff, U. (2009). Interval training normalizes cardiomyocyte function, diastolic Ca2+ control, and SR Ca2+ release synchronicity in a mouse model of diabetic cardiomyopathy. *Circ. Res., 105*(6), 527-536.

Stoylen, A., Wisloff, U., & Slordahl, S. (2003). Left ventricular mechanics during exercise: a Doppler and tissue Doppler study. *Eur. J. Echocardiogr., 4*(4), 286-291.

Strauer, B. E., Motz, W., Vogt, M., & Schwartzkopff, B. (1997). Impaired coronary flow reserve in NIDDM: a possible role for diabetic cardiopathy in humans. *Diabetes, 46 Suppl 2*, S119-124.

Strom, C. C., Aplin, M., Ploug, T., Christoffersen, T. E., Langfort, J., Viese, M., . . . Sheikh, S. P. (2005). Expression profiling reveals differences in metabolic gene expression between exercise-induced cardiac effects and maladaptive cardiac hypertrophy. *FEBS J, 272*(11), 2684-2695.

Stutts, W. C. (2002). Physical activity determinants in adults. Perceived benefits, barriers, and self efficacy. *AAOHN J., 50*(11), 499-507.

Sunni, S., Bishop, S. P., Kent, S. P., & Geer, J. C. (1986). Diabetic cardiomyopathy. A morphological study of intramyocardial arteries. *Arch. Pathol. Lab. Med., 110*(5), 375-381.

Swain, D. P., & Franklin, B. A. (2006). Comparison of cardioprotective benefits of vigorous versus moderate intensity aerobic exercise. *Am. J. Cardiol., 97*(1), 141-147.

Talbot, L. A., Morrell, C. H., Metter, E. J., & Fleg, J. L. (2002). Comparison of cardiorespiratory fitness versus leisure time physical activity as predictors of coronary events in men aged < or = 65 years and > 65 years. *Am. J. Cardiol., 89*(10), 1187-1192.

Tanasescu, M., Leitzmann, M. F., Rimm, E. B., & Hu, F. B. (2003). Physical activity in relation to cardiovascular disease and total mortality among men with type 2 diabetes. *Circulation, 107*(19), 2435-2439.

Thomas, N., Alder, E., & Leese, G. P. (2004). Barriers to physical activity in patients with diabetes. *Postgrad. Med. J., 80*(943), 287-291.

Tjonna, A. E., Lee, S. J., Rognmo, O., Stolen, T. O., Bye, A., Haram, P. M., . . . Wisloff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation, 118*(4), 346-354.

Tjonna, A. E., Leinan, I. M., Bartnes, A. T., Jenssen, B. M., Gibala, M. J., Winett, R. A., & Wisloff, U. (2013). Low- and high-volume of intensive endurance training significantly

improves maximal oxygen uptake after 10-weeks of training in healthy men. *PLoS One, 8*(5), e65382.

Trost, S. G., Owen, N., Bauman, A. E., Sallis, J. F., & Brown, W. (2002). Correlates of adults' participation in physical activity: review and update. *Med. Sci. Sports Exerc., 34*(12), 1996-2001.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., . . . Finnish Diabetes Prevention Study, G. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med., 344*(18), 1343-1350.

Tyldum, G. A., Schjerve, I. E., Tjonna, A. E., Kirkeby-Garstad, I., Stolen, T. O., Richardson, R. S., & Wisloff, U. (2009). Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *J. Am. Coll. Cardiol., 53*(2), 200-206.

Utomi, V., Oxborough, D., Whyte, G. P., Somauroo, J., Sharma, S., Shave, R., . . . George, K. (2013). Systematic review and meta-analysis of training mode, imaging modality and body size influences on the morphology and function of the male athlete's heart. *Heart, 99*(23), 1727-1733.

Vakkilainen, J., Makimattila, S., Seppala-Lindroos, A., Vehkavaara, S., Lahdenpera, S., Groop, P. H., . . . Yki-Jarvinen, H. (2000). Endothelial dysfunction in men with small LDL particles. *Circulation, 102*(7), 716-721.

Van den Bergh, A., Vanderper, A., Vangheluwe, P., Desjardins, F., Nevelsteen, I., Verreth, W., . . . Herijgers, P. (2008). Dyslipidaemia in type II diabetic mice does not aggravate contractile impairment but increases ventricular stiffness. *Cardiovasc. Res., 77*(2), 371-379.

Van Dijk, J. W., Manders, R. J., Canfora, E. E., Mechelen, W. V., Hartgens, F., Stehouwer, C. D., & Van Loon, L. J. (2013). Exercise and 24-h glycemic control: equal effects for all type 2 diabetes patients? *Med. Sci. Sports Exerc., 45*(4), 628-635.

van Dijk, J. W., Manders, R. J., Hartgens, F., Stehouwer, C. D., Praet, S. F., & van Loon, L. J. (2011). Postprandial hyperglycemia is highly prevalent throughout the day in type 2 diabetes patients. *Diabetes Res. Clin. Pract., 93*(1), 31-37.

van Hoeven, K. H., & Factor, S. M. (1990). A comparison of the pathological spectrum of hypertensive, diabetic, and hypertensive-diabetic heart disease. *Circulation, 82*(3), 848-855.

Vanninen, E., Mustonen, J., Vainio, P., Lansimies, E., & Uusitupa, M. (1992). Left ventricular function and dimensions in newly diagnosed non-insulin-dependent diabetes mellitus. *Am. J. Cardiol., 70*(3), 371-378.

Vehkavaara, S., & Yki-Jarvinen, H. (2004). 3.5 years of insulin therapy with insulin glargine improves in vivo endothelial function in type 2 diabetes. *Arterioscler. Thromb. Vasc. Biol., 24*(2), 325-330.

Vinetti, G., Mozzini, C., Desenzani, P., Boni, E., Bulla, L., Lorenzetti, I., . . . Assanelli, D. (2015). Supervised exercise training reduces oxidative stress and cardiometabolic risk in adults with type 2 diabetes: a randomized controlled trial. *Sci. Rep., 5*, 9238.

Von Bibra, H., Thrainsdottir, I. S., Hansen, A., Dounis, V., Malmberg, K., & Ryden, L. (2005). Tissue Doppler imaging for the detection and quantitation of myocardial dysfunction in patients with type 2 diabetes mellitus. *Diab. Vasc. Dis. Res., 2*(1), 24-30.

Wagner, P. D. (2000). New ideas on limitations to VO2max. *Exerc. Sport Sci. Rev., 28*(1), 10-14.

Wang, H., Bei, Y., Lu, Y., Sun, W., Liu, Q., Wang, Y., . . . Kong, X. (2015). Exercise Prevents Cardiac Injury and Improves Mitochondrial Biogenesis in Advanced Diabetic Cardiomyopathy with PGC-1alpha and Akt Activation. *Cell. Physiol. Biochem., 35*(6), 2159-2168.

Wang, J., Khoury, D. S., Yue, Y., Torre-Amione, G., & Nagueh, S. F. (2007). Left ventricular untwisting rate by speckle tracking echocardiography. *Circulation, 116*(22), 2580-2586.

Warburton, D. E., Haykowsky, M. J., Quinney, H. A., Blackmore, D., Teo, K. K., Taylor, D. A., . . . Humen, D. P. (2004). Blood volume expansion and cardiorespiratory function: effects of training modality. *Med. Sci. Sports Exerc., 36*(6), 991-1000.

Warburton, D. E., McKenzie, D. C., Haykowsky, M. J., Taylor, A., Shoemaker, P., Ignaszewski, A. P., & Chan, S. Y. (2005). Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. *Am. J. Cardiol., 95*(9), 1080-1084.

Waring, C. D., Vicinanza, C., Papalamprou, A., Smith, A. J., Purushothaman, S., Goldspink, D. F., . . . Ellison, G. M. (2014). The adult heart responds to increased workload with physiologic hypertrophy, cardiac stem cell activation, and new myocyte formation. *Eur. Heart J., 35*(39), 2722-2731.

Way, K. J., Isshiki, K., Suzuma, K., Yokota, T., Zvagelsky, D., Schoen, F. J., . . . King, G. L. (2002). Expression of connective tissue growth factor is increased in injured myocardium associated with protein kinase C beta2 activation and diabetes. *Diabetes, 51*(9), 2709-2718.

Wei, M., Gibbons, L. W., Kampert, J. B., Nichaman, M. Z., & Blair, S. N. (2000). Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. *Ann. Intern. Med., 132*(8), 605-611.

Wei, M., Gibbons, L. W., Mitchell, T. L., Kampert, J. B., Lee, C. D., & Blair, S. N. (1999). The association between cardiorespiratory fitness and impaired fasting glucose and type 2 diabetes mellitus in men. *Ann. Intern. Med., 130*(2), 89-96.

Wen, C. P., Wai, J. P., Tsai, M. K., Yang, Y. C., Cheng, T. Y., Lee, M. C., . . . Wu, X. (2011). Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. *Lancet, 378*(9798), 1244-1253.

Wende, A. R., & Abel, E. D. (2010). Lipotoxicity in the heart. *Biochim. Biophys. Acta, 1801*(3), 311-319.

Westerbacka, J., Bergholm, R., Tiikkainen, M., & Yki-Jarvinen, H. (2004). Glargine and regular human insulin similarly acutely enhance endothelium-dependent vasodilatation in normal subjects. *Arterioscler. Thromb. Vasc. Biol., 24*(2), 320-324.

Weston, K. S., Wisloff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med., 48*(16), 1227-1234.

Whiting, D. R., Guariguata, L., Weil, C., & Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res. Clin. Pract., 94*(3), 311-321.

Wilson, M., O'Hanlon, R., Prasad, S., Deighan, A., Macmillan, P., Oxborough, D., . . . Whyte, G. (2011). Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athletes. *J Appl Physiol (1985), 110*(6), 1622-1626.

Wilson, M. G., Ellison, G. M., & Cable, N. T. (2016). Basic science behind the cardiovascular benefits of exercise. *Br. J. Sports Med., 50*(2), 93-99.

Wilt, T. J., Bloomfield, H. E., MacDonald, R., Nelson, D., Rutks, I., Ho, M., . . . Sales, A. (2004). Effectiveness of statin therapy in adults with coronary heart disease. *Arch. Intern. Med., 164*(13), 1427-1436.

Wing, R. R., Bolin, P., Brancati, F. L., Bray, G. A., Clark, J. M., Coday, M., . . . Yanovski, S. Z. (2013). Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N. Engl. J. Med., 369*(2), 145-154.

Winnick, J. J., Sherman, W. M., Habash, D. L., Stout, M. B., Failla, M. L., Belury, M. A., & Schuster, D. P. (2008). Short-term aerobic exercise training in obese humans with type 2 diabetes mellitus improves whole-body insulin sensitivity through gains in peripheral, not hepatic insulin sensitivity. *J. Clin. Endocrinol. Metab., 93*(3), 771-778.

Wisloff, U., Ellingsen, O., & Kemi, O. J. (2009). High-intensity interval training to maximize cardiac benefits of exercise training? *Exerc. Sport Sci. Rev., 37*(3), 139-146.

Wisloff, U., Nilsen, T. I., Droyvold, W. B., Morkved, S., Slordahl, S. A., & Vatten, L. J. (2006). A single weekly bout of exercise may reduce cardiovascular mortality: how little pain for cardiac gain? 'The HUNT study, Norway'. *Eur. J. Cardiovasc. Prev. Rehabil., 13*(5), 798-804.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

World Health Organization. (2016). *Global report on Diabetes*. Retrieved from http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf?ua=1 Accessed: May, 2016:

Wright, J. J., Kim, J., Buchanan, J., Boudina, S., Sena, S., Bakirtzi, K., . . . Abel, E. D. (2009). Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding. *Cardiovasc. Res., 82*(2), 351-360.

Xu, F. P., Chen, M. S., Wang, Y. Z., Yi, Q., Lin, S. B., Chen, A. F., & Luo, J. D. (2004). Leptin induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured neonatal rat cardiomyocytes. *Circulation, 110*(10), 1269-1275.

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E., Jr., Drazner, M. H., . . . American Heart Association Task Force on Practice, G. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol., 62*(16), e147-239.

Yki-Jarvinen, H., & Utriainen, T. (1998). Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia, 41*(4), 369-379.

Yokoyama, I., Ohtake, T., Momomura, S., Yonekura, K., Woo-Soo, S., Nishikawa, J., . . . Omata, M. (1998). Hyperglycemia rather than insulin resistance is related to reduced coronary flow reserve in NIDDM. *Diabetes, 47*(1), 119-124.

Zabalgoitia, M., Ismaeil, M. F., Anderson, L., & Maklady, F. A. (2001). Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol., 87*(3), 320-323.

Zanesco, A., & Antunes, E. (2007). Effects of exercise training on the cardiovascular system: pharmacological approaches. *Pharmacol. Ther., 114*(3), 307-317.

Zethelius, B., Gudbjornsdottir, S., Eliasson, B., Eeg-Olofsson, K., Cederholm, J., & Swedish National Diabetes, R. (2014). Level of physical activity associated with risk of cardiovascular diseases and mortality in patients with type-2 diabetes: report from the Swedish National Diabetes Register. *Eur J Prev Cardiol, 21*(2), 244-251.

Zhou, B., Conlee, R. K., Jensen, R., Fellingham, G. W., George, J. D., & Fisher, A. G. (2001). Stroke volume does not plateau during graded exercise in elite male distance runners. *Med. Sci. Sports Exerc., 33*(11), 1849-1854.

Zierath, J. R., & Wallberg-Henriksson, H. (2015). Looking Ahead Perspective: Where Will the Future of Exercise Biology Take Us? *Cell Metab., 22*(1), 25-30.

CHAPTER 2

Thesis Methods

TABLE OF CONTENTS

CHAPTER 2

2.1 THESIS METHODS

This chapter outlines the central methodology used in this thesis by providing detailed protocols for the methods used in the two studies of this thesis (Chapters 3–5) (Table 2.1). Statistical analyses are described in the respective Chapters 3–5.

2.1.1 Clinical measurements

Clinical examinations done for Chapters 3, 4, and 5 are listed in Table 2.1.

Chapter 3 / Paper 1	Chapter 4 / Paper 2	Chapter 5 / Paper 3
▪ Echocardiography ▪ Exercise stress echocardiography* ▪ Cardiopulmonary exercise test/ $VO_{2max/peak}$ test ▪ HR_{max} ▪ Weight ▪ Height ▪ BMI ▪ WC ▪ DXA ▪ Blood sample ▪ Resting HR ▪ Resting Blood pressure ▪ FMD ▪ Self-reported physical activity* ▪ Self-reported energy intake, food diary*	▪ Echocardiography (i.e. LV twist by speckle-tracking method) * ▪ Resting HR ▪ Resting blood pressure	▪ Echocardiography ▪ Cardiopulmonary exercise test/ $VO_{2max/peak}$ test ▪ HR_{max} ▪ Weight ▪ Height ▪ BMI ▪ WC ▪ Blood sample ▪ Resting HR ▪ Resting blood pressure ▪ Self-reported energy intake*

Table 2.1. Clinical measurements for Chapters 3, 4, and 5. Abbreviations: BMI, Body mass index; DXA, dual energy x-ray absorptiometry; HR, heart rate; FMD, flow mediated dilatation of the brachial artery; LV, left ventricular; VO2max, Maximal oxygen uptake; WC, waist circumference. * This methodology will be discussed in the respective chapter only.

2.1.1.1 Echocardiography

Today, echocardiography plays a central part in the medical diagnosis of cardiac abnormalities, and is considered one of the most important noninvasive tools to evaluate cardiac function.

By echocardiography, the dynamics of cardiac function are commonly assessed by two dimensional (2D) echocardiography in combination with Doppler techniques as well as speckle-tracking imaging (i.e. regional deformation and twist/untwist), all of which will be outlined in this section.

In this thesis, echocardiography was performed according to the American Society of Echocardiography and European Society of Echocardiography (ASE/EAE) recommendations (Lang et al., 2015; Nagueh et al., 2009). Blood flow Doppler recordings were completed according to the ASE/EAE recommendations (Quinones et al., 2002).

Echocardiographic examinations were performed using the Vivid 7 scanner (phased array M4S and M3S transducers; GE Vingmed Ultrasound AS, Horten Norway). All of the recordings were performed by the same experienced clinician (CBI) in Chapter 3, as well as in Chapter 4 (CBI examined the type 2 diabetes (T2D)-group and HD examined the healthy controls). In Study 2, the majority of examinations were performed by either CBI or VM, but a few examinations were performed by other clinicians due to the complicated logistics of the project presented in Chapter 5.

Offline data echocardiographic analyses were performed using commercially available software (EchoPAC PC version 112/BT12; GE Medical Systems, Milwaukee, WI). All analyses were performed blinded to exercise group allocation and time point. Analyses of conventional echocardiographic diastolic measures as well as twist parameters were performed as previously described (Ingul et al., 2010; Molmen et al., 2012; Notomi et al., 2005) and are also described in the respective Chapters 3–5.

Before performing echocardiography, the participants had to go at least 36 hours without exercise.

Left ventricular (LV) diastolic function by echocardiography

Diastolic function can be divided into measurements of: (1) LV relaxation; (2) LV compliance; and (3) filling pressure. A detailed description of the measurements that have been used in Studies 1–2 (Chapters 3–5) will follow under "Echocardiographic measurements of diastolic function."

1) Estimation of LV relaxation

LV relaxation (See "Definitions and explanations") can be estimated directly by isovolumic relaxation time (IVRT) (Figure 2.1). In the normal heart, LV pressure falls rapidly during IVRT. When myocardial relaxation is impaired, however, the time before the LV pressure falls below the left atrial (LA) pressure is prolonged; thus, the mitral valve opening is delayed and IVRT is prolonged.

Surrogate measurements of LV relaxation are mitral inflow velocities (Figure 2.1) and tissue Doppler annular velocities (Figure 2.2) (see "Echocardiographic measurements of diastolic function" in the following section). Another measurement used was the peak late mitral inflow velocity (A)-wave duration.

When the myocardial relaxation is markedly delayed, there will be a reduction in the ratio between peak early mitral inflow velocity (E) and peak late mitral inflow velocity (A) (E/A ratio (< 1)) as well as prolongation of the deceleration time (DT) (> 220 ms) (Figures 2.3–2.4). An increase in the filling pressure can mask the changes in mitral velocities.

Peak early diastolic tissue Doppler velocity (e') is less load dependent compared to mitral inflow velocities, and therefore it serves as a more sensitive parameter for abnormal myocardial relaxation than mitral inflow velocity variables. Impaired myocardial relaxation is found in most patients with e' (lateral) < 8.5 cm/s or e' (septal) < 8 cm/s. At the time of planning for Study 1, there were no large studies reporting normal values. However, the current recommendation is to use normal values for tissue Doppler velocities. Furthermore, the e' that is less than the mean minus two standard deviations of the age group of the patient should be used as a marker of abnormal myocardial relaxation.

2) Estimation of LV compliance

Estimation of LV compliance or inversely LV stiffness (see "Definitions and explanations") can be estimated directly or by surrogate measurements such as DT of E, abbreviated mitral A-wave duration, reduced a', and prolonged atrial reversal (Ar) duration in pulmonary venous flow. The compliance of the LV is determined by the structural properties of the cardiac muscle as well as the end-diastolic pressure and end-diastolic volume (non-linear relation). DT will shorten with an increase in LV stiffness (Figure 2.3).

3) Estimation of LV filling pressures

The E/e' ratio has been correlated with pulmonary capillary wedge pressure and can be used to predict LV filling pressures (Nagueh et al., 1997).

In patients with reduced ejection fraction (EF), indicators of early diastolic LV and LA pressures are increased E/e' ratio, shortened DT, and to some extent LA enlargement, which reflects chronic rather than acute pressure changes. The mitral inflow pattern can be used to estimate filling pressures with the use of additional Doppler parameters when E/A ratio is between 1 and 2. The patients included in the projects of this thesis, however, had preserved EF.

The estimation of LV filling pressures in patients with preserved EF is more challenging than in patients with reduced EF. In patients with normal EFs the E/e' ratio can be a useful measurement; an E/e' ratio < 8 indicate normal LA pressure whereas a ratio of > 15 indicate

increased LA pressure. However, E/e' ratio between 9 and 14 requires further diagnostics including evaluation of LA volume, peak atrial reversal velocity (Ar)–A duration, Valsalva and pulmonary artery systolic pressure. Doppler velocities and time intervals reflect filling pressures at the time of measurement. LA volume reflects the effects of filling pressures over time.

Indisputably, there are severe limitations of the E/e' ratio in estimating atrial pressure and E/e' ratio has been recently challenged in patients with either very advanced heart failure (HF) or preserved LV EF (Bhella et al., 2011; Mullens et al., 2009; Park and Marwick, 2011).

E/e' ratio increases by age (Dalen et al., 2010).

Echocardiographic Doppler measurements of diastolic function

Mitral inflow velocities

In this thesis, mitral inflow velocities were obtained by pulsed-wave (pw) Doppler in the apical four-chamber view with a 2 mm to 3 mm sample volume placed between the mitral leaflet tips during diastole. Spectral gain and wall filter were set to optimize the signal and clearly display the onset and cessation of LV inflow.

IVRT was defined as the interval from the closure of the aortic valve to the opening of the mitral valve. IVRT was derived by placing the cursor of pw Doppler between the LV outflow tract and the mitral inflow, in proximity to the anterior mitral valve, to simultaneously display the outflow signal and the onset of the inflow signal.

Measurements of mitral inflow velocities included the E, A, the E/A ratio and the time intervals of early filling velocity, DT and IVRT (Figure 2.1). The mitral A-wave duration (obtained at the level of the mitral annulus) was also measured. Three cardiac cycles were averaged when measuring mitral inflow velocities and IVRT.

Limitations for the mitral variables occur during sinus tachycardia, conduction system disease, and arrhythmias. Sinus tachycardia and first-degree AV block can result in partial or complete fusion of the mitral E and A waves. However, in our material no patient with conduction system disease or arrhythmia were included. The E-wave is affected by preload and alterations in LV relaxation, the A-wave by LV compliance and LA contractile function, IVRT by preload and DT by LV relaxation and LV compliance (i.e. the relationship between LV pressure and volume). Alterations in LV end-systolic, end-diastolic volumes, LV elastic recoil, and LV diastolic pressures directly affect the early mitral inflow velocity, DT and IVRT. During E/A

fusion, DT cannot be measured, but IVRT should be unaffected. A total E/A fusion measures the combination of ventricular relaxation and atrial systole. Also the E/A estimates the peak pressure during atrial systole and not the mean pressure.

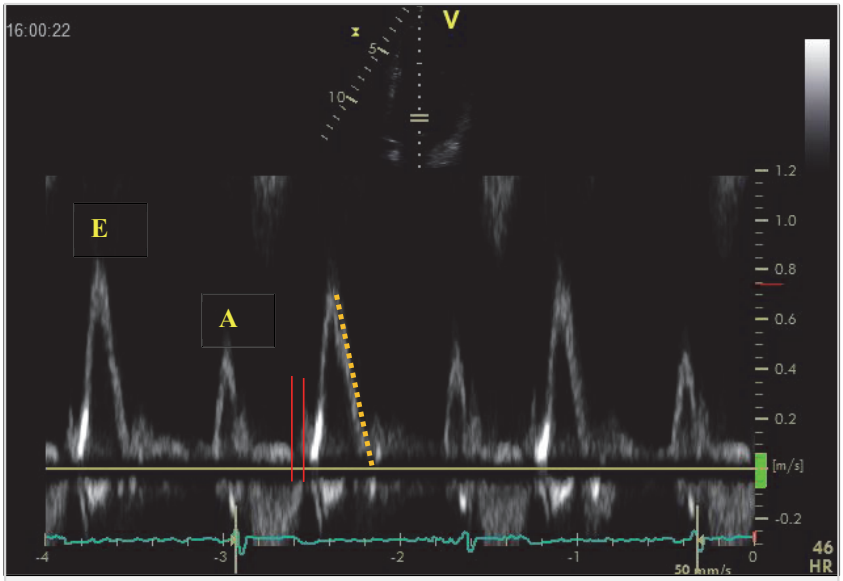


Figure 2.1. Conventional Doppler echocardiography of the LV mitral inflow velocity in an individual with normal cardiac function. E, peak early diastolic mitral inflow velocity. A, late diastolic mitral inflow velocity (atrial contribution). Red lines represent the time interval of isovolumic relaxation (IVRT). The orange dotted line represents deceleration time (DT)

Valsalva maneuver

Valsalva maneuver was used to distinguish between normal and pseudonormal mitral inflow patterns. The Valsalva maneuver was performed by asking the participant to perform a forceful expiration against a closed nose and mouth to increase the intrathoracic pressure.

The Valsalva maneuver decreases preload and a pseudonormal mitral inflow changes to a pattern of impaired relaxation with a decrease of the E-wave, a prolongation of DT and an unchanged or increased A-wave.

Pulmonary Venous Flow

Pw Doppler of pulmonary venous flow was performed in the apical four-chamber view and color flow imaging was used to guide a correct location of the sample volume in the right upper pulmonary vein. A 2 to 3mm sample volume was placed 5mm into the pulmonary vein. To display the onset and cessation of the Ar velocity wave wall filter settings were kept low.

Measurements of pulmonary venous waveforms include peak systolic (S) velocity, peak anterograde diastolic (D) velocity, the S/D ratio, the peak and duration of the Ar velocity, as well as the time difference between Ar wave and mitral A-wave duration.

One of the limitations of the pulmonary venous flow is the difficulty in obtaining high-quality recordings, especially for Ar velocity.

Tissue Doppler Annular Velocities

In tissue Doppler imaging (TDI), the velocity of blood flow is assessed by quantifying the higher-amplitude, lower-velocity signals of the myocardial tissue motion (mitral annulus). TDI can be performed in pulsed-wave (pwTDI) and color modes (cTDI). During resting images pwTDI mitral annular velocities were acquired from the apical four- and two-chamber views. The pwTDI mitral annular velocities were acquired from the base of the septal, lateral, inferior, and anterior walls. Sample volume was positioned within 10 mm of septal, lateral, inferior, and anterior insertion sites of the valve leaflets, and adjusted to cover the longitudinal excursion in both systole and diastole. The pwTDI was measured as maximal values, with a low gain setting. An angle deviation less than 20 degrees was kept between the ultrasound beam and the plane of cardiac motion.

Primary measurements included the peak systolic (S'), e', and peak late diastolic (a') tissue Doppler velocities (Figure 2.2.).

Even though TDI velocities are less load dependent compared to traditional Doppler velocities, there are limitations. For preload, LV filling pressures have little effect on e' if impaired LV relaxation is present, but with normal or enhanced LV relaxation, preload increases e'. If LA contractility increases, a' increases, and decreases with an increase in LV end diastolic pressure.

Normal values of TDI velocities are influenced by age and sex. Increased age decreases annular S' and e' and increases a' (Dalen et al., 2010).

Color TDI measures average velocities and pwTDI measures a wider spectrum of velocities including peak velocities. In a study by Dalen et al. cTDI resulted in 20% lower systolic velocities and 23% lower diastolic mitral annular velocities compared to pwTDI (Dalen et al., 2010).

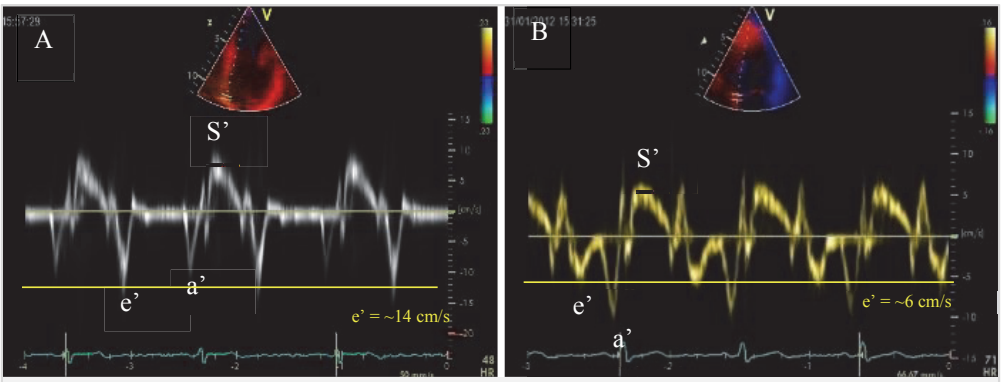


Figure 2.2. Illustration of a pulsed wave tissue Doppler image with sample size placed at septum. A: Echocardiogram of healthy individual with normal diastolic function. B: Echocardiogram of type 2 diabetes individual with diastolic dysfunction participating in Study 1.Abbreviations: a', late diastolic tissue Doppler velocity; e', peak early diastolic tissue Doppler velocity; S' peak systolic tissue Doppler velocity.

The average of three consecutive cardiac cycles was used for the measurements and a mean of the four sites was used. ASE/EAE recommend averaging indices from at least the septal and lateral sites. We used an average from four instead of two walls, which will increase the test-retest reproducibility (Thorstensen et al., 2010).

In the studies of this thesis (Studies 1–2), e' was the primary outcome measure. Our research group has previously reported the inter- and intraobserver variability for e'; by averaging the septal, lateral, inferior, and anterior walls, the mean error was reduced from 15% to 8%. E' had the lowest inter- and intraobserver variability of all the diastolic indexes (E, A, IVRT, DT, a') (Thorstensen et al., 2010).

Pulmonary Artery Systolic and Diastolic Pressures

An increase in pulmonary artery pressure may be used to indicate elevated LV filling pressure (in the absence of pulmonary disease). To derive pulmonary artery systolic pressure, the peak velocity of the tricuspid regurgitation jet by continuous wave Doppler, together with systolic right atrial pressure were used. The estimation of right atrium pressure can be derived using inferior vena cava diameter and its change with respiration. Pulmonary artery diastolic pressure can be derived from the end-diastolic velocity of the pulmonary regurgitation jet.

Limitations of Doppler measurements

Important limitations include the fact that TDI measures only motions parallel to the direction of the ultrasound beam and cannot discriminate passive motion (tethering) from active motion.

Misalignment of the ultrasound beam will cause underestimation of the tissue velocity. Sample size, gain, and filter setting will also affect the velocity measurements.

Furthermore, general limitations for Doppler measurements of diastolic function are dependent on preload, afterload (see "Definitions and explanations"), and sympathetic tone, which could cause individual variability and a day-to-day variation in the same subject. We therefore measured blood pressure, blood glucose level and resting heart rate (HR) immediately before every echocardiographic examination and if possible the same time of the day (morning, lunch, afternoon).

Grading of Diastolic Dysfunction

The grading of diastolic dysfunction was done according to EAE/ASE recommendations: grade I (impaired relaxation pattern); grade II (pseudo normal filling); and grade III (restrictive filling), see figures 2.3–2.4.

Impaired relaxation will nearly always cause diastolic dysfunction. E/A < 1 without any additional evidence of diastolic dysfunction can be normal above 60 years of age. Changes in LV relaxation should be interpreted with care since the primary effect could be changes in load rather than improved intrinsic myocardial function. When assessing diastolic dysfunction, the patient's age and HR should be taken into consideration.

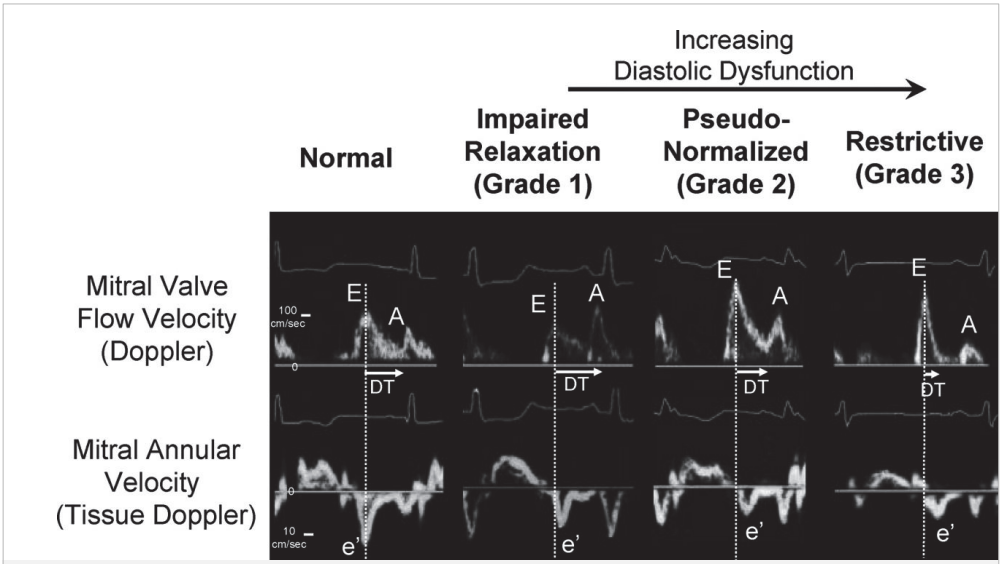


Figure 2.3. The stages of diastolic dysfunction. William C. Little and Jae K. Oh, Circulation® 2009: 120:802-809: Reprinted with permission from ADA.

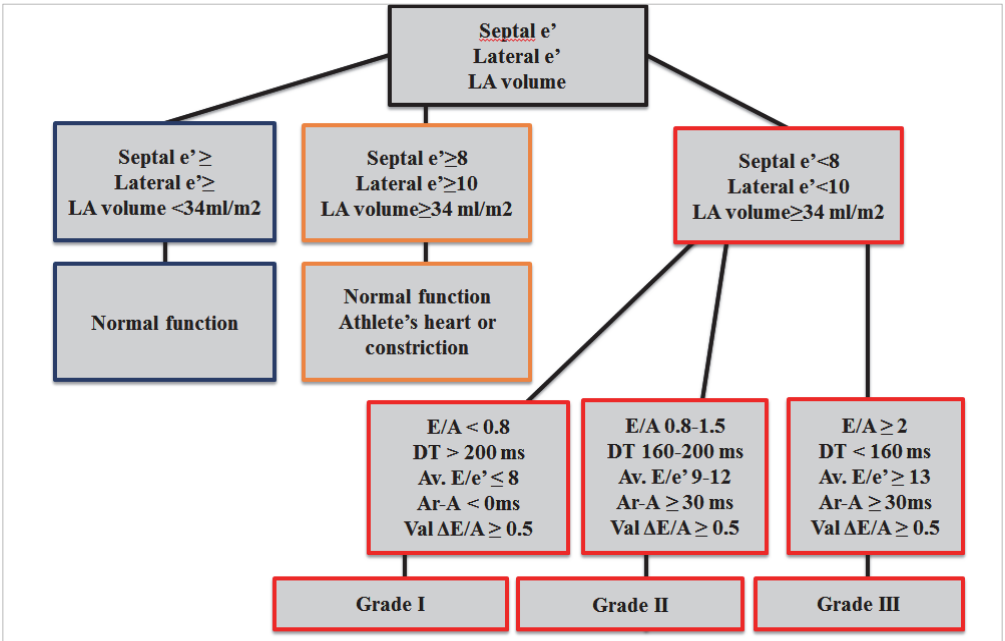


Figure 2.4. Scheme for grading diastolic dysfunction modified after Nagueh et al., J Am Soc Echocardiogr Vol. 10, 2009; 165–193. Blue frames indicate normal diastolic function; orange frames indicate normal diastolic function of an athlete's heart or constriction; red frames indicate diastolic dysfunction, grades I-III. Abbreviations: A, late diastolic mitral inflow velocity; Av, Average; Ar, peak atrial reversal velocity; DT, deceleration time; E, peak early diastolic mitral inflow velocity; e', peak early diastolic tissue Doppler velocity; LA, left atrium; Val, Valsava.

Echocardiographic measurements of left atrium volume, LV volumes and dimensions

LA volume was measured in the apical four-chamber and two-chamber views using the area-length method. Atrial cavity was traced at end-systole, at its greatest dimension. Foreshortening of the left atrium was avoided. LA volume was indexed to body surface area and considered enlarged if more than 34 ml/m^2.

Measurements of the left ventricle and its wall dimensions were performed by the use of M-mode (Figure 2.5 A) in the parasternal long-axis view, perpendicular to the LV long axis and just below the level of the mitral valve leaflet tips. Parasternal short-axis was used if the long-axis view was of poor quality.

LV volumes, end-diastolic volume (EDV) and end-systolic volume (ESV), were measured from the apical four- and two-chamber views using two-dimensional image acquisition. The biplane method of disks summation was used (modified Simpson). EF was calculated from EDV and ESV, using the formula: EF= (EDV-ESV)/EDV. Attention was paid not to foreshorten the left ventricle.

Echocardiographic measurements of systolic function

S' has been described previously in this section (under "Tissue Doppler Annular Velocities").

Stroke volume and cardiac output

Stroke volume (SV) and CO were estimated using the pw Doppler velocity-time integral (VTI) method. The LV outflow tract (LVOT) diameter was measured from the aortic annulus in the parasternal long-axis view and the LV outflow velocity, VTI, was measured from the apical five-chamber. The densest portion of the spectral tracing was traced. The sample volume was positioned proximal to the aortic valve, with the closing click of the aortic valve seen. Three cardiac cycles were averaged.

The flow volume passing through the LVOT can be calculated as the product of the VTI and the cross-sectional area of the LVOT. SV then represents the product of cross-sectional area and VTI.

$SV = \pi * (LVOT^2/2) * LVOT\ VTI$

Deformation Measurements

Strain is a measurement of deformation of an object and is defined as the change in length normalized to the original length, expressed in percent. Positive strain is lengthening or thinning and negative strain is shortening or thickening in relation to the original length (i.e., reduction to half its original length is −50% strain).

Strain rate is the rate of deformation and the unit of strain rate is /s, or s^{-1}. Strain rate is negative during shortening and positive during lengthening. Regional deformation can be assessed for each myocardial segment. Peak systolic strain rate is the non-invasive echocardiographic parameter that comes closest to measuring local contractile function. Both strain and strain rate are load dependent. However, peak systolic strain rate is an early measure of maximum systolic shortening and less load dependent than the end-systolic strain (Thorstensen et al., 2011).

Strain can be calculated from either TDI (Heimdal et al., 1998; Urheim et al., 2000) and more recently from B-mode (2D speckle-tracking) (Amundsen et al., 2006; Langeland et al., 2005; Reisner et al., 2004).

In the projects of this thesis, speckle tracking was used to measure strain at rest (supine position) (Chapters 3–5), whereas tissue Doppler strain measurement was used to achieve high enough frame rate during stress echocardiography in Chapter 3. The strain and strain rate values from these two different methods are thus not comparable. The measurements and acquisition of tissue Doppler strain during stress echocardiography is presented in Chapter 3 (Section 3.3.5.2)

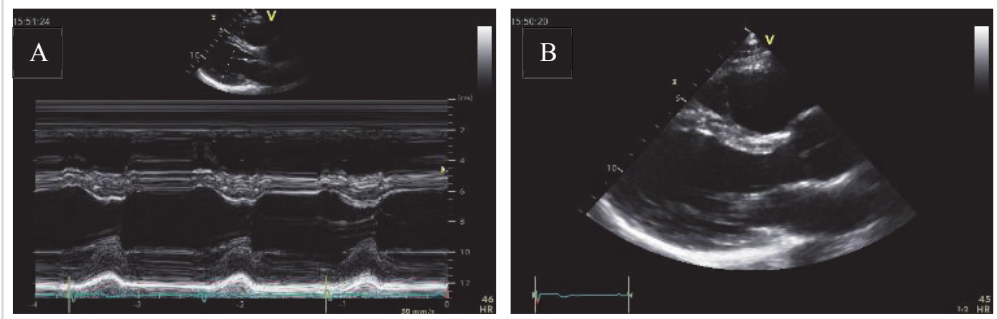


Figure 2.5. A: M-mode image, left ventricle. B: B-mode image of the left ventricle, parasternal long axis view.

2D speckle-tracking echocardiography

Speckle-tracking echocardiography (STE) is based on two-dimensional (2D) gray scale (B-mode) ultrasound images (Figure 2.5 B). The gray scale image contains many small elements, so called natural acoustic markers, which do not change their pattern significantly between adjacent frames. These natural acoustic markers are stable acoustic speckles, STE filters out random noise and the small temporally stable speckles remain. By use of STE changes in position of the speckles in the myocardium within a region of interest can be followed frame by frame throughout the cardiac cycle. During the processing, the software performs spatial and temporal smoothing of the distributed motions and deformation given on a quantitative scale that can be changed and each marker can be identified and followed accurately during several consecutive frames. Strain is then derived by changes in speckles in distance per frame in different directions by using different projections as measures of average regional deformation within a myocardial segment. STE has been validated, and the results have shown good agreements against the use of sonomicrometry in animals and MRI in humans (Behar et al., 2004; Leitman et al., 2004).

Speckle tracking is an offline technique that is applied to the acquired 2D images. The advantage of STE compared to tissue Doppler imaging is that it can measure motion in any direction within the image plane. STE is not completely angle independent, because ultrasound images normally have better resolution along the ultrasound beam compared with the perpendicular direction. Therefore, STE works better for measurements of motion and deformation in the direction along the ultrasound beam than in other directions. A disadvantage of STE compared to tissue Doppler imaging is the lower frame rate, which is necessary to capture higher HRs or events in the cardiac cycle that lasts shortly (e.g. isovolumic phases). The recommended frame rate is at least 50 frames per second. Frame rates lower than this may result in a loss of speckles, which may move out of plane or beyond the search area in successive frames. The frame rate can become too high by reducing the number of ultrasound beams in each frame, thereby reducing the spatial resolution and image quality. Any artifact that resembles speckle patterns will influence the quality of speckle tracking (Mor-Avi et al., 2011).

For assessment of 2D strain by STE, the best-quality 2D image cardiac cycle is selected. It is a semiautomatic method that requires manual definition of the myocardium by tracking the endocardium manually on a frame with well-defined border. The sampling region of interest (ROI) must be adjusted to ensure that most of the myocardium is incorporated in the analysis, while avoiding the pericardium. The computer automatically selects suitable stable objects for

tracking and then searches for them in the next frame. The 2D strain algorithm automatically evaluates the tracking quality, and provides the tracking quality of each segment as either acceptable or non-acceptable. Regions of interest must be adjusted manually until optimal tracking is achieved.

In the projects of this thesis 2D strain by STE (longitudinal strain and strain rate at rest) was used and digital loops were acquired from the three apical views at a frame rate of at least 50 frames/s. The semi-automatic method was used as described above to define the region of interest and the internal border of the myocardium was outlined. Segments that failed to track were manually adjusted, and segments that subsequently failed to track were excluded. Region of interest was adjusted manually if necessary to fit the average of the myocardial thickness. The software uses electrocardiogram (ECG) to define end-diastole or the user can define end-diastole. We defined aortic valve closure (AVC) by placing an event marker in the tissue Doppler curve at the end of the negative spike after ejection (Aase et al., 2008). 2D strain and strain rate were automatically calculated by the software algorithm at each frame throughout the heart cycle as a curve in six different segments for each apical view. LV global longitudinal strain/strain rate were defined as the average of peak longitudinal strains from a 16 LV segments model (Lang et al., 2015; Voigt et al., 2015).

Echocardiographic analyses by STE to calculate twist and twist rate are described in Chapter 4.

Limitations for 2D strain speckle tracking echocardiography

2D STE implies some methodological limitations, mostly related to image quality and through-plane motion of the myocardium since 2D STE cannot track motion occurring out of plane. Speckle-tracking is also limited by lower frame rates (as noted previously in this section).

The quality of the speckle tracking may be suboptimal if regions of the myocardium are poorly visualized or if spatial or temporal resolution of the image acquisition is insufficient. All kinds of ultrasound noise will reduce the tracking quality, so the 2D strain software has noise reduction by applied increased smoothing. The ultrasound speckle patterns are generated by the interference of the ultrasound waves reflected from tissue structures and the accumulation of small random errors in detection of speckled patterns along the tracking process can lead to inaccurate tracking results. A clear delineation of the endocardial border is also important for a reliable tracking. Even though 2D strain software includes an automated measure of tracking quality, there is no display to visually check the tracking quality by comparing the underlying

image loop with the superimposed tracking results, along with the actual curves derived from that tracking.

For the LV, the reproducibility of 2D-STE strain measurements has shown to be better than that of 1D tissue Doppler strain measurements (Ingul et al., 2005). Tissue Doppler strain is more sensitive to misalignment between the cardiac axis and the ultrasound beam. Also tissue Doppler strain is susceptible to noise. The advantages and disadvantages of using 2D STE compared to tissue Doppler strain is discussed in the previous section.

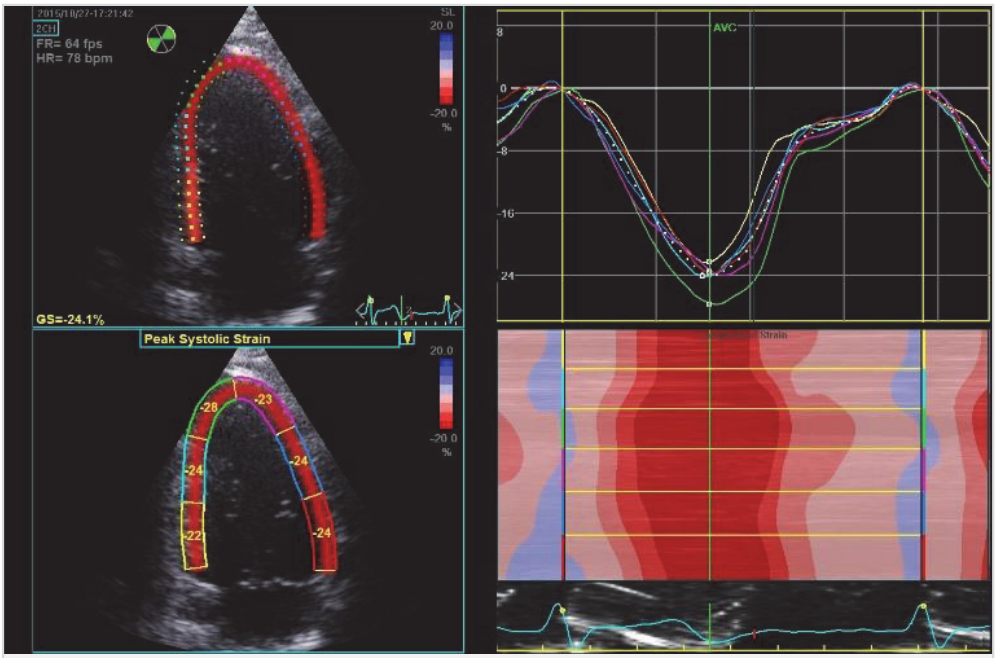


Figure 2.6 2D strain image in a normal case displaying peak longitudinal systolic strain. Top left image display parametric color overlay and the global strain (GS) value (-24.1%). Top right image show strain traces for each segment and a global value for the 6 segments (white dotted curve). Low left image show segmental peak systolic strain values. Low right image show the entire muscle with colorization according to a scale where dark red represents high negative peak strain values and blue positive strain values.

2.1.1.2 Cardiopulmonary Exercise Testing

The importance of cardiorespiratory fitness in the context of this thesis is discussed in Chapter 1, section 1.1.5.

Cardiopulmonary exercise testing (CPX) is considered the most accurate noninvasive quantification of maximal aerobic capacity (i.e. VO_{2max} or VO_{2peak}) (Balady et al., 2010; Guazzi et al., 2012).

This method has several advantages beyond the ability to assess cardiorespiratory fitness. Among others it allows for the attainment of the three primary parameters: oxygen volume (VO_2), carbon dioxide volume (VCO_2) and minute ventilation (VE), which can be used in the diagnosis of, for example, patients with HF (Balady et al., 2010; Guazzi et al., 2012). Nonetheless, in the studies comprising this thesis, CPX was carried out to determine the subjects VO_{2max} or VO_{2peak}.

In the projects of this thesis, CPX was performed by using a commercially available mixing-chamber-gas-analyzer-ergorespirometry: We chose to use the Jaeger Oxycon Pro (LE2000CE, Viasys Healthcare, Germany), which has been found valid in measuring VO_{2max} during graded exercise and time trial tests (Foss and Hallen, 2005). CPX was carried out by walking/running on an inclined treadmill. This allows for the involvement of large muscle groups and slower pace for the individual carrying out the test and thus (proposedly) increase the potential of reaching VO_{2max}. Another reason for choosing CPX through walking on an inclined treadmill was that this was a common mode of exercise in the Studies 1–2. Furthermore, this method is widely used and considered an accurate measure of VO_{2max} in the general population, including individuals with metabolic disease (Balady et al., 2010; Weston et al., 2014).

Participants performed maximal treadmill (PPS55 Med, Woodway GmbH, Germany) CPX according to a previously described individualized protocol (Rognmo et al., 2004) and the test procedure was identical to tests in previous studies carried out at our department (Aspenes et al., 2011; Tjonna et al., 2008; Tjonna et al., 2013; Wisloff et al., 2007).

A trained investigator carried out all tests. The Jaeger Oxycon Pro (LE2000 CE, Viasys Healthcare, Germany) was calibrated before testing, according to the recently updated Oxycon instruction manual at the time the projects commenced (Erich Jaeger GmbH, Hoechberg, Germany). The gas analyzer was calibrated by using the ambient air and a gas mixture of

16.0±0.04% O_2 and 5.0±0.1% CO_2 (Riessner-Gase GnbH & Co, Germany) and the calibration of inspiratory flow was performed automatically.

The subjects were asked not to ingest food within a minimum of two hours before the test and to wear comfortable shoes and clothing. Before the test began, the participants received a detailed explanation about the test procedure and purpose. They were asked to stop if chest pain or other discomfort (beyond what is considered common exercise fatigue) occurred and they were encouraged to strive towards their maximum exercise limits. Participants were also familiarized with treadmill walking, i.e. to walk steadily and to avoid grasping the handles during walking.

After familiarization with the treadmill, speed and inclination were individually adjusted during a 10-minute warm-up. After warm-up, a nose-clip was placed on the nose and a mouthpiece (connected to the Jaeger Oxygen Pro) was placed in mouth of the participants. By ventilatory expired gas analysis, VE, VO_2 and VCO_2 was captured through this mouthpiece. During the CPX, we aimed to keep the speed constant and to gradually increase the incline by two percent every second minute or when oxygen uptake stabilized, until exhaustion (VO_{2peak}) (e.g. leg fatigue and shortness of breath) or true VO_{2max} was reached. We also strived to bring the subjects to VO_{2max} within eight to 12 minutes (Froelicher, 2006). The average of the three highest consecutive 30-s measurements determined VO_{2max}.

In our studies, the criteria for VO_{2max} was considered met at exhaustion, when oxygen uptake plateaued (< 2 ml/kg/min change over the span of three 30-s successive measurements) despite increasing speed and/or inclination in combination with a respiratory-exchange-ratio (RER $=VCO_2/VO_2$) above 1.05 and subjective volitional exhaustion. There were several barriers to achieve true VO_{2max} among our study participants, such as lack of motivation, musculoskeletal fatigue, general discomfort, or myocardial ischemia. As not all participants reached VO_{2max} criteria in the Studies 1–2, the term VO_{2peak} will be used when referring to achieved maximal oxygen uptake in this thesis.

The results of this method would be limited if failing to meet the requirements listed in the former paragraphs.

2.1.1.3 Heart rate maximum

HR increases immediately in response to exercise as vagal tone is reduced and sympathetic activity increases (Balady et al., 2010) and there is a linear relationship between oxygen uptake and HR during dynamic work (Balady et al., 2010). The level of HR increase during exercise is dependent on a number of factors, such as age, body composition, exercise mode, and medication (such as beta-blockers). However, maximum HR (HR_{max}) can hardly be influenced by exercise training in the long term.

In the studies comprised in this thesis, HR_{max} was used to calculate the required exercise HR during interventions. Thus, to achieve optimal intensities (according to protocol) during the interventions (i.e. HIIE, but also the MIE intervention in Chapter 5), it was initially important that the participants achieved maximal exertion during the CPX.

HR was measured continuously during the CPX using HR monitors (Polar Electro, Finland). To determine HR_{max}, the highest achieved HR during the CPX was added by five beats per minute, as previously suggested (Ingjer, 1991). The main rationale for adding five beats per minute to HR_{max} was to increase the possibility of meeting the exercise intensity requirements.

2.1.1.4 Anthropometric measurements

Weight, height, and body mass index (BMI)

Obesity is a strong independent risk factor for T2D (Carey et al., 1997; Chan et al., 1994). Almost 75-80% of T2D individuals are overweight (body mass index (BMI), > 25 kg/m^2) or obese (BMI > 30 kg/m^2), and even if not overweight, they may have central obesity (defined in Table 2.2) (American Diabetes Association, 2013; Evert et al., 2014). There is an exponential cardiovascular risk when BMI increases > 27 (Catapano et al., 2011). Indeed, very high waist circumference (≤ 120 cm for men, ≤ 110 cm for women) is considered a substantial risk factor for mortality in adults, independent of BMI or other risk factors (Jacobs et al., 2010). Moreover, high WC has been more strongly associated with diabetes and CVD than BMI; the association being independent of BMI (Browning et al., 2010).

In Chapters 3–5, body weight was measured in the fasted state on the Seca 877 scale (Germany). Height was measured with a mechanical telescopic measuring stadiometer (Seca 222, Germany). BMI was calculated by dividing body weight (kg) by height squared (m^2) and expressed as kg/m^2.

Failure to perform body weight measurements in the fasted state and/or at the same time of day or with the same scales that are calibrated would limit the results by using this method. Furthermore, as BMI is calculated without taking muscle mass into account, individuals who have a large muscle mass can potentially be categorized as overweight or obese by solely interpreting BMI results. In the studies comprised in this thesis, the same scales were used at all time points and the participants were in the fasted state at baseline and posttest measurements (except in the postprandial state in Chapter 5). Furthermore, we also performed WC measurements and DXA measurements.

Waist circumference (WC)

In the projects of this thesis, WC was measured using a measuring tape at the level of the umbilicus after expiration. The measurement was repeated until the same measurement result (cm) was found three times.

This method has its limitations and may vary from measurement to measurement as the level of the tape can differ slightly between measurements. Furthermore, the measurements can be influenced by factors such as changes in body posture and different breathing between measurements. Moreover, the gastrointestinal content of an individual varies from day to day and between meals. Thus, a limitation in Chapter 3 is that WC was not tested in the fasted state, which could have influenced the results (Fernandez Vazquez et al., 2015) (in Chapter 5, WC was tested in the fasted state).

In the studies comprised in this thesis, the same investigator tested WC at all time points.

Table 2.2. Definition of central obesity, overweight, and obesity.

Body Mass Index		**or**	**Waist Circumference**
		Male	**Female**
≥25 kg/m^2 overweight	≥30 kg/m^2 obese	≥94cm	≥80 cm

Central obesity defined in Europeans. Modified after the International Diabetes Federation Task Force on Epidemiology and Prevention (Alberti et al., 2009).

Dual energy X-ray absorptiometry (DXA)

Although the most common indirect indicator of obesity and CVD risk is BMI, the BMI is incapable of distinguishing between fat mass and fat-free mass. Thus, to distinguish between these factors, we chose to use the DXA. DXA has emerged as a "gold standard" among body composition analysis methods in recent years, as it has generally shown to be more precise than other methods, such as underwater weighing and skinfold measurements (Laskey, 1996).

There are, however, several factors that can potentially compromise DXA results (and thus validity and reliability of the measurements), such as positioning on the DXA machine, effects of food, fluid intake, exercise sessions (Nana et al., 2015) and also age, body size and presumptions set in the equations of the DXA machine (Laskey, 1996). For example, if an individual is dehydrated, the DXA may underestimate the fat content and vice versa (Laskey, 1996).

In Chapter 3, DXA (Hologic Discovery A, version 13.3.0.1, USA) was performed to measure body fat percentage. All anthropometric measurements were performed at all time-points. The same trained investigator measured the subjects at all times.

2.1.1.5 Biochemical analysis

Poor glycemic control (measured as increased HbA_{1c}) increases the relative risk of dying from ischemic heart disease (Dale et al., 2009), and every percentage point increase in HbA_{1c} may represent an eight percent increase in risk for developing HF (Iribarren et al., 2001). Also, the fasting plasma glucose level has been shown to be a prognostic factor for HF (Held et al., 2007). Furthermore, postprandial excessive elevations in circulating glucose are associated with an increased risk of CVD (Cavalot et al., 2011; Coutinho et al., 1999; Garber, 2012; O'Keefe and Bell, 2007). Dyslipidemia associated with insulin resistance is common in T2D and is characterized by increased triglyceride levels and low HDL levels (Solano and Goldberg, 2006), which increase cardiovascular risk (Castelli et al., 1992; Catapano et al., 2011). Post-meal excessive elevations in circulating triglycerides and free fatty acids are also characteristic for T2D and associated with an increased risk of CVD (Bansal et al., 2007; Garber, 2012; Nordestgaard et al., 2007; O'Keefe and Bell, 2007). Furthermore, chronic inflammation and oxidative stress plays a major role in the pathogenesis of T2D and are considered contributors to CMR factor clustering and CVD (Dandona et al., 2004; Holt et al., 2010; Pitocco et al., 2013). C-reactive protein (CRP) is a pro-inflammatory biomarker commonly used in the evaluation of global cardiovascular risk (Pearson et al., 2003).

In the studies (1–2; Chapter 3–5) presented in this thesis, blood was obtained after at least 12 hours of fasting from food, caffeine, nicotine, and alcohol. (In Chapter 5, blood was also obtained in the postprandial state). Vials of EDTA (2x 3 ml), serum (1x5 ml) and LI – heparin (3 ml) was obtained in the morning and immediately sent for clinical chemistry analyzation at St. Olav's Hospital. In addition, vials were obtained to store (–80°C) for later analysis after treated (i.e. centrifuged) at our laboratory; serum (1x5ml vial), EDTA (2x3ml vial), and LI-heparin plasma (1x3ml vial).

HbA_{1c}, blood glucose, C-peptide, plasma triglycerides, total cholesterol, HDL, LDL, and high-sensitive C-reactive protein (hs-CRP) and hemoglobin were analyzed according to standard procedures at St. Olav's University Hospital (Trondheim). Furthermore, serum albumin, serum creatinine, potassium, and sodium were analyzed according to standard procedures at St. Olav's University Hospital (Trondheim) to reveal eventual microalbuminuria or renal failure (at study baselines only).

Specifically, blood glucose was measured using photometric hexokinase UV method (Roche Modular, Roche Diagnostics, Germany) and C-peptide was measured using chemiluminescence method (Immulite 2000, Siemens Medical Solutions, New Jersey, US). The blood lipids were measured using photometric, enzymatic colorimetric method (Roche Modular, Roche Diagnostics Germany). Hs-CRP was measured using Tina-quant CRPHS immunoturbidimetric assay (Roche Modular, Roche Diagnostics, Germany). HbA_{1c} was measured using TINIA (Turbidimetric Inhibition immunoassay) (Roche Cobas Integra 400 plus, Roche Diagnostics, Germany). Insulin sensitivity was calculated using the HOMA2 calculator (The Homeostasis Assessment Model, University of Oxford, UK); fasting blood glucose and c-peptide results were plotted into the calculator. Total antioxidant status (TAS) was analyzed at the biochemical laboratory of the HUNT Research Center (Levanger, Norway) using currently available Randox assay (Randox Laboratories Limited, United Kingdom) according to Manual NX 2332.

The results of these methods would be limited if failing to follow the protocols of the measurements listed, as well as if patients did not follow instructions of being in the fasted state at the time of collecting the blood samples.

2.1.1.6 Resting heart rate

Increased resting HRs are associated with increased CVD risk and cardiovascular mortality in the general population (Nauman et al., 2011). In the studies presented in this thesis, resting HR was measured by electrocardiography. The lowest HR registered during (i.e. at the end of) the

resting echocardiography measurements was defined as the resting HR in both studies. During an echocardiography session (including preparations), the participants rested for approximately 30–40 minutes.

Echocardiographic measurements could be experienced as stressful and increase resting HRs, thus representing a limitation in this method of measuring resting HRs. However, we did not observe any changes in resting HRs from pre- to posttest in Study 1. Furthermore, resting HR was measured the exact same way at all time points (Studies 1–2).

Resting HR can also be dependent on several other factors, such as pre-exercise. The requirements of restraining from exercise in the days before echocardiography can eliminate this if the individuals participating in the studies followed the instructions given.

2.1.1.7 Blood pressure

Resting blood pressure

Hypertension is an established cardiovascular risk factor, which substantially aggravates T2D complications and are associated with ~60% and ~70% increases in cardiovascular events and all-cause mortality, respectively (Chen et al., 2011). It is also indicated that the risk of T2D is increased in individuals with hypertension (Fukui et al., 2011).

In addition to T2D, several factors associated with this disease contribute to the development of hypertension, including lack of physical activity, unhealthy diet, overweight/obesity, central obesity, and older age. In general, hypertension prevalence in T2D are reported to be 75% to 85% (Mozaffarian et al., 2015), although findings in different populations are diverse (Colosia et al., 2013). Reducing blood pressure reduces the risk of major clinical events related to T2D (Turnbull et al., 2005). The ESC and the ADA treatment targets for blood pressure in T2D are systolic < 135/140 and diastolic < 80/85 mmHg (American Diabetes Association, 2014; Ryden et al., 2013).

We used conventional blood pressure measurements in the studies presented in this thesis. In Chapter 3 (Study 1, Paper I), supine blood pressure was measured prior to resting echocardiography (minimum 10-minute rest in supine position) using a Tango blood pressure monitor (SunTech® Tango® M2 Stress BP Monitor, Morrisville, USA). Supine blood pressure was noted as the median of three similar recordings, which did not deviate more than ± 10 mmHg from each other in regard to systolic blood pressure. The fact that blood pressure was

not obtained in a fasted state may be a limitation in this, according to the postprandial findings in Chapter 5/Paper III.

In Chapter 4 (Paper II), blood pressure measurements from Chapter 3 were used for the prospective study group (T2D). Blood pressure measurements in the retrospective study group (healthy control group) had previously been performed using Dinamap 845XT (GE Healthcare, Milwaukee, WI) (Dalen et al., 2011) after two minutes of rest with an arm on the table. Blood pressure was measured three times by trained staff and the average of the second and third blood pressure measurement was noted. The difference in blood pressure measuring methods between groups is thus a limitation in this paper.

In Chapter 5 (Study 2) upright blood pressure measurements were performed using Philips SureSigns V52 (Andover, Massachusetts, US) after resting in a sitting position for at least 10 minutes before measurements. In general, blood pressure was noted as the median of three recordings, which did not deviate more than ± 10 mmHg from each other in regard to systolic blood pressure.

Indeed, conventional blood pressure measurements can predict cardiovascular events, also after adjusting for traditional risk factors (Clement et al., 2003). However, several factors may influence blood pressure when taken conventionally as in the studies of the present thesis. For example, stress, anxiety or meals can influence the results. Certainly, ambulatory blood pressure measurements may offer additional information about future cardiovascular events compared to the conventional method (Clement et al., 2003).

Blood pressure measurement during stress echocardiography

During exercise, blood pressure is dependent upon peripheral resistance and cardiac output (CO). Normally, systolic blood pressure increases, whereas diastolic blood pressure remains relatively stable during incremental exercise (Balady et al., 2010). In general, an abnormal rise or fall in systolic blood pressure during exercise can indicate cardiac disease (Balady et al., 2010; Fletcher et al., 2001). For example, an abnormal rise in systolic blood pressure (< 20–30mmHg) can indicate presence of LV dysfunction, myocardial ischemia, or obstructed aortic outflow (Fletcher et al., 2001). However, hypotension during exercise can also occur independent of heart disease due to causes of dehydration, anti-hypertensive therapy, or prolonged vigorous exercise (Balady et al., 2010).

To assess hemodynamic changes during stress echocardiography, blood pressure was measured using a Tango blood pressure monitor (SunTech® Tango® M2 Stress BP Monitor, Morrisville, USA) at baseline (sitting on the ergometer cycle), at each workload (the last minute of each increment) and after cycling, when E and A were separated.

2.1.1.8 Flow-mediated dilatation of the brachial artery

T2D is associated with endothelial dysfunction, which is both a precursor and effect of atherosclerosis (Reyes-Soffer et al., 2010; Ryden et al., 2013). Thus, endothelial dysfunction is strongly linked to cardiovascular risk factors (Celermajer et al., 1994; Neunteufl et al., 1997; Schachinger et al., 2000; Suwaidi et al., 2000). However, in individuals with T2D, endothelial dysfunction appears to be present, even when presence of atherosclerosis is considered low (Johnstone et al., 1993; Nitenberg et al., 1993). Diabetes impairs endothelial function through several mechanisms: most CMR factors listed in Figure 1.3 (Chapter 1) are thought to be cumulatively damaging to the endothelium (Hurst and Lee, 2003; Meyers and Gokce, 2007). Improved endothelial function appears to be a whole-body response to exercise (Hawley et al., 2014), and has shown to be improved in vessels in skeletal muscles in individuals with T2D after exercise training (Montero et al., 2013; Sixt et al., 2010) and exercise training can thus have the potential to improve endothelial function in coronary vasculature (Bowles et al., 2000).

Endothelial function was assessed by the flow-mediated dilatation (FMD) technique, initially developed by Celemajer et al. in 1992 (Celermajer et al., 1992) of which guidelines were published a decade later (Corretti et al., 2002) and subsequently updated in 2005 (Pyke and Tschakovsky, 2005). After this, the FMD technique has been a commonly used research tool by endothelial researchers, in particular for studying endothelial function of the brachial artery. In general, the FMD technique is based on an ultrasound examination, which assesses the vasodilator response by changes in blood vessel diameter and blood flow related to shear stress (Thijssen et al., 2011). In this thesis (Chapter 3), changes in brachial artery diameter and blood velocity were measured according to guidelines in place at the time of conducting the study (Corretti et al., 2002; Pyke and Tschakovsky, 2005), using high-resolution, Vivid 7 ultrasound (GE Vingmed Ultrasound, Horten, Norway) with a 12MHz linear echo Doppler probe.

Several factors are important to ensure a valid FMD measurement (Harris et al., 2010) and FMD-results would indeed be limited if failing to follow the requirements listed:

Before performing FMD measurements (at all time-points), the participants were asked to complete at least 48 hours without exercise before performing FMD, according to guidelines

recommending at least 12 hours without exercise before performing FMD measurements (Harris, 2010). Furthermore, subjects were asked to fast (and thus to also abstain from caffeine, c-vitamin supplements, or c-vitamin rich foods, and nicotine) 12-hours prior to the FMD measurements. In regard to medicines that had to be administrated in some subjects, the subjects were asked to repeat the same procedure at every time point prior to FMD measurements.

The assessment room environment was kept quiet and we strove to keep the temperature at ~22°C. After placing ECG electrodes at three points of the subject's truncus (Skaug et al., 2013)), and after positioning the pneumatic cuff on the arm of the subject, the subjects were asked to rest and relax for at least ten minutes in a supine position, with a blanket over their legs and chest.

In the project (Chapter 3) of this thesis, the participants were placed in a supine position, with the arm immobilized in a slightly abducted (~30°) position; the shoulder was out-rotated, the elbow supinated and extended during the whole measurement. The subjects were also asked to refrain from talking during the measurements and to keep their head and other extremities as still as possible during the whole examination to avoid the probe coming out of position due to changes in the subject's position. The angle of the ultrasound beam is imperative during the measurement and even small shifts in the angle of the probe can influence the result of the measurement (Gill, 1985), thus the investigator needs to be experienced and the subjects have to remain still during the investigation.

To evaluate peak vasodilatation compared to resting diameters of the brachial artery, an adequate baseline measurement is imperative. At rest, before cuff occlusion, we measured baseline diameter and blood flow by placing the probe ~4.5 cm above the antecubital fossa. Baseline, pre-occlusion images and velocities were measured for three cardiac cycles.

The magnitude of FMD and blood flow responses are dependent on the placement of the cuff (Vogel et al., 2000). We chose to place the cuff on the forearm, distal to the antecubital fossa because distal occlusion appear to be mainly NO-dependent and exercise seems to improve endothelial function mainly through nitric oxide (NO) bioavailability (Green et al., 2004). Proximal occlusion (proximal and close to the measurement site) has shown to induce a greater and elongated vasodilator response and peak FMD (delay in peak dilatation (~22 sec)) compared to distal occlusion (Berry et al., 2000), probably due to: NO being released close to the occlusion site (Peretz et al., 2007); NO being released also due to other factors such as increased HR due to sympathetic nervous system activation (Doshi et al., 2001). In addition,

proximal occlusion can be more challenging than distal occlusion as images can be distorted due to brachial artery collapse and movement in soft tissue. Furthermore, distal occlusion appears to represent lower levels of discomfort for the subjects and less challenging for the researchers (Corretti et al., 2002).

The occlusion period lasted for five minutes. Indeed, the duration of occlusion affects the FMD measurements. However, five minutes' occlusion yields similar changes in diameter as ten minutes of occlusion; thus, five minutes is commonly used in research to avoid prolonged discomfort (Corretti et al., 2002).

The pneumatic cuff (SC10, Hokanson Inc., Bellevue, Washington) was inflated to 250 mm Hg (super-systolic pressure) and released after five minutes to create an ischemia-induced hyperemia. To produce an increase in shear stress and reactive hyperemia, the cuff was released abruptly to produce high blood flow. Post-occlusion images and velocities (diameter and blood flow) were subsequently collected continuously for three minutes to measure peak diameter and blood velocity.

HR is crucial when interpreting FMD measurements as timing in the cardiac cycle is important because arterial diameter can vary from one cardiac cycle to another, dependent on pulse pressure and vascular stiffness. To avoid the confounding effects, which variations in arterial compliance represent, we measured FMD at three points of the cardiac cycle (at the R-wave as well as the peak and end of the T-wave) (Chuang et al., 2002).

Flow mediated dilatation analyzes

The ultrasound images were analyzed automatically in a random order using edge-detection software (Vascular Research Tools 5, Medical Imaging Applications LLC, Coralville, IA, USA). The investigator was blinded to the patient's group allocation. An optimal region of interest was chosen on the clearest image section of the artery wall. The software tracked the anterior and posterior arterial wall, frame by frame (25 frames per second) throughout the measurement. The diameter of the brachial artery was reported both from pre-occlusion and during reactive hyperemia (post-occlusion). The software was set to automatically reject frames if the arterial wall detection was not within 70% of the confidence interval. Doppler image frames were manually readjusted by the observer if not adequately interpreted by the software. If acceptable tracking could not be obtained, image frames or the whole measurement were discarded.

Peak diameter was defined as the largest arterial diameter measured throughout the three-minute post-occlusion period. Flow mediated dilatation (FMD) peak percentage was defined as the difference between peak post-deflation diameter and resting vessel diameter (average pre-occlusion diameter), divided by resting vessel diameter multiplied by 100 (equation 2.1). Average blood flow was defined as the average flow value detected during the three-minute post-occlusion period. By calculating the area under the curve, the limitation in regard to variation in time to peak dilatation could be avoided. Share rate was calculated as the average of three minutes of blood flow velocity area under the curve relative to average diameter (mm) after deflation (equation 2.2). FMD was normalized to reduce the influence of artery diameter on the FMD response. Due to the difficulty in shear stress measurement (viscosity multiplied with velocity divided by diameter), shear rate was used as a normalizing factor (Pyke and Tschakovsky, 2005). FMD was normalized for share rate (FMD_{norm}) by dividing FMD by share rate (equation 2.3) as performed in a recent study on HF patients (Tarro Genta et al., 2013).

$$FMD\ (\%) = \frac{(\text{peak diameter post deflation (mm)} - \text{resting vessel diameter(mm)})}{\text{resting vessel diameter (mm)}} \times 100 \quad \text{(eq.2.1)}$$

$$Shearrate\ (1/s) = \frac{\text{3 minutes blood flow velocity area under curve (mm/s)}}{\text{average diameter post deflation (mm)}} \quad \text{(eq.2.2)}$$

$$FMDnorm\ (\%\ .\text{s}) = \frac{\text{FMD (\%)}}{\text{shear rate (1/s)}} \quad \text{(eq.2.3)}$$

2.1.2 Exercise protocols

2.1.2.1 High-intensity interval exercise

Participants in Chapters 3-5 performed HIIE by walking, jogging or running on an inclined treadmill.

In both studies supervised high-intensity interval exercise (HIIE) was performed by walking, jogging, or running on an inclined treadmill. HIIE sessions lasted 40 minutes all together: Following a 10-minute warm-up at 70% of HR_{max}, subjects performed four times four-minutes work bouts at 90% to 95% of HR_{max} with three-minute recovery periods between the work bouts at 70% of HR_{max}; to increase the ability perform at the same high intensity during every work bout following a recovery period. The subjects were instructed to reach a minimum of 90% of HR_{max} within the last minute of the first interval, 90% to 95% of HR_{max} within the first two minutes of the second and third interval, and 90% to 95% within the first minute of the last interval. After the fourth interval, a five-minute cool-down was performed. An illustration of HR during a bout of HIIE is presented in Figure 2.8.

2.1.2.2 Moderate-intensity exercise

In Chapters 3–4, home-based moderate-intensity exercise (MIE) was performed in accordance with the exercise guidelines of the Norwegian Directorate of Health at the time as Study 1 commenced (The Norwegian Directorate of Health, 2005), similar to international guidelines. The participants were thus instructed to be physically active at moderate intensity for a total of 210 minutes per week, divided into optional bouts of > 10 minutes (The Norwegian Directorate of Health, 2005). During the MIE sessions in Chapters 3–4, HR was not monitored. The participants were instructed to strive towards moderate breathing and moderately perceived effort: “being able to keep a conversation going, but just barely.” The activity mode during the MIE was optional: typical physical activity for the MIE group of Study 1 was walking, jogging, cycling, and swimming.

In Chapter 5 (Paper III), supervised continuous MIE was performed by walking or jogging on an inclined treadmill at 70% of HR_{peak} for 47 minutes and HR monitors (Polar RS 400, Polar

Electro, Kempele Finland) were used to ensure required exercise intensity during MIE. An example of HR during a typical bout of MIE in Chapter 5 is presented in Figure 2.8.

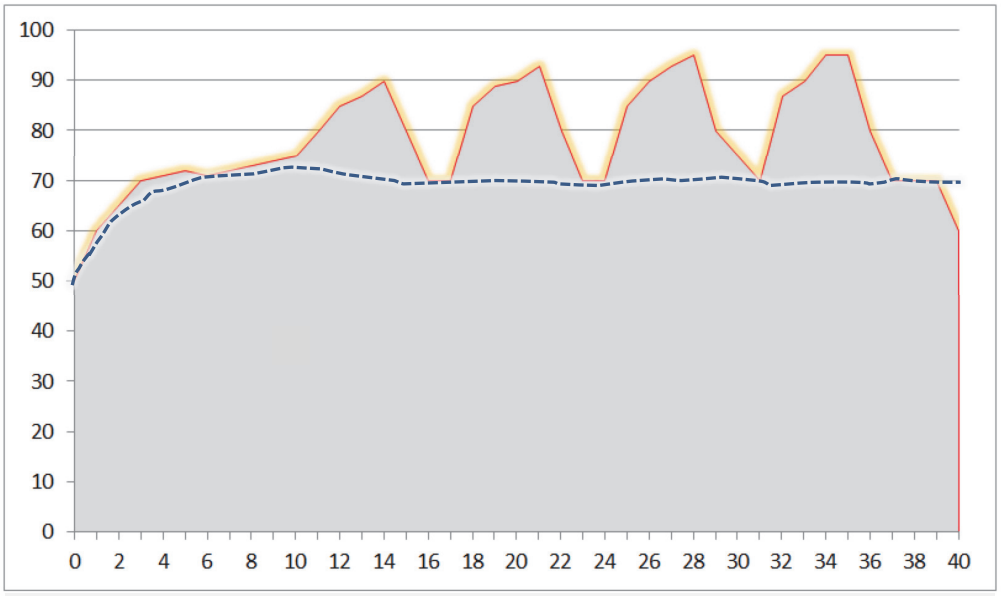


Figure 2.8. Illustration of heart rate during high intensity interval exercise (HIIE) and moderate-intensity exercise (MIE). The red line illustrates heart rate during HIIE and the blue dotted line illustrates heart rate during moderate-intensity exercise (MIE) bout. The X-axis represents percentage of maximal heart rate; y-axis represents duration in minutes.

REFERENCES

Aase, S. A., Torp, H., & Stoylen, A. (2008). Aortic valve closure: relation to tissue velocities by Doppler and speckle tracking in normal subjects. *Eur. J. Echocardiogr., 9*(4), 555-559.

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . International Association for the Study of, O. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation, 120*(16), 1640-1645.

American Diabetes Association. (2013). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 36 Suppl 1*, S67-74.

American Diabetes Association. (2014). Standards of medical care in diabetes-2014. *Diabetes Care, 37 Suppl 1*, S14-80.

Amundsen, B. H., Helle-Valle, T., Edvardsen, T., Torp, H., Crosby, J., Lyseggen, E., . . . Slordahl, S. A. (2006). Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J. Am. Coll. Cardiol., 47*(4), 789-793.

Aspenes, S. T., Nauman, J., Nilsen, T. I., Vatten, L. J., & Wisloff, U. (2011). Physical activity as a long-term predictor of peak oxygen uptake: the HUNT Study. *Med. Sci. Sports Exerc., 43*(9), 1675-1679.

Balady, G. J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G. F., . . . Outcomes, R. (2010). Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation, 122*(2), 191-225.

Bansal, S., Buring, J. E., Rifai, N., Mora, S., Sacks, F. M., & Ridker, P. M. (2007). Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA, 298*(3), 309-316.

Behar, V., Adam, D., Lysyansky, P., & Friedman, Z. (2004). The combined effect of nonlinear filtration and window size on the accuracy of tissue displacement estimation using detected echo signals. *Ultrasonics, 41*(9), 743-753.

Berry, K. L., Skyrme-Jones, R. A., & Meredith, I. T. (2000). Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery flow-mediated dilation. *Clin. Sci. (Lond.), 99*(4), 261-267.

Bhella, P. S., Pacini, E. L., Prasad, A., Hastings, J. L., Adams-Huet, B., Thomas, J. D., . . . Levine, B. D. (2011). Echocardiographic indices do not reliably track changes in left-sided filling pressure in healthy subjects or patients with heart failure with preserved ejection fraction. *Circ. Cardiovasc. Imaging, 4*(5), 482-489.

Bowles, D. K., Woodman, C. R., & Laughlin, M. H. (2000). Coronary smooth muscle and endothelial adaptations to exercise training. *Exerc. Sport Sci. Rev., 28*(2), 57-62.

Browning, L. M., Hsieh, S. D., & Ashwell, M. (2010). A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. *Nutr. Res. Rev., 23*(2), 247-269.

Carey, V. J., Walters, E. E., Colditz, G. A., Solomon, C. G., Willett, W. C., Rosner, B. A., . . . Manson, J. E. (1997). Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *Am. J. Epidemiol., 145*(7), 614-619.

Castelli, W. P., Anderson, K., Wilson, P. W., & Levy, D. (1992). Lipids and risk of coronary heart disease. The Framingham Study. *Ann. Epidemiol., 2*(1-2), 23-28.

Catapano, A. L., Reiner, Z., De Backer, G., Graham, I., Taskinen, M. R., Wiklund, O., . . . Committees. (2011). ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis, 217 Suppl 1*, S1-44.

Cavalot, F., Pagliarino, A., Valle, M., Di Martino, L., Bonomo, K., Massucco, P., . . . Trovati, M. (2011). Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. *Diabetes Care, 34*(10), 2237-2243.

Celermajer, D. S., Sorensen, K. E., Bull, C., Robinson, J., & Deanfield, J. E. (1994). Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J. Am. Coll. Cardiol., 24*(6), 1468-1474.

Celermajer, D. S., Sorensen, K. E., Gooch, V. M., Spiegelhalter, D. J., Miller, O. I., Sullivan, I. D., . . . Deanfield, J. E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet, 340*(8828), 1111-1115.

Chan, J. M., Rimm, E. B., Colditz, G. A., Stampfer, M. J., & Willett, W. C. (1994). Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care, 17*(9), 961-969.

Chen, G., McAlister, F. A., Walker, R. L., Hemmelgarn, B. R., & Campbell, N. R. (2011). Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. *Hypertension, 57*(5), 891-897.

Chuang, M. L., Douglas, P. S., Bisinov, E. A., & Stein, J. H. (2002). Effect of cardiac cycle on ultrasound assessment of endothelial function. *Vasc. Med., 7*(2), 103-108.

Clement, D. L., De Buyzere, M. L., De Bacquer, D. A., de Leeuw, P. W., Duprez, D. A., Fagard, R. H., . . . Office versus Ambulatory Pressure Study, I. (2003). Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N. Engl. J. Med., 348*(24), 2407-2415.

Colosia, A. D., Palencia, R., & Khan, S. (2013). Prevalence of hypertension and obesity in patients with type 2 diabetes mellitus in observational studies: a systematic literature review. *Diabetes Metab. Syndr. Obes., 6*, 327-338.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., . . . Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol., 39*(2), 257-265.

Coutinho, M., Gerstein, H. C., Wang, Y., & Yusuf, S. (1999). The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care, 22*(2), 233-240.

Dale, A. C., Midthjell, K., Nilsen, T. I., Wiseth, R., & Vatten, L. J. (2009). Glycaemic control in newly diagnosed diabetes patients and mortality from ischaemic heart disease: 20-year follow-up of the HUNT Study in Norway. *Eur. Heart J., 30*(11), 1372-1377.

Dalen, H., Thorstensen, A., Romundstad, P. R., Aase, S. A., Stoylen, A., & Vatten, L. J. (2011). Cardiovascular risk factors and systolic and diastolic cardiac function: a tissue Doppler and speckle tracking echocardiographic study. *J. Am. Soc. Echocardiogr., 24*(3), 322-332 e326.

Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A., & Stoylen, A. (2010). Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ. Cardiovasc. Imaging, 3*(5), 614-622.

Dandona, P., Aljada, A., & Bandyopadhyay, A. (2004). Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol., 25*(1), 4-7.

Doshi, S. N., Naka, K. K., Payne, N., Jones, C. J., Ashton, M., Lewis, M. J., & Goodfellow, J. (2001). Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin. Sci. (Lond.), 101*(6), 629-635.

Evert, A. B., Boucher, J. L., Cypress, M., Dunbar, S. A., Franz, M. J., Mayer-Davis, E. J., . . . Yancy, W. S., Jr. (2014). Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care, 37 Suppl 1*, S120-143.

Fernandez Vazquez, R., Martinez Blanco, J., Garcia Vega Mdel, M., Barbancho, M. A., & Alvero-Cruz, J. R. (2015). Effects of Food and Drink Ingestion on Body Composition Variables of Abdominal Bioelectrical Impedance. *Nutr. Hosp., 32*(5), 2269-2273.

Fletcher, G. F., Balady, G. J., Amsterdam, E. A., Chaitman, B., Eckel, R., Fleg, J., . . . Bazzarre, T. (2001). Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. *Circulation, 104*(14), 1694-1740.

Foss, O., & Hallen, J. (2005). Validity and stability of a computerized metabolic system with mixing chamber. *Int. J. Sports Med., 26*(7), 569-575.

Froelicher, V. F. M., J. N. (2006). *Exercise and the heart* (5 edition ed.): WB Saunders Company.

Fukui, M., Tanaka, M., Toda, H., Senmaru, T., Sakabe, K., Ushigome, E., . . . Nakamura, N. (2011). Risk factors for development of diabetes mellitus, hypertension and dyslipidemia. *Diabetes Res. Clin. Pract., 94*(1), e15-18.

Garber, A. J. (2012). Postprandial dysmetabolism and the heart. *Heart Fail. Clin., 8*(4), 563-573.

Gill, R. W. (1985). Measurement of blood flow by ultrasound: accuracy and sources of error. *Ultrasound Med. Biol., 11*(4), 625-641.

Green, D. J., Maiorana, A., O'Driscoll, G., & Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J. Physiol., 561*(Pt 1), 1-25.

Guazzi, M., Adams, V., Conraads, V., Halle, M., Mezzani, A., Vanhees, L., . . . American Heart, A. (2012). EACPR/AHA Scientific Statement. Clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *Circulation, 126*(18), 2261-2274.

Harris, R. A., Nishiyama, S. K., Wray, D. W., & Richardson, R. S. (2010). Ultrasound assessment of flow-mediated dilation. *Hypertension, 55*(5), 1075-1085.

Harris, R. A. N., S.K.; Wray W.; Richardson, R.S. (2010). Ultrasound assessment of flow-mediated dilatation: A tutorial. *Hypertension, 55*(5), 10.

Hawley, J. A., Hargreaves, M., Joyner, M. J., & Zierath, J. R. (2014). Integrative biology of exercise. *Cell, 159*(4), 738-749.

Heimdal, A., Stoylen, A., Torp, H., & Skjaerpe, T. (1998). Real-time strain rate imaging of the left ventricle by ultrasound. *J. Am. Soc. Echocardiogr., 11*(11), 1013-1019.

Held, C., Gerstein, H. C., Yusuf, S., Zhao, F., Hilbrich, L., Anderson, C., . . . Investigators, O. T. (2007). Glucose levels predict hospitalization for congestive heart failure in patients at high cardiovascular risk. *Circulation, 115*(11), 1371-1375.

Holt, R. I. G., Cockram, C. S., Flyvbjerg, A., & Goldstein, B. J. (2010). *Textbook of Diabetes. -4th ed.* (4 ed.): Blackwell Publishing Ltd.

Hurst, R. T., & Lee, R. W. (2003). Increased incidence of coronary atherosclerosis in type 2 diabetes mellitus: mechanisms and management. *Ann. Intern. Med., 139*(10), 824-834.

Ingjer, F. (1991). Factors influencing assessment of maximal heart rate. *Scand. J. Med. Sci. Sports, 1*, 134-140.

Ingul, C. B., Tjonna, A. E., Stolen, T. O., Stoylen, A., & Wisloff, U. (2010). Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch. Pediatr. Adolesc. Med., 164*(9), 852-859.

Ingul, C. B., Torp, H., Aase, S. A., Berg, S., Stoylen, A., & Slordahl, S. A. (2005). Automated analysis of strain rate and strain: feasibility and clinical implications. *J. Am. Soc. Echocardiogr., 18*(5), 411-418.

Iribarren, C., Karter, A. J., Go, A. S., Ferrara, A., Liu, J. Y., Sidney, S., & Selby, J. V. (2001). Glycemic control and heart failure among adult patients with diabetes. *Circulation, 103*(22), 2668-2673.

Jacobs, E. J., Newton, C. C., Wang, Y., Patel, A. V., McCullough, M. L., Campbell, P. T., . . . Gapstur, S. M. (2010). Waist circumference and all-cause mortality in a large US cohort. *Arch. Intern. Med., 170*(15), 1293-1301.

Johnstone, M. T., Creager, S. J., Scales, K. M., Cusco, J. A., Lee, B. K., & Creager, M. A. (1993). Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation, 88*(6), 2510-2516.

Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., . . . Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr., 28*(1), 1-39 e14.

Langeland, S., D'Hooge, J., Wouters, P. F., Leather, H. A., Claus, P., Bijnens, B., & Sutherland, G. R. (2005). Experimental validation of a new ultrasound method for the simultaneous assessment of radial and longitudinal myocardial deformation independent of insonation angle. *Circulation, 112*(14), 2157-2162.

Laskey, M. A. (1996). Dual-energy X-ray absorptiometry and body composition. *Nutrition, 12*(1), 45-51.

Leitman, M., Lysyansky, P., Sidenko, S., Shir, V., Peleg, E., Binenbaum, M., . . . Vered, Z. (2004). Two-dimensional strain-a novel software for real-time quantitative echocardiographic assessment of myocardial function. *J. Am. Soc. Echocardiogr., 17*(10), 1021-1029.

Meyers, M. R., & Gokce, N. (2007). Endothelial dysfunction in obesity: etiological role in atherosclerosis. *Curr. Opin. Endocrinol. Diabetes Obes., 14*(5), 365-369.

Molmen, H. E., Wisloff, U., Aamot, I. L., Stoylen, A., & Ingul, C. B. (2012). Aerobic interval training compensates age related decline in cardiac function. *Scand. Cardiovasc. J., 46*(3), 163-171.

Montero, D., Walther, G., Benamo, E., Perez-Martin, A., & Vinet, A. (2013). Effects of Exercise Training on Arterial Function in Type 2 Diabetes Mellitus : A Systematic Review and Meta-Analysis. *Sports Med.*

Mor-Avi, V., Lang, R. M., Badano, L. P., Belohlavek, M., Cardim, N. M., Derumeaux, G., . . . Zamorano, J. L. (2011). Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. *Eur. J. Echocardiogr., 12*(3), 167-205.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., . . . Stroke Statistics, S. (2015). Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation, 131*(4), e29-322.

Mullens, W., Borowski, A. G., Curtin, R. J., Thomas, J. D., & Tang, W. H. (2009). Tissue Doppler imaging in the estimation of intracardiac filling pressure in decompensated patients with advanced systolic heart failure. *Circulation, 119*(1), 62-70.

Nagueh, S. F., Appleton, C. P., Gillebert, T. C., Marino, P. N., Oh, J. K., Smiseth, O. A., . . . Evangelisa, A. (2009). Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur. J. Echocardiogr., 10*(2), 165-193.

Nagueh, S. F., Middleton, K. J., Kopelen, H. A., Zoghbi, W. A., & Quinones, M. A. (1997). Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *J. Am. Coll. Cardiol., 30*(6), 1527-1533.

Nana, A., Slater, G. J., Stewart, A. D., & Burke, L. M. (2015). Methodology review: using dual-energy X-ray absorptiometry (DXA) for the assessment of body composition in athletes and active people. *Int. J. Sport Nutr. Exerc. Metab., 25*(2), 198-215.

Nauman, J., Janszky, I., Vatten, L. J., & Wisloff, U. (2011). Temporal changes in resting heart rate and deaths from ischemic heart disease. *JAMA, 306*(23), 2579-2587.

Neunteufl, T., Katzenschlager, R., Hassan, A., Klaar, U., Schwarzacher, S., Glogar, D., . . . Weidinger, F. (1997). Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis, 129*(1), 111-118.

Nitenberg, A., Valensi, P., Sachs, R., Dali, M., Aptecar, E., & Attali, J. R. (1993). Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes, 42*(7), 1017-1025.

Nordestgaard, B. G., Benn, M., Schnohr, P., & Tybjaerg-Hansen, A. (2007). Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA, 298*(3), 299-308.

Notomi, Y., Lysyansky, P., Setser, R. M., Shiota, T., Popovic, Z. B., Martin-Miklovic, M. G., . . . Thomas, J. D. (2005). Measurement of ventricular torsion by two-dimensional ultrasound speckle tracking imaging. *J. Am. Coll. Cardiol., 45*(12), 2034-2041.

O'Keefe, J. H., & Bell, D. S. (2007). Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. *Am. J. Cardiol., 100*(5), 899-904.

Park, J. H., & Marwick, T. H. (2011). Use and Limitations of E/e' to Assess Left Ventricular Filling Pressure by Echocardiography. *J. Cardiovasc. Ultrasound, 19*(4), 169-173.

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., 3rd, Criqui, M., . . . American Heart, A. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation, 107*(3), 499-511.

Peretz, A., Leotta, D. F., Sullivan, J. H., Trenga, C. A., Sands, F. N., Aulet, M. R., . . . Kaufman, J. D. (2007). Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. *BMC Cardiovasc. Disord., 7*, 11.

Pitocco, D., Tesauro, M., Alessandro, R., Ghirlanda, G., & Cardillo, C. (2013). Oxidative stress in diabetes: implications for vascular and other complications. *Int. J. Mol. Sci., 14*(11), 21525-21550.

Pyke, K. E., & Tschakovsky, M. E. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J. Physiol., 568*(Pt 2), 357-369.

Quinones, M. A., Otto, C. M., Stoddard, M., Waggoner, A., Zoghbi, W. A., Doppler Quantification Task Force of the, N., & Standards Committee of the American Society of, E. (2002). Recommendations for quantification of Doppler echocardiography: a report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. *J. Am. Soc. Echocardiogr., 15*(2), 167-184.

Reisner, S. A., Lysyansky, P., Agmon, Y., Mutlak, D., Lessick, J., & Friedman, Z. (2004). Global longitudinal strain: a novel index of left ventricular systolic function. *J. Am. Soc. Echocardiogr., 17*(6), 630-633.

Reyes-Soffer, G., Holleran, S., Di Tullio, M. R., Homma, S., Boden-Albala, B., Ramakrishnan, R., . . . Ginsberg, H. N. (2010). Endothelial function in individuals with coronary artery disease with and without type 2 diabetes mellitus. *Metabolism, 59*(9), 1365-1371.

Rognmo, O., Hetland, E., Helgerud, J., Hoff, J., & Slordahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur. J. Cardiovasc. Prev. Rehabil., 11*(3), 216-222.

Ryden, L., Grant, P. J., Anker, S. D., Berne, C., Cosentino, F., Danchin, N., . . . Xuereb, R. G. (2013). ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur. Heart J., 34*(39), 3035-3087.

Schachinger, V., Britten, M. B., & Zeiher, A. M. (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation, 101*(16), 1899-1906.

Sixt, S., Beer, S., Bluher, M., Korff, N., Peschel, T., Sonnabend, M., . . . Niebauer, J. (2010). Long- but not short-term multifactorial intervention with focus on exercise training improves coronary endothelial dysfunction in diabetes mellitus type 2 and coronary artery disease. *Eur. Heart J., 31*(1), 112-119.

Skaug, E. A., Aspenes, S. T., Oldervoll, L., Morkedal, B., Vatten, L., Wisloff, U., & Ellingsen, O. (2013). Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. *Eur J Prev Cardiol, 20*(4), 531-540.

Solano, M. P., & Goldberg, R. B. (2006). Management of dyslipidemia in diabetes. *Cardiol. Rev., 14*(3), 125-135.

Suwaidi, J. A., Hamasaki, S., Higano, S. T., Nishimura, R. A., Holmes, D. R., Jr., & Lerman, A. (2000). Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation, 101*(9), 948-954.

Tarro Genta, F., Eleuteri, E., Temporelli, P. L., Comazzi, F., Tidu, M., Bouslenko, Z., . . . Giallauria, F. (2013). Flow-mediated dilation normalization predicts outcome in chronic heart failure patients. *J. Card. Fail., 19*(4), 260-267.

The Norwegian Directorate of Health. (2005). *Norske anbefalinger for ernæring og fysisk aktivitet*. Helsedirektoratet Retrieved from http://helsedirektoratet.no/publikasjoner/norske-anbefalinger-for-ernering-og-fysisk-aktivitet/Publikasjoner/norske-anbefalinger-for-ernering-og-fysisk-aktivitet.pdf Accessed: May, 2014.

Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., . . . Green, D. J. (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am. J. Physiol. Heart Circ. Physiol., 300*(1), H2-12.

Thorstensen, A., Dalen, H., Amundsen, B. H., Aase, S. A., & Stoylen, A. (2010). Reproducibility in echocardiographic assessment of the left ventricular global and regional function, the HUNT study. *Eur. J. Echocardiogr., 11*(2), 149-156.

Thorstensen, A., Dalen, H., Amundsen, B. H., & Stoylen, A. (2011). Peak systolic velocity indices are more sensitive than end-systolic indices in detecting contraction changes assessed by echocardiography in young healthy humans. *Eur. J. Echocardiogr., 12*(12), 924-930.

Tjonna, A. E., Lee, S. J., Rognmo, O., Stolen, T. O., Bye, A., Haram, P. M., . . . Wisloff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation, 118*(4), 346-354.

Tjonna, A. E., Leinan, I. M., Bartnes, A. T., Jenssen, B. M., Gibala, M. J., Winett, R. A., & Wisloff, U. (2013). Low- and high-volume of intensive endurance training significantly improves maximal oxygen uptake after 10-weeks of training in healthy men. *PLoS One, 8*(5), e65382.

Turnbull, F., Neal, B., Algert, C., Chalmers, J., Chapman, N., Cutler, J., . . . Blood Pressure Lowering Treatment Trialists, C. (2005). Effects of different blood pressure-lowering regimens on major cardiovascular events in individuals with and without diabetes mellitus: results of prospectively designed overviews of randomized trials. *Arch. Intern. Med., 165*(12), 1410-1419.

Urheim, S., Edvardsen, T., Torp, H., Angelsen, B., & Smiseth, O. A. (2000). Myocardial strain by Doppler echocardiography. Validation of a new method to quantify regional myocardial function. *Circulation, 102*(10), 1158-1164.

Vogel, R. A., Corretti, M. C., & Plotnick, G. D. (2000). A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. *Clin. Cardiol., 23*(8), 571-575.

Voigt, J. U., Pedrizzetti, G., Lysyansky, P., Marwick, T. H., Houle, H., Baumann, R., . . . Badano, L. P. (2015). Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *Eur. Heart J. Cardiovasc. Imaging, 16*(1), 1-11.

Weston, K. S., Wisloff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med., 48*(16), 1227-1234.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

CHAPTER 3

The effect of high-intensity interval exercise versus moderate-intensity exercise on cardiac function and cardiometabolic risk factors in individuals with type 2 diabetes and diastolic dysfunction

TABLE OF CONTENTS
CHAPTER 3

3.1 ABSTRACT

Background Type 2 diabetes (T2D) is associated with diastolic dysfunction, which represents the earliest pre-clinical manifestation of diabetic cardiomyopathy and lacks effective treatment strategies. **Objectives** To compare the effects of moderate-intensity exercise (MIE; 210 min/week) and high-intensity interval exercise (HIIE; 4x4 minutes, 90-95% HR_{max}, 3/week) on left ventricular diastolic function and cardiovascular risk factors in individuals with T2D and diastolic dysfunction. **Methods** Eighty-three individuals with T2D-duration < 10 years without known cardiovascular disease were pre-screened for diastolic dysfunction, defined as early diastolic tissue Doppler velocity (e'< 8 cm/s). Inclusion criteria were fulfilled by 47 individuals (55.9 ± 6.0 years; 36 % female) randomized to MIE (n=23) or HIIE (n=24). Subjects tested at baseline, 12 weeks (MIE, n=17; HIIE, n=20) and one year (MIE, n=16; HIIE, n=16). The primary outcome measure was diastolic dysfunction (e'). Several secondary cardiovascular and metabolic end-points were also studied. **Results** After 12 weeks, HIIE was superior to MIE ($p \leq 0.05$; test for group difference) in improving diastolic function (e' Δ 1.8±1.1, $p<0.001$ vs. 0.5±0.7 cm/s, p=0.02), systolic function (peak systolic tissue Doppler velocity, S' Δ 0.9±1.4, p=0.007 vs. -0.1±1.1 cm/s, p=0.76; global strain Δ -1.0±1.9, p=0.03 vs. 0.3±1.6 %, p=0.51; global strain rate Δ -0.10±0.17, p=0.03 vs. 0.06±0.23 s^{-1}, p=0.14), peak oxygen uptake (VO_{2peak} Δ 4.1±2.9, $p<0.001$ vs. 1.2±2.2 ml/kg/min, p=0.04) and flow-mediated dilatation (FMD Δ 9.2±11.2, p=0.004, vs. 0.0±6.2 percentage points (pp), p=0.99). Only HIIE improved filling-pressure (E/e') and reduced glycosylated hemoglobin (HbA_{1c}) and body mass index (test for group difference n.s.). Compared to baseline, the HIIE group, but not the MIE group, sustained improved diastolic function after one year. **Conclusions** In early stages of type 2 diabetes and diastolic dysfunction, high-intensity interval exercise can be an effective exercise treatment option.

3.2 INTRODUCTION

The following manuscript (Paper I) is based on this chapter:

Hollekim-Strand, S.M.; Bjørgaas, M.R.; Albrektsen, G.; Tjønna, A.E.; Wisløff, U.; Ingul, C.B. High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: A randomized controlled trial. **J Am Coll Cardiol**, 2014. 64(16): p. 1758–60.

Parts of this chapter was also presented at the

1) ESC congress, Amsterdam, 1st September 2013. Oral presentation. Abstract title: The effect of exercise on cardiac function in patients with type 2 diabetes and diastolic dysfunction (Abstract session, Spotlight of the Congress: Heart failure with preserved ejection fraction: beyond the heart).
2) World Diabetes Congress, Melbourne, December 2013. Poster presentation. Abstract title: The effect of exercise on cardiac function in patients with type 2 diabetes and diastolic dysfunction.
3) EuroEcho Imaging Congress, Istanbul, December 2013. Poster presentation. Abstract title: Aerobic interval training improves diastolic dysfunction in patients with type 2 diabetes, a tissue Doppler study.
4) 11th annual Center for Heart Failure Research (CHRF) Symposium, Oslo, September 2013. Poster presentation. Abstract title: The effect of exercise on cardiac function in patients with type 2 diabetes and diastolic dysfunction.

Type 2 diabetes (T2D) is associated with cardiomyopathy, which may lead to heart failure (HF) (Nichols et al., 2004). Early diabetic cardiomyopathy is often characterized by diastolic dysfunction (i.e. impaired relaxation and pseudonormal filling), which has been detected in 20% to 60% of T2D individuals without overt coronary artery disease (Di Bonito et al., 2005; Fang, Schull-Meade, et al., 2005; Hare et al., 2011; Poirier et al., 2001; Redfield et al., 2003; Von Bibra et al., 2005; Zabalgoitia et al., 2001). Compared to people without diabetes, individuals with T2D have a two times higher risk of cardiovascular mortality and a two- to five times higher risk of developing heart failure (HF) (Go et al., 2014; Kannel et al., 1974; Nichols et al., 2004). The prevalence of T2D increases rapidly worldwide and it is critical to reduce cardiovascular complications and reduce mortality by adequate prevention and treatment (Go et al., 2014; Ryden et al., 2013).

Exercise training is a cornerstone of T2D management, and can improve factors such as metabolic control, cardiorespiratory fitness, blood pressure and body composition, but little is known about the effect of exercise on diastolic function in T2D (Marwick et al., 2009). Numerous public health institutions worldwide recommend that individuals with T2D perform at least 150 minutes of moderate (50% to 70% of HR_{max}) intensity aerobic activity per week, for prevention of cardiovascular complications (Alberti et al., 2009; American Diabetes Association, 2014; Buse et al., 2007; Colberg et al., 2010; International Diabetes Federation; Mendes et al., 2015). However, MIE in accordance with these recommendations seems to be insufficient to improve myocardial function in T2D (Hordern et al., 2009).

Studies have shown that high-intensity interval exercise (HIIE) reduces cardiovascular risk factors more than MIE in patients with cardiometabolic disease, such as metabolic syndrome, coronary artery disease and HF (Rognmo et al., 2004; Tjønna et al., 2008; Weston et al., 2014; Wisloff et al., 2007) and exercise intensity is found to be of importance for reversing left ventricular (LV) remodeling in patients with post-infarction HF (Wisloff et al., 2007). It is suggested that HIIE have additional benefits in patients with T2D (Boule et al., 2003), but it is still unclear whether HIIE yields more benefit than MIE (Balducci et al., 2012; Hordern et al., 2009). No intervention study has compared the effect of HIIE and MIE on diastolic function in patients with T2D and diastolic dysfunction.

Aim

To investigate and compare the effects of HIIE and MIE, in accordance with current exercise recommendations, on diastolic function defined as peak early diastolic tissue Doppler velocity (e') in patients with T2D and diastolic dysfunction (defined as e'<8cm/s). Furthermore, to compare the effectiveness of HIIE and MIE on other diastolic echocardiographic measures (peak late diastolic tissue Doppler velocity, a'; peak early diastolic mitral inflow velocity, E; late diastolic mitral inflow velocity, A; filling pressure, E/e'; E/A-ratio; isovolumic relaxation time, IVRT; deceleration time, DT), systolic echocardiographic measures (peak systolic tissue Doppler velocity, S'; global strain; strain rate) as well as cardiorespiratory fitness (peak oxygen uptake, VO_{2peak}), resting heart rate (HR), resting blood pressure, endothelial function (measured as flow mediated dilatation [FMD] of the brachial artery), glycemic control (measured as glycosylated hemoglobin [HbA_{1c}]), insulin sensitivity (measured by homeostatic model assessment [HOMA-ir]), circulating lipids (triglycerides, HDL and LDL-cholesterol), chronic inflammation (hs-CRP) and body composition (body mass index [BMI], waist circumference and body fat percentage (assessed by dual-energy X-ray absorptiometry [DXA]) in patients with T2D and diastolic dysfunction.

Hypothesis

a. HIIE is more effective than MIE in improving diastolic function (measured as e') in T2D individuals with diastolic dysfunction (defined as e'< 8cm/s) (at rest and during exercise).
b. HIIE is more effective than MIE in improving other measures of diastolic function as well as systolic function (at rest and during exercise), cardiorespiratory fitness, resting HR, blood pressure, glycemic control, insulin sensitivity, circulating lipids,

chronic inflammation and body composition in T2D individuals with diastolic dysfunction.

3.3 MATERIALS AND METHODS

3.3.1 Study design

We performed a randomized controlled trial with a 12-week exercise intervention and a one-year follow-up in which we investigated the effect of exercise on LV cardiac function and CMR factors in individuals with T2D and diastolic dysfunction.

3.3.2 Participants

The T2D participants were recruited through the local newspaper (*Adresseavisen*, Trondheim) and from the outpatient population at St. Olav's University Hospital, Trondheim, Norway from August 2010 to March 2013.

The inclusion and exclusion criteria are presented in Table 3.1 and characteristics for the included participants are presented in Table 3.2.

Table 3.1. Inclusion and exclusion criteria for the participants described in Chapter 3

Paper	Study population	N	Eligibility criteria	Exclusion criteria
I	Individuals with T2D and diastolic dysfunction	47	Age 20–65 T2D duration <10 yrs. Diastolic function (e'<8cm/s)	-Overt CVD -Atrial fibrillation or other significant cardiac arrhythmia -Untreated hypertension -Left ventricular EF <40% -Ischemia at exercise echocardiography -Body mass index >35 kg/m^2 -Diabetic retinopathy and/or neuropathy -Albuminuria -Drug or alcohol abuse -Pregnancy -Unable to exercise -Habitual exercise > guidelines for patients with type 2 diabetes.

Abbreviations: CVD, cardiovascular disease; EF, ejection fraction; T2D, type 2 diabetes.

Table 3.2 Baseline characteristics for the included T2D individuals described in Chapter 3.

	Papers I/Chapter 3	
Group allocation	MIE	HIIE
N	23	24
Female	8 (34.8)	9 (37.5)
Age, years	54.6±5.6	57.1±6.2
T2D-duration, years	3.1±2.5	4.3±2.3
BMI, kg/m^2	29.7±3.3	30.4±3.0
WC, cm	107.6±9.0	109.4±9.1
Female, ≥80cm	8 (100)	9 (100)
Male, ≥94cm	14 (93)	14 (93)
HbA_{1c}		
%	6.8±0.7	7.2±1.3
mmol/mol	51.0±5.3	55.0±9.9

Results are presented as mean±SD or No (%). Abbreviations: BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; T2D, type 2 diabetes; WC, waist circumference.

3.3.3 Study intervention

The exercise intervention period was 12 weeks, with a one-year follow-up (Figure 3.1): The participants in the HIIE-group exercised in a supervised setting at our laboratory, whereas the MIE group performed non-supervised home-based exercise.

3.3.3.1 High-intensity interval exercise (HIIE)

HIIE was performed by walking, jogging or running on an inclined treadmill as described in Chapter 2 (Section 2.1.2.1) and the rationale for using this method is discussed in Chapter 1 (Section 1.1.6).

The participants performed HIIE three times a week, altogether 120 minutes per week. After the cessation of the supervised exercise intervention period of 12 weeks, the HIIE-group was encouraged to continue exercising as prescribed for the following 40 weeks.

3.3.3.2 Moderate-intensity exercise (MIE)

Home-based, unsupervised MIE was performed in accordance with the exercise guidelines of the Norwegian Directorate of Health at the time this study commenced (The Norwegian Directorate of Health, 2005).

The participants were instructed to be physically active at moderate intensity for a total of 210 minutes per week, divided into work bouts of >10 minutes (The Norwegian Directorate of Health, 2005). The activity mode during the MIE was optional: Typical physical activity for the MIE group was walking, jogging, cycling and swimming. At the 12-week post-test, the

MIE group was encouraged to continue home-based unsupervised exercise, as performed during the first 12 weeks, for the following 40 weeks.

Exercise intensity control

During the supervised HIIE sessions, all participants exercised with HR monitors (Polar RS 400, Polar Electro, Kempele Finland) to ensure that the required exercise intensity was achieved and maintained. Instructions in HR monitoring were given during HIIE before the participants started exercising and throughout the 12-week intervention period, when needed. If the target HR was not achieved, the treadmill speed or incline was increased to reach it. Most participants were self-administered after the first couple of exercise sessions, but a trained exercise physiologist was present during all exercise sessions.

During the unsupervised home-based MIE sessions, HR was not monitored as we did not have equipment available to save HRs recorded at home. The MIE participants were instructed to strive towards moderate breathing and moderately perceived effort: "being able to keep a conversation going, but just barely" as instructed in the guidelines (The Norwegian Directorate of Health, 2005).

After 12 weeks, exercise intensity was not monitored in any of the exercise groups.

3.3.4 Outcome measures

The primary outcome measure was change in LV peak early diastolic tissue Doppler velocity (e'). The secondary outcome measures were changes in other measures of diastolic function (a'; E; A; E/e'; E/A-ratio; IVRT; DT) and systolic function (S'; global strain; global strain rate) as VO_{2peak}, resting HR, blood pressure, FMD (of the brachial artery), HbA_{1c}, insulin sensitivity (HOMA-ir), triglycerides, HDL-cholesterol, LDL-cholesterol, hs-CRP, BMI, waist circumference and body fat percentage (dual-energy X-ray absorptiometry, DXA).

Although lipid profile (triglycerides, LDL and HDL-cholesterol) was initially a secondary endpoint measure in this study, these measures were not reported in Paper I due to the word limit of the research letter in JACC (see attachments: Paper I). These measures are, however, presented and discussed in this chapter.

The choice of primary outcome measure (diastolic dysfunction defined as e') (i.e. < 8 cm/s) was based on the findings of von Bibra et al. who reported that T2D individuals (n=43; 60.6±7 years; both with and without evidence of coronary artery disease) had compromised relaxation

measured as mean e' (in septal, anteroseptal, anterior, lateral, posterior and inferior walls) compared to healthy controls at rest (8.5+1.7 vs. 9.6+1.9 cm/sec, respectively; $p < 0.02$) (Von Bibra et al., 2005). At the time this study commenced, no other study had to our knowledge presented reference data such as this, although an increasing amount of evidence showed a high prevalence of reduced e' in T2D individuals (further discussed in Chapter 1, section 1.1.3.3).

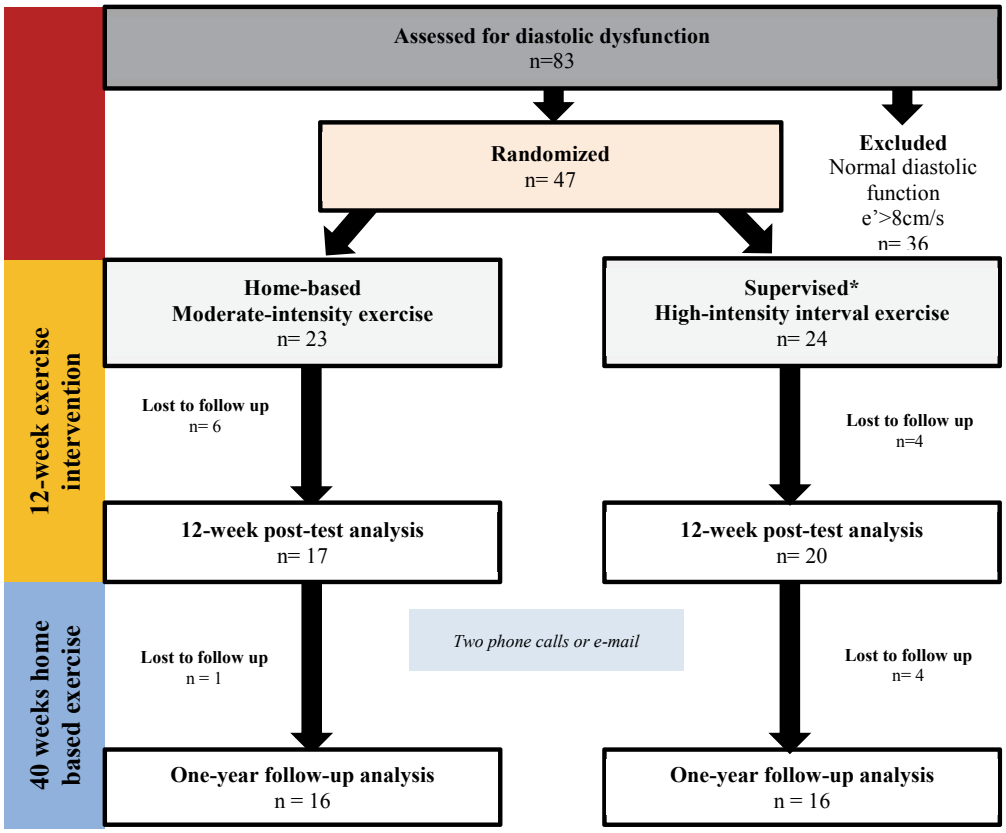


Figure 3.1. Flow chart of intervention and participants described in Chapter 3.

3.3.5 Clinical measurements

Detailed protocols of the main clinical measurement methods in this study are presented and discussed further in Chapter 2.

3.3.5.1 Pre-screening

Before inclusion, all subjects underwent a pre-screening by a medical doctor (CBI), including patient history, auscultation of the lung and heart, resting blood pressure, resting electrocardiogram (ECG) and echocardiography. After pre-screening, it was stated to the participant whether or not the inclusion criteria were met, including diastolic dysfunction (mean e'< 8cm/s).

3.3.5.2 Echocardiography

All participants obtained an echocardiogram at rest and during exercise at baseline, at 12 weeks and after one year.

We used a Vivid 7 scanner (GE Vingmed, Horten, Norway) with a phased array transducer (M4S and M3S probe). Images were digitally stored on a hard disk for offline analysis using commercially available software (EchoPAC version BT12, GE Vingmed Ultrasound, Horten, Norway). Echocardiographic recordings were performed by CBI only.

Resting echocardiography

CBI performing resting echocardiography on a study participant, lying in the left lateral decubitus position.

Resting echocardiography was performed with subjects in the left lateral decubitus position as described previously (Ingul et al., 2010; Molmen et al., 2012) as well as in Chapter 2 (Section 2.1.1.1).

Three consecutive cycles in B-mode (brightness mode; 2D echocardiography) acquisitions and color tissue Doppler imaging (TDI) were recorded from the three apical views (four chambers, two chambers and long axis) and B-mode from the parasternal view (short axis). The loop with the best quality was chosen for analysis.

LV mitral inflow velocity was measured by conventional Doppler echocardiography, including E, A, IVRT and DT (Figure 2.1). E, A and DT measures were obtained with the sample volume between the tips of the mitral leaflet. IVRT was measured with the sample volume between the

aortic and the mitral annulus, imaging the aortic valve click and the start of the mitral inflow. LV volumes and ejection fraction (EF) were calculated from apical four- and two-chamber recordings by biplane modified Simpson's method (Lang et al., 2006).

Pulsed wave (pw) tissue Doppler velocities were measured with the sample volume placed in four sites of the mitral ring in the four-chamber and two-chamber view: The septal and lateral in the four-chamber view and inferior and anterior in the two-chamber view. The mean velocity of the four mitral annular points was used for S', e' and a' (Figure 2.2). The E/e' was calculated as an estimate of LV filling pressure (Ommen et al., 2000).

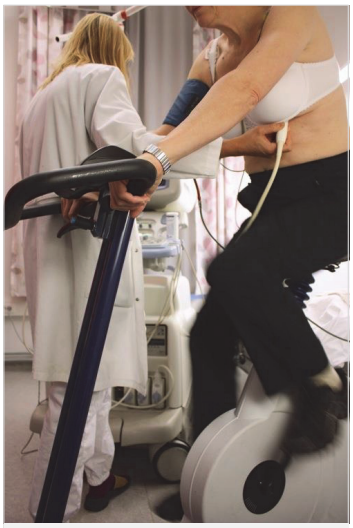
The grading criteria for classifying diastolic dysfunction by Doppler techniques are presented in Chapter 2 (Figure 2.4) (Nagueh et al., 2009).

Echocardiographic measurements obtained, as well as calculations of LV mass and relative wall thickness, are in accordance with standard procedures recommended by the American Society of Echocardiography (Nagueh et al., 2009). Indexation of the LV mass was made to the body surface area, and left ventricular hypertrophy was defined as > 95g/m^2 for women and > 115g/m^2 for men.

Before performing echocardiography, the participants had to have at least 36 hours without exercise.

Stress echocardiography

Stress echocardiography was performed on a cycle ergometer in upright position as described in detail in a previous study (Ingul et al., 2010).



CBI performing stress echocardiography on participant bicycling on an ergometer cycle.

The echocardiography was first obtained at rest (sitting on the cycle ergometer). Then, the participants started cycling at 25 watt and workload was increased by 25 watt every three minutes, until the subject was not able to cycle harder (90–95 % of HR_{max}). Recordings were performed at baseline, at each workload (the last minute of each increment) and at rest when E and A were separated. Apical four-chamber and two-chamber in B-mode and color TDI and mitral/aortic flow were assessed. Color TDI was used to record myocardial tissue velocities and analyses were done

post-processing. Global strain rate and strain were calculated as the mean of 12 segments using TDI.

No subjects were excluded due to ischemia during stress echocardiography.

To assess hemodynamic changes during stress echocardiography, blood pressure was measured using a Tango blood pressure monitor (SunTech® Tango® M2 Stress BP Monitor, Morrisville, USA) at baseline, at each workload (the last minute of each increment) and after cycling, when E and A were separated.

Echocardiographic analyses

Off-line data analyses were performed by using commercially available software (Echopac PC, Version 112, GE Medical Systems). All analyses were performed blinded for group allocation. Analyses of conventional echocardiographic diastolic measures were performed as described in Chapter 2 and as published previously (Ingul et al., 2010; Molmen et al., 2012).

3.3.5.3 Cardiopulmonary Exercise Testing

The VO_{2peak} test used is described in detail in Chapter 2 (section 2.1.1.2) and was performed according to a previously described individualized protocol (Rognmo et al., 2004). The Jaeger Oxycon Pro (LE2000CE, Viasys Healthcare, Germany) was used to measure VO_{2peak}. Absolute and relative VO_{2peak} is reported: Both absolute body weight (mL/min/kg) and body weight exponent 0.75 (mL/min/$kg^{0.75}$) were used to assess relative VO_{2peak}. The rationale for reporting the latter was to take disproportional increase in muscle mass with body size into consideration when comparing relative VO_{2peak} within and between the groups (Åstrand, 2003).

At baseline, 12-lead ECG was recorded during the VO_{2peak} test at baseline to detect ischemia or arrhythmia during the test. Two subjects developed ischemia at ECG during VO_{2peak} test and was sent for coronary angiography.

HR was measured continuously during the VO_{2peak} test using HR monitors (Polar, Finland), and HR_{max} was determined by adding five beats to the highest HR observed during the VO_{2peak} test (see Chapter 2, section 2.1.1.3).

The fact that only the HIIE participants, but not all participants in the MIE-group, performed exercise training on treadmill could have influenced the results as the MIE-group might not have been as familiarized with the treadmill as the HIIE-group at post-test. Furthermore, even though the VO_{2peak} tests were performed by the same person at all time points, the investigator was not blinded to group allocation.

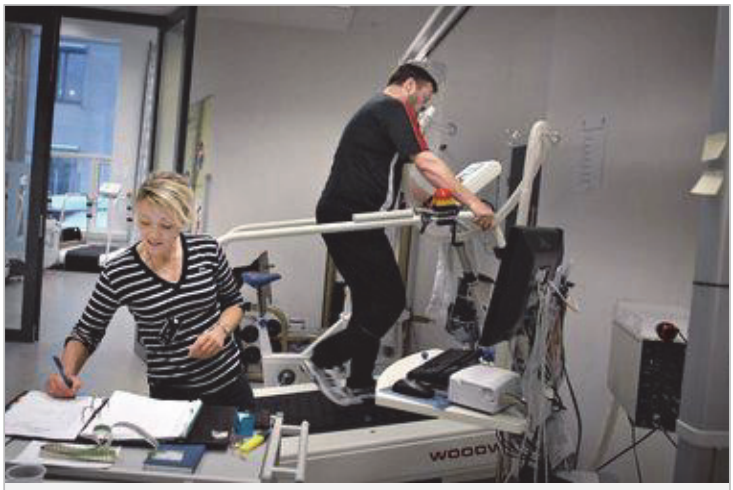


Illustration of a VO_2 test situation with one of the study participants.
Photo: Adresseavisen 2011.

3.3.5.4 Resting heart rate and blood pressure

Resting heart rate (HR) and blood pressure was measured according to Chapter 2 (sections 2.1.1.7-2.1.1.8).

Resting HR was measured by ECG. The lowest HR observed during resting echocardiography was noted as resting HR.

Blood pressure was measured by oscillometry using a Tango blood pressure monitor (SunTech® Tango® M2 Stress BP Monitor, Morrisville, USA). Supine blood pressure was measured prior to resting echocardiography. After a minimum 10-minute rest in supine position, supine blood pressure was noted as the median of three similar recordings, which did not deviate more than ± 10 mmHg from each other in regard to systolic blood pressure.

The fact that resting heart rate and blood pressure measurements were not obtained in the fasted state, represent a limitation in this thesis due to the postprandial findings of Chapter 5 (Paper III).

3.3.5.5 Flow mediated dilatation of the brachial artery

Brachial artery diameter and blood velocity were measured according to guidelines in place at the time of conducting this study (Corretti et al., 2002; Harris, 2010), using high-resolution

Vivid 7 ultrasound (GE Vingmed Ultrasound, Horten, Norway) with a 12MHz linear echo Doppler probe (see Chapter 2, section 2.1.1.8)

A pneumatic cuff (SC10, Hokanson Inc., Bellevue, Washington) was placed on the forearm, distal to the antecubital fossa, where it was inflated to 250 mm Hg and released abruptly after five minutes to create an ischemia-induced hyperemia. Baseline, pre-occlusion images and velocities were measured for three cardiac cycles before deflation of the occlusion cuff. Post-occlusion images and velocities were collected continuously for three minutes to measure peak diameter and blood velocity. The ultrasound images were analyzed using edge-detection software (Vascular Research Tools 5, Medical Imaging Applications LLC, Coralville, IA, USA).

All ultrasound images were analyzed in a random order by the same investigator blinded to patient's group allocation. The software was set to automatically reject frames if the arterial wall detection was not within 70% of the confidence interval. Doppler image frames were manually readjusted by the observer if not adequately interpreted by the software. If acceptable tracking could not be obtained, image frames or the whole measurement were discarded.

Peak diameter was defined as the largest arterial diameter measured throughout the three-minute post-occlusion period. Flow mediated dilatation (FMD) was defined as the difference between peak post-occlusion diameter and average pre-occlusion diameter, divided by average pre-occlusion diameter (multiplied by 100). Average blood flow was defined as the average flow value detected during the three-minute post-occlusion period. Share rate was calculated as average blood flow relative to average diameter (mm) after deflation. FMD was normalized for share rate (FMD_{norm}) by dividing FMD by share rate.

SMH performing FMD measurement.

Before performing FMD measurements (at all time-points), the participants had to fast from food (including caffeine, nicotine and alcohol) for at least 12 hours and to have at least 48 hours without exercise before performing FMD, according to guidelines recommending at least 12 hours without exercise (Harris, 2010; Thijssen et al., 2011).

3.3.5.6 Biochemical analysis

Blood was obtained after at least 12 hours of fasting. The following analyses were performed at St. Olav's University Hospital with standard procedures as described in Chapter 2 (section 2.1.1.5): HbA_{1c}, blood glucose, insulin C-peptide, plasma triglycerides, total cholesterol, HDL-cholesterol, LDL, high-sensitive C-reactive protein (hs-CRP). At baseline, hemoglobin, serum albumin, serum creatinine, potassium and sodium was also measured to reveal eventual microalbuminuria or renal failure.

Insulin resistance was calculated from fasting blood glucose and insulin C-peptide (HOMA2 calculator, The Homeostasis Assessment Model, University of Oxford, UK).

3.3.5.7 Anthropometric measurements

Anthropometric measurements were performed as described in Chapter 2 (section 2.1.1.4). BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference was measured by the same investigator at all time-points using a measuring tape at the level of the umbilicus after expiration. The measure that could be reproduced three times was noted as waist circumference.

In the present study, DXA (Hologic Discovery A, version 13.3.0.1, USA) was performed to measure body fat percentage. All anthropometric measurements were performed at all time-points. The same, trained investigator measured the subjects at all times. However, the participants were not asked to abstain from eating or drinking nor exercising prior to the DXA measurements, which represents a potential for both false conclusions (Type I error) or failure to identify real differences (Type II error) in regard to body composition in this study.

3.3.5.8 Self-reported physical activity and energy intake

Physical activity diary

Both exercise groups (HIIE and MIE) kept a diary during the 12-week intervention to register all physical activities characterized as "a little strenuous", "strenuous" and "very strenuous" as well as duration of the different activities (walking, running, bicycling, swimming, strength training and "other"). Potential limitations when interpreting physical activity diaries are the variation between subjects in regard to perceived effort as well as the potential for over- or underreporting physical activity.

Four-days food diary

Subjects filled out a four-day food diary representing their eating habits of three weekdays and one day of the weekend. They were asked to register on days characterized as "normal days" at all time-points. Energy intake was analyzed using a nutrition software program (Mat på Data 5.1, the Norwegian Food Safety Authority, Norway). Self-reporting (over- or underreporting), the limited days of registering food intake; using household measures instead of weighed food measurements; and translation of the registrations into the nutrition software program represents some limitations which has to be taken into account when interpreting the results of these food diaries.

3.3.6 Statistical analysis

The analyses were carried out using a standard statistical software program (IBM SPSS Statistics version 21.0; SPSS Inc., Chicago, Illinois). Statistical differences were considered significant at $p<0.05$.

Analysis of variance (ANOVA) with repeated measurements was used to analyze the mean change in cardiac function and other physiological, anthropometrical and biochemical variables during the 12-week intervention period. The number of subjects with complete information is reported in the papers tables as well as in the results section of the present thesis. An interaction term between time (baseline, 12 weeks) and group (MIE, HIIE) was included in the statistical model to examine whether potential change in the outcome measures differed between the MIE and HIIE groups. To assess subsequent changes at one-year follow-up, and also compare with initial baseline values, additional analyses with time (baseline, 12 weeks, one year) defined as categorical variable, were carried out. The analyses were performed by means of the general linear model (GLM) and linear mixed model (LMM) modules in SPSS. Pearson's correlation coefficient (r) was calculated to evaluate associations between changes in the different measurements during the 12-week intervention (total sample).

The chi-square test was used to compare the two intervention groups with respect to demographic characteristics (Table 3.3, not reported in Paper I). In table 3.3, the variables presented are mainly categorical and hence the choice of chi-square test. As Table 3.3 had several categorical variables, we also chose to present age, T2D duration and BMI as categorized variables in this table. However, mean baseline values for the latter variables are also presented this Chapter 3 (sections 3.4.1-3.4.2).

Sample size calculations

Sample size was calculated a priori from the primary outcome variable e'. We anticipated that to assess a clinically significant overall increase in e' (1.1 cm/s) in patients with T2D with a power of 0.80 and an α of 0.05, 38 patients would be needed in total. In the expectation of a 15% dropout or failure to exercise adequately, we aimed to recruit 22 patients for each group (n=44).

3.4 SUMMARY OF RESULTS

3.4.1 Baseline characteristics and compliance

Participants in the MIE group were younger (mean 54.7±5.3 versus 58.6±5.0 years, p=0.02), had a better systolic function (S' and LV strain rate), higher HR at rest and lower hs-CRP compared to the HIIE group (Table 3.3). There was no difference between groups in regard to severity of diastolic dysfunction (chi-square test) (Table 3.3). Diastolic dysfunction (as defined in Figure 2.4) grade 1 was found in 16%, grade 2 in 81% and grade 3 in 3% of the individuals (Table 3.3).

All participants complied with their medical treatment regimen during the 12-week intervention period. Medical agents are presented in Table 3.3 and the concomitant antihypertensive medication subclass is presented in Textbox 1.

The HIIE group performed 93.7% of the scheduled exercise sessions. In the MIE group, 16 of 17 (94%) reported physical activity to be $\geqslant$ 210 minutes/week.

Table 3.3. Baseline Characteristics for the individuals completing the 12-week exercise period.

Characteristics		MIE (n=17)	HIIE (n=20)	*P* Value*
Gender				
	Male	11 (64.7)	12 (60.0)	0.77
	Female	6 (35.3)	8 (40.0)	
Age				
	40–55 years	9 (52.9)	4 (20.0)	
	55–60 years	7 (41.2)	7 (35.0)	0.02
	60–65 years	1 (5.9)	9 (45.0)	
Type 2 diabetes duration				
	<5 years	13 (76.5)	14 (70.0)	0.66
	5–10 years	4 (23.5)	6 (30.0)	
Body Mass Index				
	18.5–25 kg/m^2	2 (11.8)	1 (5.0)	
	25–30 kg/m^2	6 (35.3)	9 (45.0)	0.69
	30–35 kg/m^2	9 (52.9)	10 (50.0)	
Diastolic dysfunction classification				
Mild (Grade I)		5 (29.4)	1 (5.0)	0.16
Moderate (Grade II)		12 (70.6)	18 (90)	
Severe (Grade III)		0 (0.0)	1 (5.0)	
LVH				
	LVH	6 (35.3)	11 (55.0)	
	Abnormal RWT	14 (82.4)	11 (55.0)	0.16
	Eccentric LVH	1 (5.9)	5 (25.0)	
	Concentric LVH	5 (29.4)	6 (30.0)	
	Concentric remodeling	8 (47.1)	5 (25.0)	
Medical agents				
	Anti-hypertension	6 (35)	7 (35)	0.99
	Anti-platelet	3 (18)	4 (20)	0.86
	Statins	7 (41)	4 (20)	0.16
	Anti-diabetic	12 (71)	17(85)	0.29

Data are presented as No. (%) of sample at baseline. Abbreviations: LVH, left ventricular hypertrophy; RWT, relative wall thickness.* Pearson chi-squared cross-table.

Textbox 1. Reported concomitant antihypertensive medication sub-class.

HIIE- group (n=2):
n=1: Calcium antagonist + beta blocker
n=1: Calcium antagonist + ACE-inhibitor + angiotensin II-receptor antagonist and diuretic + alpha blocker
MIE group (n=2):
n=2: Angiotensin II-receptor antagonist and diuretic + calcium antagonist

3.4.2 Clinical measurements

3.4.2.1 Diastolic and systolic function at rest

After 12 weeks exercise intervention, 19 (51%) individuals had grade 1 and 18 (49%) had grade 2 diastolic dysfunction.

Individuals with T2D and diastolic dysfunction improved diastolic function (e') after both MIE and HIIE, but HIIE was superior to MIE ($p<0.001$) in improving e'. Only HIIE improved other diastolic measurements (E, E/e' and E/A) and systolic function (S', global strain, global strain rate) (Table 3.4). Figure 3.2 illustrates a baseline and 12-week post-test echocardiogram (pw-TDI) with exercise-induced improvements.

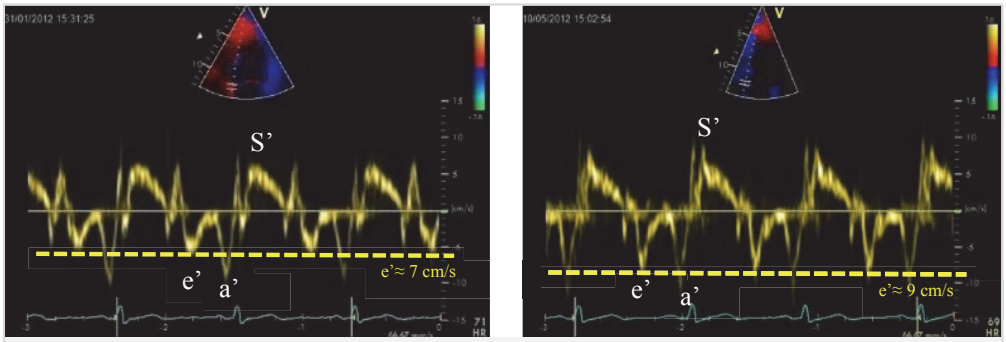


Figure 3.2. Echocardiographic image from baseline (left panel) and 12-week post-test (right panel) measurement of one of the participants of the present study. Abbreviations: S', peak systolic tissue Doppler velocity; e', peak early diastolic tissue Doppler velocity; a', peak late diastolic tissue Doppler velocity.

Table 3.4. Baseline and 12 week post-test results for diastolic and systolic echocardiographic variables at rest.

	MIE Mean ± SD				HIIE Mean ± SD				MIE vs. HIIE
Supine values	**n**	**Baseline**	**Post**	**Difference**	**p†**	**n**	**Baseline**	**Post**	**Difference**	**p†**	**p‡**
Diastolic variables											
E, cm/s	14	63.4±9.5	64.4±13.4	1.0±14.0	0.78	20	64.8±10.7	75.1±15.8	10.3±12.7	0.002	0.05
e`, cm/s	17	7.1±0.7	7.6±1.1	0.5±0.7	0.02	20	7.0±0.7	8.8±1.2	1.8±1.1	<0.001	<0.001
E/e'	14	9.1±1.8	8.6±1.9	-0.5±1.6	0.28	20	9.3±1.7	8.6±1.9	-0.7±1.5	0.05	0.67
E/A, ratio	14	0.92±0.18	0.99±0.21	0.07±0.23	0.28	20	0.93±0.21	1.07±0.26	0.13±0.18	0.003	0.36
A, m/s	14	70.2±9.8	67.1±11.0	- 3.1±9.2	0.23	20	71.1±15.0	72.7±17.4	1.6±13.9	0.61	0.28
DT, ms	16	194.2±36.9	194.7±39.7	0.5±51.7	0.97	20	191.9±38.4	183.1±31.8	-8.8±41.2	0.35	0.55
IVRT, ms	15	66.9±10.6	69.7±12.5	2.7±16.2	0.52	20	70.0±9.6	62.4±8.7	-7.6±11.2	0.39	0.03
Systolic variables											
S', cm/s	14	7.7±1.2*	7.6 ±1.0	-0.1±1.1	0.76	20	6.8±0.8*	7.8±1.5	0.9±1.4	0.007	0.03
Global strain, %	15	-16.7±2.2	-16.4±2.1	0.3±1.6	0.51	20	-17.2±1.9	-18.2±2.2	-1.0±1.9	0.03	0.05
Global strain rate, s^{-1}	16	-1.00±0.15*	-0.95±0.10	0.06±0.23	0.14	20	-0.87±0.11*	-0.97±0.13	-0.10±0.17	0.03	0.009

Data are presented as mean ± SD. Abbreviations: A, peak late diastolic mitral inflow velocity; DT, deceleration time; e', peak early diastolic tissue Doppler velocity; E, peak early diastolic mitral inflow velocity; HIIE, high-intensity interval exercise; IVRT, isovolumic relaxation time; MIE, moderate-intensity exercise; S', peak systolic tissue Doppler velocity. †p-value: within group difference.‡ p-value: difference in mean change between groups.

Table 3.5 Baseline and 12-week post-test results for exercise stress echocardiography with tissue Doppler and Doppler flow velocities during cycle ergometer.

	MIE Mean ± SD					HIIE Mean ± SD					MIE vs. HIIE
Upright Values	n	Baseline	Post	Diff.	p†	n	Baseline	Post	Diff.	p†	p‡
$Watt_{peak}$, W	11	181±54	184±49	3±18	0.58	17	183±43	176±38	-7±26	0.30	0.28
Test duration, min	11	14.9±2.2	15.2±2.2	0.4±2.3	0.62	16	16.1±2.3	16.0±1.9	-0.1±2.1	0.84	0.59
Global strain rate, s^{-1} at:											
0 Watt	16	-1.08±0.09	-1.11±0.09	-0.03±0.13	0.30	20	-1.07±0.07	-1.14±0.16	-0.07±0.15	0.04	0.42
$Watt_{peak}$	14	-2.03±0.28*	-1.96±0.17	0.08±0.27	0.32	20	-1.80±0.22*	-2.14±0.29	-0.34±0.39	0.001	0.002
E, cm/s at:											
0 Watt	16	50.1±10.2	52.9±7.6	2.7±10.0	0.29	20	50.6±10.2	54.7±11.3	4.2±10.0	0.08	0.68
25 Watt	13	65.6±11.5	71.1±15.2	5.5±17.5	0.28	18	68.11±11.8	75.3±10.1	7.2±9.7	0.006	0.73
50 Watt	14	82.4±20.3	77.1±14.8	14.3±22.7	0.25	18	72.3±11.4	85.8±14.4	9.5±13.4	0.003	0.51
75 Watt	14	92.1±16.8	90.0±14.3	-2.1±13.2	0.55	18	82.5±19.2	98.4±17.8	15.9±20.2	0.004	0.007
$Watt_{peak}$	13	114.8±25.6	102.8±15.3	-11.9±24.6	0.11	16	121.1±19.2	121.3±16.9	0.3±15.6	0.95	0.12
Recovery	14	67.1±16.2	75.7±9.4	0.08±0.17	0.07	18	67.4±10.8	74.6±0.11	0.07±0.12	0.02	0.77
SV, mL at:											
0 Watt	11	70.9±25.0	78.5±30.0	7.6±16.9	0.17	15	70.6±12.2	77.2±15.0	6.6±11.7	0.05	0.86
25 Watt	11	87.5±22.5	89.6±29.3	2.1±19.8	0.73	15	84.9±13.4	91.6±29.3	6.7±11.0	0.03	0.45
50 Watt	11	90.3±20.3	104.5±32.9	14.3±22.7	0.06	15	86.6±18.6	96.1±21.5	9.5±13.4	0.02	0.51
75 Watt	11	93.3±23.9	101.1±23.1	7.8±13.2	0.08	15	86.9±19.6	103.5±31.8	16.6±24.1	0.02	0.29
$Watt_{peak}$	11	90.1±28.8	96.7±21.3	6.6±25.6	0.41	15	86.2±20.8	93.7±20.4	7.5±11.7	0.03	0.91
CO, L/min at:											
0 Watt	11	5.4±1.7	5.9±2.0	0.5±1.3	0.23	15	5.0±0.9	5.5±1.1	0.6±0.9	0.03	0.90
25 Watt	11	7.9±2.0	7.9±1.6	-0.1±1.7	0.87	15	7.5±1.4	8.1±1.5	0.6±0.9	0.02	0.19
50 Watt	11	8.8±1.3	10.3±2.9	1.5±2.8	0.11	15	8.3±1.7	9.5±2.5	1.2±2.1	0.04	0.77
75 Watt	11	10.2±2.3	11.0±2.6	0.8±2.0	0.23	15	9.2±2.3	11.0±2.6	1.7±2.3	0.01	0.28
$Watt_{peak}$	11	13.2±4.5	14.4±3.3	1.2±2.7	0.29	15	12.4±2.8	13.7±2.4	1.2±1.9	0.02	0.99

Data are presented as mean ± SD. Abbreviations: CO, cardiac output; Diff., difference; E, peak early diastolic mitral inflow velocity; HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise; SV, stroke volume; $Watt_{peak}$, peak watt achieved during stress echocardiography. *Difference between groups at baseline, $p<0.05$. †p-value; within group difference. ‡p-interaction value; difference in mean change between groups.

3.4.2.2 Diastolic and systolic function during exercise

Only HIIE improved diastolic and systolic function during stress echocardiography: E at 75 watt and global strain rate at $watt_{peak}$ improved significantly after HIIE, but not after MIE ($p<0.01$, Table 3.4). In addition, the HIIE group also improved E at 25 and 50 watt, global strain rate at 0 watt, as well as SV and cardiac output (CO) at all workloads, but there was no significant group difference ($p > 0.4$, Table 3.5). No significant change in other diastolic or systolic measures, blood pressure or HR during stress echocardiography was found from baseline to 12-week post-test.

The strain and strain rate values from supine resting values and stress echocardiographic values are not comparable due to different techniques used (see Chapter 2, Section 2.1.1.1 Echocardiography), hence they are not compared.

3.4.2.3 Cardiorespiratory fitness

Individuals with T2D and diastolic dysfunction had reduced cardiorespiratory fitness (measured as VO_{2peak}) compared to previous findings in healthy counterparts from the HUNT Study (Aspenes et al., 2011). HIIE and MIE both improved VO_{2peak}, but HIIE was superior to MIE ($p=0.002$) (Table 3.6).

3.4.2.4 Heart rate and blood pressure

There was no change in resting blood pressure, resting HR or in HR_{max} in either group (Table 3.6).

3.4.2.5 Endothelial function

HIIE was superior to MIE in improving endothelial function (FMD) in T2D individuals with diastolic dysfunction (Table 3.6). However, FMD results were incomplete due to errors that occurred while transferring ultrasound images to CD/DVD, and results must thus be interpreted with caution.

Table 3.6. Baseline and 12 week post- test results for oxygen uptake, endothelial function, heart rate, blood pressure.

		MIE Mean ± SD					HIIE Mean ± SD					MIE vs. HIIE
		n	**Baseline**	**Post**	**Difference**	**p †**	**n**	**Baseline**	**Post**	**Difference**	**p †**	**p ‡**
$VO2_{peak}$												
	mL/kg/min	16	33.2 ± 7.4	34.4 ± 7.7	1.2 ± 2.2	0.04	20	31.5 ± 6.1	35.6 ± 6.3	4.1 ± 2.9	<0.001	0.002
	$mL/kg^{-0.75}/min$	16	102.0 ± 23.8	105.2 ± 23.9	3.2 ± 6.1	0.06	20	98.6 ± 17.7	110.4 ± 20.0	11.8 ± 9.5	<0.001	0.003
	L/min	16	2.96 ± 0.81	3.0 ± 0.79	0.06 ± 0.17	0.17	20	2.96 ± 0.57	3.29 ± 0.68	0.33 ± 0.29	<0.001	0.003
FMD												
	FMD, %	10	13.0 ± 9.8	13.0 ± 9.9	0.0 ± 6.2	0.99	17	9.2 ± 9.6	18.5 ± 9.6	9.2 ± 11.2	0.004	0.03
	FMD_{norm}, %	10	13.7 ± 12.6	14.6 ±19.3	1.0 ± 10.0	0.76	16	8.9 ±11.4	22.8 ±15.1	13.9 ± 18.5	0.009	0.05
Heart rate, bpm at:												
	Rest	16	74 ± 10*	72 ± 8	2 ± 8	0.39	19	68 ± 8*	66 ± 9	1 ± 8	0.48	0.84
	Maximum	16	168 ± 17	170 ± 14	2 ± 4	0.10	20	167 ± 10	169 ± 10	2 ± 4	0.08	0.85
Blood pressure, mmHg												
	Systolic	17	135.4 ± 11.9	134.9 ±14.8	-0.5 ±11.9	0.87	20	142.1 ± 18.3	142.5 ± 20.6	-0.5 ±15.8	0.90	0.85
	Diastolic	17	80.9 ± 7.1	80.8 ± 6.6	-0.1 ± 5.8	0.97	20	81.7 ± 6.9	78.3 ± 9.0	-3.4 ± 9.3	0.12	0.21

Data is presented as mean ± SD. Abbreviations: FMD, brachial artery flow-mediated dilatation; FMD_{norm}, FMD normalized= FMD/shear rate (average flow/average diameter); HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise; VO_{2peak}, peak oxygen uptake. *Difference between groups at baseline, $p<0.05$. †p-value: within group difference. ‡p-value: difference in mean change between groups.

Table 3.7. Baseline and 12 week post- test results for biochemical values and body composition.

	MIE Mean ± SD					HIIE Mean ± SD					MIE vs. HIIE
	n	**Baseline**	**Post**	**Difference**	**p†**	**n**	**Baseline**	**Post**	**Difference**	**p†**	**p‡**
HbA_{1c} %	16	6.7 ± 0.7	6.5 ± 0.6	-0.2 ± 0.7	0.30	20	7.0 ±1.2	6.6 ± 0.9	-0.4 ± 0.5	0.007	0.36
HOMA-ir	15	2.6 ± 1.0	2.5 ± 0.9	-0.1 ± 0.5	0.32	19	2.7 ± 0.7	2.7 ± 1.0	0.0 ± 0.8	1.0	0.61
Total cholesterol, mmol/L	16	5.25 ± 0.92	5.30 ±1.20	0.06 ± 0.55	0.69	20	5.24 ± 1.02	5.27 ± 1.27	0.04 ± 1.00	0.88	0.94
LDL, mmol/L	16	3.21 ± 0.91	3.30 ±1.00	0.09 ± 0.42	0.40	20	3.12 ± 0.97	3.16 ± 1.00	0.04 ± 0.77	0.84	0.80
HDL	16					20					
mmol/L		1.19 ± 0.28	1.26 ± 0.33	0.06 ± 0.09	0.01		1.30 ± 0.43	1.37 ± 0.45	0.07 ± 0.16	0.07	0.93
%		23.0 ± 4.8	24.3 ± 5.5	1.2 ± 2.6	0.07		25.1 ± 7.1	26.0 ± 5.6	0.9 ± 4.0	0.33	0.78
Triglycerides, mmol/L	16	1.88 ± 0.77	1.65 ± 0.66	-0.23 ± 0.50	0.09	20	1.80 ± 0.78	1.64 ± 0.76	-0.17 ± 0.86	0.39	0.81
hs-CRP, mg/L	16	1.6 ± 1.2*	1.6 ±1.2	-0.1 ±1.3	0.86	19	3.7 ± 2.8*	2.1 ± 1.3	-1.6 ± 2.6	0.02	0.04
Body mass index, m/kg^2	17	29.7 ± 3.7	29.4 ± 3.8	-0.3 ± 0.8	0.13	20	30.2 ± 2.8	29.7 ± 2.4	-0.5 ± 0.7	0.009	0.52
Waist circumference, cm	17	106.5 ± 8.7	104.5 ± 7.3	-2.0 ± 2.5	0.005	20	108.6 ±7.7	106.0 ± 6.8	-2.6 ± 2.9	0.001	0.55
Body fat, %	15	27.5 ± 7.3	27.2 ± 6.7	-0.3 ± 2.1	0.58	19	27.9 ± 7.7	27.6 ± 8.5	-0.28 ± 1.3	0.36	0.97

Data is presented as mean ± SD. Abbreviations: HIIE, high-intensity interval exercise; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; HDL%, HDL as percent of total cholesterol; HOMA-ir, homeostatic model assessment; hs-CRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; MIE, moderate-intensity exercise. *Difference between groups at baseline, $p<0.05$.†p-value: within group difference. ‡p-value: difference in mean change between groups.

3.4.2.6 Biochemical analysis

Glycemic control

Only HIIE improved glycemic control (HbA_{1c}) after 12 weeks, but insulin sensitivity (HOMA-ir) did not change in either group (Table 3.7). Altogether at baseline, 14 individuals had HbA_{1c} above treatment target (>7%) and 23 individuals had HbA_{1c} at treatment target (≤7%) (American Diabetes Association, 2014; Ryden et al., 2013). During the 12-week exercise intervention, the number of individuals with HbA_{1c} > 7% reduced to eight individuals (Table 3.8).

Table 3.8 Proportions of individuals with HbA_{1c} ≤ and > 7% at baseline, post-test and after one year.

	Baseline n=37			**Post-test n=37**		
HbA_{1c}	**MIE**	**HIIE**	**MIE+HIIE**	**MIE***	**HIIE**	**MIE+HIIE***
>7%	5 (29.4)	9 (45.0)	14 (37.8)	3 (18.8)	5 (25.0)	8 (22.2)
≤7%	12 (70.6)	11 (55.0)	23 (62.2)	13 (81.3)	15 (75.0)	28 (77.8)

Data are presented as No. (%). Abbreviations: HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise. *n=1 missing.

Lipid profile

HDL-cholesterol increased only after MIE (p=0.01), though with a similar pattern in the HIIE group (p=0.07) (Table 3.7). Mean LDL levels did not change from baseline to post-test in either exercise group. Proportions of individuals above and within target treatment goals are presented in Table 3.9. In general, compared to lipid treatment targets, the participants had high mean triglyceride and LDL levels and low HDL levels at baseline.

Table 3.9 Proportion of participants at the low range or below lipid treatment target guidelines at baseline and post-test.

		Baseline		**12-week post-test**	
		MIE (n=17)	HIIE (n=20)	MIE (n=16)	HIIE (n=20)
Triglycerides	> 1.7 mmol/L	10 (56.0)	10 (50.0)	6 (38.0)	9 (45.0)
HDL*	< 1.3 mmol/L	13 (76.0)	13 (65.0)	11 (69.0)	11 (55.0)
LDL					
	< 1.8 mmol/L	2 (12.0)	1 (5.0)	2 (12.5)	2 (10.0)
	1,8–2,5 mmol/L	2 (12.0)	4 (20.0)	2(12.5)	1 (5.0)
	> 2.5 mmol/L	13 (76.0)	15 (75.0)	12 (75.0)	17 (85.0)

Data are presented as No. (%). Treatment targets are based on Brunzell et al. (Brunzell et al., 2008), ESC guidelines (Catapano et al., 2011) and the Nordic Reference Interval Project 2000 (Rustad et al., 2004). *ADA guidelines for women (Evert et al., 2014). Abbreviations: HDL, high-density lipoprotein cholesterol; HIIE, high-intensity interval exercise; LDL, low-density lipoprotein cholesterol; MIE, moderate-intensity exercise.

Hs-CRP

Only the HIIE group improved hs-CRP over 12 weeks (p=0.04) (Table 3.7).

Diverse baseline analysis

Biochemical measurements only performed at baseline (hemoglobin, serum albumin, serum creatinine, potassium and sodium) were considered normal in all the participants, but two (MIE, n=1; HIIE, n=1) had microalbuminuria, i.e. a slightly elevated urine albumin/creatinine ratio. In addition, we had five missing urine samples.

3.4.2.7 Body composition

Body composition (BMI, WC and DXA) was measured according to Chapter 2 (section 2.1.1.4). At baseline, the majority of the T2D participants were overweight (~40%) or obese (~50%). Only HIIE improved BMI in 12 weeks, whereas both HIIE and MIE improved waist circumference. Twelve weeks of exercise did not modulate fat percentage measured by DXA.

Self-reported energy intake

There was no significant difference between the MIE group (n=13) and the HIIE group (n=19) in average reported energy intake at baseline, and no significant difference from baseline to post-test was found in either group (Figure 3.3). The macronutrient composition in the diet was on average in accordance with current recommendations (Protein, 10–20% of total energy intake (E%); Fat, 25–40 E%; Carbohydrate, 45–60 E%) (The Norwegian Directorate of Health, 2014). However, total energy intake from fat was on average at the upper range of what is recommended, and reported saturated fat intakes were on average >10 E%. Proportions of mono- and polyunsaturated fat intakes were reported to be in the low range of current

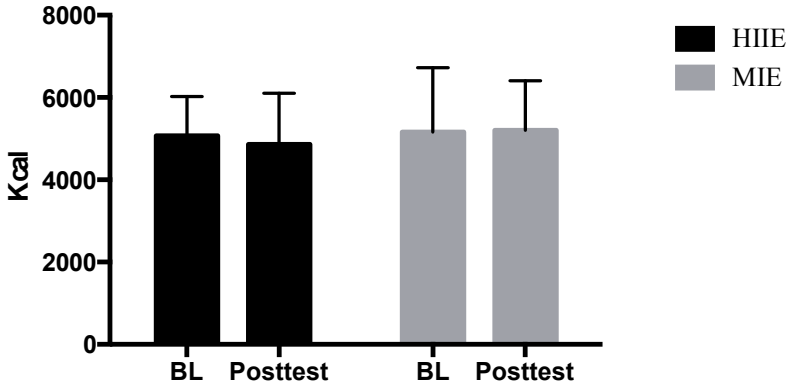


Figure 3.3. Average self-reported total daily dietary energy intake of participants in the present study. Abbreviations: BL, baseline; HIIE, high-intensity interval exercise group; kcal, kilocalories; MIE, moderate-intensity exercise group.

recommendations (monounsaturated fat, 10–20 E%; polyunsaturated fat, 5–10 E%). There was no significant change in dietary intake during intervention, except for a significant increase in energy intake from protein (19±4 at baseline vs. 21±4 E% at 12-week post-test, p=0.02) and a proposedly compensatory decrease in energy intake from carbohydrate intake (39±7 at baseline vs. 36±8 E% at 12-week post-test, p=0.03) in the HIIE group. However, there was no significant difference in change from baseline to 12 weeks in any of the macronutrients between the HIIE and MIE group. Analysis of fiber, salt and added sugar intake was discarded due to the limitations in the method used to analyze dietary intake.

3.4.2.8 Correlation between outcome measures

There was a correlation between changes in S' and e' (r=0.54, p=0.001), E (r=0.38, p=0.026), A (r=0.53, p=0.001) and IVRT (r=-0.44, p=0.01). There was also a correlation between changes in global strain and IVRT (r=0.38, p=0.03). No other correlations were seen between diastolic and systolic variables.

Improvement in diastolic function (e') correlated significantly ($p<0.05$) with improvements in HbA_{1c} (r=-0.39, p=0.009) and diastolic blood pressure (r=-0.40, p=0.02), but with less of an increase in HDL (r=-0.40, p=0.02).

3.4.2.9 One-year follow-up

After one year, the HIIE group had sustained improvements in cardiac function, cardiorespiratory fitness, body composition and biochemical measurements, in contrast to the MIE group.

Individual changes in e' during the year are presented in Figure 3.4. The HIIE group, but not the MIE group, had on average better e' after one year compared to baseline. The initial improvements (baseline to post-test) in E, S' and the strain rate were sustained after one year in the HIIE group. In the MIE group, the 12-week exercise-induced improvements in cardiac function had returned to baseline levels. No significant difference from baseline was seen for other variables describing cardiac function.

Furthermore, the HIIE group sustained improvement in VO_{2peak}, BMI, waist circumference and hs-CRP, whereas improvements in FMD and HbA_{1c} were lost after one year. Body fat percentage had decreased from baseline to one year and HDL improved slightly and differed significantly from baseline at the one-year follow-up (mean increase of 0.09 mmol/L). In the MIE group, waist circumference was increased from the 12-week post-test to the one-year follow-up and HDL returned to baseline-levels after one year.

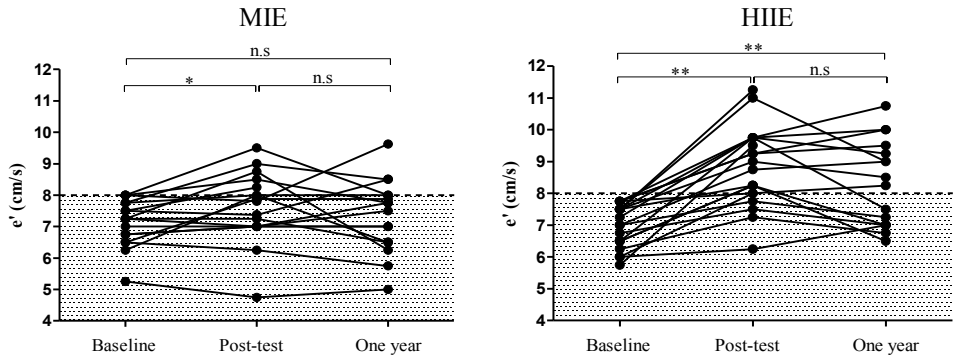


Figure 3.4. Individual changes in diastolic function during one year in the present study**.** Lines represent diastolic function (e') at baseline, post-test and one-year follow-up. Grey area represents diastolic dysfunction (e' < 8 cm/s). Results from statistical tests on mean change (between two time points) within each group are shown. *p<0.05; **p<0.001 Abbreviations: e', peak early diastolic tissue Doppler velocity; HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise; n.s. not significant at 5% level.

3.5 DISCUSSION

3.5.1 Effect of exercise on cardiac function in T2D

3.5.1.1 Diastolic function

The improvement in diastolic function (e') of 1.8 cm/s (~26% increase from baseline) in the HIIE group compared to 0.5 cm/s (~7% increase from baseline) in the MIE group, as well as significant differences between groups in improvement in E and E/e', reflects the importance of exercise intensity for improving diastolic function.

Although it is not yet known whether or not exercise can prevent the development of diastolic dysfunction in T2D over time (Hare et al., 2011), the findings suggest it may be possible to slow down the progression of diastolic dysfunction if performing regular MIE in general or HIIE in particular.

This study is one of the very first to investigate the effect of high intensities at 90 to 95% of HR_{max} on diastolic function in T2D. Continuous exercise training at moderate intensities has been more frequently used to investigate the effect of exercise on diastolic function in T2D. The few previous studies reporting the effect of exercise on diastolic function have conflicting results (Brassard et al., 2007; Friis Schmidt et al., 2013; Hare et al., 2011; Hordern et al., 2009; Loimaala et al., 2007; Ofstad et al., 2014). Due to different characteristics of diastolic dysfunction, different measurement methods and exercise modalities, it is hard to compare studies. However, one study observed improvements in diastolic function (E/A-ratio) of similar magnitude to our MIE group (E), and better than our HIIE group after 12 weeks MIE at 60 to 70% of VO_{2max} (corresponding to 70 to 80% of HR_{max}) in subjects with a slightly older age (58±5 years), longer T2D duration (5±7 years), and poorer baseline E/A-ratio (0.76±0.11) than the participants of the present study (Brassard et al., 2007). Another study performed a one-year intervention program, including MIE according to current guidelines, and observed improvements in diastolic function after intervention, but only in a subgroup that had a larger exercise volume or exercised at greater intensities (Hordern et al., 2009). Only one, non-randomized, study (Friis Schmidt et al., 2013) has, to our knowledge, investigated the effect of high-intensity intermittent exercise (soccer; twice weekly) on diastolic function. Subjects (n=12) in that study (Friis Schmidt et al., 2013) exercised 43%±22% of the exercise time above 85% of HR_{max} (average intensity of 82%±6% of HR_{max}) and showed improvements in diastolic function (e', E/e', E/A) during a 12-week period, similar to our HIIE group. The

soccer playing group of Friis Schmidt et al. (Friis Schmidt et al., 2013) improved e' by 18% in 24 weeks, giving approximately the same average results as the HIIE group of the present study, which improved e' by 26% in 12 weeks. In the soccer study (Friis Schmidt et al., 2013), the subjects seem to have had the largest exercise impact from baseline to 12 weeks, while less change happened from 12 weeks to 24 weeks. The lack of substantial improvement from 12 to 24 weeks in the study by Friis Schmidt et al. (Friis Schmidt et al., 2013) might have been a result of reduced relative exercise intensity during the course of intervention.

Even though both intervention groups improved diastolic function (e'), the HIIE group also improved several other measures of diastolic function during 12 weeks of exercise. On average, these parameters were still significantly different from the healthy control group in Chapter 4 at the 12-week post-test. This suggests that 12 weeks of MIE or HIIE is not sufficient to normalize diastolic function in middle-aged T2D individuals with T2D for less than 10 years. This calls upon future research to further investigate whether increased exercise volume, frequency, or longitude can achieve normalization (under supervision; either done as in the present studies, or e.g. via telemedicine approaches incorporating more specific and long-term exercise programs in apps, such as diabetesdagboka.no, that have been shown to improve attitudes in regard to disease management in individuals with T2D (El-Gayar et al., 2013)).

Indeed, it may be argued that the selection criterion for the major outcome measure (e' < 8 cm/s) in this study gives a significant potential for regression to the mean. It is acknowledged that the selected individuals in this study had e' values in the lower range of the distribution for the population of interest, and an increase in e' after intervention might be (partly) due to regression to the mean. However, the same issue exists for both the HIIE and the MIE group, but since the individuals were randomized into one of two interventions, the comparison of the mean change from baseline for HIIE and MIE will in any case demonstrate the effect of interest.

In addition, the HIIE group had more individuals with grade II diastolic dysfunction at baseline, which can potentially explain group differences as one might speculate whether these patients (with grade II) have a greater capacity for improvement.

Long-term adherence to 12-week exercise intervention

The lack of additional improvement in diastolic function (e') after 12 weeks is consistent with previous observations (Friis Schmidt et al., 2013; Hare et al., 2011; Hordern et al., 2009). This may reflect difficulties with long-term adherence to exercise in people with T2D (Schneider et al., 1992), but it may also be due to the absence of supervision from 12 weeks to one year

(Colberg et al., 2010). We did not control exercise after 12 weeks in any of the groups, and our observations of reductions in improved parameters (from 12 weeks to 12 months) may reflect the lack of motivation to exercise in this particular patient group. It was previously reported that up to two-thirds of patients with T2D attend no regular physical activity at all (Thomas et al., 2004), and few achieve the recommended amount of weekly exercise (Egan et al., 2013; Nelson et al., 2002; Thomas et al., 2004). However, the improved diastolic function in the HIIE group after one year (compared to baseline) indicates that continued home-based HIIE could be more achievable. The HIIE group sustained improved diastolic function to some extent in the long term, but the sustained improvements may also have been due to the significant superior improvements in the HIIE group compared to the MIE group, which may have allowed for sustained results in spite of assumed lower volumes of exercise. To our knowledge, only Hordern et al. (Hordern et al., 2009) observed improvements in diastolic function over one year of exercise, but only in a subgroup that increased moderate or vigorous activity. The present study supports those findings (Hordern et al., 2009) if we presume that both exercise groups kept exercising to some extent (as reported verbally to the exercise physiologist by phone and after one year).

The reason for the adaptations in diastolic function due to exercise is not fully understood as the development of diastolic dysfunction is complex (see Chapter 1, sections 1.1.3-1.1.4). However, due to the short duration of diabetes in the participants of this study, structural changes in the myocardium (fibrosis) might not have come far. Even though this was not tested, we could expect the exercise-induced improvements to partially reflect an improved calcium homeostasis in the cardiomyocytes (Loganathan et al., 2007; Stolen et al., 2009), improving contractility and elastance and thus improved recoil and suction in early diastole (also reflected by the improved time to untwist rate [UTR] discussed in Chapter 4). However, the improvements in systolic measures (strain rate and S') reflecting improved contractile function were only evident after HIIE. Nevertheless, this additional effect to systolic function may also partially explain the superior effect of HIIE in improving diastolic function (e'). Earlier studies have shown an association between diastolic and systolic function by a correlation (~0.6) between S' and e' in healthy subjects (n=1266) (Dalen et al., 2010).

Even though HIIE seems to be superior to MIE in improving diastolic function in the short term (Hordern et al., 2009), it is unclear which is more effective to achieve adherence to exercise and potentially prevent the development of diastolic dysfunction in T2D in the long term. Although individuals who have exercised throughout adulthood have better diastolic

function than those who have been less physically active, the development of diastolic dysfunction due to aging does not seem possible to prevent. A recent invasive study measuring LV distensibility and compliance in healthy seniors showed that a high dose of exercise 4-5 times per week throughout adulthood can slow down age-related LV stiffness (Bhella et al., 2014). More research in warranted to determine whether exercise can slow down the development of diastolic dysfunction in T2D, and which exercise regimen is the most effective in the long run.

3.5.1.2 Systolic function

Our findings suggest that MIE is not sufficient to improve systolic function. The observed improvements in systolic function in the HIIE group (S', global strain, and strain rate) are in line with previous observations in exercise studies of asymptomatic T2D patients with normal LV EF (Friis Schmidt et al., 2013; Nakai et al., 2009). Abnormal myocardial deformation in systole (LV strain) is associated with reduced cardiorespiratory fitness and has recently been found to be a potentially important diagnostic marker for ACC/AHA stage B HF (i.e. diastolic dysfunction) (Kosmala et al., 2015). Due to the putative additive effect of increased systolic function to improve diastolic filling properties (Støylen), our findings strengthen the importance of performing exercise at higher intensities to also affect systolic function when aiming to achieve cardiac remodeling in individuals with T2D and diastolic dysfunction.

Furthermore, global strain was lower in the T2D group of the present study compared to normal values reported in a previous study using the same speckle tracking method for analysis and might serve as another early marker of cardiomyopathy.

A recent study has suggested a reduction in systolic strain to be an even earlier marker of diabetic cardiomyopathy than diastolic dysfunction (Ernande et al., 2011) and has recently been introduced as a possible important marker for stage B HF (Kosmala et al., 2015).

3.5.1.3 Stress echocardiographic measurements

We also observed improved CO and SV from baseline to 12 weeks post-test during exercise (stress echocardiography) as well as at rest (sitting upright on the bicycle without pedaling) after HIIE but not MIE. Thus, our findings indicate that HIIE is superior to MIE in improving contraction (global strain rate) at maximum effort and work rates (independent of the Frank-Starling relationship). This superior effect of HIIE is confirmed by the finding of improved filling velocity with incremental exercise as well as the substantial improvement of VO_{2peak} after HIIE compared to MIE (as discussed in the next section 2.5.2.1).

3.5.2 The effect of aerobic exercise on cardiometabolic risk factors

3.5.2.1 Cardiorespiratory fitness

This study confirms previous findings showing that T2D subjects have reduced cardiorespiratory fitness compared to healthy individuals (Aspenes et al., 2011). Our findings are also in line with previous studies reporting that diastolic dysfunction is associated with reduced cardiorespiratory fitness (Edelmann et al., 2011; Kosmala et al., 2015; Poirier et al., 2000).

The lack of a strong correlation between change in diastolic function and change in VO_{2peak} supports a recent study finding that aerobic capacity is relatively independent of diastolic function (Gurdal et al., 2015). However, previous studies also suggest that improvement in VO_{2peak} may be due to improved diastolic function (Edelmann et al., 2011; Poirier et al., 2000). This relation has to be further investigated to better understand the mechanisms mediating improved diastolic dysfunction in relation to improved VO_{2peak}.

As in this study, several randomized controlled trials (RCT) have shown improvements in VO_{2peak} following aerobic exercise in T2D (Belli et al., 2011; Bjorgaas et al., 2005; Cuff et al., 2003; Kadoglou et al., 2007). The observed superior effect of HIIE (~13% increase in VO_{2peak}; Δ4.1 ml/kg/min) compared to MIE (~4% increase in VO_{2peak}; Δ1.2 ml/kg/min) in improving VO_{2peak} is also in line with findings in several patient populations, such as HF and metabolic syndrome (Tjonna et al., 2008; Wisloff et al., 2007). Our findings also support the meta-analysis of Boulé et al. (Boule et al., 2003), reporting VO_{2peak} increase up to 11.8% following aerobic exercise for at least eight weeks in T2D. Indeed, a recent meta-analysis found that when compared to MIE, HIIE produced a significantly higher increase in VO_{2peak} (9.1%; 3.03 mL/kg/min difference, 95% CI 2.00 to 4.07).

The increase in VO_{2peak} in the HIIE group, in particular, can be considered a substantial and clinically significant increase, which may translate into a decrease in risk of morbidity and all-cause mortality. Cardiorespiratory fitness has been characterized as a more powerful predictor of mortality than other established risk factors for CVD; both Meyers et al. (Myers et al., 2002) and Nes et al. (Nes et al., 2014) have reported that a ~3.5 ml/kg/min (1 MET) higher VO_{2peak} level represents a substantial risk reduction in all-cause and CVD mortality. Meyers et al. (Myers et al., 2002) showed a 12% increase of survival in men with 1 MET higher cardiorespiratory fitness, whereas Nes et al. (Nes et al., 2014) reported a 21% reduction in CVD

mortality risk in both genders as well as 15% and 8% all-cause mortality risk in men and women, respectively. The persistent excess cardiovascular and overall mortality risk seen in T2D, in spite of aggressive cardiovascular risk-factor prevention (Pi-Sunyer et al., 2007), may thus be due to reduced cardiorespiratory fitness.

Resting heart rate

HR at rest did not change in either exercise group in spite of improved VO_{2peak}. This is consistent with the findings of Friis Schmidt et al. (Friis Schmidt et al., 2013), and may be explained by CAN, a common form of autonomic dysfunction associated with diastolic dysfunction in T2D (Mythri, 2015; Poanta et al., 2011) (Chapter 1, section 1.1.3.4)

On average, the participants had relatively high resting HRs, which is associated with reduced cardiorespiratory fitness and is considered a predictor for CVD (Nauman et al., 2012). CAN in T2D leads to increased resting HR (Tang et al., 2014), reduced HR variability, and reduced HR recovery after exercise (Sacre et al., 2012), the last of which has also been associated with reduced cardiorespiratory fitness in T2D (Fang, Sharman, et al., 2005). Although not tested, it is possible that the relatively high resting HRs and the inability to reduce resting HR can be indications of CAN. However, Sacre et al. (Sacre et al., 2014) found reduced resting HRs after six months of exercise intervention with improved VO_{2peak} in T2D individuals with reduced tissue Doppler velocities despite a lack of exercise induced improvement in other CAN measures such as HR variability, baroreflex sensitivity, and exercise recovery HR. The difference in findings may be due to differences in study populations, severity of diastolic dysfunction, or exercise modalities, among other factors. Future studies should include measures of CAN when assessing the effect of exercise in this patient group to better understand the relationship between diastolic cardiomyopathy and CAN as well as the potential modifying effects of exercise on the variables affecting HR.

The fact that the improvement in diastolic function (e') from baseline to 12 weeks and one year was present without a change in resting HR indicates that there were mechanisms mediating improvements in e' other than exercise training induced changes in HR.

3.5.2.2 Glycemic control

Glycosylated hemoglobin (HbA_{1c})

The improvements in glycemic control after HIIE were similar to, or somewhat smaller than, previous studies (Boule et al., 2001; Marwick et al., 2009). The relatively good mean baseline

metabolic control (American Diabetes Association, 2014; Ryden et al., 2013) in the study participants of the present study may explain the modest changes observed in HbA_{1c}.

The mean HbA_{1c} reduction in the HIIE group may not be of great clinical significance. However, improvements were clinically significant on an individual level in ~43% of the participants with $HbA_{1c} \geq 7\%$, reaching treatment target goals during the 12-week intervention. The improved glycemic control in individuals with HbA_{1c} levels above target for CVD prevention in T2D ($HbA_{1c} > 7\%$) in this study coincides with previous exercise studies (Boule et al., 2001). The observed reductions in HbA_{1c} may be transferred into risk reduction as even relatively small reductions in HbA_{1c} have been shown to yield cardiovascular and all-cause mortality risk reduction (Advance Collaborative Group: Patel, 2008; ten Brinke et al., 2008; Zhang et al., 2012). Every percentage point increase in HbA_{1c} has been shown to represent an 8% increase in risk for developing HF (Iribarren et al., 2001) and fasting plasma glucose levels have been shown to be a prognostic factor for HF (Held et al., 2007). Moreover, poor glycemic control (measured as increased HbA_{1c}) increases the relative risk of dying from ischemic heart disease (Dale et al., 2009). On the contrary, improved HbA_{1c} levels are associated with reduced risk of CVD and mortality in T2D (Dale et al., 2009; Gerstein et al., 2008; Zhang et al., 2012).

The lack of change in HbA_{1c} in the MIE group is partly in line with Umpierre et al. (Umpierre et al., 2011), who concluded that supervised exercise reduces HbA_{1c} more than exercise advice alone, which is also stated by the ADA (Colberg et al., 2010). In a recent systematic review with meta-regression analysis (Umpierre et al., 2013), an association was also observed between exercise volume and change in glycemic control. However, the MIE group had increases in VO_{2peak} in line with previous interventions with supervised MIE, which indicates that exercise volume was sufficient. Thus, volume or lack of supervision is probably not the main reason for lack of improvements in glycemic control in this group, but rather exercise intensity.

This study indicates that intensity plays an important role in improving glycemic control in T2D, which is in line with a previous meta-analysis (Boule et al., 2003) concluding that exercise at higher intensities could have additional benefits in regard to HbA_{1c} in T2D (Mourier et al., 1997). Our findings also support the few available trials that have compared high-intensity exercise with MIE in T2D, which show beneficial results of high-intensity exercise on glycemic control (HbA_{1c}) as well as on other CMR factors (Backx et al., 2011; Mitranun et al., 2013; Terada et al., 2013); for example, Karstoft et al. (Karstoft et al., 2013) showed greater

effect on glycemic control with HIIE at 70 to 85% of VO_{2peak} compared to continuous walking at lower intensities. It has also been speculated that the concept of intermittent exercise protocols of intensities higher than 75% of VO_{2peak} could be more effective in improving cardiovascular risk factors in T2D than current recommendations of moderate-intensity exercise (Balducci et al., 2012; Hansen et al., 2009). Nevertheless, our findings in regard to glycemic control indicate that higher intensities can compensate for a reduced use of time when aiming to improve glycemic control.

The observation of greater improvements in subjects with $HbA_{1c} \geq 7\%$ compared to subjects with $HbA_{1c} < 7\%$ contrasts with previous findings; Solomon et al. (Solomon et al., 2013) predicted that every 1% increase in pre-exercise HbA_{1c} above 6.2% would induce a 0.2% loss of exercise improvement in HbA_{1c}. Furthermore, the Look AHEAD study found that diabetes remission due to exercise and diet intervention is more likely in individuals with short T2D duration and lower pre-HbA_{1c} (Gregg et al., 2012). However, our findings are in line with a recent pilot performed at our lab where even extremely short bouts of high-intensity exercise improved HbA_{1c} in individuals with $HbA_{1c} > 7\%$ (Revdal et al., 2016). Due to this and previous studies showing the effectiveness of HIIE on insulin sensitivity, one might speculate whether intensity plays a role in this.

Although a group of individuals with T2D in the HUNT study (Dale et al., 2009) had an overall increased risk of dying from coronary artery disease compared to individuals without T2D, the individuals with the poorest glycemic control ($HbA_{1c} \geq 8.21$) were at a particularly increased relative risk (hazard ratio 4.2) of dying from coronary artery disease (Dale et al., 2009). This underlines the importance of exercise to improve glycemic control in T2D individuals with $HbA_{1c} > 7\%$ in particular and indicates that the potential of higher-intensity exercise to reduce glycemic control in this group should be further investigated.

Insulin sensitivity

Although not tested, several mechanisms could have contributed to the improved glycemic control observed in the HIIE group, such as increased muscle blood flow, improved capillary density, and increased glycogen storage (Colberg et al., 2010). The fact that the HIIE group reduced BMI more than the MIE group may also explain the additional effect on glycemic control in the HIIE group (Pi-Sunyer et al., 2007). However, we failed to detect expected improvements in insulin resistance measured by the homeostasis model assessment (HOMA-ir).

The reason for this inconsistent finding (improved HbA_{1c} without improvements in insulin sensitivity) is not clear. Indeed, exercise induced improvements in insulin sensitivity and β-cell function are suggested to be due to enhanced skeletal muscle glucose transport capacity, which is partially mediated by the protein GLUT4 (Gibala et al., 2012; Koval et al., 1999). The HOMA-ir, however, mostly reflects hepatic insulin resistance, as opposed to the hyperinsulinemic-euglycemic clamp technique, which to a greater extent reflects peripheral (skeletal muscle) insulin sensitivity (Bonora et al., 2000; DeFronzo, 1999). Thus, potential exercise-induced improvements in insulin sensitivity in muscle tissue or in β-cell function do not necessarily need to be entirely reflected by this assessment method. However, HOMA-ir was found to correlate significantly to the clamp technique in T2D patients and is considered a useful method for evaluating insulin sensitivity in this patient group (Katsuki et al., 2001). Nevertheless, we might have missed true changes in insulin sensitivity due to the choice of assessment model.

Our findings contrast with several previous studies investigating the effect of moderate or high-intensity exercise of relatively high volume on insulin sensitivity (Bruce et al., 2006; Houmard et al., 2004; O'Donovan et al., 2005; Tjønna et al., 2008). Indeed, using the same HIIE protocol as in the present study, Tjønna et al. found HIIE to improve insulin sensitivity (measured by HOMA-ir) and fasting blood glucose more than MIE in individuals with metabolic syndrome (Tjønna et al., 2008). Furthermore, insulin sensitivity has been shown to be improved by 35% (measured by HOMA-ir) during only two weeks (six sessions a week) of low-volume HIIE in middle-aged sedentary adults (n=7) (Hood et al., 2011). Furthermore, Little et al. demonstrated improved GLUT4 content, reduced 24 h blood glucose concentration, and postprandial glucose excursion after two weeks (six sessions a week) of low-volume HIIE in T2D individuals (n=8) (Little et al., 2011), indicating that HIIE is a time-efficient method to improve insulin sensitivity in this patient group. However, the latter studies were of small sample size and the mechanism for improved insulin action induced by HIIE is not entirely clear. Thus, further research in larger studies is warranted to investigate the role of intensity in this context.

Finally, the participants in the present study had relatively good mean baseline metabolic control (American Diabetes Association, 2014; Ryden et al., 2013) and most of the participants used metformin or other oral anti-diabetic agents, which may also partly explain the lack of change in insulin sensitivity (Giannarelli et al., 2003).

3.5.2.3 Body composition

The sustained reduction in BMI and waist circumference from baseline to one year and reduction in body fat percentage from 12 weeks to the one-year follow-up in the HIIE group indicate that HIIE yields an additional effect on body composition compared to MIE. There has been conflicting evidence regarding whether or not exercise can contribute to reduced BMI and waist circumference (Boule et al., 2003; Chudyk and Petrella, 2011; Thomas et al., 2006), but the present study supports the findings of the meta-analysis of Hayashino et al., which concluded that exercise has the potential to improve body composition (Hayashino et al., 2012).

The exercise volumes applied in this study were substantially lower than what is suggested to contribute to weight reduction when only relying on physical activity (~60 min/day) (Ross et al., 2000; Ross et al., 2004). Our findings thus suggest that higher intensities can compensate for less use of time when aiming to reduce weight in T2D. Although not tested, the mechanisms explaining the excess weight loss in the HIIE group may be an increased excess post-oxygen consumption (EPOC) due to HIIE compared to MIE. Larsen et al. (Larsen et al., 2014) showed that the same HIIE protocol used in the present study gave a significantly greater EPOC than 47 minutes of MIE (Larsen et al., 2014). One may also speculate whether an effective increase in VO_{2peak} could increase total energy expenditure due to increased capacity to perform daily activities. Another speculation is whether HIIE and MIE induce different appetite controls, which subsequently can explain the weight loss favoring HIIE; however, a recent study conducted on obesity indicates that this might not be the case (Martins et al., 2015).

The reduced waist circumference in the MIE group without reduction in BMI may be explained by reduced intra-abdominal fat. This assumption supports previous studies showing that intra-abdominal fat (Cuff et al., 2003; Lehmann et al., 1995; Mourier et al., 1997) and waist circumference (Chudyk and Petrella, 2011) can be reduced even though weight was sustained through exercise interventions in T2D.

The observed reduction in BMI together with reduced HbA_{1c} in the HIIE group confirms what was previously observed: even modest weight reductions, including reductions in waist circumference, in T2D are associated with improved glycemic control (Catapano et al., 2011; Pi-Sunyer et al., 2007). However, the role of weight reduction in cardiovascular health is unclear as reduced weight has not yet been shown to reduce cardiovascular events in adults with T2D (Wing et al., 2013).

3.5.2.4 Blood pressure

The lack of improvement in blood pressure in this study is both in contrast to (Friis Schmidt et al., 2013; Hayashino et al., 2012; Marwick et al., 2009; Pi-Sunyer et al., 2007; Wing et al., 2013) and in line with previous observations in T2D (Sigal et al., 2007; Wycherley et al., 2008). This may be explained by the fact that 35% of our subjects were on anti-hypertensive medication.

In contrast to our findings, Friis Schmidt et al. (Friis Schmidt et al., 2013) observed reduction in both systolic and diastolic blood pressures, which were closely associated with increases in VO_{2peak}. The authors (Friis Schmidt et al., 2013) suggested that reduction in blood pressure contributed to improved diastolic function. This may be a partial explanation, but our study shows that other mechanisms, independent of HR and blood pressure, mediate improvement in diastolic function in T2D.

3.5.2.5 Dyslipidemia

The effects of MIE on HDL-cholesterol and the similar pattern in the HIIE group are in line with previous small, randomized trials involving T2D (Kadoglou et al., 2007; Ronnemaa et al., 1988), but most have found no effect of exercise on lipids (Gordon et al., 2008; Sigal et al., 2007; Tudor-Locke et al., 2004; Vanhees et al., 2012). Weight reduction has also been shown to reduce HDL with 0.01 mmol/L per kg reduction in body mass (Catapano et al., 2011), which may partly explain the slight increase in HDL after exercise. Nevertheless, neither HIIE nor MIE improved lipid levels to a clinically significant level.

3.5.2.6 Chronic inflammation

The participants, and the HIIE group in particular, had chronic inflammation (measured as hs-CRP) that only HIIE reduced. In general, the mean hs-CRP levels in light of the lipid profiles in both the HIIE and MIE group represent high and average cardiovascular risk (Pearson et al., 2003). Thus, the decrease in this cardiovascular risk marker in the HIIE group represents a reduction in cardiovascular risk category from high to average (Pearson et al., 2003), whereas the MIE group sustained hs-CRP levels (average risk category) throughout the exercise period.

Indeed, the significant improvement in the HIIE group may be due to the fact that this group had substantially higher hs-CRP at baseline, and the improvements may thus be an effect of regression to the mean. However, the improved hs-CRP in the HIIE group supports previous studies finding that exercise can play a role in reducing CRP in T2D (Hayashino et al., 2014) and hs-CRP in overweight individuals (when improved cardiorespiratory fitness) (Pathak et al.,

2015), and that higher intensities (i.e. MIE) play a role over low-intensity exercise in improving inflammatory biomarkers (Abd El-Kader et al., 2013; Balducci et al., 2010; Krause et al., 2014).

Overall, our findings indicate that higher hs-CRP levels are more susceptible to exercise response than average levels and/or that HIIE is more effective in reducing chronic inflammation. However, the impact of exercise on chronic inflammation in T2D is uncertain (Krause et al., 2014).

3.5.2.7 Endothelial function

Mean FMD was substantially greater in our participants at both baseline and post-test compared to the normal FMD distribution reported in the HUNT3 fitness study (n=4739; Total mean FMD=4.8±4.2%) (Skaug et al., 2013). However, the analyzing method in HUNT3 did not involve continuous measurements, making findings difficult to compare. Whether the participants of the present study had endothelial dysfunction is thus also uncertain. However, previous studies have shown that exercise training has little effect on endothelial function when function is normal at baseline (Moyna and Thompson, 2004), which may indicate that the participants in the present study had endothelial dysfunction due to the response observed in the HIIE group.

The improved FMD in the HIIE group conforms to a recent meta-analysis (Montero et al., 2013) concluding that exercise has the potential to improve endothelial function in T2D. Furthermore, the magnitude of change observed after HIIE is similar to previous findings on individuals with metabolic syndrome in our department (Tjønna et al., 2008). However, in contrast to the findings in the meta-analysis of Montero et al. (Montero et al., 2013) and the findings of Tjønna et al. (Tjønna et al., 2008), we did not observe any significant effect of MIE on FMD. HIIE has also been shown to be superior to MIE in improving FMD in HF (Wisloff et al., 2007).

The reason for the potential differences in response to exercise training intensities in regard to FMD is not fully understood. It might, however, be speculated whether the different responses in shear stress on the walls of the blood vessels (and thus different molecular responses) induced by different exercise intensities can be an explanation. Consistent with this, HIIE but not MIE improved blood glucose levels (measured as HbA_{1c}), which directly influence NO bioavailability (Davignon and Ganz, 2004). Moreover, the improvements in FMD may partly explain the observed improvements in cardiorespiratory fitness (Reusch et al., 2013).

Nevertheless, the observed mean improvements in FMD are greater than what was observed in previous studies in T2D (Barone Gibbs et al., 2012; Kwon et al., 2011; Montero et al., 2013; Okada et al., 2010). However, studies investigating the effect of exercise on FMD have different exercise protocols, mostly at lower intensities than in the present HIIE group, and are difficult to compare. Although there are strong indications of intensity being important to achieve an exercise response in FMD, there is wide diversity in the findings, and further studies are needed to determine the effects of exercise on FMD in T2D (Hwang and Kim, 2014).

3.5.3 Improved diastolic function and cardiovascular outcomes

Although diastolic function might be an early marker of future CVD in T2D individuals, it is not known whether improvement in this parameter can reduce cardiovascular outcomes. However, mortality benefits have been observed in individuals who demonstrated improved diastolic function compared to those whose diastolic function remained the same or worsened (Halley et al., 2011). On the other hand, the Look AHEAD study found that reduction of traditional cardiovascular risk factors after lifestyle intervention (minimum 175 minutes of MIE per week) did not reduce the risk of cardiac events in T2D subjects (Wing et al., 2013). Further research is thus necessary to investigate whether HIIE is more effective than MIE in reducing cardiac events in general in T2D patients, but also to investigate the effect of exercise modes on the development of cardiomyopathy and the development of HF, in particular.

3.5.4 Study limitations and strengths

Results from this study are based on a relatively low number of subjects, and our study participants were generally physically fit compared to participants of similar age and T2D duration in other studies (Hare et al., 2011). Thus, our results need to be confirmed in larger studies.

The classification of diastolic dysfunction is complicated due to the many parameters that must be considered (Nagueh et al., 2009). Although e' might seem like a simple inclusion criteria for reduced diastolic function, which could potentially represent a limitation in the present study, it was appropriate since the majority of the included subjects had diastolic dysfunction grade 2 or 3 according to the guidelines (Nagueh et al., 2009). The American Society of Echocardiography (ASE) and European Association of Cardiovascular Imaging (EACVI) have recently published new guidelines for diastolic function assessment to simplify the approach (Nagueh et al., 2016).

It might be argued that including patients with diastolic dysfunction solely based on e'-measurements was a simple approach that represents a limitation of this study. However, after the patients were included, the grade of diastolic function was analyzed according to the guidelines and it was confirmed that all included patients (with e' < 8 cm/s) had diastolic dysfunction, with the majority having diastolic dysfunction grade II.

The effect of MIE might have been better if all subjects had received supervised exercise, and closer follow-up after 12 weeks in both groups might have influenced results at the one-year follow-up. Although all study participants reported partial adherence to the exercise protocol from the 12-week post-test to the one-year follow-up, no registration of compliance to the exercise protocol was conducted after 12 weeks and the results of the one-year follow-up must be interpreted with caution.

FMD results must also be interpreted with caution because of incomplete data due to impaired ultrasound image quality and lost recordings. The missing rate was higher in the MIE group than the HIIE group. Indeed, the FMD improvements observed in the HIIE group are of greater magnitude than expected and may be due to a type I error.

Even though the groups in this study were randomized, the age difference between groups was not considered in the statistical analysis. However, the analysis performed in Chapter 4 showed that, when adjusting for age in regard to diastolic measures relevant for this chapter, a significant decrease with age was found for e' as expected, but age could not explain all variability between groups in the material of Chapter 4. Thus, it is not likely that the relatively small difference in age between groups in the present study can be expected to have a major impact on the results.

The strengths of this study include the prospective study design with pre-specified and controlled exercise training and test regimens in addition to the comprehensive physiological evaluations and a clearly defined T2D patient group. We also performed blood pressure measurements and blood glucose measurements before every echocardiographic measurement to control for hemodynamic status variation and other loading conditions that could potentially contribute to variations in echocardiographic measurements.

3.6 CONCLUSION

HIIE was superior to MIE in improving diastolic function (e') in T2D individuals with diastolic dysfunction. The findings of the present study emphasize the importance of targeting exercise guidelines towards higher intensities particularly when aiming to improve diastolic and systolic function; cardiorespiratory fitness (VO_{2peak}); glycemic control (HbA_{1c}), and body composition (BMI, waist circumference, and body fat percentage) in T2D individuals with diastolic dysfunction.

REFERENCES

Abd El-Kader, S., Gari, A., & Salah El-Den, A. (2013). Impact of moderate versus mild aerobic exercise training on inflammatory cytokines in obese type 2 diabetic patients: a randomized clinical trial. *Afr. Health Sci., 13*(4), 857-863.

Advance Collaborative Group: Patel, A. M., S.; Chalmers, J.; Neal, B.; Billot, L.; Woodward, M.; Marre, M.; Cooper, M.; Glasziou, P.; Grobbee, D.; Hamet, P.; Harrap, S.; Heller, S.; Liu, L.; Mancia, G.; Mogensen, C. E.; Pan, C.; Poulter, N.; Rodgers, A.; Williams, B.; Bompoint, S.; de Galan, B. E.; Joshi, R.; Travert, F. (2008). Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med., 358*(24), 2560-2572.

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . International Association for the Study of, O. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation, 120*(16), 1640-1645.

American Diabetes Association. (2014). Standards of medical care in diabetes-2014. *Diabetes Care, 37 Suppl 1*, S14-80.

Aspenes, S. T., Nilsen, T. I., Skaug, E. A., Bertheussen, G. F., Ellingsen, O., Vatten, L., & Wisloff, U. (2011). Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med. Sci. Sports Exerc., 43*(8), 1465-1473.

Backx, K., McCann, A., Wasley, D., Dunseath, G., Luzio, S., & Owens, D. (2011). The effect of a supported exercise programme in patients with newly diagnosed Type 2 diabetes: a pilot study. *J. Sports Sci., 29*(6), 579-586.

Balducci, S., Zanuso, S., Cardelli, P., Salvi, L., Bazuro, A., Pugliese, L., . . . Italian Diabetes Exercise Study, I. (2012). Effect of high- versus low-intensity supervised aerobic and resistance training on modifiable cardiovascular risk factors in type 2 diabetes; the Italian Diabetes and Exercise Study (IDES). *PLoS One, 7*(11), e49297.

Balducci, S., Zanuso, S., Nicolucci, A., Fernando, F., Cavallo, S., Cardelli, P., . . . Pugliese, G. (2010). Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutrition, metabolism, and cardiovascular diseases : NMCD, 20*(8), 608-617.

Barone Gibbs, B., Dobrosielski, D. A., Bonekamp, S., Stewart, K. J., & Clark, J. M. (2012). A randomized trial of exercise for blood pressure reduction in type 2 diabetes: effect on flow-mediated dilation and circulating biomarkers of endothelial function. *Atherosclerosis, 224*(2), 446-453.

Belli, T., Ribeiro, L. F., Ackermann, M. A., Baldissera, V., Gobatto, C. A., & Galdino da Silva, R. (2011). Effects of 12-week overground walking training at ventilatory threshold velocity in type 2 diabetic women. *Diabetes Res. Clin. Pract., 93*(3), 337-343.

Bhella, P. S., Hastings, J. L., Fujimoto, N., Shibata, S., Carrick-Ranson, G., Palmer, M. D., . . . Levine, B. D. (2014). Impact of lifelong exercise "dose" on left ventricular compliance and distensibility. *J. Am. Coll. Cardiol., 64*(12), 1257-1266.

Bjorgaas, M., Vik, J. T., Saeterhaug, A., Langlo, L., Sakshaug, T., Mohus, R. M., & Grill, V. (2005). Relationship between pedometer-registered activity, aerobic capacity and self-reported activity and fitness in patients with type 2 diabetes. *Diabetes Obes. Metab., 7*(6), 737-744.

Bonora, E., Targher, G., Alberiche, M., Bonadonna, R. C., Saggiani, F., Zenere, M. B., . . . Muggeo, M. (2000). Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care, 23*(1), 57-63.

Boule, N. G., Haddad, E., Kenny, G. P., Wells, G. A., & Sigal, R. J. (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA, 286*(10), 1218-1227.

Boule, N. G., Kenny, G. P., Haddad, E., Wells, G. A., & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia, 46*(8), 1071-1081.

Brassard, P., Legault, S., Garneau, C., Bogaty, P., Dumesnil, J.-G., & Poirier, P. (2007). Normalization of diastolic dysfunction in type 2 diabetics after exercise training. *Med. Sci. Sports Exerc., 39*(11), 1896-1901.

Bruce, C. R., Thrush, A. B., Mertz, V. A., Bezaire, V., Chabowski, A., Heigenhauser, G. J., & Dyck, D. J. (2006). Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am. J. Physiol. Endocrinol. Metab., 291*(1), E99-E107.

Brunzell, J. D., Davidson, M., Furberg, C. D., Goldberg, R. B., Howard, B. V., Stein, J. H., . . . American College of Cardiology, F. (2008). Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care, 31*(4), 811-822.

Buse, J. B., Ginsberg, H. N., Bakris, G. L., Clark, N. G., Costa, F., Eckel, R., . . . American Diabetes, A. (2007). Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation, 115*(1), 114-126.

Catapano, A. L., Reiner, Z., De Backer, G., Graham, I., Taskinen, M. R., Wiklund, O., . . . Committees. (2011). ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis, 217 Suppl 1*, S1-44.

Chudyk, A., & Petrella, R. J. (2011). Effects of exercise on cardiovascular risk factors in type 2 diabetes: a meta-analysis. *Diabetes Care, 34*(5), 1228-1237.

Colberg, S. R., Albright, A. L., Blissmer, B. J., Braun, B., Chasan-Taber, L., Fernhall, B., . . . American Diabetes, A. (2010). Exercise and type 2 diabetes: American College of

Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Med. Sci. Sports Exerc., 42*(12), 2282-2303.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., . . . Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol., 39*(2), 257-265.

Cuff, D. J., Meneilly, G. S., Martin, A., Ignaszewski, A., Tildesley, H. D., & Frohlich, J. J. (2003). Effective exercise modality to reduce insulin resistance in women with type 2 diabetes. *Diabetes Care, 26*(11), 2977-2982.

Dale, A. C., Midthjell, K., Nilsen, T. I., Wiseth, R., & Vatten, L. J. (2009). Glycaemic control in newly diagnosed diabetes patients and mortality from ischaemic heart disease: 20-year follow-up of the HUNT Study in Norway. *Eur. Heart J., 30*(11), 1372-1377.

Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A., & Stoylen, A. (2010). Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ. Cardiovasc. Imaging, 3*(5), 614-622.

Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation, 109*(23 Suppl 1), III27-32.

DeFronzo, R. A. (1999). Pathogenesis of type 2 diabetes: implications for metformin. *Drugs, 58 Suppl 1*, 29-30; discussion 75-82.

Di Bonito, P., Moio, N., Cavuto, L., Covino, G., Murena, E., Scilla, C., . . . Sibilio, G. (2005). Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging. *Diabet. Med., 22*(12), 1720-1725.

Edelmann, F., Gelbrich, G., Dungen, H. D., Frohling, S., Wachter, R., Stahrenberg, R., . . . Pieske, B. (2011). Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J. Am. Coll. Cardiol., 58*(17), 1780-1791.

Egan, A. M., Mahmood, W. A., Fenton, R., Redziniak, N., Kyaw Tun, T., Sreenan, S., & McDermott, J. H. (2013). Barriers to exercise in obese patients with type 2 diabetes. *QJM*.

El-Gayar, O., Timsina, P., Nawar, N., & Eid, W. (2013). Mobile applications for diabetes self-management: status and potential. *J. Diabetes Sci. Technol., 7*(1), 247-262.

Ernande, L., Bergerot, C., Rietzschel, E. R., De Buyzere, M. L., Thibault, H., Pignonblanc, P. G., . . . Derumeaux, G. (2011). Diastolic dysfunction in patients with type 2 diabetes mellitus: is it really the first marker of diabetic cardiomyopathy? *J. Am. Soc. Echocardiogr., 24*(11), 1268-1275 e1261.

Evert, A. B., Boucher, J. L., Cypress, M., Dunbar, S. A., Franz, M. J., Mayer-Davis, E. J., . . . Yancy, W. S., Jr. (2014). Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care, 37 Suppl 1*, S120-143.

Fang, Z. Y., Schull-Meade, R., Downey, M., Prins, J., & Marwick, T. H. (2005). Determinants of subclinical diabetic heart disease. *Diabetologia, 48*(2), 394-402.

Fang, Z. Y., Sharman, J., Prins, J. B., & Marwick, T. H. (2005). Determinants of exercise capacity in patients with type 2 diabetes. *Diabetes Care, 28*(7), 1643-1648.

Friis Schmidt, J., Rostgaard Andersen, T., Horton, J., Brix, J., Tarnow, L., Krustrup, P., . . . Riis Hansen, P. (2013). Soccer Training Improves Cardiac Function in Men with Type 2 Diabetes. *Med. Sci. Sports Exerc.*

Gerstein, H. C., Miller, M. E., Byington, R. P., Goff, D. C., Jr., Bigger, J. T., Buse, J. B., . . . Friedewald, W. T. (2008). Effects of intensive glucose lowering in type 2 diabetes. *N. Engl. J. Med., 358*(24), 2545-2559.

Giannarelli, R., Aragona, M., Coppelli, A., & Del Prato, S. (2003). Reducing insulin resistance with metformin: the evidence today. *Diabetes Metab., 29*(4 Pt 2), 6S28-35.

Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol., 590*(Pt 5), 1077-1084.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Blaha, M. J., . . . Stroke Statistics, S. (2014). Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation, 129*(3), e28-e292.

Gordon, L. A., Morrison, E. Y., McGrowder, D. A., Young, R., Fraser, Y. T., Zamora, E. M., . . . Irving, R. R. (2008). Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. *BMC Complement. Altern. Med., 8*, 21.

Gregg, E. W., Chen, H., Wagenknecht, L. E., Clark, J. M., Delahanty, L. M., Bantle, J., . . . Look, A. R. G. (2012). Association of an intensive lifestyle intervention with remission of type 2 diabetes. *JAMA, 308*(23), 2489-2496.

Gurdal, A., Kasikcioglu, E., Yakal, S., & Bugra, Z. (2015). Impact of diabetes and diastolic dysfunction on exercise capacity in normotensive patients without coronary artery disease. *Diab. Vasc. Dis. Res.*

Halley, C. M., Houghtaling, P. L., Khalil, M. K., Thomas, J. D., & Jaber, W. A. (2011). Mortality rate in patients with diastolic dysfunction and normal systolic function. *Arch. Intern. Med., 171*(12), 1082-1087.

Hansen, D., Dendale, P., Jonkers, R. A., Beelen, M., Manders, R. J., Corluy, L., . . . van Loon, L. J. (2009). Continuous low- to moderate-intensity exercise training is as effective as moderate- to high-intensity exercise training at lowering blood HbA(1c) in obese type 2 diabetes patients. *Diabetologia, 52*(9), 1789-1797.

Hare, J. L., Hordern, M. D., Leano, R., Stanton, T., Prins, J. B., & Marwick, T. H. (2011). Application of an exercise intervention on the evolution of diastolic dysfunction in

patients with diabetes mellitus: efficacy and effectiveness. *Circ. Heart Fail., 4*(4), 441-449.

Harris, R. A. N., S.K.; Wray W.; Richardson, R.S. (2010). Ultrasound assessment of flow-mediated dilatation: A tutorial. *Hypertension, 55*(5), 10.

Hayashino, Y., Jackson, J. L., Fukumori, N., Nakamura, F., & Fukuhara, S. (2012). Effects of supervised exercise on lipid profiles and blood pressure control in people with type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. *Diabetes Res. Clin. Pract., 98*(3), 349-360.

Hayashino, Y., Jackson, J. L., Hirata, T., Fukumori, N., Nakamura, F., Fukuhara, S., . . . Ishii, H. (2014). Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Metabolism, 63*(3), 431-440.

Held, C., Gerstein, H. C., Yusuf, S., Zhao, F., Hilbrich, L., Anderson, C., . . . Investigators, O. T. (2007). Glucose levels predict hospitalization for congestive heart failure in patients at high cardiovascular risk. *Circulation, 115*(11), 1371-1375.

Hood, M. S., Little, J. P., Tarnopolsky, M. A., Myslik, F., & Gibala, M. J. (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Med. Sci. Sports Exerc., 43*(10), 1849-1856.

Hordern, M. D., Coombes, J. S., Cooney, L. M., Jeffriess, L., Prins, J. B., & Marwick, T. H. (2009). Effects of exercise intervention on myocardial function in type 2 diabetes. *Heart, 95*(16), 1343-1349.

Houmard, J. A., Tanner, C. J., Slentz, C. A., Duscha, B. D., McCartney, J. S., & Kraus, W. E. (2004). Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol (1985), 96*(1), 101-106.

Hwang, M. H., & Kim, S. (2014). Type 2 Diabetes: Endothelial dysfunction and Exercise. *J Exerc Nutrition Biochem, 18*(3), 239-247.

Ingul, C. B., Tjonna, A. E., Stolen, T. O., Stoylen, A., & Wisloff, U. (2010). Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch. Pediatr. Adolesc. Med., 164*(9), 852-859.

International Diabetes Federation. (2012). *Global Guideline for Type 2 Diabetes* Available at: http://www.idf.org/sites/default/files/IDF%20T2DM%20Guideline.pdf Accessed: October, 2015.

Iribarren, C., Karter, A. J., Go, A. S., Ferrara, A., Liu, J. Y., Sidney, S., & Selby, J. V. (2001). Glycemic control and heart failure among adult patients with diabetes. *Circulation, 103*(22), 2668-2673.

Kadoglou, N. P., Iliadis, F., Angelopoulou, N., Perrea, D., Ampatzidis, G., Liapis, C. D., & Alevizos, M. (2007). The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups*

on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology, 14(6), 837-843.

Kannel, W. B., Hjortland, M., & Castelli, W. P. (1974). Role of diabetes in congestive heart failure: the Framingham study. *Am. J. Cardiol., 34*(1), 29-34.

Karstoft, K., Winding, K., Knudsen, S. H., Nielsen, J. S., Thomsen, C., Pedersen, B. K., & Solomon, T. P. (2013). The effects of free-living interval-walking training on glycemic control, body composition, and physical fitness in type 2 diabetic patients: a randomized, controlled trial. *Diabetes Care, 36*(2), 228-236.

Katsuki, A., Sumida, Y., Gabazza, E. C., Murashima, S., Furuta, M., Araki-Sasaki, R., . . . Adachi, Y. (2001). Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care, 24*(2), 362-365.

Kosmala, W., Jellis, C. L., & Marwick, T. H. (2015). Exercise limitation associated with asymptomatic left ventricular impairment: analogy with stage B heart failure. *J. Am. Coll. Cardiol., 65*(3), 257-266.

Koval, J. A., Maezono, K., Patti, M. E., Pendergrass, M., DeFronzo, R. A., & Mandarino, L. J. (1999). Effects of exercise and insulin on insulin signaling proteins in human skeletal muscle. *Med. Sci. Sports Exerc., 31*(7), 998-1004.

Krause, M., Rodrigues-Krause, J., O'Hagan, C., Medlow, P., Davison, G., Susta, D., . . . De Vito, G. (2014). The effects of aerobic exercise training at two different intensities in obesity and type 2 diabetes: implications for oxidative stress, low-grade inflammation and nitric oxide production. *European journal of applied physiology, 114*(2), 251-260.

Kwon, H. R., Min, K. W., Ahn, H. J., Seok, H. G., Lee, J. H., Park, G. S., & Han, K. A. (2011). Effects of Aerobic Exercise vs. Resistance Training on Endothelial Function in Women with Type 2 Diabetes Mellitus. *Diabetes Metab. J., 35*(4), 364-373.

Lang, R. M., Bierig, M., Devereux, R. B., Flachskampf, F. A., Foster, E., Pellikka, P. A., . . . European Association of Echocardiography, E. S. o. C. (2006). Recommendations for chamber quantification. *Eur. J. Echocardiogr., 7*(2), 79-108.

Larsen, I., Welde, B., Martins, C., & Tjonna, A. E. (2014). High- and moderate-intensity aerobic exercise and excess post-exercise oxygen consumption in men with metabolic syndrome. *Scand. J. Med. Sci. Sports, 24*(3), e174-179.

Lehmann, R., Vokac, A., Niedermann, K., Agosti, K., & Spinas, G. A. (1995). Loss of abdominal fat and improvement of the cardiovascular risk profile by regular moderate exercise training in patients with NIDDM. *Diabetologia, 38*(11), 1313-1319.

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol (1985), 111*(6), 1554-1560.

Loganathan, R., Bilgen, M., Al-Hafez, B., Zhero, S. V., Alenezy, M. D., & Smirnova, I. V. (2007). Exercise training improves cardiac performance in diabetes: in vivo

demonstration with quantitative cine-MRI analyses. *J Appl Physiol (1985), 102*(2), 665-672.

Loimaala, A., Groundstroem, K., Rinne, M., Nenonen, A., Huhtala, H., & Vuori, I. (2007). Exercise training does not improve myocardial diastolic tissue velocities in Type 2 diabetes. *Cardiovasc. Ultrasound, 5*, 32.

Martins, C., Stensvold, D., Finlayson, G., Holst, J., Wisloff, U., Kulseng, B., . . . King, N. A. (2015). Effect of moderate- and high-intensity acute exercise on appetite in obese individuals. *Med. Sci. Sports Exerc., 47*(1), 40-48.

Marwick, T. H., Hordern, M. D., Miller, T., Chyun, D. A., Bertoni, A. G., Blumenthal, R. S., . . . Rocchini, A. (2009). Exercise training for type 2 diabetes mellitus: impact on cardiovascular risk: a scientific statement from the American Heart Association. *Circulation, 119*(25), 3244-3262.

Mendes, R., Sousa, N., Almeida, A., Subtil, P., Guedes-Marques, F., Reis, V. M., & Themudo-Barata, J. L. (2015). Exercise prescription for patients with type 2 diabetes-a synthesis of international recommendations: narrative review. *Br. J. Sports Med.*

Mitranun, W., Deerochanawong, C., Tanaka, H., & Suksom, D. (2013). Continuous vs interval training on glycemic control and macro- and microvascular reactivity in type 2 diabetic patients. *Scand. J. Med. Sci. Sports*.

Molmen, H. E., Wisloff, U., Aamot, I. L., Stoylen, A., & Ingul, C. B. (2012). Aerobic interval training compensates age related decline in cardiac function. *Scand. Cardiovasc. J., 46*(3), 163-171.

Montero, D., Walther, G., Benamo, E., Perez-Martin, A., & Vinet, A. (2013). Effects of Exercise Training on Arterial Function in Type 2 Diabetes Mellitus : A Systematic Review and Meta-Analysis. *Sports Med.*

Mourier, A., Gautier, J. F., De Kerviler, E., Bigard, A. X., Villette, J. M., Garnier, J. P., . . . Cathelineau, G. (1997). Mobilization of visceral adipose tissue related to the improvement in insulin sensitivity in response to physical training in NIDDM. Effects of branched-chain amino acid supplements. *Diabetes Care, 20*(3), 385-391.

Moyna, N. M., & Thompson, P. D. (2004). The effect of physical activity on endothelial function in man. *Acta Physiol. Scand., 180*(2), 113-123.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002). Exercise capacity and mortality among men referred for exercise testing. *N. Engl. J. Med., 346*(11), 793-801.

Mythri, S. R., H. (2015). Left Ventricular Diastolic Dysfunction (LVDD) & Cardiovascular Autonomic Neuropathy (CAN) in Type 2 Diabetes Mellitus (DM): A Cross-Sectional Clinical Study. *J Clin Diagn Res, 9*(1), OC18-22.

Nagueh, S. F., Appleton, C. P., Gillebert, T. C., Marino, P. N., Oh, J. K., Smiseth, O. A., . . . Evangelisa, A. (2009). Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur. J. Echocardiogr., 10*(2), 165-193.

Nagueh, S. F., Smiseth, O. A., Appleton, C. P., Byrd, B. F., 3rd, Dokainish, H., Edvardsen, T., . . . Waggoner, A. D. (2016). Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr., 29*(4), 277-314.

Nakai, H., Takeuchi, M., Nishikage, T., Lang, R. M., & Otsuji, Y. (2009). Subclinical left ventricular dysfunction in asymptomatic diabetic patients assessed by two-dimensional speckle tracking echocardiography: correlation with diabetic duration. *Eur. J. Echocardiogr., 10*(8), 926-932.

Nauman, J., Aspenes, S. T., Nilsen, T. I., Vatten, L. J., & Wisloff, U. (2012). A prospective population study of resting heart rate and peak oxygen uptake (the HUNT Study, Norway). *PLoS One, 7*(9), e45021.

Nelson, K. M., Reiber, G., Boyko, E. J., & Nhanes, III. (2002). Diet and exercise among adults with type 2 diabetes: findings from the third national health and nutrition examination survey (NHANES III). *Diabetes Care, 25*(10), 1722-1728.

Nes, B. M., Vatten, L. J., Nauman, J., Janszky, I., & Wisloff, U. (2014). A simple nonexercise model of cardiorespiratory fitness predicts long-term mortality. *Med. Sci. Sports Exerc., 46*(6), 1159-1165.

Nichols, G. A., Gullion, C. M., Koro, C. E., Ephross, S. A., & Brown, J. B. (2004). The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes Care, 27*(8), 1879-1884.

O'Donovan, G., Kearney, E. M., Nevill, A. M., Woolf-May, K., & Bird, S. R. (2005). The effects of 24 weeks of moderate- or high-intensity exercise on insulin resistance. *Eur. J. Appl. Physiol., 95*(5-6), 522-528.

Ofstad, A. P., Johansen, O. E., Gullestad, L., Birkeland, K. I., Orvik, E., Fagerland, M. W., . . . Aakhus, S. (2014). Neutral impact on systolic and diastolic cardiac function of 2 years of intensified multi-intervention in type 2 diabetes: the randomized controlled Asker and Baerum Cardiovascular Diabetes (ABCD) study. *Am. Heart J., 168*(3), 280-288 e282.

Okada, S., Hiuge, A., Makino, H., Nagumo, A., Takaki, H., Konishi, H., . . . Miyamoto, Y. (2010). Effect of exercise intervention on endothelial function and incidence of cardiovascular disease in patients with type 2 diabetes. *J Atheroscler Thromb, 17*(8), 828-833.

Ommen, S. R., Nishimura, R. A., Appleton, C. P., Miller, F. A., Oh, J. K., Redfield, M. M., & Tajik, A. J. (2000). Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation, 102*(15), 1788-1794.

Pathak, R. K., Elliott, A., Middeldorp, M. E., Meredith, M., Mehta, A. B., Mahajan, R., . . . Sanders, P. (2015). Impact of CARDIOrespiratory FITness on Arrhythmia Recurrence in Obese Individuals With Atrial Fibrillation: The CARDIO-FIT Study. *J. Am. Coll. Cardiol., 66*(9), 985-996.

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., 3rd, Criqui, M., . . . American Heart, A. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation, 107*(3), 499-511.

Pi-Sunyer, X., Blackburn, G., Brancati, F. L., Bray, G. A., Bright, R., Clark, J. M., . . . Yanovski, S. Z. (2007). Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care, 30*(6), 1374-1383.

Poanta, L., Porojan, M., & Dumitrascu, D. L. (2011). Heart rate variability and diastolic dysfunction in patients with type 2 diabetes mellitus. *Acta Diabetol., 48*(3), 191-196.

Poirier, P., Bogaty, P., Garneau, C., Marois, L., & Dumesnil, J. G. (2001). Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. *Diabetes Care, 24*(1), 5-10.

Poirier, P., Garneau, C., Bogaty, P., Nadeau, A., Marois, L., Brochu, C., . . . Dumesnil, J. G. (2000). Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol., 85*(4), 473-477.

Redfield, M. M., Jacobsen, S. J., Burnett, J. C., Jr., Mahoney, D. W., Bailey, K. R., & Rodeheffer, R. J. (2003). Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA, 289*(2), 194-202.

Reusch, J. E., Bridenstine, M., & Regensteiner, J. G. (2013). Type 2 diabetes mellitus and exercise impairment. *Rev. Endocr. Metab. Disord., 14*(1), 77-86.

Revdal, A., Hollekim-Strand, S. M., & Ingul, C. B. (2016). Can Time Efficient Exercise Improve Cardiometabolic Risk Factors in Type 2 Diabetes? A Pilot Study. *J. Sports Sci. Med., 15*(2), 308-313.

Rognmo, O., Hetland, E., Helgerud, J., Hoff, J., & Slordahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur. J. Cardiovasc. Prev. Rehabil., 11*(3), 216-222.

Ronnemaa, T., Marniemi, J., Puukka, P., & Kuusi, T. (1988). Effects of long-term physical exercise on serum lipids, lipoproteins and lipid metabolizing enzymes in type 2 (non-insulin-dependent) diabetic patients. *Diabetes Res., 7*(2), 79-84.

Ross, R., Dagnone, D., Jones, P. J., Smith, H., Paddags, A., Hudson, R., & Janssen, I. (2000). Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann. Intern. Med., 133*(2), 92-103.

Ross, R., Janssen, I., Dawson, J., Kungl, A. M., Kuk, J. L., Wong, S. L., . . . Hudson, R. (2004). Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obes. Res., 12*(5), 789-798.

Rustad, P., Felding, P., Franzson, L., Kairisto, V., Lahti, A., Martensson, A., . . . Uldall, A. (2004). The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scand. J. Clin. Lab. Invest., 64*(4), 271-284.

Ryden, L., Grant, P. J., Anker, S. D., Berne, C., Cosentino, F., Danchin, N., . . . Xuereb, R. G. (2013). ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur. Heart J., 34*(39), 3035-3087.

Sacre, J. W., Jellis, C. L., Coombes, J. S., & Marwick, T. H. (2012). Diagnostic accuracy of heart-rate recovery after exercise in the assessment of diabetic cardiac autonomic neuropathy. *Diabet. Med., 29*(9), e312-320.

Sacre, J. W., Jellis, C. L., Jenkins, C., Haluska, B. A., Baumert, M., Coombes, J. S., & Marwick, T. H. (2014). A six-month exercise intervention in subclinical diabetic heart disease: effects on exercise capacity, autonomic and myocardial function. *Metabolism, 63*(9), 1104-1114.

Schneider, S. H., Khachadurian, A. K., Amorosa, L. F., Clemow, L., & Ruderman, N. B. (1992). Ten-year experience with an exercise-based outpatient life-style modification program in the treatment of diabetes mellitus. *Diabetes Care, 15*(11), 1800-1810.

Sigal, R. J., Kenny, G. P., Boule, N. G., Wells, G. A., Prud'homme, D., Fortier, M., . . . Jaffey, J. (2007). Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Ann. Intern. Med., 147*(6), 357-369.

Skaug, E. A., Aspenes, S. T., Oldervoll, L., Morkedal, B., Vatten, L., Wisloff, U., & Ellingsen, O. (2013). Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. *Eur J Prev Cardiol, 20*(4), 531-540.

Solomon, T. P., Malin, S. K., Karstoft, K., Haus, J. M., & Kirwan, J. P. (2013). The influence of hyperglycemia on the therapeutic effect of exercise on glycemic control in patients with type 2 diabetes mellitus. *JAMA Intern Med, 173*(19), 1834-1836.

Stolen, T. O., Hoydal, M. A., Kemi, O. J., Catalucci, D., Ceci, M., Aasum, E., . . . Wisloff, U. (2009). Interval training normalizes cardiomyocyte function, diastolic Ca2+ control, and SR Ca2+ release synchronicity in a mouse model of diabetic cardiomyopathy. *Circ. Res., 105*(6), 527-536.

Støylen, A. Strain rate imaging. Retrieved from http://folk.ntnu.no/stoylen/strainrate/ Accessed: May, 2015

Tang, Z. H., Zeng, F., Li, Z., & Zhou, L. (2014). Association and predictive value analysis for resting heart rate and diabetes mellitus on cardiovascular autonomic neuropathy in general population. *J Diabetes Res, 2014*, 215473.

ten Brinke, R., Dekker, N., de Groot, M., & Ikkersheim, D. (2008). Lowering HbA1c in type 2 diabetics results in reduced risk of coronary heart disease and all-cause mortality. *Prim. Care Diabetes, 2*(1), 45-49.

Terada, T., Friesen, A., Chahal, B. S., Bell, G. J., McCargar, L. J., & Boule, N. G. (2013). Feasibility and preliminary efficacy of high intensity interval training in type 2 diabetes. *Diabetes Res. Clin. Pract., 99*(2), 120-129.

The Norwegian Directorate of Health. (2005). *Norske anbefalinger for ernæring og fysisk aktivitet*. Helsedirektoratet Retrieved from http://helsedirektoratet.no/publikasjoner/norske-anbefalinger-for-ernering-og-fysisk-aktivitet/Publikasjoner/norske-anbefalinger-for-ernering-og-fysisk-aktivitet.pdf Accessed: May, 2014.

The Norwegian Directorate of Health. (2014). *Anbefalinger om kosthold, ernæring og fysisk aktivitet*. (IS-2170). Retrieved from https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/806/Anbefalinger-om-kosthold-ernering-og-fysisk-aktivitet-IS-2170.pdf Accessed: May, 2016.

Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A., . . . Green, D. J. (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am. J. Physiol. Heart Circ. Physiol., 300*(1), H2-12.

Thomas, D. E., Elliott, E. J., & Naughton, G. A. (2006). Exercise for type 2 diabetes mellitus. *Cochrane Database Syst Rev*(3), CD002968.

Thomas, N., Alder, E., & Leese, G. P. (2004). Barriers to physical activity in patients with diabetes. *Postgrad. Med. J., 80*(943), 287-291.

Tjonna, A. E., Lee, S. J., Rognmo, O., Stolen, T. O., Bye, A., Haram, P. M., . . . Wisloff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation, 118*(4), 346-354.

Tjønna, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., . . . Wisløff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation, 118*(4), 346-354.

Tudor-Locke, C., Bell, R. C., Myers, A. M., Harris, S. B., Ecclestone, N. A., Lauzon, N., & Rodger, N. W. (2004). Controlled outcome evaluation of the First Step Program: a daily physical activity intervention for individuals with type II diabetes. *Int. J. Obes. Relat. Metab. Disord., 28*(1), 113-119.

Umpierre, D., Ribeiro, P. A., Kramer, C. K., Leitao, C. B., Zucatti, A. T., Azevedo, M. J., . . . Schaan, B. D. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA, 305*(17), 1790-1799.

Umpierre, D., Ribeiro, P. A., Schaan, B. D., & Ribeiro, J. P. (2013). Volume of supervised exercise training impacts glycaemic control in patients with type 2 diabetes: a systematic review with meta-regression analysis. *Diabetologia, 56*(2), 242-251.

Vanhees, L., Geladas, N., Hansen, D., Kouidi, E., Niebauer, J., Reiner, Z., . . . Vanuzzo, D. (2012). Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II. *Eur J Prev Cardiol, 19*(5), 1005-1033.

Von Bibra, H., Thrainsdottir, I. S., Hansen, A., Dounis, V., Malmberg, K., & Ryden, L. (2005). Tissue Doppler imaging for the detection and quantitation of myocardial dysfunction in patients with type 2 diabetes mellitus. *Diab. Vasc. Dis. Res., 2*(1), 24-30.

Weston, K. S., Wisloff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med., 48*(16), 1227-1234.

Wing, R. R., Bolin, P., Brancati, F. L., Bray, G. A., Clark, J. M., Coday, M., . . . Yanovski, S. Z. (2013). Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N. Engl. J. Med., 369*(2), 145-154.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

Wycherley, T. P., Brinkworth, G. D., Noakes, M., Buckley, J. D., & Clifton, P. M. (2008). Effect of caloric restriction with and without exercise training on oxidative stress and endothelial function in obese subjects with type 2 diabetes. *Diabetes Obes. Metab., 10*(11), 1062-1073.

Zabalgoitia, M., Ismaeil, M. F., Anderson, L., & Maklady, F. A. (2001). Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol., 87*(3), 320-323.

Zhang, Y., Hu, G., Yuan, Z., & Chen, L. (2012). Glycosylated hemoglobin in relationship to cardiovascular outcomes and death in patients with type 2 diabetes: a systematic review and meta-analysis. *PLoS One, 7*(8), e42551.

Åstrand, P. O. R., K. (2003). *Textbook of work physiology - physiological bases of exercise* (Fourth Edition ed.): Human Kinetics.

CHAPTER 4

The effect of moderate-intensity exercise and high-intensity interval exercise training on left ventricular untwist parameters in individuals with type 2 diabetes and diastolic dysfunction.

TABLE OF CONTENTS
CHAPTER 4

4.1 ABSTRACT

Background There is limited information regarding the role of left ventricular (LV) twist and the effect of exercise in patients with type 2 diabetes (T2D). **Objectives** To compare LV twist parameters in patients with T2D versus healthy control subjects and the effects of high-intensity interval exercise (HIIE) and moderate-intensity exercise (MIE) on LV twist in T2D patients with diastolic dysfunction. **Methods** This study, which included both prospective and retrospective components, included 47 patients with T2D and diastolic dysfunction and 37 healthy individuals. Patients with T2D were randomized to HIIE (4 x 4 min at 90%–95% of maximal heart rate (HR_{max}), three times a week, 120 min/week; n=24) or MIE (210 min/week; n=23) for 12 weeks and examined with echocardiography (LV twist by speckle-tracking method) at baseline and post-test. The control subjects received no intervention and were matched according to age, gender, and body mass index to those completing the intervention. **Results** In total, 37 subjects completed 12 weeks of MIE (n=17) or HIIE (n=20). The LV peak untwist rate (UTR) was similar in patients with T2D and control subjects ($p>0.05$). At baseline, LV peak UTR, relative to total diastolic period, occurred 5.8later in patients with T2D compared with control subjects ($p=0.004$). Time to peak UTR was shortened by 6.5 percentage ($p=0.002$) and 7.7 percentage points ($p<0.001$) after MIE and HIIE, respectively. Time to peak UTR was similar to that in control subjects after exercise interventions. **Conclusions** In patients with T2D and diastolic dysfunction, LV peak UTR was similar, but time to peak LV UTR was delayed compared with control subjects. Twelve weeks of endurance exercise normalized the timing of UTR.

4.2 INTRODUCTION

The following manuscript (Paper II) is based on this chapter:

Hollekim-Strand, S.M.; Høydahl, S.F.; Follestad, T.; Dalen, H.; Bjørgaas, M.R.; Wisløff, U; Ingul, C.B. Exercise training normalizes timing of left ventricular untwist rate, but not peak untwist rate, in individuals with type 2 diabetes and diastolic dysfunction: A pilot study. **J Am Soc Echocardiogr**. 2016. 29(5): p.421–430.

Type 2 diabetes (T2D) is associated with cardiomyopathy, which ultimately can lead to heart failure (HF) (Nichols et al., 2004). Early diabetic cardiomyopathy is often characterized by diastolic dysfunction, which has been detected in 20% to 60% of individuals with T2D without overt coronary artery disease (Di Bonito et al., 2005; Fang et al., 2005; Hare et al., 2011; Poirier et al., 2001; Redfield et al., 2003; Von Bibra et al., 2005; Zabalgoitia et al., 2001).

Although not commonly evaluated, a fundamental component in normal diastolic function is left ventricular (LV) untwist in early diastole, which contributes to LV relaxation and suction (Notomi et al., 2006; Notomi et al., 2007).The sequence of twisting and untwisting events establishes the peak early diastolic blood flow velocity across the mitral valve (Notomi et al., 2007).

LV twist and untwist (see Definitions and explanations) is the wringing and unwringing motion of the LV around the long axis of the ventricle, induced by contraction and active relaxation of myofibers in the LV wall, respectively. Untwisting is the earliest mechanical manifestation of diastole. It starts in late systole, but most of the untwisting occurs during the isovolumetric relaxation period and is largely completed at the time of mitral valve opening (Rademakers et al., 1992). When released, the recoil created during systole (due to the elastic energy stored in the collagen matrix and cytoskeletal proteins [titin] during contraction) causes a rapid untwist during isovolumic relaxation period (Notomi et al., 2006; Rademakers et al., 1992). This elastic recoil/untwisting and active myocardial relaxation generate active suction of blood from the atria (Arts et al., 1982; Bell et al., 2000; Rademakers et al., 1992; Sengupta et al., 2008; Wu and Kovacs, 2006); creating a rapid LV pressure fall during the isovolumetric relaxation period, which promote early LV filling (Oh et al., 2006; Quinones, 2005). Untwisting appear to be most important for the early diastolic LV filling, not later diastolic events (Burns et al., 2009). Indeed, the recoil mechanism is a major determinant of myocardial compliance (see Definitions and

explanations) and links systolic contraction to diastolic filling (Dong et al., 2001; Notomi et al., 2006; Rademakers et al., 1992).

Overall, the physiological role of LV twist and untwist are well established and the LV untwisting and untwisting rate may provide additional insight and promising indexes into early diastolic function (Burns et al., 2009; Opdahl et al., 2012); especially since the untwisting occurs before the myocardial and blood flow changes.

A reduction in LV untwisting has been associated with attenuation or loss of diastolic suction; contributing to diastolic dysfunction in diseased hearts (Bell et al., 2000; DeAnda et al., 1995; Fuchs et al., 2004). Furthermore, worsening of diastolic relaxation and reduced early diastolic suction has been associated with reduction in the rate and magnitude of untwist (Burns et al., 2009; Notomi et al., 2006).

Peak LV systolic twist and twist rate have shown to be greater and peak UTR similar between T2D patients (with diastolic dysfunction and normal ejection fraction [EF]) and controls; indicating impaired relaxation in the T2D patients (Fonseca et al., 2004). It can be speculated whether an increase in LV twist represents a compensatory mechanism for reduced myocardial relaxation or whether it is a consequence of reduced filling in the early stage of diastolic dysfunction. However, there is limited information regarding the role of LV twist in T2D. Furthermore, there is limited data on the clinical application of the UTR for the assessment of LV diastolic function and more research is needed as well as more robust techniques.

The accuracy of two-dimensional (2D) speckle tracking echocardiography (STE) for the measurements of LV twist parameters has been validated in several studies (Helle-Valle et al., 2005) and allows for a more accessible, less expensive, and less time consuming measurements compared to MRI; the gold standard for LV twist evaluation (Lorenz et al., 2000).

Exercise training is a cornerstone in T2D management to reduce CV disease and mortality (Hordern et al., 2009; International Diabetes Federation, 2012, 2013; Mitka, 2013) and several studies, including Paper I (Chapter 3), have shown that high-intensity interval exercise (HIIE) is superior to moderate-intensity exercise (MIE) according to current exercise guidelines in reducing CV risk factors (Boule et al., 2003; Rognmo et al., 2004; Rognmo et al., 2012; Tjonna et al., 2008; Weston et al., 2014). Furthermore, twelve weeks of endurance exercise training has previously shown to increase apical rotation and peak early diastolic untwisting rate in male rowers (Weiner et al., 2010). Moreover, competitive athletes (cyclists, soccer players and

basketball players) have increased twist compared to non-athletes (De Luca et al., 2011). This suggests that LV twist and UTR augmentation might contribute to exercise-induced cardiac remodeling (Weiner et al., 2015). However, to our knowledge, no study has investigated the effects of exercise on LV untwist and UTR in patients with T2D and diastolic dysfunction.

Aims

The aims of this study were (1) to determine the effect of HIIE and MIE on LV untwist and UTR as well as time to peak UTR in individuals with T2D (duration <10 years) and diastolic dysfunction, and; (2) to compare these twist variables with those of healthy individuals with no exercise intervention.

Hypothesis

The hypothesis of this article was based on the initial finding in Paper I (Chapter 3) where exercise improved early diastolic filling. We therefore sought to investigate if even earlier diastolic events where affected by exercise using LV untwist that occurs mainly during the isovolumetric relaxation period:

a. Exercise training (HIIE and MIE) improves LV peak UTR.
b. Exercise training (HIIE and MIE) improves time to LV peak UTR, LV peak twist rate, LV peak twist.
c. Individuals with T2D and diastolic function have reduced untwist variables compared to healthy controls.

4.3 MATERIALS AND METHODS

4.3.1 Study design

The T2D group was recruited prospectively, and the healthy controls were recruited retrospectively (Figure 4.1).

4.3.2 Participants

The T2D study population in this chapter is also described in Chapter 3 and was recruited through a local newspaper and from the outpatient population at St. Olav's Hospital, Trondheim, Norway (August 2010-March 2013). The healthy individuals were selected from the Echocardiography substudy in Nord-Trøndelag Health study (HUNT 3) (Dalen et al., 2010) to serve as a control group. These were selected based on age, sex and body mass index (BMI), and they were matched to the extent that was possible with the baseline records of the T2D group reporting for the 12-week post-test.

The study population, inclusion and exclusion criteria are presented in Table 4.1 and characteristics for the included participants are presented in Table 4.2.

Table 4.1. Inclusion and exclusion criteria for the participants in Paper II/Chapter 4.

Paper/ Chapter	Study population	N	Eligibility criteria	Exclusion criteria
II/4	T2D individuals with diastolic dysfunction (e'<8cm/s)	47	Same as in Chapter 3/Paper I: Age 20-65, T2D duration < 10 yrs. Diastolic dysfunction (e'< 8 cm/s)	-Overt CVD -Atrial fibrillation or other significant cardiac arrhythmia -Untreated hypertension -LV EF <40% -BMI >35 kg/m^2 -Diabetic retinopathy and/or neuropathy -Albuminuria -Drug or alcohol abuse -Pregnancy -Unable to exercise -Habitual exercise > guidelines for patients with T2D.
	Age, gender and BMI matched healthy controls	37	HUNT3 Study participants	Diabetes Hypertension Known CVD Arrhythmias Valvular pathology Any kind of myocardial pathology

Abbreviations: BMI, Body mass index; CVD, cardiovascular disease; HUNT3, Echocardiography substudy in Nord-Trøndelag Health study; LV, left ventricular; T2D, Type 2 diabetes.

Table 4.2. Characteristics of the study participants included in Chapter 4.

Paper II			
Group allocation	**MIE**	**HIIE**	**Controls**
N	23	24	37
Female	8 (34.8)	9 (37.5)	18 (48.6)
Age, yrs.	54.6±5.6	57.1±6.2	52.0±9.4
T2D-duration	3.1±2.5	4.3±2.3	-
BMI, kg/m^2	29.7±3.3	30.4±3.0	27.0±3.8
WC, cm	107.6±9.0	109.4±9.1	-
Female, ≥80cm	8 (100)	9 (100)	
Male, ≥94cm	14 (93)	14 (93)	
HbA_{1c}			
%	6.8±0.7	7.2±1.3	
mmol/mol	51.0±5.3	55.0±9.9	

Results are presented as mean ± SD or No (%). Abbreviations: MIE, moderate-intensity exercise; HIIE, high-intensity interval exercise; T2D, Type 2 diabetes; BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; waist circumference, WC.

4.3.3 Study intervention

The exercise intervention period for the T2D participants was 12 weeks (Figure 4.1).

The exercise interventions are previously described in Chapter 2 (section 2.1.2). The HIIE-group exercised in a supervised setting at our laboratory and performed exercise training by walking or jogging on an inclined treadmill three times/week for 12 weeks (120 minutes/week). Following 10 minutes warm up at 70% of HR_{max}, the HIIE group performed four work bouts at 90-95% of HR_{max} with three minutes recovery periods between work bouts at 70% of HR_{max} and five minutes cool down, altogether 40 minutes. During the HIIE sessions, HR monitors (Polar RS 400, Polar Electro, Kempele Finland) were used to ensure that the required exercise intensity was achieved and maintained.

The MIE group performed home-based exercise in accordance with guidelines from The Norwegian Diabetes Association (2005), recommending MIE for 210 minutes/week (exercise bouts ≥ 10 min) (The Norwegian Diabetes Association), similar to the guidelines of the International Diabetes Federation (International Diabetes Federation, 2012). At 4 and 8 weeks of the intervention period, the MIE group was contacted by phone or e-mail to motivate to continue with MIE as prescribed.

The individuals in the MIE and HIIE group used an activity diary to record daily physical activity (see Chapter 3, section 3.3.5.8).

The control group had no intervention.

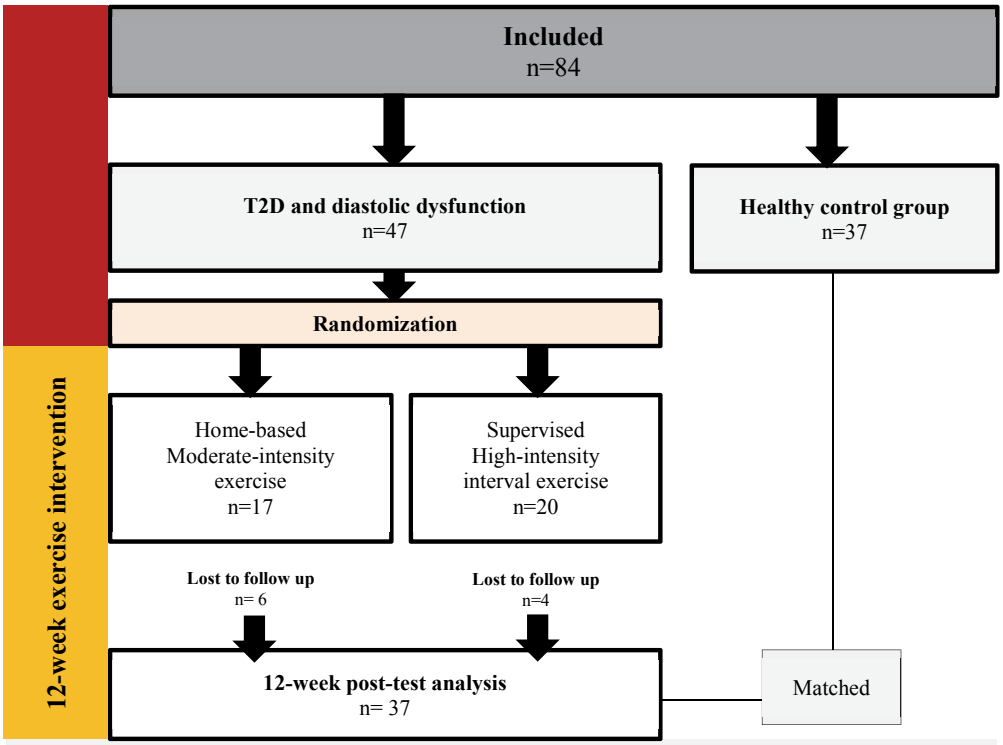


Figure 4.1. Flow chart of intervention and participants described in Chapter 4 (Paper II).

4.3.4 Outcome measures

The primary outcome measure was LV peak UTR. Secondary outcome measures were time to LV peak UTR, LV peak twist rate and LV peak twist.

The primary outcome variable (LV UTR) were chosen since studies suggest that LV UTR best reflects the link between systolic compression and early diastolic recoil.

The secondary variables, time to peak UTR, was chosen because in several studies pathologies have shown either no change, a decrease or an increase in untwisting, but all of them consistently show delayed time to peak untwist (Mor-Avi et al., 2011). Time to peak UTR during early diastole has also been suggested to be a valuable parameter in identifying diastolic dysfunction in hypertensive and hypertrophic ventricles (Salehi et al., 2014).

4.3.5 Clinical measurements

All clinical measurements performed at baseline and 12-week post-test on the T2D subjects are also described in Chapter 2.

4.3.5.1 Resting echocardiography

The T2D participants obtained an echocardiogram during rest at baseline and at the 12-week post-test. In addition, echocardiogram from the healthy controls from the HUNT Study was utilized for analysis.

The same operator (CBI) performed the echocardiographic recordings at both time points for the T2D population and the same operator (HD) performed the echocardiographic recordings in the healthy control group.

As also described in Chapter 2 (section 2.1.1.1), full resting echocardiography was performed using a Vivid 7 scanner with a phased-array matrix (M4S and M3S transducers, GE Vingmed, Horten, Norway) at baseline and 12 weeks posttest for the MIE and HIIE group. The same method and equipment was used for the controls, who were examined only once (Dalen et al., 2010). A detailed description of the echocardiographic recordings has previously been published (Ingul et al., 2010; Molmen et al., 2012).

The images were obtained in parasternal and three standard apical views (4-chamber, 2-chamber and apical long axis), both in B-mode and with color tissue Doppler imaging, and three loops were acquired for each image. The images were digitally stored on a hard disk for offline analysis. All recordings were performed with the individuals in left lateral supine position during breath holds at end expiration. Basal and apical LV short axis levels were recorded for the analysis of twist and standardized to two anatomical landmarks. The basal level was recorded from parasternal position, using the mitral valve leaflets location in the middle of the LV cavity as the landmark. The apical level was recorded more distal towards the apex, just proximal to the level with luminal closure at end systole. All loops were recorded with the LV cross sections as circular as possible to obtain. To optimize image quality, the sector depth and width were adjusted in each subject resulting in a mean frame rate of 69±13 frames/s (range 51-113 frames/s). To determine the timing of cardiac events, tissue Doppler velocity curves with the sample volume at the basal septum were conducted immediately before the acquisition of the short-axis images, to minimize differences in HR. Aortic valve closure (AVC) was set at the end of the negative spike after ejection (Aase et al., 2008).

Echocardiographic analyses by speckle tracking

Off line data analyses were performed using commercially available two-dimensional strain software (Echopac PC, Version 112, GE Medical Systems). All analyses were performed blinded to exercise group allocation as well as baseline- and posttest. Analyses of conventional echocardiographic diastolic measures were performed as previously published (Ingul et al., 2010; Molmen et al., 2012).

The analyses of the twist parameters have been described previously (Notomi et al., 2005). To analyze twist parameters, one cardiac cycle from the basal and apical short axis data set with the best-quality 2D images and a well-defined endocardial border during late systole were selected. Regions of interest of the LV were adjusted to include most of the myocardium, but not the pericardium. The endocardial borders of the apical and basal short axis plane in end systole were manually traced and subsequently tracked by the software. When the software or the observer signaled poor tracking quality, the observer readjusted the region of interest until an acceptable tracking was obtained. The computer automatically selected suitable stable objects for tracking and then searched for them in the next frame using the sum of absolute differences algorithm (Leitman et al., 2004). For the twist analysis, default spatial and temporal smoothing was used. If this was not adjustable, the segment of poor tracking was discarded.

The twist variables are presented in Tables 4.3-4.6. In addition, the timing of AVC and the length of cardiac cycle (i.e. time interval between the R waves on the electrocardiogram) were measured. LV rotation, twist and twist rates were automatically calculated by the software algorithm at each frame throughout the heart cycle as a curve in six different segments in each short-axis view.

LV twist was calculated (Excel, Microsoft Corporation, Redmond, Washington) as apical LV rotation relative to the base. Counterclockwise rotation as viewed from the LV apex was expressed as a positive value, whereas clockwise rotation as a negative value (Figure 4.2). Peak UTR was defined as the first negative peak after AVC.

To adjust for intra and inter subject differences in HR, the time sequences from AVC to peak UTR were normalized to the percentage of total diastolic duration. Diastole was expressed as a percentage of the total duration of diastole and the diastolic period was defined from 0% (AVC) to 100% (end-diastole) (Figure 4.2) (Nakai et al., 2006). Time to peak UTR was measured from time of AVC (0%) to peak UTR in percentage of length of diastole (100%) (Figure 4.2).

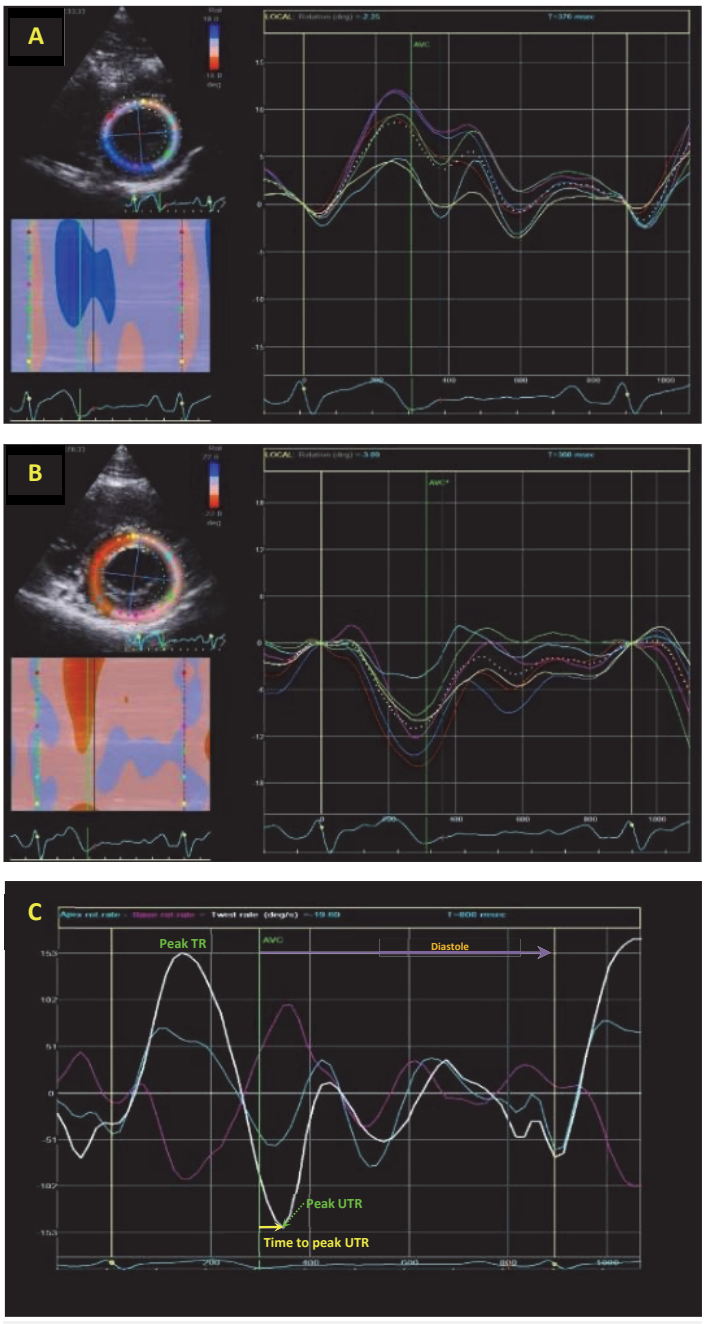


Figure 4.2. Left ventricular basal and apical rotation, twist and untwist rate. **A**. Apical counterclockwise rotation expressed as a positive value. Six curves in different colors represent different myocardial segments. White dashed line is the average value of the six segments. **B**. Basal rotation expressed as a negative value. **C**. Curves showing peak twist rate (TR) and untwist rate (UTR). Time to peak UTR is measured from time of aortic valve closure (AVC) to peak UTR (yellow arrow) in percentage of length of diastole (orange arrow).

Variability of echocardiographic measurements

In total, 15 individuals were used for measurements of intra- and interobserver variability, five randomly selected from each group (five T2D baseline, five T2D posttest and five nondiabetic subjects) of LV twist parameters. Intraobserver variability was performed by a single investigator by blinded assessment on two separate occasions. Interobserver variability was assessed by two investigators who were unaware of each other's measurements and of the study time point.

The percentage of diastole was used in the analysis of reproducibility. The reproducibility data is expressed by the coefficients of variation, and as the mean percent error (mean error). Coefficient of variation was calculated as the ratio of the standard deviation of the repeated measurements for each individual to the mean. Mean error was derived as the absolute difference between the two sets of observations, divided by the mean of the observations (Thorstensen et al., 2010). The average values of the 15 individuals are presented.

4.3.5.2 Resting heart rate and blood pressure

Resting heart rate (HR) and blood pressure was measured according to Chapter 2 (sections 2.1.1.7-2.1.1.8). Resting HR was measured by electrocardiography at the end of the resting echocardiography measurement in both groups. Blood pressure was measured by oscillometry using SunTech® Tango® M2 Stress BP Monitor (USA) and Dinamap 845XT (GE Healthcare, Milwaukee, WI) in the T2D group and controls, respectively, as previously described (Dalen et al., 2011; Hollekim-Strand et al., 2014).

4.3.6 Statistical analyses

The measurements are presented as mean ± standard deviation unless otherwise stated. Differences between the HIIE and MIE intervention groups, and within-group effects of intervention, were analyzed using linear mixed models (LMMs). Within subject correlations were taken into account by using a random intercept in the LMM. The baseline means were restricted to be equal for the two interventions, due to randomization to intervention group. Tests for between-group and within-group differences were performed as post-hoc t-tests for the appropriate pairs of model parameters. Differences between the T2D group and the healthy controls were analyzed using two-sample t-tests for the full T2D group at baseline and for the MIE and HIIE groups separately after intervention. Given the limited population available for matching, the matching of the control group aimed for no significant differences between

groups with respect to age, gender and BMI. Therefore, the T2D individuals and healthy controls were treated as independent groups, and not paired, in the statistical analysis.

For all tests a significance level of 0.05 was used. No formal adjustments for multiple testing due to the number of variables were made, but the risk of incidental findings was kept in mind when interpreting the results.

The analyses were carried out using SPSS version 21.0 (IBM SPSS Inc., New York) and the R statistical package (R Core Team, 2014).

Sample size calculations

Sample size of the healthy control group was set from the exercise group completing the 12-week post-test in Paper I, described in Chapter 3.

4.4 SUMMARY OF RESULTS

In this chapter, the results for the LV twist parameters, isovolumic relaxation time (IVRT) and the analysis comparing T2D baseline values as well as HIIE and MIE posttest variables with healthy controls, will be presented.

Data from 37 T2D participants were available for posttests; 17 in MIE and 20 in HIIE. In total 10 subjects withdrew from different reasons (Figure 4.1). In total 8.4% of the segments in basal and apical short-axis views were discarded, because of inadequate tracking.

Before intervention six of the T2D participants were classified as having diastolic dysfunction grade 1, 30 were classified as having diastolic dysfunction grade 2 and one participant had diastolic dysfunction grade 3 (Chapter 3; Table 3.3). After exercise intervention, 18 T2D participants had diastolic dysfunction grade 2, and 19 had diastolic dysfunction grade 1.

4.4.1 Subject characteristics

At baseline, the participants in the T2D group were significantly older than the control group (56.5 versus 51.0 years, $p<0.004$) and BMI was higher in the T2D group compared to the control group (29.9 versus 27.0 kg/m^2; $p<0.001$). As reported in Chapter 3, there was no significant difference in age, gender distribution or BMI between the MIE and HIIE group. The use of medication was similar in the MIE and HIIE groups (Chapter 3; Table 3.3).

4.4.1.1 Exercise compliance

As reported in Chapter 3, the HIIE group performed 94 % of the scheduled training sessions and 94% of the MIE subjects reported (by activity diary) physical activity to be ≥ 210 minutes/ week.

4.4.2 Clinical measurements

4.4.2.1 Left ventricular twist parameters

LV twist results at baseline and 12-week posttest are described in Table 4.3. Comparison between the exercise intervention groups (at baseline and 12-week posttest) and the healthy controls are given in Table 4.4. The effects of HIIE and MIE are shown in Table 4.5.

There were no differences between T2D and controls in LV peak UTR, peak twist rate or peak twist, except for basal twist rate ($p=0.02$) (Table 4.4).

At baseline, time to peak LV UTR relative to total diastolic period occurred 5.8 percentage points (pp) later in T2D than in controls (p=0.004) (Table 4.4, Figure 4.3a-c). When normalized for resting HR, time to peak UTR from AVC in the T2D group was significantly delayed compared to controls at baseline (mean difference 0.53 ms/beat, 95% CI: 0.20 to 0.86, p=0.002).

Twelve weeks of HIIE and MIE, respectively, reduced time to peak UTR by 7.7 pp (p<0.001) and 6.5 pp (p=0.002) (Table 4.5, Figure 4.3b). When normalized for resting HR HIIE and MIE reduced time to peak UTR by 0.41 ms/beat (95% CI: 0.13 to 0.69, p=0.006) and 0.46 ms/beat (95% CI: 0.16 to 0.77, p=0.004), respectively.

Time to peak apical UTR was reduced on average with 10.0 pp after MIE (p<0.001) and 8.8 pp after HIIE (p<0.001) (Table 4.5). Time to peak basal UTR was reduced by 4.6 pp after MIE (p=0.02) and 5.5 pp after HIIE (p=0.003), and time to peak UTR was reduced by 6.5 pp after MIE (p=0.002) and 7.7 pp after HIIE (p<0.001) (Table 4.5). There were no significant changes after MIE or HIIE for other twist variables (Table 4.5). At follow-up after 12 weeks, there were no significant differences between the exercise groups and controls for any twist variables (Table 4.4, Figure 4.3c).

At baseline, peak UTR occurred during IVRT in 27% of the T2D participants, whereas it occurred after IVRT in 73% of the T2D participants. After intervention, peak UTR occurred during IVRT in 85.5% and after IVRT in 13.5% of the T2D individuals. Peak UTR occurred during IVRT in all participants in the control group.

4.4.2.2 Conventional left ventricular diastolic and systolic echocardiographic variables

Conventional and tissue Doppler LV diastolic and systolic variables are presented in Chapter 3 (section 3.4.2.1). Diastolic dysfunction grade I was found in 16% (n=6), grade II in 81% (n=30) and grade III in 3 % (n=3) of these subjects (measured according to Chapter 2, section 2.1.1.1)

At baseline, the values for e', peak early diastolic mitral inflow (E), and E/A-ratio were lower, while filling pressure (E/e'-ratio), and late diastolic mitral inflow (A) were higher in the T2D group compared to the controls (all p<0.001) (Table 4.6).

Both exercise groups, but HIIE significantly more than MIE, improved diastolic function measured by e' from baseline to posttest by 1.8±1.1 cm/s (p<0.001) and 0.5±0.7 cm/s (p=0.02), respectively, (difference between exercise groups, p<0.001). At 12-week posttest, there were still significant differences in diastolic function between the MIE group and the controls (e', E,

E/e', A and E/A) and the HIIE group and the controls (e', E/e', A, E/A and IVRT), but not between the HIIE group and the controls for E (Table 4.6).

There was no significant difference in IVRT between the T2D group and the control group at baseline (p=0.11) (Table 4.6). IVRT reduced from baseline to posttest in the HIIE group (-9.5 ms, 95% CI: -18.2 to -0.9; p=0.03), but not in the MIE group (-4.7 ms, 95% CI: -14.3 to 5.0; p=0.33), but there was no difference in change between groups (MIE vs. HIIE: -4.9 ms, 95% CI: -16.1 to 6.2; p=0.37).

At baseline, the peak systolic tissue Doppler velocity (S') was significantly lower in the T2D group than in the control group (p<0.001) and was still reduced compared to controls in the MIE group (p=0.005) and HIIE group (p=0.04) after intervention (Table 4.6). The HIIE group, but not the MIE group, significantly improved systolic function (S') from baseline to posttest (0.9 ± 1.4 cm/s versus -0.1 ± 1.1 cm/s, respectively; p=0.03).

4.4.2.3 Left ventricular dimensions and volumes

Left interventricular septum (IVS) and posterior wall (PW) were significantly thicker both in diastole (d) and systole (s) among the individuals with T2D vs. controls at baseline (IVSd 1.07±0.17 vs. 0.89±0.15 cm; p=0.001), (IVSs 1.62±0.31 vs. 1.29±0.21 cm, respectively; p<0.001), (PWd 1.10±0.15 vs. 0.88±0.16 cm; p<0.001), and (PWs 1.79±0.28 vs.1.52±0.22 cm; p=0.001). There was no difference in LV internal dimension between the controls and T2D individuals. Intervention did not change LV dimensions. Left atrium volume index was 35.1 ± 9.9 ml/m^2 in T2D individuals at baseline and remained similar after intervention (p=0.09).

At baseline, the end diastolic volume (EDV) was lower in the control group than in the T2D group (122.7 ml vs. 155.6 ml, respectively; p<0.001) whereas there was no significant difference in end systolic volume (ESV) between groups (67.3 ml vs. 68.8 ml, respectively). EDV and ESV did not change after exercise in the T2D group (155.6 vs. 153.1 ml and 68.9 vs. 68.8 ml, respectively). The left atrial volume index decreased from baseline to posttest in the T2D group by 4.3 ml/m2 (p<0.001).

4.4.2.4 Resting heart rate and blood pressure

At baseline, the mean resting HR was significantly higher in the T2D group than in the control group (p=0.008; Table 4.6). The diastolic blood pressure was higher in T2D than controls at baseline (4.6 mmHg 95% CI: 0.7 to 8.5; p=0.02), but systolic blood pressure was not (4.8 mmHg, 95% CI: -1.7 to 11.4; p=0.15).

At 12-week posttest, there was no significant difference in resting HR between the HIIE group and controls, whereas HR was still higher in the MIE group ($p=0.007$; Table 4.6). There was no significant difference between the intervention groups and controls in systolic or diastolic blood pressure at posttest (Table 4.6).

Table 4.3. Summary of the LV twist- and untwist data for the healthy controls and intervention groups.

	Control		T2D, baseline		MIE, baseline		MIE, post		HIIE, baseline		HIIE, post
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
LV Base												
Heart rate basal, beats/minute	37	66 (11.3)	36	71.2 (9.8)	17	72.9 (10.3)	15	72.1 (9.1)	19	69.8 (9.3)	19	65.2 (9.3)
Peak basal rotation	37	-5.2 (2.7)	36	-5.5 (3.2)	17	-5.3 (3.8)	15	-4.3 (2.5)	19	-5.6 (2.7)	19	-4.2 (2.2)
Basal TR, degrees/s	37	-42.1 (17.2)	36	-54.2 (26.1)	17	-54.4 (24.8)	15	-54 (23.1)	19	-54.1 (27.9)	19	-45.4 (17.8)
Basal UTR, degrees/s	37	52.3 (16.4)	36	57.2 (25.6)	17	60.9 (29.6)	15	52.3 (28.4)	19	54 (21.8)	19	47.4 (23.6)
Time to peak basal UTR, % of diastole	37	8 (4.7)	35	13.9 (7.2)	16	14.4 (6)	15	9.4 (4.8)	19	13.5 (8.3)	19	8.4 (4.6)
LV Apex												
Heart rate apical, beats/minute	37	64.8 (10.5)	36	70.2 (9.6)	17	73.2 (10.2)	15	71.7 (9.7)	19	67.5 (8.4)	19	65.7 (9.1)
Peak apical rotation	37	8.5 (3.5)	36	8.5 (3.6)	17	8.9 (3.7)	15	8.2 (4.2)	19	8 (3.6)	19	9.2 (4)
Apical TR, degrees/s	37	40.8 (11.9)	36	49.3 (24.1)	17	52 (29.6)	15	52.9 (24.3)	19	46.9 (18.3)	19	45.7 (23.4)
Apical UTR, degrees/s	37	-61.4 (18.5)	36	-59.8 (25.3)	17	-56.9 (28.1)	15	-76 (27.1)	19	-62.5 (23)	19	-61.9 (23.4)
Time to peak apical UTR, % of diastole	37	8.8 (6.3)	35	19.4 (9.4)	16	21 (9.2)	16	9.7 (8.5)	19	18.1 (9.6)	19	10.2 (5)
LV Twist												
Peak twist	37	13.1 (3.6)	36	12.3 (5.1)	17	12.5 (5.7)	15	10.5 (4.7)	19	12.2 (4.7)	19	11.3 (4.2)
Peak TR, degrees/s	37	65.4 (20.9)	36	72.7 (31.8)	17	72 (33.8)	15	67.9 (26.2)	19	73.3 (30.9)	19	70.1 (29.4)
Peak UTR, degrees/s	37	-92.8 (23.4)	36	-83.2 (33.4)	17	-86.5 (34.9)	15	-89.4 (33.8)	19	-80.2 (32.6)	19	-77.5 (25.1)
Time to peak UTR, % of diastole	37	10.5 (7.4)	35	16.3 (8.9)	16	16.1 (9)	15	10.2 (7.7)	19	16.5 (9)	19	8.8 (4.1)

Abbreviations: Control, healthy control group; HIIE, high-intensity exercise (type 2 diabetes) group; MIE, moderate-intensity exercise (type 2 diabetes) group; LV, left ventricular; T2D, type 2 diabetes; TR, twist rate; UTR, untwist rate.

Table 4.4 Results for comparison of the type 2 diabetes groups to the healthy controls for LV twist- and untwist variables.

	T2D vs Control, baseline				MIE vs Control, post				HIIE vs Control , post			
		95% CI				95% CI				95% CI		
	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value
LV Base												
Heart rate basal, beats/minute	5.2	0.3	10.2	0.04	6.1	0.0	12.2	0.05	-0.8	-6.5	4.9	0.78
Peak basal rotation	-0.2	-1.6	1.2	0.74	0.9	-0.6	2.5	0.23	1.0	-0.3	2.3	0.13
Basal TR, degrees/s	-12.1	-22.5	-1.7	0.02	-11.9	-25.6	1.9	0.09	-3.3	-13.4	6.7	0.51
Basal UTR, degrees/s	5.0	-5.1	15.1	0.33	0.0	-16.4	16.4	0.99	-4.9	-17.3	7.5	0.43
Time to peak basal UTR, % of diastole	5.9	3.0	8.9	<0.001	1.5	-1.6	4.5	0.33	0.5	-2.2	3.1	0.72
LV Apex												
Heart rate apical, beats/minute	5.4	0.7	10.1	0.03	6.9	0.7	13.2	0.03	1.0	-4.5	6.4	0.73
Peak apical rotation	-0.1	-1.7	1.6	0.94	-0.3	-2.9	2.2	0.79	0.7	-1.5	2.9	0.52
Apical TR, degrees/s	8.5	-0.5	17.4	0.06	12.1	-1.8	25.9	0.08	4.9	-6.9	16.7	0.40
Apical UTR, degrees/s	1.6	-8.8	12.0	0.76	-14.6	-30.5	1.4	0.07	-0.6	-13.2	12.1	0.93
Time to peak apical UTR, % of diastole	10.6	6.8	14.4	<0.001	0.9	-4.0	5.7	0.72	1.4	-1.7	4.5	0.38
LV Twist												
Peak rotation (twist)	-0.7	-2.8	1.3	0.47	-2.6	-5.4	0.2	0.07	-1.8	-4.1	0.5	0.12
Peak TR, degrees/s	7.3	-5.3	20.0	0.25	2.5	-13.3	18.3	0.74	4.8	-10.8	20.3	0.53
Peak UTR, degrees/s	9.6	-3.9	23.1	0.16	3.4	-16.5	23.3	0.73	15.2	1.2	29.3	0.03
Time to peak UTR, % of diastole	5.8	1.9	9.7	0.004	-0.3	-5.1	4.5	0.91	-1.7	-4.8	1.4	0.27

Abbreviations: HIIE, high-intensity interval exercise; LV, left ventricular; MIE, moderate-intensity exercise; T2D, type 2 diabetes; TR, twist rate; UTR, untwist rate. The table shows results from two-sample t-tests for comparing the T2D group and controls at baseline, and the HIIE and MIE groups post intervention to controls, for resting heart rate and the variables describing diastolic and systolic function. Estimate = estimated mean difference, Lower = lower limit of a 95% CI, Upper = upper limit of a 95% CI.

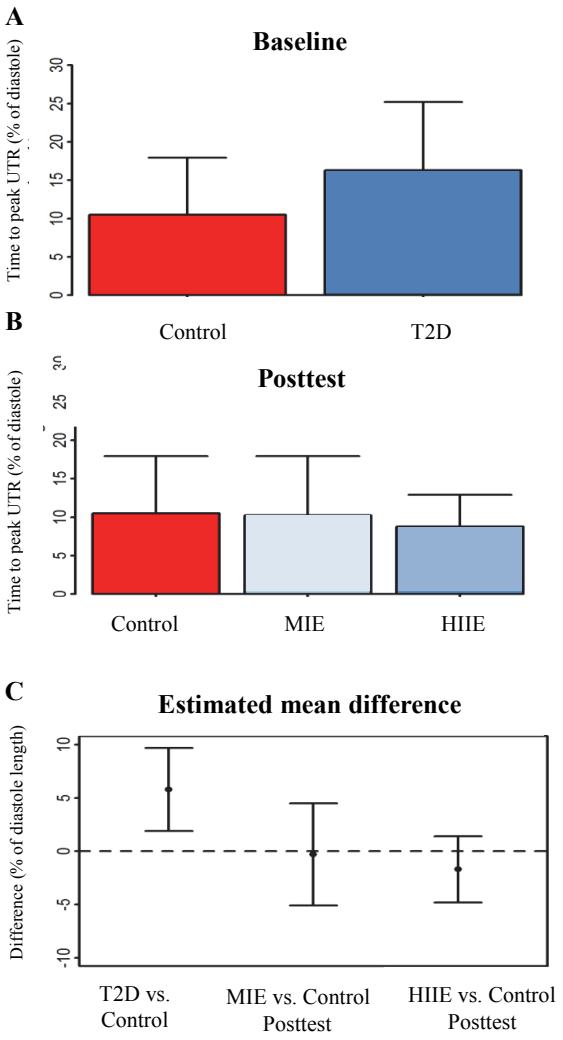


Figure 4.3. Effect of endurance exercise training on time to left ventricular (LV) peak untwist rate (UTR). **A.** Mean and SD for time to peak UTR for the healthy control group (Control) and the T2D group (T2D) at baseline. **B.** Mean and SD for the moderate-intensity exercise (MIE) and high-intensity interval exercise (HIIE) group at posttest, and controls. **C.** Estimated mean difference between T2D and controls at baseline, and between the MIE and HIIE group and controls at posttest, and 95% CI for the differences.

Table 4.5. Estimated exercise intervention effects on twist- and untwist variables.

	MIE, post - baseline				HIIE, post-baseline				HIIE vs MIE			
		95% CI				95% CI				95% CI		
	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value
LV Base												
Heart rate basal, beats/minute	-0.1	-4.8	4.6	0.96	-5.3	-9.5	-1.1	0.02	-5.2	-11.1	0.6	0.08
Peak basal rotation	1.2	-0.6	2.9	0.19	1.2	-0.4	2.9	0.13	0.1	-1.9	2.1	0.94
Basal TR, degrees/s	0.2	-14.3	14.7	0.98	8.8	-4.6	22.1	0.19	8.6	-8.0	25.1	0.30
Basal UTR, degrees/s	-5.9	-20.6	8.8	0.42	-9.0	-22.4	4.3	0.18	-3.2	-20.7	14.4	0.72
Time to peak basal UTR, % of diastole	-4.6	-8.4	-0.8	0.020	-5.5	-9.0	-2.0	0.003	-0.9	-5.2	3.4	0.67
LV Apex												
Heart rate apical, beats/minute	-0.8	-4.6	2.9	0.65	-2.5	-5.9	0.8	0.13	-1.7	-6.5	3.1	0.48
Peak apical rotation	-0.4	-2.3	1.4	0.64	1.0	-0.7	2.7	0.25	1.4	-0.9	3.8	0.23
Apical TR, degrees/s	2.8	-11.1	16.8	0.68	-3.1	-15.9	9.7	0.63	-5.9	-22.5	10.6	0.47
Apical UTR, degrees/s	-16.8	-31.5	-2.0	0.03	-1.6	-15.1	11.9	0.81	15.1	-2.3	32.6	0.09
Time to peak apical UTR, % of diastole	-10.0	-14.5	-5.6	<0.001	-8.8	-12.9	-4.7	<0.001	1.2	-4.1	6.6	0.64
LV Twist												
Peak rotation (twist), degrees	-1.9	-4.1	0.4	0.10	-1.0	-2.9	1.0	0.33	0.9	-1.9	3.7	0.52
Peak TR, degrees/s	-4.5	-22.3	13.2	0.61	-2.7	-18.9	13.6	0.74	1.8	-19.0	22.7	0.86
Peak UTR, degrees/s	-4.1	-19.7	11.4	0.59	4.3	-9.8	18.3	0.54	8.4	-11.0	27.8	0.39
Time to peak UTR, % of diastole	-6.5	-10.6	-2.5	0.002	-7.7	-11.3	-4.1	<0.001	-1.2	-6.1	3.8	0.64

Abbreviations: HIIE, high-intensity interval exercise; LV, left ventricular; MIE, moderate-intensity exercise; TR, twist rate; UTR, untwist rate. The table shows estimated effects of interventions HIIE and MIE based on linear mixed models. Estimate = estimated mean difference, Lower = lower limit of a 95% CI, Upper = upper limit of a 95% CI.

Table 4.6 Results for comparison between the type 2 diabetes groups and controls for LV longitudinal diastolic- and systolic function as well as resting heart rate and blood pressure.

	T2D vs Control, baseline				MIE vs Control, post				HIIE vs Control , post			
		95% CI				95% CI				95% CI		
	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value	Estimate	Lower	Upper	p-value
Resting HR	6.4	1.7	11.1	0.008	8.8	2.6	15.1	0.007	1.3	-3.9	6.4	0.62
BP Systolic, mmHg	4.8	-1.7	11.4	0.15	2.9	-5.8	11.7	0.50	10.5	-0.0	21.1	0.05
BP Diastolic, mmHg	4.6	0.7	8.5	0.02	4.6	-0.1	9.3	0.06	2.0	-3.2	7.3	0.44
E, cm/s	-11.7	-17.8	-5.6	<0.001	-9.6	-18.3	-0.9	0.03	-0.6	-9.4	8.2	0.89
A, cm/s	0.1	0.1	0.2	<0.001	0.1	0.1	0.2	0.003	0.2	0.1	0.3	0.001
E/A, ratio	-0.4	-0.6	-0.3	<0.001	-0.4	-0.6	-0.2	<0.001	-0.3	-0.5	-0.1	0.005
e', cm/s	-4.0	-4.8	-3.1	<0.001	-3.4	-4.4	-2.4	<0.001	-2.2	-3.1	-1.2	<0.001
E/e', ratio	2.6	1.7	3.5	<0.001	2.2	1.0	3.4	0.001	2.0	0.9	3.1	0.001
IVRT, ms	-8.2	-18.2	1.8	0.11	-13.2	-23.8	-2.7	0.02	-18.1	-30.1	-6.1	0.004
S', cm/s	-1.4	-1.9	-0.8	<0.001	-1.0	-1.6	-0.3	0.005	-0.8	-1.6	-0.1	0.04

Abbreviations: A, peak late diastolic mitral inflow velocity; BP, blood pressure; E, peak early diastolic transmitral flow velocity; e', peak early diastolic tissue Doppler velocity; IVRT, isovolumic relaxationa time; MIE, moderate-intensity exercise; HIIE, high-intensity interval exercise; HR, heart rate; S', peak systolic tissue Doppler velocity; T2D, type 2 diabetes. The table shows results from two-sample t-tests for comparing the T2D group and controls at baseline, and the HIIE and MIE groups post intervention to controls, for the variables describing diastolic and systolic function and resting heart rate. Estimate = estimated mean difference, Lower = lower limit of a 95% CI, Upper = upper limit of a 95% CI.

4.4.6 Intraobserver and interobserver variability for echocardiographic measurements

The intra- and interobserver variability was low for most twist variables, especially for the time to apical UTR (Table 4.7).

Table 4.7 Intraobserver and interobserver variability of left ventricular twist variables.

	COV (mean), %	Mean error, %	Bias	LoA, lower (-2SD)	LoA, upper (+2SD)
Intraobserver					
Time to apical UTR	2.45	3.46	-2.27	-60.11	55.47
Time to basal UTR	0.89	1.26	1.27	-17,82	20.35
Time to peak UTR	2.21	3.13	4.00	-38.72	46.72
Peak twist	7.50	10.61	0.77	-2.28	3.82
Peak UTR	11.62	16.43	0.80	-33.15	34.76
Interobserver					
Time to apical UTR	1.45	2.05	-1.33	-26.77	24.11
Time to basal UTR	6.82	9.65	26.73	-72.13	125.60
Time to peak UTR	2.36	3.34	-0.93	-42.28	40.41
Peak twist	7.38	10.44	0.97	-2.04	3.99
Peak UTR	6.41	9.07	0.36	-19.48	20.19

Abbreviations: Bias, the mean of the differences measured between the two repeated measurements (intra and inter); COV, coefficient of variation; LoA, 95% limits of agreement (lower and upper limit); Mean error, the mean of the errors in each patient; UTR, untwist rate.

4.5 DISCUSSION

The main findings of the present study were that individuals with T2D and diastolic dysfunction had normal LV peak UTR, twist rate and peak twist, but delayed time to peak UTR at rest, when compared to healthy individuals. Furthermore, we found that 12 weeks MIE and HIIE normalized time to peak UTR in T2D subjects. This is to our knowledge the first study to evaluate the effect of exercise training on LV twist parameters in T2D individuals with diastolic dysfunction.

4.5.1 Untwist and twist rate

The lack of difference between the control and T2D group in regard to LV rotation, twist and UTR are in accordance with Wang et al. (Wang et al., 2007) who found reduced LV twist and UTR in patients with LV systolic dysfunction and depressed EF, but not in those with diastolic dysfunction and normal EF. The onset of LV untwisting and the magnitude of peak untwisting velocities either remained normal or reduced and were significantly delayed (Wang et al., 2007). Our results also support Fonseca et al. (Fonseca et al., 2004) who compared healthy and T2D subjects (with abnormal filling pattern) with MRI and echocardiography; and found no difference in peak UTR between groups [34].

In the present T2D study population (also presented in Chapter 3), the majority of participants had moderate diastolic dysfunction. These findings of apparently normal LV twist and UTR in T2D support Park et al. (Park et al., 2008) who found that diastolic untwisting is increased in mild diastolic dysfunction, but normalized or reduced in individuals with more severe diastolic dysfunction.

4.5.2 Time to peak untwist rate

The finding of reduced time to peak UTR in the present study is consistent with several previous studies observing delayed early diastolic untwisting in states associated with worsened LV relaxation, such as in diastolic dysfunction with normal EF (Wang et al., 2007), diastolic dysfunction due to aortic stenosis (Stuber et al., 1999), severe LV hypertrophy (Takeuchi et al., 2007), and age (Nakai et al., 2006). Thus, the present study also support the suggestion of delayed untwist reflecting an ineffective relaxation of the ventricle (Notomi et al., 2007). However, due to different assessment modes and point of time in which timing is measured (i.e.

onset of untwist or time to peak UTR), and different patient groups, results are difficult to compare.

Nevertheless, timing seems to be important in diastolic dysfunction as delayed diastolic untwisting may be caused by less radial displacement during early diastolic period (Nakai et al., 2006). Wang et al. (Wang et al., 2007) also found that a subgroup of patients with diastolic dysfunction, preserved EF and delayed onset of untwisting also had delayed peak untwisting. Furthermore, Park et al. found that patients with HF, preserved systolic function and normal peak untwist may have delayed onset of untwist (Park et al., 2008; Wang et al., 2007). This indicates that time to peak UTR also reflects the onset of untwisting, which coincides with the onset of relaxation, preceding suction due to elastic recoil (Notomi et al., 2007). Furthermore, in individuals with diastolic dysfunction, preserved EF and delayed peak untwisting, peak untwist happened almost simultaneously with peak early mitral inflow velocity (E) (Wang et al., 2007). This indicate that time to peak UTR is a useful marker of early diastolic function, which potentially can serve as a therapeutic target for improving diastolic function.

The observation that exercise normalizes time to peak UTR is novel and highlights the usefulness of exercise training as an effective tool in secondary prevention in T2D individuals. The fact that time to peak UTR was the only diastolic variable measured, which became normalized after exercise training in T2D patients with diastolic dysfunction also indicate that time to peak UTR might be a more sensitive parameter than LV UTR to evaluate early diastolic changes. The magnitude of UTR is dependent on many factors such as change in preload, afterload and contractility (Sengupta et al., 2008), which makes it difficult to compare studies. For example, the magnitude of twist and untwist can substantially increase after short term exercise (Neilan et al., 2006) but can be reduced at rest in endurance-trained individuals after long-term exercise (Zocalo et al., 2007). The end-diastolic volume was larger among the diabetes group compared to the controls, but there was no difference in end-systolic volume. There was no change in end-diastolic or end-systolic volume after intervention, but both global strain rate and S' increased significantly after intervention indicating an increased contractility (Hollekim-Strand et al., 2014). An increased contractility would contribute to an increase in recoil and suction, and therefore, an increased relaxation rate that would contribute to an earlier onset of untwist. However, the effect of load is unknown.

Time to peak UTR has not been extensively used in the evaluation of diastolic function and other indices such as negative torsion acceleration incorporating UTR has been proposed to be

as useful and might be more reliable than time to peak UTR (Burns et al., 2008). However, this needs to be tested in future studies. Furthermore, the present results indicate that time to apical UTR has higher reproducibility than basal UTR. The differences in HR or frame rate between apical and basal short-axis images could result in an incorrect value of LV twist, which could be avoided by only using the apical short-axis images. Apical rotation by STE has earlier been suggested as a simplified index of LV twist, since apical rotation is the dominant contributor to LV twist (Opdahl et al., 2008).

Reduced diastolic function can be reversible, but early identification and treatment intervention is essential in order to prevent the development of HF. The time to peak, apical UTR appears to be sensitive, not only for identification, but also for follow-up after exercise training intervention.

LV UTR and time to peak UTR can be useful in studying the effects of suction on LV filling and the link between LV systolic and diastolic function and should be further studied.

4.5.3 Study limitations and strengths

Limitations include the relatively small sample size, and information bias due to self-reporting of exercise in the MIE group. Supervised exercise only in the HIIE group could have implied better compliance in this group. However, the improvement of diastolic function in the MIE group is in line with previous MIE studies (Brassard et al., 2007).

The control group had lower BMI than the exercise groups and was significantly younger than the HIIE group. However, there was no difference between groups in gender distribution and the difference in BMI was relatively small between groups (2.9 kg/m2). According to Aljaroudi et al. (Aljaroudi et al., 2012) the severity of diastolic dysfunction increases more with BMI >30 kg/m2 compared to 25-29.9 kg/m2. Thus, the relatively small difference in BMI between groups in the present paper can only be expected to have minor impact on results.

Nevertheless, the matching between the T2D group and controls is considered suboptimal in relation to age. Aging is associated with an increase in LV twist, mainly because of a gradual increase in apical rotation with age (Notomi et al., 2006). The increase in LV twist angle can be explained by less opposed apical rotation, resulting from a gradual decrease in subendocardial function with aging. Aging also reduce and delay diastolic untwisting (Takeuchi et al., 2006), and increasing age delays the onset of UTR (Lumens et al., 2006; Takeuchi et al., 2006), which might be associated with decreased myocardial compliance. However,

controversy exists regarding the observed reduction in untwist with aging (Hees et al., 2004; Oxenham et al., 2003). The reduction in early diastolic filling occurring with aging is thought to be partly a result of the presence of a partially rotated LV that impairs blood flow into the LV during early diastole. When adjusting for age for diastolic variables (e', E, A, IVRT, UTR and time to peak UTR) and S', using controls and baseline data for T2D, a significant increase with age was found for A ($p<0.001$) and decrease with age for e' ($p<0.001$) and S' ($p=0.001$). However, after adjusting for age, significant differences between groups (T2D vs. controls) were still found (mean differences -3.3, and -1.1 for e´ and S', respectively ($p<0.001$), and 0.1 for A ($p=0.01$)). Thus, suboptimal age matching does not explain all variability between groups for these variables. Furthermore, a previous study has shown no significant change with age in LV twist in healthy individuals between 31 and 60 years old (Kim et al., 2007).

Two-dimensional STE implies some methodological limitations, mostly related to image quality and through-plane motion of the myocardium since 2D STE cannot track motion occurring out of plane (see also Chapter 2, section 2.1.1.1). To ensure accuracy and compatibility of the echocardiographic recordings, the same operator conducted the recordings within each group, the levels of the basal and apical short axis views were well defined, equal frame rate and HR were used within each patient. Twist obtained in 2D echocardiography is imperfectly defined since the exact position of the image planes relative to the left ventricle and relative to each other is unknown and this off-plane limitation is critical in the short axis views (Saito et al., 2009). According to the latest task force recommendations both twist and torsion are poorly defined in 2D echocardiography and caution is urged in their use (Voigt et al., 2015). Furthermore, reverberations (stationary image artifacts) can impair the speckle recognition and if tracked they will interfere with the frame-by-frame tracking, resulting in drift or incorrect calculation of twist parameters (Ferferieva et al., 2009). The image quality of basal LV short-axis recordings can also be a limitation, because of acoustic problems related to the depth of the basal part of the ventricle and to the wide sector angle that is necessary to visualize the entire LV base. Measurements are also complicated by out-of-plane motion when the base descends toward the apex in systole.

LV UTR will be affected by changes in LV contractility, end systolic volume and LV preload (Bell et al., 2000; Moon et al., 1994; Wang et al., 2007). Exercise can affect both LV preload and afterload and we could not control for this variable without invasive measurements of loading conditions before and after intervention. Preload and afterload affect LV twist and untwist because of the changes in LV end-diastolic volume and end-systolic volume. For

example, increased preload reduces early UTR, while increased afterload delays the onset of UTR (Dong et al., 1999; Hansen et al., 1991; MacGowan et al., 1996). Furthermore, an increase in contractility will increase early UTR (Gibbons Kroeker et al., 1995; Hansen et al., 1991; Moon et al., 1994).

Optimal image quality and acquisition of standardized image planes are essential for a low inter- and intraobserver variability. To ensure a high reproducibility, interobserver variability in imaging acquisition (test-retest) should have been provided in this study. Temporal normalization and interpolation of the raw data were not performed and could have influenced the calculation of twist and twist rates since basal and apical images are acquired at different cardiac cycles. However, time to apical UTR would not have been affected. During the acquisition, the recordings of the basal and apical short-axis images were repeated until the same heartrate was achieved and a difference in HR of more than five beats was detected in only four individuals.

In four individuals there was a difference in HR of more than five beats between the apical and basal recordings, which may have led to inaccuracy of the final results.
Strengths of this study are the prospective study design of the intervention, high compliance to exercise, blinded data analysis and a high reproducibility for major LV twist variables.

4.6 CONCLUSION

In individuals with T2D and diastolic dysfunction, LV twist parameters were similar to healthy subjects except for time to peak UTR, which was delayed at baseline. Both MIE and HIIE normalized time to peak UTR after 12 weeks in individuals with T2D and diastolic dysfunction.

REFERENCES

Aase, S. A., Torp, H., & Stoylen, A. (2008). Aortic valve closure: relation to tissue velocities by Doppler and speckle tracking in normal subjects. *Eur. J. Echocardiogr., 9*(4), 555-559.

Aljaroudi, W., Halley, C., Houghtaling, P., Agarwal, S., Menon, V., Rodriguez, L., . . . Jaber, W. A. (2012). Impact of body mass index on diastolic function in patients with normal left ventricular ejection fraction. *Nutr. Diabetes, 2*, e39.

Arts, T., Veenstra, P. C., & Reneman, R. S. (1982). Epicardial deformation and left ventricular wall mechanisms during ejection in the dog. *Am. J. Physiol., 243*(3), H379-390.

Bell, S. P., Nyland, L., Tischler, M. D., McNabb, M., Granzier, H., & LeWinter, M. M. (2000). Alterations in the determinants of diastolic suction during pacing tachycardia. *Circ. Res., 87*(3), 235-240.

Boule, N. G., Kenny, G. P., Haddad, E., Wells, G. A., & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia, 46*(8), 1071-1081.

Brassard, P., Legault, S., Garneau, C., Bogaty, P., Dumesnil, J. G., & Poirier, P. (2007). Normalization of diastolic dysfunction in type 2 diabetics after exercise training. *Med. Sci. Sports Exerc., 39*(11), 1896-1901.

Burns, A. T., La Gerche, A., MacIsaac, A. I., & Prior, D. L. (2008). Augmentation of left ventricular torsion with exercise is attenuated with age. *J. Am. Soc. Echocardiogr., 21*(4), 315-320.

Burns, A. T., La Gerche, A., Prior, D. L., & Macisaac, A. I. (2009). Left ventricular untwisting is an important determinant of early diastolic function. *JACC Cardiovasc. Imaging, 2*(6), 709-716.

Dalen, H., Thorstensen, A., Romundstad, P. R., Aase, S. A., Stoylen, A., & Vatten, L. J. (2011). Cardiovascular risk factors and systolic and diastolic cardiac function: a tissue Doppler and speckle tracking echocardiographic study. *J. Am. Soc. Echocardiogr., 24*(3), 322-332 e326.

Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A., & Stoylen, A. (2010). Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ. Cardiovasc. Imaging, 3*(5), 614-622.

De Luca, A., Stefani, L., Pedrizzetti, G., Pedri, S., & Galanti, G. (2011). The effect of exercise training on left ventricular function in young elite athletes. *Cardiovasc. Ultrasound, 9*, 27.

DeAnda, A., Jr., Komeda, M., Nikolic, S. D., Daughters, G. T., 2nd, Ingels, N. B., & Miller, D. C. (1995). Left ventricular function, twist, and recoil after mitral valve replacement. *Circulation, 92*(9 Suppl), II458-466.

Di Bonito, P., Moio, N., Cavuto, L., Covino, G., Murena, E., Scilla, C., . . . Sibilio, G. (2005). Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging. *Diabet. Med., 22*(12), 1720-1725.

Dong, S. J., Hees, P. S., Huang, W. M., Buffer, S. A., Jr., Weiss, J. L., & Shapiro, E. P. (1999). Independent effects of preload, afterload, and contractility on left ventricular torsion. *Am. J. Physiol., 277*(3 Pt 2), H1053-1060.

Dong, S. J., Hees, P. S., Siu, C. O., Weiss, J. L., & Shapiro, E. P. (2001). MRI assessment of LV relaxation by untwisting rate: a new isovolumic phase measure of tau. *Am. J. Physiol. Heart Circ. Physiol., 281*(5), H2002-2009.

Fang, Z. Y., Schull-Meade, R., Downey, M., Prins, J., & Marwick, T. H. (2005). Determinants of subclinical diabetic heart disease. *Diabetologia, 48*(2), 394-402.

Ferferieva, V., Claus, P., Vermeulen, K., Missant, C., Szulik, M., Rademakers, F., & D'Hooge, J. (2009). Echocardiographic assessment of left ventricular untwist rate: comparison of tissue Doppler and speckle tracking methodologies. *Eur. J. Echocardiogr., 10*(5), 683-690.

Fonseca, C. G., Dissanayake, A. M., Doughty, R. N., Whalley, G. A., Gamble, G. D., Cowan, B. R., . . . Young, A. A. (2004). Three-dimensional assessment of left ventricular systolic strain in patients with type 2 diabetes mellitus, diastolic dysfunction, and normal ejection fraction. *Am. J. Cardiol., 94*(11), 1391-1395.

Fuchs, E., Muller, M. F., Oswald, H., Thony, H., Mohacsi, P., & Hess, O. M. (2004). Cardiac rotation and relaxation in patients with chronic heart failure. *Eur. J. Heart Fail., 6*(6), 715-722.

Gibbons Kroeker, C. A., Tyberg, J. V., & Beyar, R. (1995). Effects of load manipulations, heart rate, and contractility on left ventricular apical rotation. An experimental study in anesthetized dogs. *Circulation, 92*(1), 130-141.

Hansen, D. E., Daughters, G. T., 2nd, Alderman, E. L., Ingels, N. B., Stinson, E. B., & Miller, D. C. (1991). Effect of volume loading, pressure loading, and inotropic stimulation on left ventricular torsion in humans. *Circulation, 83*(4), 1315-1326.

Hare, J. L., Hordern, M. D., Leano, R., Stanton, T., Prins, J. B., & Marwick, T. H. (2011). Application of an exercise intervention on the evolution of diastolic dysfunction in patients with diabetes mellitus: efficacy and effectiveness. *Circ. Heart Fail., 4*(4), 441-449.

Hees, P. S., Fleg, J. L., Dong, S. J., & Shapiro, E. P. (2004). MRI and echocardiographic assessment of the diastolic dysfunction of normal aging: altered LV pressure decline or load? *Am. J. Physiol. Heart Circ. Physiol., 286*(2), H782-788.

Helle-Valle, T., Crosby, J., Edvardsen, T., Lyseggen, E., Amundsen, B. H., Smith, H. J., . . . Smiseth, O. A. (2005). New noninvasive method for assessment of left ventricular rotation: speckle tracking echocardiography. *Circulation, 112*(20), 3149-3156.

Hollekim-Strand, S. M., Bjorgaas, M. R., Albrektsen, G., Tjonna, A. E., Wisloff, U., & Ingul, C. B. (2014). High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: a randomized controlled trial. *J. Am. Coll. Cardiol., 64*(16), 1758-1760.

Hordern, M. D., Coombes, J. S., Cooney, L. M., Jeffriess, L., Prins, J. B., & Marwick, T. H. (2009). Effects of exercise intervention on myocardial function in type 2 diabetes. *Heart, 95*(16), 1343-1349.

Ingul, C. B., Tjonna, A. E., Stolen, T. O., Stoylen, A., & Wisloff, U. (2010). Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch. Pediatr. Adolesc. Med., 164*(9), 852-859.

International Diabetes Federation. (2012). *Global Guideline for Type 2 Diabetes* Available at: http://www.idf.org/sites/default/files/IDF%20T2DM%20Guideline.pdf Accessed: October, 2015.

International Diabetes Federation. (2013). *IDF Diabetes Atlas 6th ed. 2013*. Available at: https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf Accessed September, 2015.

Kim, H. K., Sohn, D. W., Lee, S. E., Choi, S. Y., Park, J. S., Kim, Y. J., . . . Choi, Y. S. (2007). Assessment of left ventricular rotation and torsion with two-dimensional speckle tracking echocardiography. *J. Am. Soc. Echocardiogr., 20*(1), 45-53.

Leitman, M., Lysyansky, P., Sidenko, S., Shir, V., Peleg, E., Binenbaum, M., . . . Vered, Z. (2004). Two-dimensional strain-a novel software for real-time quantitative echocardiographic assessment of myocardial function. *J. Am. Soc. Echocardiogr., 17*(10), 1021-1029.

Lorenz, C. H., Pastorek, J. S., & Bundy, J. M. (2000). Delineation of normal human left ventricular twist throughout systole by tagged cine magnetic resonance imaging. *J. Cardiovasc. Magn. Reson., 2*(2), 97-108.

Lumens, J., Delhaas, T., Arts, T., Cowan, B. R., & Young, A. A. (2006). Impaired subendocardial contractile myofiber function in asymptomatic aged humans, as detected using MRI. *Am. J. Physiol. Heart Circ. Physiol., 291*(4), H1573-1579.

MacGowan, G. A., Burkhoff, D., Rogers, W. J., Salvador, D., Azhari, H., Hees, P. S., . . . Shapiro, E. P. (1996). Effects of afterload on regional left ventricular torsion. *Cardiovasc. Res., 31*(6), 917-925.

Mitka, M. (2013). Study: Exercise may match medication in reducing mortality associated with cardiovascular disease, diabetes. *JAMA, 310*(19), 2026-2027.

Molmen, H. E., Wisloff, U., Aamot, I. L., Stoylen, A., & Ingul, C. B. (2012). Aerobic interval training compensates age related decline in cardiac function. *Scand. Cardiovasc. J., 46*(3), 163-171.

Moon, M. R., Ingels, N. B., Jr., Daughters, G. T., 2nd, Stinson, E. B., Hansen, D. E., & Miller, D. C. (1994). Alterations in left ventricular twist mechanics with inotropic stimulation and volume loading in human subjects. *Circulation, 89*(1), 142-150.

Mor-Avi, V., Lang, R. M., Badano, L. P., Belohlavek, M., Cardim, N. M., Derumeaux, G., . . . Zamorano, J. L. (2011). Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. *Eur. J. Echocardiogr., 12*(3), 167-205.

Nakai, H., Takeuchi, M., Nishikage, T., Kokumai, M., Otani, S., & Lang, R. M. (2006). Effect of aging on twist-displacement loop by 2-dimensional speckle tracking imaging. *J. Am. Soc. Echocardiogr., 19*(7), 880-885.

Neilan, T. G., Ton-Nu, T. T., Jassal, D. S., Popovic, Z. B., Douglas, P. S., Halpern, E. F., . . . Wood, M. J. (2006). Myocardial adaptation to short-term high-intensity exercise in highly trained athletes. *J. Am. Soc. Echocardiogr., 19*(10), 1280-1285.

Nichols, G. A., Gullion, C. M., Koro, C. E., Ephross, S. A., & Brown, J. B. (2004). The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes Care, 27*(8), 1879-1884.

Notomi, Y., Lysyansky, P., Setser, R. M., Shiota, T., Popovic, Z. B., Martin-Miklovic, M. G., . . . Thomas, J. D. (2005). Measurement of ventricular torsion by two-dimensional ultrasound speckle tracking imaging. *J. Am. Coll. Cardiol., 45*(12), 2034-2041.

Notomi, Y., Martin-Miklovic, M. G., Oryszak, S. J., Shiota, T., Deserranno, D., Popovic, Z. B., . . . Thomas, J. D. (2006). Enhanced ventricular untwisting during exercise: a mechanistic manifestation of elastic recoil described by Doppler tissue imaging. *Circulation, 113*(21), 2524-2533.

Notomi, Y., Popovic, Z. B., Yamada, H., Wallick, D. W., Martin, M. G., Oryszak, S. J., . . . Thomas, J. D. (2007). Ventricular untwisting: a temporal link between left ventricular relaxation and suction. *Am. J. Physiol. Heart Circ. Physiol., 294*(1), H505-513.

Oh, J. K., Hatle, L., Tajik, A. J., & Little, W. C. (2006). Diastolic heart failure can be diagnosed by comprehensive two-dimensional and Doppler echocardiography. *J. Am. Coll. Cardiol., 47*(3), 500-506.

Opdahl, A., Helle-Valle, T., Remme, E. W., Vartdal, T., Pettersen, E., Lunde, K., . . . Smiseth, O. A. (2008). Apical rotation by speckle tracking echocardiography: a simplified bedside index of left ventricular twist. *J. Am. Soc. Echocardiogr., 21*(10), 1121-1128.

Opdahl, A., Remme, E. W., Helle-Valle, T., Edvardsen, T., & Smiseth, O. A. (2012). Myocardial relaxation, restoring forces, and early-diastolic load are independent determinants of left ventricular untwisting rate. *Circulation, 126*(12), 1441-1451.

Oxenham, H. C., Young, A. A., Cowan, B. R., Gentles, T. L., Occleshaw, C. J., Fonseca, C. G., . . . Sharpe, N. (2003). Age-related changes in myocardial relaxation using three-dimensional tagged magnetic resonance imaging. *J. Cardiovasc. Magn. Reson., 5*(3), 421-430.

Park, S. J., Miyazaki, C., Bruce, C. J., Ommen, S., Miller, F. A., & Oh, J. K. (2008). Left ventricular torsion by two-dimensional speckle tracking echocardiography in patients with diastolic dysfunction and normal ejection fraction. *J. Am. Soc. Echocardiogr., 21*(10), 1129-1137.

Poirier, P., Bogaty, P., Garneau, C., Marois, L., & Dumesnil, J. G. (2001). Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. *Diabetes Care, 24*(1), 5-10.

Quinones, M. A. (2005). Assessment of diastolic function. *Prog. Cardiovasc. Dis., 47*(5), 340-355.

R Core Team. (2014). *R: A Language and Environment for Statistical Computing*: R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Rademakers, F. E., Buchalter, M. B., Rogers, W. J., Zerhouni, E. A., Weisfeldt, M. L., Weiss, J. L., & Shapiro, E. P. (1992). Dissociation between left ventricular untwisting and filling. Accentuation by catecholamines. *Circulation, 85*(4), 1572-1581.

Redfield, M. M., Jacobsen, S. J., Burnett, J. C., Jr., Mahoney, D. W., Bailey, K. R., & Rodeheffer, R. J. (2003). Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA, 289*(2), 194-202.

Rognmo, O., Hetland, E., Helgerud, J., Hoff, J., & Slordahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur. J. Cardiovasc. Prev. Rehabil., 11*(3), 216-222.

Rognmo, O., Moholdt, T., Bakken, H., Hole, T., Molstad, P., Myhr, N. E., . . . Wisloff, U. (2012). Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. *Circulation, 126*(12), 1436-1440.

Saito, K., Okura, H., Watanabe, N., Hayashida, A., Obase, K., Imai, K., . . . Yoshida, K. (2009). Comprehensive evaluation of left ventricular strain using speckle tracking echocardiography in normal adults: comparison of three-dimensional and two-dimensional approaches. *J. Am. Soc. Echocardiogr., 22*(9), 1025-1030.

Salehi, H. R., Syed, W., Sugeng, L., Patel, A., & Pandian, N. (2014). Early diastolic untwist rate for assesment of diastolic function; A three-dimensional speckle tracking echocardiography study in patients with hypertension and hypertrophic ventricles with preserved ejection fraction. *JACC Cardiovasc. Imaging, 63*(12).

Sengupta, P. P., Tajik, A. J., Chandrasekaran, K., & Khandheria, B. K. (2008). Twist mechanics of the left ventricle: principles and application. *JACC Cardiovasc. Imaging, 1*(3), 366-376.

Stuber, M., Scheidegger, M. B., Fischer, S. E., Nagel, E., Steinemann, F., Hess, O. M., & Boesiger, P. (1999). Alterations in the local myocardial motion pattern in patients suffering from pressure overload due to aortic stenosis. *Circulation, 100*(4), 361-368.

Takeuchi, M., Borden, W. B., Nakai, H., Nishikage, T., Kokumai, M., Nagakura, T., . . . Lang, R. M. (2007). Reduced and delayed untwisting of the left ventricle in patients with hypertension and left ventricular hypertrophy: a study using two-dimensional speckle tracking imaging. *Eur. Heart J., 28*(22), 2756-2762.

Takeuchi, M., Nakai, H., Kokumai, M., Nishikage, T., Otani, S., & Lang, R. M. (2006). Age-related changes in left ventricular twist assessed by two-dimensional speckle-tracking imaging. *J. Am. Soc. Echocardiogr., 19*(9), 1077-1084.

The Norwegian Diabetes Association. Type 2-diabetes og fysisk aktivitet.

Thorstensen, A., Dalen, H., Amundsen, B. H., Aase, S. A., & Stoylen, A. (2010). Reproducibility in echocardiographic assessment of the left ventricular global and regional function, the HUNT study. *Eur. J. Echocardiogr., 11*(2), 149-156.

Tjonna, A. E., Lee, S. J., Rognmo, O., Stolen, T. O., Bye, A., Haram, P. M., . . . Wisloff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation, 118*(4), 346-354.

Voigt, J. U., Pedrizzetti, G., Lysyansky, P., Marwick, T. H., Houle, H., Baumann, R., . . . Badano, L. P. (2015). Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *Eur. Heart J. Cardiovasc. Imaging, 16*(1), 1-11.

Von Bibra, H., Thrainsdottir, I. S., Hansen, A., Dounis, V., Malmberg, K., & Ryden, L. (2005). Tissue Doppler imaging for the detection and quantitation of myocardial dysfunction in patients with type 2 diabetes mellitus. *Diab. Vasc. Dis. Res., 2*(1), 24-30.

Wang, J., Khoury, D. S., Yue, Y., Torre-Amione, G., & Nagueh, S. F. (2007). Left ventricular untwisting rate by speckle tracking echocardiography. *Circulation, 116*(22), 2580-2586.

Weiner, R. B., DeLuca, J. R., Wang, F., Lin, J., Wasfy, M. M., Berkstresser, B., . . . Baggish, A. L. (2015). Exercise-Induced Left Ventricular Remodeling Among Competitive Athletes: A Phasic Phenomenon. *Circ. Cardiovasc. Imaging, 8*(12).

Weiner, R. B., Hutter, A. M., Jr., Wang, F., Kim, J., Weyman, A. E., Wood, M. J., . . . Baggish, A. L. (2010). The impact of endurance exercise training on left ventricular torsion. *JACC Cardiovasc. Imaging, 3*(10), 1001-1009.

Weston, K. S., Wisloff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med., 48*(16), 1227-1234.

Wu, Y., & Kovacs, S. J. (2006). Frequency-based analysis of the early rapid filling pressure-flow relation elucidates diastolic efficiency mechanisms. *Am. J. Physiol. Heart Circ. Physiol., 291*(6), H2942-2949.

Zabalgoitia, M., Ismaeil, M. F., Anderson, L., & Maklady, F. A. (2001). Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol., 87*(3), 320-323.

Zocalo, Y., Bia, D., Armentano, R. L., Arias, L., Lopez, C., Etchart, C., & Guevara, E. (2007). Assessment of training-dependent changes in the left ventricle torsion dynamics of professional soccer players using speckle-tracking echocardiography. *Conf. Proc. IEEE Eng. Med. Biol. Soc., 2007*, 2709-2712.

CHAPTER 5

The effect of two different types of acute exercise 16-18 hours prior to a fast food meal on postprandial cardiac function in individuals with type 2 diabetes and healthy counterparts

TABLE OF CONTENTS

CHAPTER 5

5.1 ABSTRACT

Background Type 2 diabetes (T2D) aggravates the postprandial metabolic effects of food, which contributes to reduced glycemic control and increased cardiovascular risk, including risk of heart failure. Fluctuating excessive elevations in circulating glucose, triglycerides and free-fatty acids throughout the day is highly prevalent in T2D, but there is a lack of knowledge about how excess postprandial circulating glucose and triglycerides levels induced by a single meal acutely influence postprandial cardiac workload. **Objectives** To study the acute effects of an energy-dense meal rich in processed carbohydrates and saturated fats (fast food) on postprandial left ventricular (LV) workload and to investigate whether a single bout of pre-exercise can modulate potential fast-food induced changes in postprandial cardiac workload in T2D individuals. **Methods** This crossover study includes 10 T2D individuals (7 males and 3 females; 53.4±8.1 years; 28.3±3.8 kg/m2; T2D duration 3.1±1.8 years) and 10 controls (7 males and 3 females; 52.8±10.1 years; 28.5±4.2 kg/m2) performing high-intensity interval exercise (HIIE; 40 min, 4 × 4 min work bouts, 90–95% HRmax), moderate-intensity exercise (MIE; 47 min, 70% HRmax), and no exercise (NE) in a random order 16–18 hours prior to fast food ingestion. Baseline echocardiography, blood pressure, and biochemical measurements were recorded prior to and 16–18 hours after exercise, and 30 minutes, two hours, and four hours after fast food ingestion. **Results** LV diastolic (peak early diastolic tissue Doppler velocity [e'], peak early diastolic mitral inflow velocity [E]), and systolic workload (global strain rate, peak systolic tissue Doppler velocity [S'], rate pressure product) increased after consumption of fast food in both groups. In contrast to controls, the T2D group had prolonged elevations in resting heart rate (HR) and indications of prolonged elevations in diastolic workload (e') as well as reduced systolic blood pressure after fast food consumption. No significant modifications due to exercise in the postprandial phase were seen in any group. **Conclusions** Our findings indicate that fast food induces increased postprandial cardiac workload in both T2D individuals and body mass index and age-matched controls. The postprandial increase in resting HR was prolonged in T2D individuals versus controls. Exercise 16–18 hours pre-meal had no acute effects on the postprandial phase. The acute interaction of food on cardiac workload and the effect of acute exercise in T2D need further study.

5.2 INTRODUCTION

The following manuscript (Paper III) is based on this chapter:

Hollekim-Strand, S.M.; Malmo, V.; Follestad, T.; Wisløff, U.; Ingul, C.B.
Fast food increases postprandial cardiac workload in type 2 diabetes independent of pre-exercise: A pilot study. **Nutr J**, 2015. 14(1): p. 79-90.

Most of the day is spent in the postprandial state and frequent ingestion of energy-dense food that is rich in processed carbohydrates and saturated fats (fast food) increases the risk of cardiometabolic diseases (Cavalot et al., 2011; Eberly et al., 2003; Pereira et al., 2005).

Type 2 diabetes (T2D) aggravates the postprandial metabolic effects of food (Stephenson et al., 2014). Fluctuating excessive elevations in circulating glucose, triglycerides, and free-fatty acids (hereafter referred to as postprandial dysmetabolism), which is associated with an increased risk of cardiovascular disease, is thus highly prevalent in T2D (Bansal et al., 2007; Cavalot et al., 2011; Cavalot et al., 2006; Ceriello et al., 2008; Coutinho et al., 1999; Garber, 2012; Meigs et al., 2002; Monnier et al., 2006; Nordestgaard et al., 2007; O'Keefe and Bell, 2007; van Dijk et al., 2011). Furthermore, postprandial hyperglycemia contributes to the overall glycemic control in T2D individuals (Monnier et al., 2003), which when increased (measured as glycosylated hemoglobin, HbA_{1c}) is associated with increased risk of heart failure (HF) (de Simone et al., 2010; Iribarren et al., 2001). Consequently, postprandial dysmetabolism is considered an important treatment target in T2D, although treatment-target guidelines in regard to these factors in T2D have not been fully addressed (Boren et al., 2014; Ryden et al., 2013). Indeed, long-term improvements in postprandial metabolic control (induced by anti-diabetic medication) have been shown to reduce cardiovascular risk in individuals with impaired glucose tolerance (Chiasson, 2006). Thus, there is a need for exercise physiologists to search for an exercise prescription (i.e. timing in relation to meals, duration, and intensity) to produce improved postprandial metabolic control in T2D. Furthermore, there is a need to better understand the acute consequences of postprandial dysmetabolism in regard to the cardiovascular system, to target exercise prescriptions to modify these potential consequences in every single exercise bout, and thus possibly add to the effect of chronic exercise training.

Indeed, there is a lack of knowledge concerning the direct role of postprandial dysmetabolism on cardiac workload in T2D. Interestingly, von Bibra et al. (2009) observed improved diastolic function (measured as peak early diastolic tissue Doppler velocity, e') and improved vascular

function in T2D individuals when postprandial glycemic control was improved (after insulin administration) in the long term (von Bibra et al., 2009). The few studies that have investigated acute postprandial cardiac function have shown that diastolic function can be altered in the postprandial phase after carbohydrate ingestion in T2D individuals (von Bibra et al., 2013), and that acute elevations in circulating triglycerides can give compensatory increase in left ventricular (LV) systolic workload in healthy individuals (Holland et al., 2011).

Previous studies have also reported acute effects of fast food on vascular function. These studies indirectly indicate that cardiac workload can be affected acutely by a meal. It has been shown that endothelial function can be impaired due to acute increases in circulating glucose, triglycerides, or elevated oxidative stress (Ceriello et al., 2004; Gill et al., 2004; Ikeda et al., 2015; Kobayashi et al., 2015; Tyldum et al., 2009). For example, postprandial hyperglycemia has been shown to reduce coronary microvascular function in T2D individuals (Ikeda et al., 2015) and increased arterial stiffness has been observed after glucose ingestion in untrained men (Kobayashi et al., 2015). Furthermore, an excessive increase in circulating triglycerides has been associated with reduced endothelial function in the hours after a fast food meal (Gill et al., 2004; Tyldum et al., 2009).

Regular exercise has the potential to improve cardiac function (Chapters 3-4), and even single bouts of exercise can reduce postprandial glucose elevations in patients with T2D and improve endothelial function, triglycerides, and/or oxidative stress in healthy individuals (Gillen et al., 2012; Karstoft et al., 2014; Tyldum et al., 2009). For example, a single bout of exercise undertaken in conjunction with meals can impact blood glucose and insulin sensitivity in the short term, which may even last into the next day (Colberg et al., 2010; Francois et al., 2014; Gillen et al., 2012; Little et al., 2011). The molecular mechanism for the improvements in glucose metabolism after acute exercise is not entirely understood, but exercise before a meal may promote glucose transporter (GLUT4) translocation to the surface of the myocytes to increase glucose uptake and thus reduce the levels of circulating glucose (Holloszy, 2003) (see Chapter 1; section 1.1.6.2). Interestingly, high-intensity interval exercise (HIIE) appears to be a time efficient method to reduce postprandial glucose excursions and improve insulin sensitivity compared to moderate-intensity exercise (MIE) (Francois et al., 2014; Gillen et al., 2012; Hawley and Gibala, 2009). Furthermore, acute exercise may modify postprandial lipemic response (Burns et al., 2015; Gill et al., 2003; Petitt and Cureton, 2003), which appears to last at least 16 hours post-exercise and may prevent fatty acid induced insulin resistance even the day after exercise (Schenk, 2007). Furthermore, a single bout of MIE and HIIE 16-18 hours

pre-meal has been previously shown to induce improvements in total antioxidant status (TAS) and endothelial function rather than reduce the circulating glucose or triglycerides (Tyldum et al., 2009). In this setting, HIIE was more effective in improving postprandial endothelial function than MIE (Tyldum et al., 2009). However, no study has investigated whether a potential fast-food induced change in cardiac function can be modified by pre-exercise as observed for endothelial function.

The purpose of this study was thus to explore whether a single fast food meal affects LV function in the four-hour postprandial phase and whether exercise (HIIE or MIE) 16-18 hours prior to a single fast food meal could modulate LV workload, resting heart rate (HR), blood pressure, circulating glucose, triglycerides, and other biochemical measures in individuals with T2D.

Aim

To investigate the acute effects of fast food on LV diastolic function (peak early diastolic tissue Doppler velocity, e'; peak late diastolic tissue Doppler velocity, a'; peak diastolic early mitral inflow velocity, E; late diastolic mitral inflow velocity, A; filling pressure, E/e'; E/A-ratio; isovolumic relaxation time, IVRT; deceleration time, DT), systolic function (peak systolic tissue Doppler velocity, S'; global strain; strain rate), HR, and blood pressure, and to explore whether pre-exercise of high intensity or moderate intensity could modulate putative effects to these outcome measures and to biochemical measures (circulating glucose; triglycerides; total cholesterol; low density lipoprotein, LDL; high density lipoprotein, HDL; c-peptide; TAS; high sensitive c-reactive protein, hs-CRP) in T2D individuals versus nondiabetic counterparts.

Hypotheses

a. Fast food induces greater diastolic workload in the postprandial phase in T2D individuals than in controls.
b. Fast food induces a greater systolic workload in the postprandial phase in T2D individuals than in controls.
c. A single bout of HIIE or MIE 16 to 18 hours before fast food ingestion modulates postprandial cardiac response in T2D individuals and controls.
d. A single bout of HIIE or MIE 16 to 18 hours before fast food ingestion modulates postprandial response in biochemical measures in T2D individuals.

5.3 MATERIAL AND METHODS

5.3.1 Study design

This study was a two-group crossover trial that investigated the acute effects of fast food on LV cardiac workload, blood pressure, HR, and biochemical measurements in T2D versus healthy counterparts. This study also investigated the potential effects of pre-exercise to the postprandial state after fast food ingestion.

5.3.2 Participants

The participants were recruited through the local newspaper (*Adresseavisen*, Trondheim) and advertisement at St. Olav's University Hospital, Trondheim, Norway. The study was performed from February to June 2012. The inclusion and exclusion criteria are presented in Table 5.1 and characteristics for the included participants are presented in Table 5.2.

5.3.3 Study intervention

The intervention protocol was based on the study of Tyldum et al. (Tyldum et al., 2009).

The participants performed one exercise bout of HIIE, MIE, or no exercise (NE) with at least one week of wash out between the three intervention periods. In addition, the participants ingested a fast food meal 16 to 18 hours after exercise (Figure 5.1).

Table 5.1. Inclusion and exclusion criteria for the participants described in Chapter 5.

Paper	Study population	N	Eligibility criteria	Exclusion criteria
III	T2D individuals	10	Age 20–65 T2D duration < 10 yrs.	-Overt CVD -Atrial fibrillation or other significant cardiac arrhythmia -Untreated hypertension -Left ventricular EF <40% -Body mass index >35 kg/m^2 -Diabetic retinopathy or neuropathy -Albuminuria -Drug or alcohol abuse -Pregnancy -Unable to exercise -Habitual exercise > guidelines for patients with T2D.
	Healthy individuals	10	Age, BMI, and gender matched with T2D group	-Diabetes -Untreated hypertension -Known CVD -Habitual exercise > national exercise guidelines

Abbreviations: CVD, cardiovascular disease; EF, ejection fraction; T2D, type 2 diabetes.

Table 5.2 Characteristics of the study participants described in Chapter 5.

	Paper III	
Group allocation	T2D MIE HIIE NE	Healthy MIE HIIE NE
N	10	10
Female	3 (30.0)	3 (30.0)
Age, yrs.	53.4±8.1	52.8±10.1
T2D-duration	3.1±1.8	-
Body mass, kg	87.7±19.1	87.7±15.2
BMI, kg/m^2	28.3±3.8	28.5±4.2
WC, cm	107.1±27.5	104.4±13.8
Female, ≥80cm	3 (100)	2 (67)
Male, ≥94cm	3 (43)	7 (100)
HbA_{1c}		
%	6.4±1.0	5.5±0.2
mmol/mol	46.0±7.2	37.0±1.3

Results are presented as mean±SD or No. (%). Abbreviations: BMI, body mass index; HbA1c, glycosylated hemoglobin; HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise; NE, no exercise (sedate behavior); T2D, type 2 diabetes; WC, waist circumference.

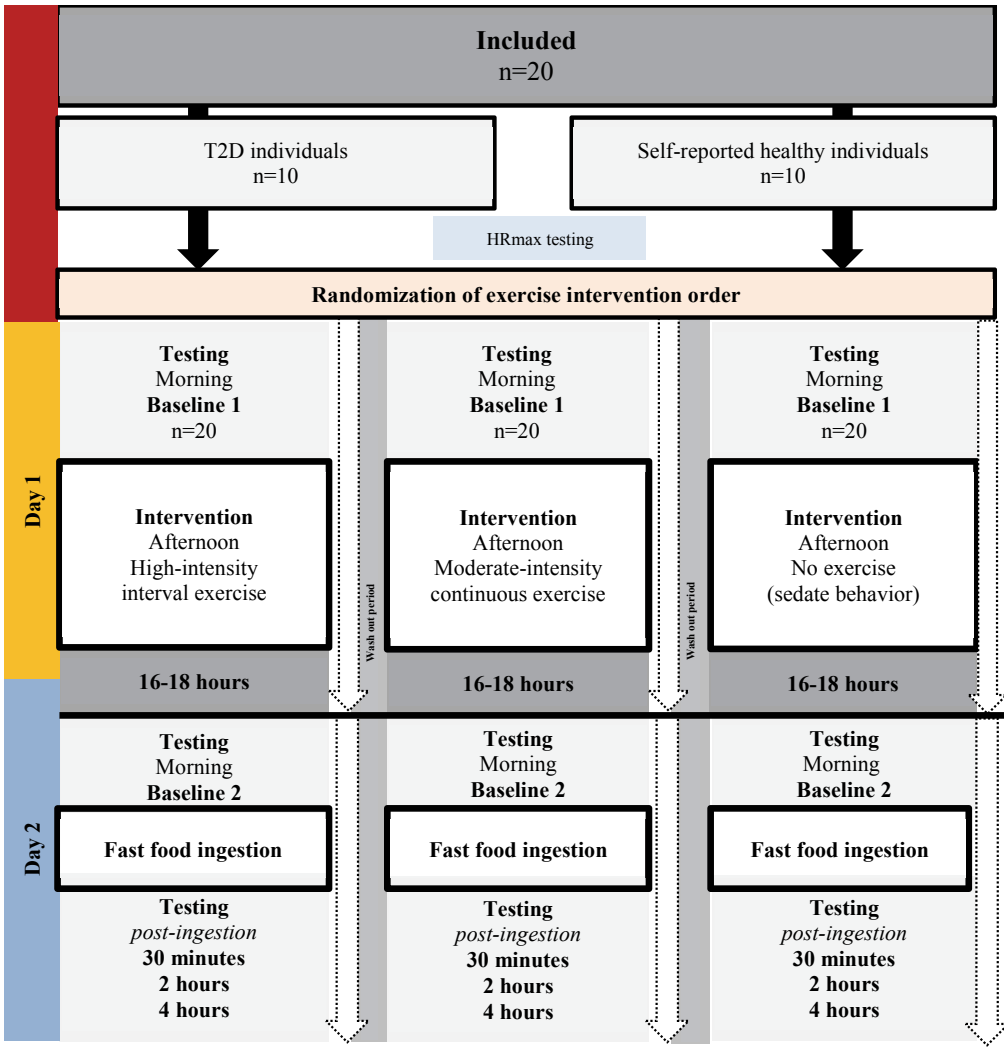


Figure 5.1. Flow chart of intervention and participants described in Chapter 5.

5.3.3.1 The exercise protocols

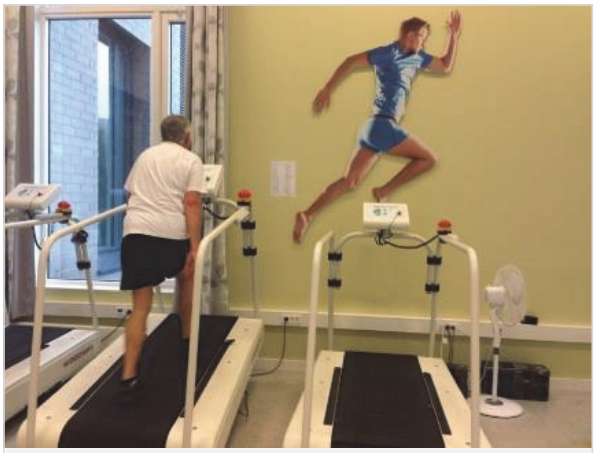
High-intensity interval exercise

HIIE was performed by walking, jogging, or running on an inclined treadmill at our laboratory. The HIIE session comprised a 10-minute warm up at 70% of HR_{max}, four 4-minute work bouts at 90-95% of HR_{max} with three minutes active recovery between work bouts at 70% of HR_{max}. The HIIE bout lasted 40 minutes and is described in detail in Chapter 2 (section 2.1.2.1). The participants were instructed not to perform any moderate intensity or strenuous physical activity or exercise 48 hours prior to the exercise bout performed on Day 2 (Figure 5.1).

Moderate-intensity exercise (MIE)

MIE was performed by walking or jogging on an inclined treadmill (at our laboratory) at 70% of HR_{max} and the MIE bout lasted for 47 minutes. The participants were instructed not to perform any moderate intensity or strenuous physical activity or exercise 48 hours prior to the exercise bout performed on Day 2 (Figure 5.1).

The exercise protocols were designed to be approximately isoenergetic according to the calculation procedure previously explained by Rognmo et al. (Rognmo et al., 2004). When using the mean baseline VO_{2peak} values of the present study (~3.40 L/min) in the calculations (Rognmo et al., 2004), the participants consumed ~3.06 liters of O_2 per minute at 90% of VO_{2peak} and ~2.04 liters of O_2 per minute at 60% of VO_{2peak}. Considering that every liter of O_2 consumed approximates the expenditure of 5 kcal (McArdle et al., 2010), the total kcal expenditure for the HIIE bout (including warm up, work bouts, active recovery, and cool down) would amount to ~490 kcal and the total kcal expenditure for the 47-minute continuous MIE bout at 60% of VO_{2peak} would amount to ~480 kcal. So, in theory, we can assume that the exercise protocols were approximately isoenergetic, as stated in Paper III.



Participants in performed HIIE and MIE by walking, jogging or running on an inclined treadmill in our training laboratory.

Exercise intensity control

During the HIIE and MIE sessions, participants exercised with HR monitors (Polar RS 400, Polar Electro, Kempele Finland) to ensure that the required exercise intensity was achieved and maintained. Participants were continuously supervised by a trained exercise physiologist to ensure that target HRs were achieved at all times.

No exercise (NE)

In particular, participants were instructed not to perform any physical activity in the period 16-18 hours prior to fast food ingestion. In this period, they were encouraged to watch TV, read a book, or to perform other activities that do not require anything but sitting or lying down. The participants were instructed not to perform any moderate intensity or strenuous physical activity or exercise at all 48 hours prior to Day 2 (Figure 5.1).

No intensity control was performed to ensure that these requirements were met, except self-reporting on Day 2 (Figure 5.1).

5.3.3.2 Fast food ingestion

The participants ingested a pizza previously used in a trial using the same protocol with healthy individuals of normal weight (Tyldum et al., 2009). The vegetarian mozzarella pizza (Dr. Oetker) consisted of: 335g (874kcal/ 3655kJ), 83.4g carbohydrates, 44.2g fat, and 34.8g protein (5.6g fiber and 1.4g sodium; salt equivalent 3.5g). The meal in the evening on day 1, prior to fasting before fast food ingestion on day 2 (Figure 10), had the same content (energy and composition) before every intervention.

The pizza ingested by the participants on Day 2.

5.3.4 Outcome measures

The primary outcome measure was LV e'. Secondary outcome measures were other measures of LV diastolic (a', E, A, E/e', E/A, DT, and IVRT) and LV systolic function (S', global strain, and strain rate) as well as resting HR, blood pressure, circulating glucose, triglycerides, total cholesterol, LDL, HDL, c-peptide, total antioxidant status, and hs-CRP.

The primary outcome measure was chosen on the basis of the findings of von Bibra et al. being the only paper that, to our knowledge, had reported postprandial effects of a carbohydrate meal on cardiac function (Δ e') in T2D individuals at the time when this study commenced (von Bibra et al., 2009). Tissue Doppler velocities are sensitive to changes and have high reproducibility, are easy to measure, and are less load dependent than other traditional diastolic variables.

5.3.5 Clinical measurements

5.3.4.1 Pre-testing

All participants conducted a VO_{2peak} test (as described in Chapter 2, section 2.1.1.2) to estimate HR_{max}, the latter to be able to achieve the exercise intensity levels prescribed in the protocol. The Jaeger (LE2000CE, Hochberg, Germany) was used to measure VO_{2peak}. HR was measured continuously during the VO_{2peak} test using HR monitors (Polar, Finland), and HR_{max} was determined by adding five beats to the highest HR observed during the VO_{2peak} test (Ingjer, 1991).

5.3.4.2 Resting echocardiography

All participants obtained an echocardiogram at each time-point (before and after fast food ingestion) using a Vivid 7 scanner (GE Vingmed, Horten, Norway) with a phased array transducer (M4S and M3S probe). Images were digitally stored on a hard disk for offline analysis using commercially available software (EchoPAC version BT12, GE Vingmed Ultrasound, Horten, Norway).

Echocardiographic recordings were performed mainly by two operators (CBI and VM). However, due to the challenging logistics, a few single recordings had to be performed by cardiologists at St. Olav's University Hospital, Trondheim, Norway to obtain all recordings.

Resting echocardiography was performed with subjects in the left lateral decubitus position as described previously (Ingul et al., 2010; Molmen et al., 2012).

Three consecutive cycles in B-mode acquisitions (mean frame rate 53/sec) and color tissue Doppler imaging (TDI) (mean frame rate 159.9/sec) were recorded from the three apical views (four-chamber, two-chamber, and long-axis) and B-mode from the parasternal view.

Measurements included E, A, IVRT, and DT. Pulsed wave tissue Doppler velocities were measured at the four mitral annular sites in the four-chamber and two-chamber views. The mean of these points was used for S', e', and a' (Thorstensen et al., 2010). The E/e' was calculated as an estimate of LV filling pressure (Ommen et al., 2000). Global strain and strain rate were calculated from 2-dimensional strain echocardiography (Reisner et al., 2004). Measurements obtained in this study were in accordance with standard procedures recommended by the American Society of Echocardiography (Lang et al., 2005), and no subjects were excluded because of impaired echocardiographic image quality. Images were analyzed offline using EchoPAC version BT12 (GE Vingmed Ultrasound, Horten, Norway). The observer was blinded to group participation, trial, and point in time during ultrasound analysis.

Before performing echocardiography at baseline 1, the participants had to have at least 36 hours without exercise.

5.3.4.3 Resting heart rate and blood pressure

The lowest HR observed during echocardiography was defined as the resting HR. Upright blood pressure measurements were performed using Philips SureSigns V52 (Andover, Massachusetts, US). Before measuring baseline 1 and baseline 2, participants rested in a sitting position for at least 10 minutes. Blood pressure at these time points was noted as the median of three recordings. At the remaining time points, upright blood pressure was measured only once. Rate pressure product (RPP; HR x systolic blood pressure) was calculated to determine myocardial workload.

5.3.4.4 Biochemical analysis

At baselines 1 and 2, blood was obtained after at least 12 hours of fasting from food, caffeine, nicotine, and alcohol. The remaining blood samples were selected in the postprandial state, after ingesting pizza. The participants had a right peripheral intravenous catheter inserted upon arrival at the laboratory on day two to avoid the discomfort of taking multiple blood samples.

Blood was collected after blood pressure measurements and before echocardiography. Blood glucose, C-peptide, plasma triglycerides, total cholesterol, HDL, LDL, and high-sensitive C-

reactive protein (hs-CRP) were analyzed according to standard procedures at the St.Olavs University Hospital (Trondheim) at all time points (Chapter 2, section 2.1.1.5). HbA_{1c} and hemoglobin was measured only at baseline 1. Insulin sensitivity was calculated using the HOMA2 calculator (The Homeostasis Assessment Model, University of Oxford, UK). Total antioxidant status (TAS) was analyzed as previously described (Wisloff et al., 2007).

5.3.4.5 Anthropometric measurements

Anthropometric measurements were performed as described in Chapter 2 (section 2.1.1.4). BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured by the same investigator at all time-points using a measuring tape at the level of the umbilicus after expiration. The anthropometric measurements were performed only at inclusion.

5.3.4.6 Self-reported meals

The subjects registered their afternoon meals before fasting overnight. This was necessary to ensure that they ate similar amounts and content (food composition) before every intervention. If a subject reported eating differently from the agreed upon standardized meal in the afternoon before baseline tests, the intervention was terminated and the subject was asked to come back another day to restart the intervention. This happened twice during the project.

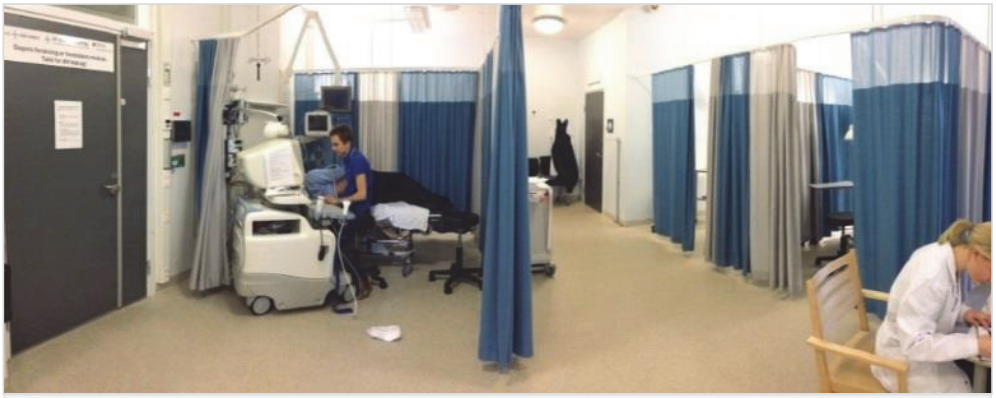
5.3.5 Statistical analysis

Statistical analysis was performed with linear mixed models (LMMs). Within-subject correlations were considered using a random intercept in the LMM. A full model included group (T2D or controls), trial (HIIE, MIE, or NE), and time (baseline 1, baseline 2, and 30 minutes, two hours, and four hours post-meal). Models with two levels of the trial factor (HIIE and MIE combined or NE) were also considered. Tests for overall effects of factors and factor interactions were done by likelihood ratio tests using a significance level of 0.05. Post hoc comparisons specified and tested appropriate linear combinations (contrasts) of the estimated model parameters for the selected models. In all models, the baseline means (baseline 1) for each group were restricted to be equal for the three exercise trials due to randomization to trials within each group (Fitzmaurice, 2004). Outcome variables not meeting the normal assumptions of the LMM were log transformed prior to the statistical analysis in cases where this transformation improved the approximation to the normal distribution. The analyses were performed with the R statistical package (R Core Team, 2014).

This study is explorative rather than confirmative, and thus we did not perform any formal adjustment for multiple testing. The results were considered statistically significant at $p<0.01$.

Sample size calculations

Sample size was not calculated as this study was considered a pilot study.



The research environment at the research unit (Forskningsposten) where this study was performed.

5.4 SUMMARY OF RESULTS

5.4.1 Subject characteristics

Subject characteristics are reported in Table 5.1. Twenty participants completed all trials (HIIE, MIE, and NE), exercised according to prescribed exercise HRs, and reported to have followed instructions of sedentary behavior during the NE trial. No adverse events were reported.

5.4.2 Clinical measurements

Neither exercise program influenced any of the outcome measures. Since no statistically significant effects of pre-exercise were found, the results are from linear mixed models including the factors group and time.

Table 5.1 Subject characteristics

	Type 2 diabetes	Control	p
n	10	10	
Male/Female, n (%)	7/3 (70/30)	7/3 (70/30)	
Diabetes duration, years	3.1±1.8	-	
Age, years	53.4±8.1	52.8±10.1	0.89
Body mass index, kg/m^2	28.3±3.8	28.5±4.2	0.91
Waist circumference, cm	107.1±27.5	104.4±13.8	0.78
HbA_{1c}, % (mmol/mol)	6.4±1.0 (46.0±7.0)	5.5±0.2 (36.0±1.5)	0.01
HOMA-ir	2.2±0.7	1.7±0.7	0.10
VO_{2peak}, ml/kg/min	38.8±7.8	36.2±8.8	0.51
VO_{2peak}, L/min	3.39±0.84	3.36±0.92	0.93
Medical agents, n (%)			
Anti-diabetic	6 (60)	0 (0)	
Statins	1 (10)	1 (10)	
Anti-hypertension	6 (60)	1 (10)	

Data are means±SD unless otherwise indicated. Abbreviations: HbA1c, glycosylated hemoglobin; HOMA-ir, homeostatic assessment model- insulin resistance; VO_{2peak}, peak oxygen uptake. T-tests were used to test for differences between groups at baseline.

5.4.2.1 Echocardiographic measures

Diastolic measures

The LV diastolic responses to fast food are illustrated in Figure 5.2 A-D. The T2D group had an overall poorer diastolic function (e') and higher filling pressure (E/e') than the controls (Figure 5.2 A-B).

In general, diastolic workload increased (higher e', a', E, and A) within 30 minutes after the meal in both groups. Subsequently, diastolic workload reversed towards baseline 2 levels.

Although not significant (see section 5.3.5), the T2D group, in contrast to controls (p=0.10), showed a tendency towards increased diastolic workload as measured by e' that persisted four hours after the meal (p=0.02). Late diastolic filling (a') remained elevated four hours after fast food in both groups.

Filling pressure (E/e') was reduced and isovolumic relaxation time (IVRT) shorter within two hours after the meal in the T2D group; this was not significantly different after fast food in the controls. Limitations in regard to the use of E/e' in this context are discussed in Chapter 2, section 2.1.1.1. Although not significant, there was a tendency towards differences between groups in change from baseline 2 to two hours post-meal for IVRT (p=0.03), but not for filling pressure (p=0.08). No effect of time was observed for the E/A ratio because both E and A increased. Supernormal filling is associated with vigorous recoil of the ventricle during early diastole with an increase of negative pressure in the ventricle and evacuation of blood from the atrium. This causes a high E wave, shortening of the IVRT, and normal deceleration time. The shortened IVRT and lack of changes to deceleration time might be due to the same mechanism.

Pre-exercise did not influence postprandial early diastolic velocity (e') (Figure 5.2 E) or any other diastolic echocardiographic variables (Figure 5.2 F-H).

Systolic measures

The LV systolic responses to fast food are illustrated in Figure 5.3 A-B. In general, systolic workload (global strain rate and S') increased 30 minutes after the meal in both groups. Systolic workload was subsequently reversed, but remained significantly elevated (global strain rate and S') versus baseline 2 after four hours in both groups (Figure 5.3 A-B). Pre-exercise did not affect systolic function (Figure 5.3 C-D).

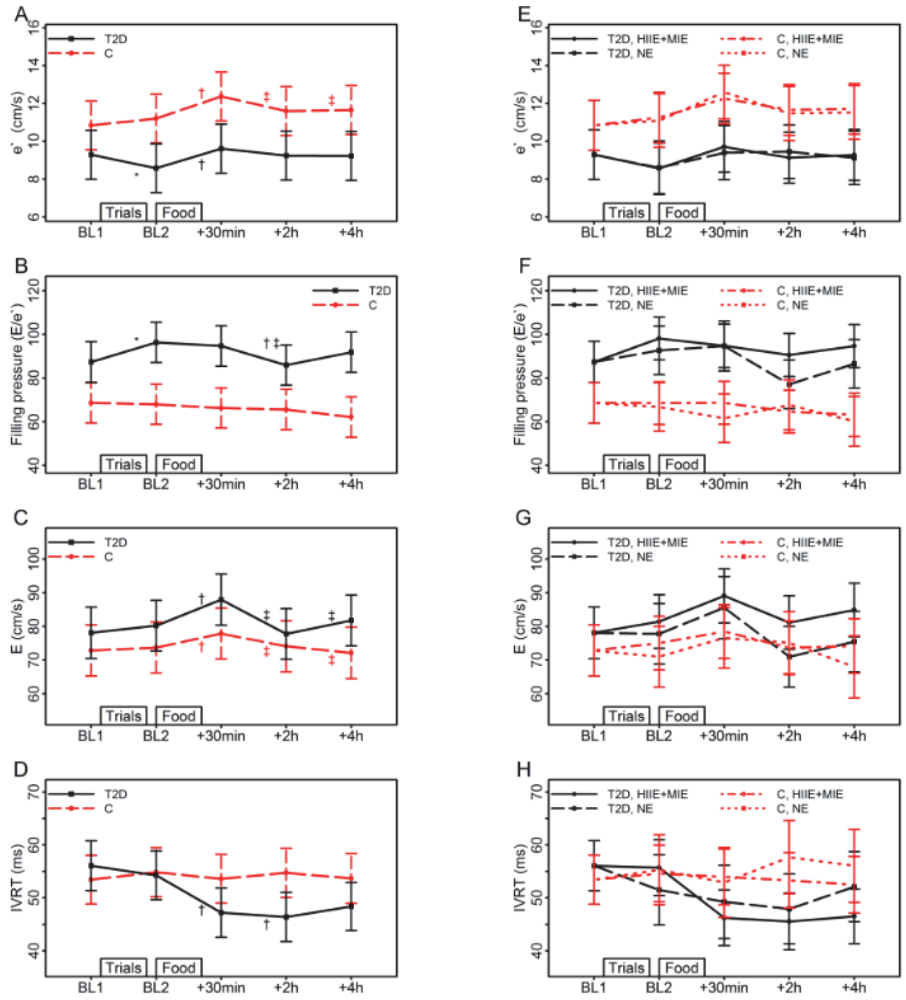


Figure 5.2 Effects of fast food (left panel; all trials combined) and exercise (right panel; high-intensity interval exercise+moderate-intensity exercise vs. no exercise) on left ventricular diastolic function. Abbreviations: BL, baseline; C, control group; e', peak early diastolic tissue Doppler velocity; E/e', filling pressure; E, peak early filling velocity; HIIE, high-intensity interval exercise; HIIE+MIE, exercise combined; IVRT, isovolumic relaxation time; MIE, moderate-intensity exercise; NE, no exercise; T2D, type 2 diabetes group. Estimated means and 95% CIs from LMMs with the factors time, group, and their interaction (left panel, figures A-D), and with the factors time, group, trial, and their interactions (right panel, figures E-H). In the left panel, significant ($p<0.01$) time differences are indicated by * (from BL1), † (from BL2), ‡ (from food+30min), and § (from food +2h). For peak early filling velocity (E), there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups.

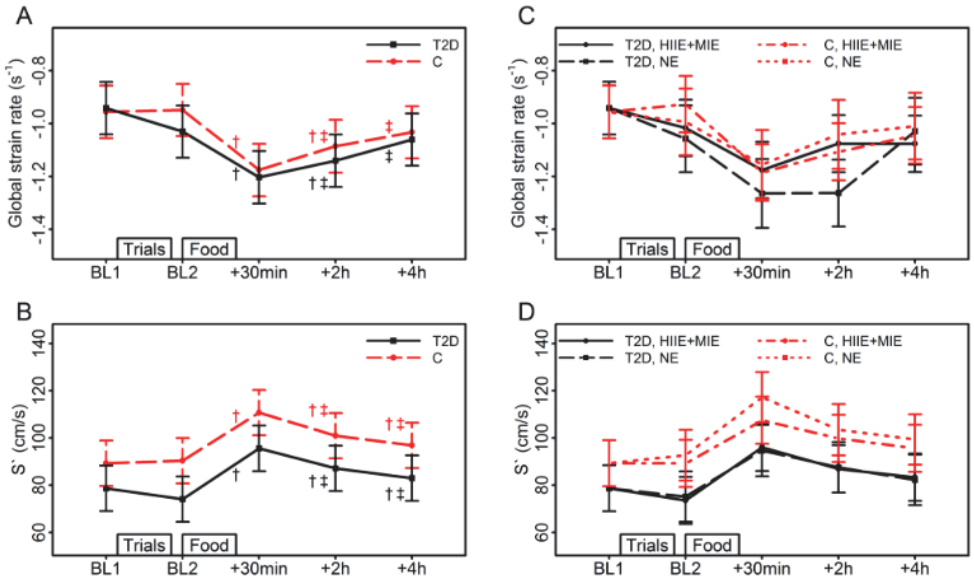


Figure 5.3 Effects of fast food (left panel; all trials combined) and exercise (right panel; high-intensity interval exercise+moderate-intensity exercise vs. no exercise) on left ventricular systolic function. Abbreviations: BL, baseline; C, control group; high-intensity interval exercise, HIIE; HIIE+MIE, exercise combined; moderate-intensity exercise, MIE; NE, no exercise; S', peak systolic tissue Doppler velocity; T2D, type 2 diabetes group. Estimated means and 95% CIs from LMMs with the factors time, group and their interaction (left panel, figures A-B), and with the factors time, group, trial, and their interactions (right panel, figures C-D). In the left panel, significant ($p<0.01$) time differences are indicated by * (from BL1), † (from BL2), ‡ (from food+30min), and § (from food +2h). For S' and global strain rate, there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups.

5.4.2.2 Resting heart rate and blood pressure

The HR, blood pressure, and RPP responses to fast food are illustrated in Figure 5.4 A-D.

The T2D group had an increased HR versus controls ($p<0.01$ or $p<0.05$) at all time points except 30 minutes after ingesting fast food ($p=0.06$). Resting HR increased within 30 minutes after the meal and subsequently decreased in both groups; it decreased to a larger extent in the controls than in the type 2 diabetes individuals. Only the controls regained baseline resting HR after four hours (Figure 5.4 A).

From baseline 1 to baseline 2, systolic blood pressure decreased in T2D, but not in controls. Within 2 hours after fast food, the mean systolic blood pressure in the T2D group decreased and subsequently reversed within four hours post-meal. In contrast, systolic blood pressure in the controls did not change after ingestion of fast food (Figure 5.4 B).

Overall, RPP was higher in the T2D group than in the controls (baseline 1 and four hours, $p<0.01$; baseline 2 and two hours: $p<0.05$; 30 min, $p=0.05$). The RPP was increased post-meal in both controls and T2D ($p<0.01$ and $p<0.05$, respectively). It subsequently reduced within two hours ($p<0.01$ and $p<0.05$, respectively), but returned to baseline 2 levels after four hours only in the controls (Figure 5.4 D). Pre-exercise did not influence HR, blood pressure response, or RPP (Figure 5.4 E-H).

5.4.2.3 Biochemical analysis

The response of circulating glucose, C-peptide, triglycerides, and TAS to fast food is illustrated in Figure 5.5 A-D, respectively. Fast food increased the glucose levels within 30 minutes after the meal in both groups. The subsequent drop in mean glucose levels was delayed in the T2D group versus the control group. This was indicated by the fact that the difference of two hours versus 30 minutes post-meal is significant for controls ($p<0.001$) but not for the T2D group ($p=0.5$); only the controls returned to baseline glucose levels four hours post-meal (Figure 5.5 A). Concurrently, the C-peptide levels peaked at 30 minutes post-meal in controls versus two hours post-meal in the T2D individuals (Figure 5.5 B).

The T2D group had a higher overall triglyceride level than the controls ($p<0.05$, Figure 5.5 C). The effect of fast food on triglyceride levels was similar in both groups. The TAS response to fast food was similar for the two groups—TAS decreased within four hours in both the control and T2D group ($p<0.01$ and $p<0.05$, respectively; Figure 5.5 D). The hs-CRP, HDL, and LDL did not change in the postprandial phase in either group. Pre-exercise did not influence any biochemical variables measured at any time-point (Figure 5.5 E-H).

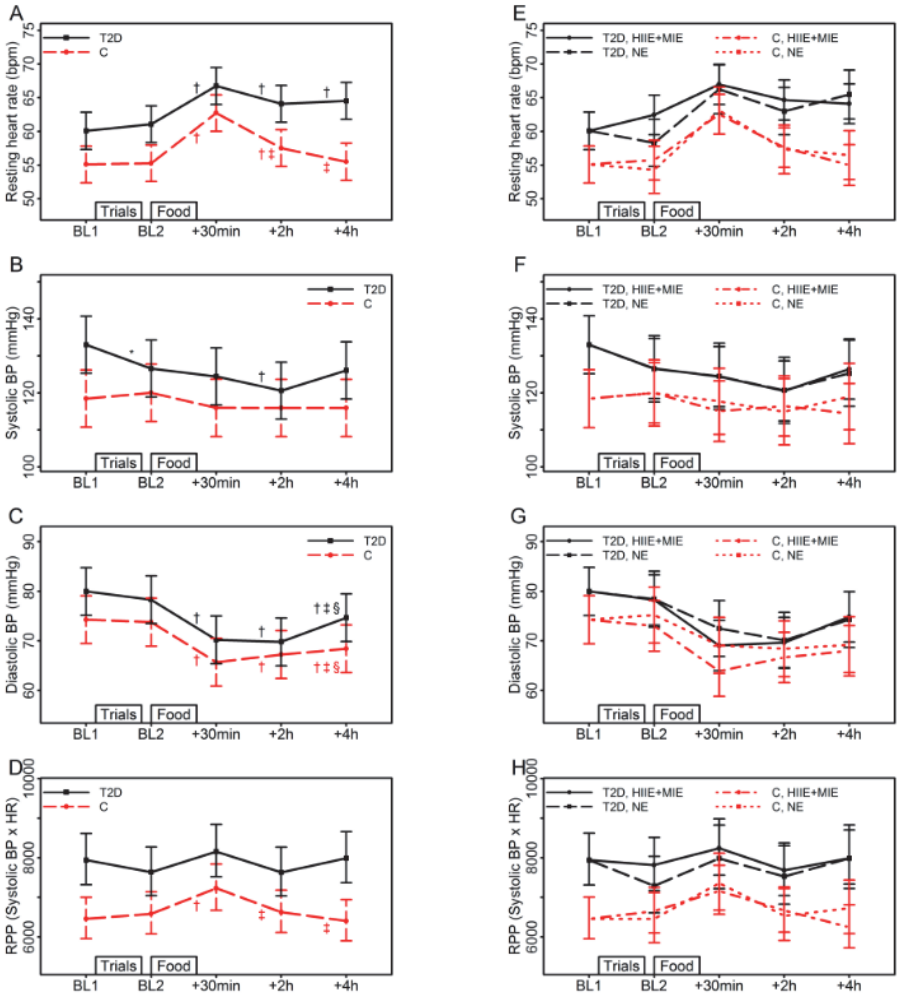


Figure 5.4 Effects of fast food (left panel; all trials combined) and exercise (right panel; high-intensity interval exercise+moderate-intensity exercise vs. no exercise) on resting heart rate and blood pressure. Abbreviations: BL, baseline; BP, blood pressure; C, control group; HIIE, high intensity exercise; HIIE+MIE, exercise combined; HR, resting heart rate; MIE; moderate-intensity exercise; NE, no exercise; RPP, rate pressure product; T2D, type 2 diabetes group. Estimated means and 95% CIs from LMMs with the factors time, group, and their interaction (left panel, figures A-D), and with the factors time, group, trial, and their interactions (right panel, figures E-H). In the left panel, significant ($p<0.01$) time differences are indicated by * (from BL1), † (from BL2), ‡ (from food+30min), and § (from food +2h). For diastolic BP, there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups. For RPP, the means and CIs are shown as back-transformed values, computed by direct exponentiation of the means and CIs from the LMM based on log-transformed data.

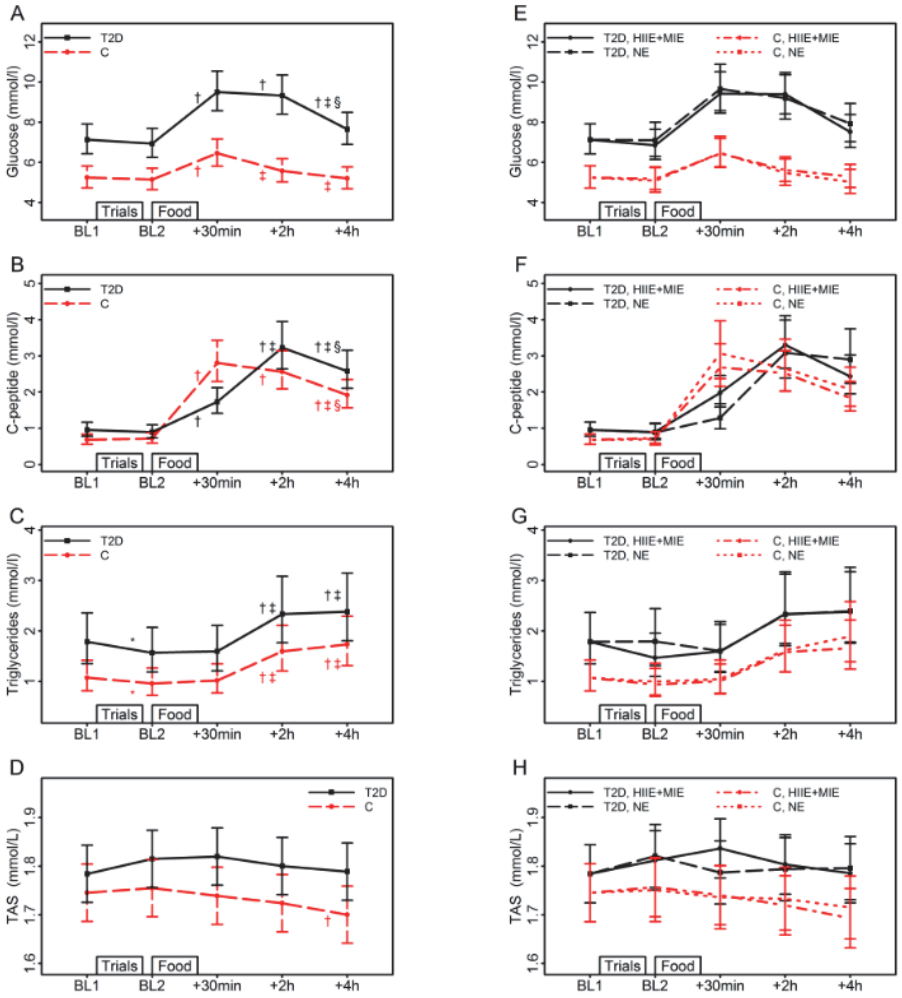


Figure 5.5 Effects of fast food (left panel, trials combined) and exercise (right panel; high-intensity interval exercise+moderate-intensity exercise vs. no exercise) on blood glucose, C-peptide, triglycerides, and total antioxidant status. Abbreviations: BL, baseline; C, control group; high intensity exercise, HIIE; moderate-intensity exercise; HIIE+MIE, exercise combined; TAS, total antioxidant status; T2D, type 2 diabetes group. Estimated means and 95% CIs from LMMs with the factors time, group, and their interaction (left panel, figures A-D), and with the factors time, group, trial, and their interactions (right panel, figures E-H). In the left panel significant ($p<0.01$) time differences are indicated by * (from BL1), † (from BL2), ‡ (from food+30min), and § (from food +2h). For triglycerides, there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups. Except for TAS, the means and CIs are shown as back-transformed values, computed by direct exponentiation of the means and CIs from the LMMs based on log-transformed data.

5.5 DISCUSSION

The findings of this study show that a fast food meal can induce increased cardiac workload (increased cardiac function and resting HR) in both T2D individuals and healthy, BMI- and age-matched controls. Furthermore, this study indicates that fast food interacts with LV diastolic function to a greater extent in T2D individuals than BMI- and age-matched controls. Pre-exercise did not modify fast-food induced changes in LV function, resting HR, blood pressure, blood glucose, triglycerides, or total antioxidant status.

5.5.1 Effects of fast food

5.5.1.1 Cardiac function

The observed postprandial increase in diastolic workload in both T2D and healthy overweight individuals is novel. Our data contrasts with the few previous studies that investigated the effects of lipid infusions or a carbohydrate-rich meal on cardiac function (Holland et al., 2011; von Bibra et al., 2013). Further study is needed to determine whether this is a result of the "combined meal" used here. The other studies (Holland et al., 2011; von Bibra et al., 2013) might also have missed an initial increase in diastolic workload due to measuring postprandial response one or two hours after infusion or ingestion, respectively. In contrast to our finding of increased diastolic workload (measured as e') after the meal, von Bibra et al. (von Bibra et al., 2013) observed a significantly reduced diastolic workload (e') 2 hours after ingesting a pure carbohydrate meal (48 g). Nielsen et al. (Nielsen, Norrelund, Kampmann, Botker, et al., 2013) found no changes in diastolic function after short-term hyperglycemia by insulin discontinuation in insulin-dependent patients with T2D. However, these participants (Nielsen, Norrelund, Kampmann, Botker, et al., 2013; von Bibra et al., 2013) had longer history of T2D and were insulin-dependent. The systolic responses to fast food have been observed previously after lipid infusions (Holland et al., 2011; Nielsen, Norrelund, Kampmann, Botker, et al., 2013). However, previous findings are diverse, probably due to the diversity in protocols (Dencker et al., 2011; Holland et al., 2011; Nielsen, Norrelund, Kampmann, Botker, et al., 2013; von Bibra et al., 2013).

In general, the mechanisms causing the change in cardiac function after a meal are not fully understood. It is, however, reasonable to think that the changes observed in cardiac function are partly due to changes in HR and loading conditions. The indications of fast food interacting with LV diastolic function to a greater extent in T2D individuals than in controls could be

explained by the prolonged postprandial increase in HR in the T2D group. An increased HR increases diastolic function (E, e') and shortens IVRT. The decrease in filling pressure in the T2D group within two hours post-meal could be explained by the indication of sustained increase in e' at this time point in this group while E is reduced. In young, healthy individuals, alterations in CO within 30 minutes post-meal have shown to increase flow (E, A) and tissue velocities (S', a') and to reduce DT, with no change in E/A, e', or E/e' (Dencker et al., 2011). Moreover, food ingestion has been shown to increase ventricular wall stress during the hours after a meal in healthy individuals, which indicates increased load (Gardinger et al., 2014).

We could speculate whether the postprandial diastolic compensations observed in the T2D group is an early sign of diastolic dysfunction. However, further research is needed to investigate the progress and interaction of food ingestion and diastolic compensations in T2D across different disease stages.

The increased LV systolic workload after fast food ingestion is in line with Holland et al. (Holland et al., 2011), who demonstrated increased systolic workload (LV global strain rate) induced by increased circulating triglycerides after intravenous administration of a fat emulsion in healthy individuals, and Nielsen et al. (Nielsen, Norrelund, Kampmann, Botker, et al., 2013), who observed increased systolic workload (S' and strain rate) due to hyperglycemia in T2D individuals with and without HF. However, our data contrasts with von Bibra et al. (von Bibra et al., 2013), who observed no postprandial change in systolic function (S') in insulin-dependent T2D individuals with a longer duration after ingesting carbohydrates. The diverse findings may be due to different measurement times or differences in the methods used to increase circulating glucose and/or triglycerides.

Indeed, the postprandial alterations in cardiac function observed are in contrast with observations in healthy individuals (Dencker et al., 2011). The difference between middle aged T2D and overweight individuals in this study versus young healthy individuals may be due to several factors, such as: age, meals ingested, metabolic status (T2D duration, level of hyperglycemia), antidiabetic drug treatment, and exercise/physical activity status (Solomon, Malin, Karstoft, Haus, et al., 2013). Furthermore, many of these factors may explain differences between groups in the present study, even within subjects, but they may also explain the similarity between the groups.

5.5.1.2 Heart rate, blood pressure, and rate pressure product

The higher HR at rest and prolonged increases in resting HR after fast food consumption by T2D individuals relative to controls may be due to several factors including CAN that can cause abnormalities in HR control by reduced vagal activity and/or high sympathetic activity (Valensi et al., 2013). The postprandial increase in HR is in line with previous findings. It has been suggested that the HR increase after meals is due to increased blood flow to the gastrointestinal system (Dencker et al., 2011; Gardinger et al., 2014; Waaler et al., 1991). Although increased HR is commonly observed during euglycemic clamp in this patient group as well as those with metabolic syndrome (Valensi et al., 2013), von Bibra et al. (von Bibra et al., 2013) observed no particular increase in resting HR two hours after a carbohydrate-rich meal in insulin-dependent patients with T2D. This may be due to the long standing T2D (von Bibra et al., 2013), which increases the possibility of depressed sympathetic activity (Valensi et al., 2013). Nielsen et al. (Nielsen, Norrelund, Kampmann, Kim, et al., 2013) observed an increased HR ($p=0.08$) due to high levels of lipid infusion versus low lipid infusion controls, which indicates that fast food may increase HR more than healthy foods. Furthermore, both glucose ingestion (Paolisso, Manzella, Rizzo, Barbieri, et al., 2000) and elevated plasma fatty acid concentrations (Paolisso, Manzella, Rizzo, Ragno, et al., 2000) may stimulate the cardiac autonomic nervous system with a possible increase in catecholamines. Thus, the LV effects of fast food seen here may be due to catecholamine-induced increases in inotropy that result in increased contractility of the cardiac muscle as well as increased dromotropic and chronotropic effects that increase the HR. Furthermore, circulating glucose and insulin levels, as well as glucagon-like peptide-1 (GLP-1) and ghrelin seem to influence cardiac activity (Baron, 1994; Hlebowicz et al., 2011). Due to the characteristics of T2D, it is thus also reasonable to think that differences in postprandial hormonal response could contribute to differences in postprandial cardiac function between T2D and healthy counterparts. However, although this study measured postprandial c-peptide levels, it did not investigate the effect of fast food or pre-exercise on hormonal changes (i.e. insulin, GLP-1, or catecholamines) or the presence of CAN in the participants.

The observed postprandial response in systolic blood pressure in the T2D group contrasted with healthy counterparts in the present study as well as with young, fit, and healthy individuals in a previous study (Dencker et al., 2011). The postprandial reduction in systolic blood pressure in the T2D group might be an early stage of postprandial systolic hypotension, which is a common hemodynamic condition in patients with diabetes (Jansen and Lipsitz, 1995) and is associated with an increased risk of cerebrovascular disease (Tabara et al., 2014). The

mechanisms mediating postprandial reductions in blood pressure are not fully understood, but food-induced neurohormonal changes leading to reduced vascular resistance in the splanchnic vasculature as well as cardiovascular autonomic neuropathy (CAN) resulting in impaired sympathetic nervous activity has been suggested (Lipsitz et al., 1993; Luciano et al., 2010; Parati and Bilo, 2014). Nevertheless, due to the low number of subjects participating in this study and the fact that only one blood pressure measurement was performed in the postprandial state, further study is needed to confirm these findings.

The reduced postprandial diastolic blood pressure observed in both groups in the postprandial state was in line with previous findings in healthy individuals (Dencker et al., 2011). Diastolic blood pressure decrease within 30 minutes, whereas systolic blood pressure seem to be unaffected in the postprandial state in healthy individuals (Dencker et al., 2011; Gardinger et al., 2014).

The RPP differences seen between groups suggest that the T2D group had greater stress to the cardiac muscle than the controls. However, the change in RPP after ingesting fast food was the same for both groups. Hence, there was no difference in postprandial stress to the cardiac muscle in response to fast food.

5.5.1.3 Biochemical measures

Gudmundsdottir et al. (Gudmundsdottir, 2012) investigated the postprandial changes after a healthy meal versus a junk food meal and found small changes in blood lipids and hsCRP with no differences between meals. The present study supports these findings with no changes in cholesterol and hsCRP.

In the present study, pre-exercise did not modify TAS in T2D or controls. This is in contrast to Tyldum et al. (Tyldum et al., 2009), who observed a significant exercise-induced improvement in TAS associated with improvements in endothelial function in healthy normal weight men (42±4 years) using the same protocol as described here. This indicates that the participants in the present study had a poorer response to exercise than lean and healthy individuals (Tyldum et al., 2009). This may possibly be due to central obesity and poorer metabolic control in our study participants versus normal weight individuals.

The lack of exercise-induced improvements in postprandial TAS in the present study may be explained by a lack of exercise-induced postprandial changes in circulating glucose and triglyceride levels. Although the lack of exercise-induced TAS changes contrasts with the

findings of Tyldum et al. (Tyldum et al., 2009), the lack of exercise induced changes in glucose and triglyceride levels did concur. Nevertheless, exercise-induced changes have previously been observed due to acute exercise on postprandial triglyceride levels and hyperglycemia (Gill et al., 2004; van Dijk et al., 2012; Zhang et al., 2004).

However, studies are difficult to compare due to different measurement methods; meal composition and size; timing of exercise; exercise mode; intensity; and duration. Inadequate energy expenditure (Peddie et al., 2012) and/or inadequate exercise timing relative to the meal may explain the lack of exercise-induced reductions in postprandial glucose (Poirier et al., 2000), and/or triglyceride excursion (Zhang et al., 1998), etc. The acute effect of different exercise modes and the timing of these on the postprandial response of the LV certainly needs to be further investigated in patients with T2D as the time course of adaptation may be different in the heart/endothelium than normal body weight persons.

5.5.2 Effects of exercise to the postprandial phase

Pre-exercise did not influence any of the changes observed in the postprandial phase. Our findings thus indicate that T2D and overweight individuals do not respond to pre-exercise in the same way as healthy, lean, younger individuals with better aerobic capacity do (Tyldum et al., 2009). There may be several reasons for this, such as factors mentioned in the previous section, as well as the timing of exercise, exercise mode, intensity, and duration. Furthermore, it may be speculated whether the lack of exercise response can be explained by metabolic status (Knudsen et al., 2015; Solomon, Malin, Karstoft, Haus, et al., 2013); i.e. there are indications of fasting glucose levels, HbA_{1c}, and antidiabetic drug being determinants of pancreatic endocrine response to acute aerobic exercise (MIE) in T2D, favoring subjects with low-fasting glucose and subjects who do not use antidiabetic drugs (even when antidiabetic drug use is paused) (Knudsen et al., 2015). There is increasing evidence to suggest that metabolic status (i.e. degree of hyperglycemia, β-cell dysfunction, and antidiabetic drug use) determines exercise effect (Dela et al., 2004; Malin et al., 2012; Solomon, Malin, Karstoft, Haus, et al., 2013; Solomon, Malin, Karstoft, Kashyap, et al., 2013). The lack of pre-exercise effect in the present study may thus be due to the potential of large inter-subject variability in exercise responses due to the relatively heterogeneous groups as well as the small sample size in this study. Further research is thus warranted to investigate the potential of exercise to improve postprandial cardiac function in T2D.

5.5.3 Study limitations and strengths

This study was explorative, rather than confirmative, and was intended to lay the groundwork for a more complete research study in the future. An important limitation in this study is the small sample size and results must thus be interpreted with caution. In future studies, the study sample must be expanded to ensure that the potential flaws due to the small study sample are overcome.

The choice of meal is also an important limitation in this study. The meal represents a different relative energy and macronutrient intake between subjects: For example, as it is, the glycemic load for each subject is different, making comparisons between the exercise bouts difficult. The meal should have been normalized to give the same relative energy to each subject (i.e., as grams of macronutrients per kg body mass or even better, per kg lean body mass). Furthermore, the choice of having self-reported meals, after the exercise bout prior to day 2 (Figure 5.1), is a weakness in this study; and pre-prepared meals should have been given at least on day 1 (Figure 5.1), if not also the day prior to day 1.

Several factors were tested in this study and the problem of multiple comparisons is acknowledged. However, multiple testing was to some extent accounted for by considering the results to be statistically significant at $p<0.01$. Overall, we found few significant interactions between time and group (the presence of T2D), indicating that the effect of fast food is the same for both groups. Nonetheless, due to the explorative character of this study, we chose to include apparently reasonable time-trends, which did not reach statistical significance due to the small sample size and the small effects relative to the standard deviations. Future studies are required to investigate whether healthy food induces the same postprandial changes as fast food and to better understand the role of postprandial cardiac function changes in the development of cardiovascular disease in T2D.

In an attempt to account for important factors that could have been unevenly distributed between groups, and thus could influence the results, the T2D individuals available for testing were matched with a group of individuals with the same gender, age, and BMI distribution. However, factors other than BMI and age, such as muscle mass versus body fat mass as well as cardiorespiratory fitness, could have influenced the results. In regard to the effects of interventions within groups, the crossover design of this study represents a strength, as variability tends to be smaller within-subjects than between-subject (Machin, 2010).

Furthermore, the similarity (BMI and WC) between groups may represent a limitation as the similarity in BMI and WC between groups excludes potentially confounding effects of adiposity. Moreover, three of the self-reported healthy participants had $HbA_{1c} \geq 5.7\%$ at, at least, one of the three baseline-1 measurements during the trial. According to Chapter 1 (Table 1.1), $HbA_{1c} \geq 5.7\%$ is characterized as impaired fasting glucose/prediabetes. Another three of the self-reported healthy participants also had HbA_{1c} of 5.6% at one or more of the three baseline 1 measurements. A more thorough pre-examination of the healthy participants could have avoided this important limitation, preferably by performing an oral glucose tolerance test at pre-test.

In this study, three participants did not reach the required RER levels for the test to be considered a VO_{2max}-test (RER < 1.05) at pre-testing and the RER values of four participants were lost (due to a technical error hindering us from saving and printing the VO_{2max} test results after testing. Although the results were written down manually, the RER values were not noted). The HR_{max} was calculated from this test to set the intensity level during MIE and HIIE, meaning that some participants might not have exercised according to the intensity requirements in the protocol. Although the exercise physiologists supervising the exercise sessions increased intensity levels if they observed that the participants did not perceive the expected effort, this is an important potential limitation in the present study.

The choice of exercise intensities during intervention and the timing of exercise relative to the meal influenced the results. Indeed, several research projects have investigated the effect of acute exercise on postprandial glucose levels and triglycerides, but timing and exercise interventions are diverse, making it challenging to pick a protocol. The choice of protocol in this study was based on a previous research project performed at our department (Tyldum et al., 2009), in which the rationale for the exercise intervention timing and intensity was to affect circulating lipids and thus endothelial function, not circulating glucose. Although insulin sensitivity has been shown to be affected between 2-72 hours after an exercise session, most intervention studies investigating the effect of exercise on postprandial circulating glucose in T2D individuals have been performed closer to the meal (within hours before or after). The lack of exercise effects might have been due to this, and the fact that we investigated the second meal after exercise rather than the meal subsequent to the exercise bout. Further studies should investigate different exercise strategies and exercise timing relative to meals. For example, low-volume HIIE has been shown to be particularly time efficient in reducing excessive postprandial elevations in circulating glucose. However, further research is required to

investigate whether the acute effects on circulating glucose has an impact on cardiac workload as well.

The logistics and the many outcome measures in this study represent potential limitations. Even though the study environment was well controlled and calm, the stress produced during the fast and effective measurements taken at the exact time points, for example, might have stressed the participants more at the first intervention than the last (e.g. when performing blood pressure measurements or resting HR measurements during echocardiography). The logistics were strict and the timing was imperative, and even though the measurements were taken within the time limits at most of the time points, some of the measurements might have been slightly delayed or postponed in time, which could represent failure to properly compare between groups and interventions.

This study evaluated the effect of fast food on LV function, so the individual effects of carbohydrates, fat, and salt cannot be evaluated. A recent study demonstrated no effect on diastolic function in normotensive healthy men after one week of high dietary sodium intake (Mak et al., 2013). However, a previous study found that one week of high dietary sodium intake impaired myocardial relaxation (Tzemos et al., 2008). This study used a mixed meal to simulate reality rather than separating the macronutrients to study their separate effects, though a separation might have been useful to better understand the role of the macronutrients. However, the most important priority would be to study a healthy meal as opposed to the fast food meal in the future to reveal whether there is a clinical significant difference in cardiac workload after fast food compared to healthy food according to nutritional guidelines.

The strengths of this study include the strictly controlled study environment during Day 2 and supervised exercise interventions on Day 1. However, in this study, the energy intake and meal composition the day prior to baseline 1 and baseline 2; the exercise and physical activity performed 48 hours before baseline 2; and the sedate behavior in the NE intervention-arm of the trial were self-reported. This could not be independently verified and the participants had to be trusted to tell the truth. Potential sources of bias in this concern are indeed present. A future study should monitor HRs or use activity monitors prior to the intervention. Furthermore, the participants should be provided with a standardized evening meal, and perhaps provided all meals for the three days prior to baseline 2. However, this study did not have the equipment or funding to make this possible. The optimal situation would have been to monitor the

participants at the laboratory throughout the intervention or to perform continuous blood glucose measurements, but this was not practically possible to carry out at the time.

The fact that several medical doctors, with diverse levels of experience in performing echocardiography, performed the measurements is a limitation in this study. Ideally, only one, experienced investigator should have performed these measurements, but again, this was not logistically possible.

5.6 CONCLUSION

The findings of this study indicate that fast food induces increased postprandial cardiac workload in both T2D individuals and BMI- and age-matched controls. The postprandial increase in resting HR was more prolonged in T2D individuals than in controls. Pre-exercise had no acute effects to the postprandial phase. The acute interaction of food on cardiac function in T2D needs further study. More research is also needed on the effects of other exercise methods and exercise timing on postprandial cardiac function and other cardiovascular risk markers in this patient group.

REFERENCES

Bansal, S., Buring, J. E., Rifai, N., Mora, S., Sacks, F. M., & Ridker, P. M. (2007). Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA, 298*(3), 309-316.

Baron, A. D. (1994). Hemodynamic actions of insulin. *Am. J. Physiol., 267*(2 Pt 1), E187-202.

Boren, J., Matikainen, N., Adiels, M., & Taskinen, M. R. (2014). Postprandial hypertriglyceridemia as a coronary risk factor. *Clin. Chim. Acta, 431C*, 131-142.

Burns, S. F., Miyashita, M., & Stensel, D. J. (2015). High-Intensity Interval Exercise and Postprandial Triacylglycerol. *Sports Med., 45*(7), 957-968.

Cavalot, F., Pagliarino, A., Valle, M., Di Martino, L., Bonomo, K., Massucco, P., . . . Trovati, M. (2011). Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. *Diabetes Care, 34*(10), 2237-2243.

Cavalot, F., Petrelli, A., Traversa, M., Bonomo, K., Fiora, E., Conti, M., . . . Trovati, M. (2006). Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *J. Clin. Endocrinol. Metab., 91*(3), 813-819.

Ceriello, A., Esposito, K., Piconi, L., Ihnat, M. A., Thorpe, J. E., Testa, R., . . . Giugliano, D. (2008). Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes, 57*(5), 1349-1354.

Ceriello, A., Quagliaro, L., Piconi, L., Assaloni, R., Da Ros, R., Maier, A., . . . Giugliano, D. (2004). Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. *Diabetes, 53*(3), 701-710.

Chiasson, J. L. (2006). Acarbose for the prevention of diabetes, hypertension, and cardiovascular disease in subjects with impaired glucose tolerance: the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) Trial. *Endocr. Pract., 12 Suppl 1*, 25-30.

Colberg, S. R., Albright, A. L., Blissmer, B. J., Braun, B., Chasan-Taber, L., Fernhall, B., . . . American Diabetes, A. (2010). Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Med. Sci. Sports Exerc., 42*(12), 2282-2303.

Coutinho, M., Gerstein, H. C., Wang, Y., & Yusuf, S. (1999). The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care, 22*(2), 233-240.

de Simone, G., Devereux, R. B., Chinali, M., Lee, E. T., Galloway, J. M., Barac, A., . . . Howard, B. V. (2010). Diabetes and incident heart failure in hypertensive and normotensive participants of the Strong Heart Study. *J. Hypertens., 28*(2), 353-360.

Dela, F., von Linstow, M. E., Mikines, K. J., & Galbo, H. (2004). Physical training may enhance beta-cell function in type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab., 287*(5), E1024-1031.

Dencker, M., Bjorgell, O., & Hlebowicz, J. (2011). Effect of food intake on commonly used pulsed Doppler and tissue Doppler measurements. *Echocardiography, 28*(8), 843-847.

Eberly, L. E., Stamler, J., Neaton, J. D., & Multiple Risk Factor Intervention Trial Research, G. (2003). Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. *Arch. Intern. Med., 163*(9), 1077-1083.

Fitzmaurice, G. M. L., N.M.; Ware, J.H. . (2004). *Applied Longitudinal Analysis*. Page 126-132: Wiley , New York.

Francois, M. E., Baldi, J. C., Manning, P. J., Lucas, S. J., Hawley, J. A., Williams, M. J., & Cotter, J. D. (2014). 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia, 57*(7), 1437-1445.

Garber, A. J. (2012). Postprandial dysmetabolism and the heart. *Heart Fail. Clin., 8*(4), 563-573.

Gardinger, Y., Hlebowicz, J., Bjorgell, O., & Dencker, M. (2014). Effect of food intake on left ventricular wall stress. *Cardiovasc. Ultrasound, 12*, 2.

Gill, J. M., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Packard, C. J., Sattar, N., . . . Caslake, M. J. (2004). Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J. Am. Coll. Cardiol., 44*(12), 2375-2382.

Gill, J. M., Herd, S. L., Vora, V., & Hardman, A. E. (2003). Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and postprandial states. *Eur. J. Appl. Physiol., 89*(2), 184-190.

Gillen, J. B., Little, J. P., Punthakee, Z., Tarnopolsky, M. A., Riddell, M. C., & Gibala, M. J. (2012). Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes Obes. Metab., 14*(6), 575-577.

Gudmundsdottir, F. D. (2012). *Effects of two different types of fast food on postprandial metabolism in normal- and overweight subjects.* (MSc MSc thesis), University of Iceland Reykjavík. (P.6)

Hawley, J. A., & Gibala, M. J. (2009). Exercise intensity and insulin sensitivity: how low can you go? *Diabetologia, 52*(9), 1709-1713.

Hlebowicz, J., Lindstedt, S., Bjorgell, O., & Dencker, M. (2011). Relationship between postprandial changes in cardiac left ventricular function, glucose and insulin concentrations, gastric emptying, and satiety in healthy subjects. *Nutr. J., 10*, 26.

Holland, D. J., Erne, D., Kostner, K., Leano, R., Haluska, B. A., Marwick, T. H., & Sharman, J. E. (2011). Acute elevation of triglycerides increases left ventricular contractility and alters ventricular-vascular interaction. *Am. J. Physiol. Heart Circ. Physiol., 301*(1), H123-128.

Holloszy, J. O. (2003). A forty-year memoir of research on the regulation of glucose transport into muscle. *Am. J. Physiol. Endocrinol. Metab., 284*(3), E453-467.

Ikeda, H., Uzui, H., Morishita, T., Fukuoka, Y., Sato, T., Ishida, K., . . . Tada, H. (2015). Effect of postprandial hyperglycaemia on coronary flow reserve in patients with impaired glucose tolerance and type 2 diabetes mellitus. *Diab. Vasc. Dis. Res., 12*(6), 405-410.

Ingjer, F. (1991). Factors influencing assessment of maximal heart rate. *Scand. J. Med. Sci. Sports, 1*, 134-140.

Ingul, C. B., Tjonna, A. E., Stolen, T. O., Stoylen, A., & Wisloff, U. (2010). Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch. Pediatr. Adolesc. Med., 164*(9), 852-859.

Iribarren, C., Karter, A. J., Go, A. S., Ferrara, A., Liu, J. Y., Sidney, S., & Selby, J. V. (2001). Glycemic control and heart failure among adult patients with diabetes. *Circulation, 103*(22), 2668-2673.

Jansen, R. W., & Lipsitz, L. A. (1995). Postprandial hypotension: epidemiology, pathophysiology, and clinical management. *Ann. Intern. Med., 122*(4), 286-295.

Karstoft, K., Christensen, C. S., Pedersen, B. K., & Solomon, T. P. (2014). The acute effects of interval- Vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a crossover, controlled study. *J. Clin. Endocrinol. Metab., 99*(9), 3334-3342.

Knudsen, S. H., Karstoft, K., Winding, K., Holst, J. J., Pedersen, B. K., & Solomon, T. P. (2015). Effects of acute exercise on pancreatic endocrine function in subjects with type 2 diabetes. *Diabetes Obes. Metab., 17*(2), 207-210.

Kobayashi, R., Yoshida, S., & Okamoto, T. (2015). Arterial stiffness after glucose ingestion in exercise-trained versus untrained men. *Appl. Physiol. Nutr. Metab., 40*(11), 1151-1156.

Lang, R. M., Bierig, M., Devereux, R. B., Flachskampf, F. A., Foster, E., Pellikka, P. A., . . . Stewart, W. J. (2005). Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J. Am. Soc. Echocardiogr., 18*(12), 1440-1463.

Lipsitz, L. A., Ryan, S. M., Parker, J. A., Freeman, R., Wei, J. Y., & Goldberger, A. L. (1993). Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. *Circulation, 87*(2), 391-400.

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol (1985), 111*(6), 1554-1560.

Luciano, G. L., Brennan, M. J., & Rothberg, M. B. (2010). Postprandial hypotension. *Am. J. Med., 123*(3), 281 e281-286.

Machin, D. F. P. M. (2010). *Randomized clinical trials: Design, practice and reporting*: Wiley-Blackwell.

Mak, G. S., Sawaya, H., Khan, A. M., Arora, P., Martinez, A., Ryan, A., . . . Scherrer-Crosbie, M. (2013). Effects of subacute dietary salt intake and acute volume expansion on diastolic function in young normotensive individuals. *Eur. Heart J. Cardiovasc. Imaging, 14*(11), 1092-1098.

Malin, S. K., Gerber, R., Chipkin, S. R., & Braun, B. (2012). Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes. *Diabetes Care, 35*(1), 131-136.

McArdle, W. D., Katch, F. I., & Katch, L. V. (2010). *Exercise Physiology; Nutrition, Energy and Human Performance* (P. Williams Ed. 7th ed.): Lippincott Williams & Wilkins.

Meigs, J. B., Nathan, D. M., D'Agostino, R. B., Sr., Wilson, P. W., & Framingham Offspring, S. (2002). Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. *Diabetes Care, 25*(10), 1845-1850.

Molmen, H. E., Wisloff, U., Aamot, I. L., Stoylen, A., & Ingul, C. B. (2012). Aerobic interval training compensates age related decline in cardiac function. *Scand. Cardiovasc. J., 46*(3), 163-171.

Monnier, L., Lapinski, H., & Colette, C. (2003). Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care, 26*(3), 881-885.

Monnier, L., Mas, E., Ginet, C., Michel, F., Villon, L., Cristol, J. P., & Colette, C. (2006). Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA, 295*(14), 1681-1687.

Nielsen, R., Norrelund, H., Kampmann, U., Botker, H. E., Moller, N., & Wiggers, H. (2013). Effect of acute hyperglycemia on left ventricular contractile function in diabetic patients with and without heart failure: two randomized cross-over studies. *PLoS One, 8*(1), e53247.

Nielsen, R., Norrelund, H., Kampmann, U., Kim, W. Y., Ringgaard, S., Schar, M., . . . Wiggers, H. (2013). Failing heart of patients with type 2 diabetes mellitus can adapt to extreme short-term increases in circulating lipids and does not display features of acute myocardial lipotoxicity. *Circ. Heart Fail., 6*(4), 845-852.

Nordestgaard, B. G., Benn, M., Schnohr, P., & Tybjaerg-Hansen, A. (2007). Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA, 298*(3), 299-308.

O'Keefe, J. H., & Bell, D. S. (2007). Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. *Am. J. Cardiol., 100*(5), 899-904.

Ommen, S. R., Nishimura, R. A., Appleton, C. P., Miller, F. A., Oh, J. K., Redfield, M. M., & Tajik, A. J. (2000). Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation, 102*(15), 1788-1794.

Paolisso, G., Manzella, D., Rizzo, M. R., Barbieri, M., Gambardella, A., & Varricchio, M. (2000). Effects of glucose ingestion on cardiac autonomic nervous system in healthy centenarians: differences with aged subjects. *Eur. J. Clin. Invest., 30*(4), 277-284.

Paolisso, G., Manzella, D., Rizzo, M. R., Ragno, E., Barbieri, M., Varricchio, G., & Varricchio, M. (2000). Elevated plasma fatty acid concentrations stimulate the cardiac autonomic nervous system in healthy subjects. *Am. J. Clin. Nutr., 72*(3), 723-730.

Parati, G., & Bilo, G. (2014). Postprandial blood pressure fall: another dangerous face of blood pressure variability. *J. Hypertens., 32*(5), 983-985.

Peddie, M. C., Rehrer, N. J., & Perry, T. L. (2012). Physical activity and postprandial lipidemia: are energy expenditure and lipoprotein lipase activity the real modulators of the positive effect? *Prog. Lipid Res., 51*(1), 11-22.

Pereira, M. A., Kartashov, A. I., Ebbeling, C. B., Van Horn, L., Slattery, M. L., Jacobs, D. R., Jr., & Ludwig, D. S. (2005). Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. *Lancet, 365*(9453), 36-42.

Petitt, D. S., & Cureton, K. J. (2003). Effects of prior exercise on postprandial lipemia: a quantitative review. *Metabolism, 52*(4), 418-424.

Poirier, P., Tremblay, A., Catellier, C., Tancrede, G., Garneau, C., & Nadeau, A. (2000). Impact of time interval from the last meal on glucose response to exercise in subjects with type 2 diabetes. *J. Clin. Endocrinol. Metab., 85*(8), 2860-2864.

R Core Team. (2014). *R: A Language and Environment for Statistical Computing*: R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Reisner, S. A., Lysyansky, P., Agmon, Y., Mutlak, D., Lessick, J., & Friedman, Z. (2004). Global longitudinal strain: a novel index of left ventricular systolic function. *J. Am. Soc. Echocardiogr., 17*(6), 630-633.

Rognmo, O., Hetland, E., Helgerud, J., Hoff, J., & Slordahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur. J. Cardiovasc. Prev. Rehabil., 11*(3), 216-222.

Ryden, L., Grant, P. J., Anker, S. D., Berne, C., Cosentino, F., Danchin, N., . . . Xuereb, R. G. (2013). ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur. Heart J., 34*(39), 3035-3087.

Schenk, S. H., J.F. (2007). Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid–induced insulin resistance. *The Journal of Clinical Investigation, 117*(6).

Solomon, T. P., Malin, S. K., Karstoft, K., Haus, J. M., & Kirwan, J. P. (2013). The influence of hyperglycemia on the therapeutic effect of exercise on glycemic control in patients with type 2 diabetes mellitus. *JAMA Intern Med, 173*(19), 1834-1836.

Solomon, T. P., Malin, S. K., Karstoft, K., Kashyap, S. R., Haus, J. M., & Kirwan, J. P. (2013). Pancreatic beta-cell function is a stronger predictor of changes in glycemic control after an aerobic exercise intervention than insulin sensitivity. *J. Clin. Endocrinol. Metab., 98*(10), 4176-4186.

Stephenson, E. J., Smiles, W., & Hawley, J. A. (2014). The Relationship between Exercise, Nutrition and Type 2 Diabetes. *Med. Sport Sci., 60*, 1-10.

Tabara, Y., Okada, Y., Uetani, E., Nagai, T., Igase, M., Kido, T., . . . Miki, T. (2014). Postprandial hypotension as a risk marker for asymptomatic lacunar infarction. *J. Hypertens., 32*(5), 1084-1090; discussion 1090.

Thorstensen, A., Dalen, H., Amundsen, B. H., Aase, S. A., & Stoylen, A. (2010). Reproducibility in echocardiographic assessment of the left ventricular global and regional function, the HUNT study. *Eur. J. Echocardiogr., 11*(2), 149-156.

Tyldum, G. A., Schjerve, I. E., Tjonna, A. E., Kirkeby-Garstad, I., Stolen, T. O., Richardson, R. S., & Wisloff, U. (2009). Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *J. Am. Coll. Cardiol., 53*(2), 200-206.

Tzemos, N., Lim, P. O., Wong, S., Struthers, A. D., & MacDonald, T. M. (2008). Adverse cardiovascular effects of acute salt loading in young normotensive individuals. *Hypertension, 51*(6), 1525-1530.

Valensi, P., Chiheb, S., & Fysekidis, M. (2013). Insulin- and glucagon-like peptide-1-induced changes in heart rate and vagosympathetic activity: why they matter. *Diabetologia, 56*(6), 1196-1200.

van Dijk, J. W., Manders, R. J., Hartgens, F., Stehouwer, C. D., Praet, S. F., & van Loon, L. J. (2011). Postprandial hyperglycemia is highly prevalent throughout the day in type 2 diabetes patients. *Diabetes Res. Clin. Pract., 93*(1), 31-37.

van Dijk, J. W., Manders, R. J., Tummers, K., Bonomi, A. G., Stehouwer, C. D., Hartgens, F., & van Loon, L. J. (2012). Both resistance- and endurance-type exercise reduce the prevalence of hyperglycaemia in individuals with impaired glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic patients. *Diabetologia, 55*(5), 1273-1282.

von Bibra, H., Siegmund, T., Ceriello, A., Volozhyna, M., & Schumm-Draeger, P. M. (2009). Optimized postprandial glucose control is associated with improved cardiac/vascular function - comparison of three insulin regimens in well-controlled type 2 diabetes. *Horm. Metab. Res., 41*(2), 109-115.

von Bibra, H., St John Sutton, M., Schuster, T., Ceriello, A., Siegmund, T., & Schumm-Draeger, P. M. (2013). Oxidative stress after a carbohydrate meal contributes to the deterioration of diastolic cardiac function in nonhypertensive insulin-treated patients with moderately well controlled type 2 diabetes. *Horm. Metab. Res., 45*(6), 449-455.

Waaler, B. A., Eriksen, M., & Toska, K. (1991). The effect of meal size on postprandial increase in cardiac output. *Acta Physiol. Scand., 142*(1), 33-39.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

Zhang, J. Q., Ji, L. L., Nunez, G., Feathers, S., Hart, C. L., & Yao, W. X. (2004). Effect of exercise timing on postprandial lipemia in hypertriglyceridemic men. *Can. J. Appl. Physiol., 29*(5), 590-603.

Zhang, J. Q., Thomas, T. R., & Ball, S. D. (1998). Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. *J Appl Physiol (1985), 85*(4), 1516-1522.

CHAPTER 6

Thesis Summary
Future Recommendations
and
Main Conclusions

TABLE OF CONTENTS

CHAPTER 6

6.1 THESIS SUMMARY

This thesis has contributed to important areas relevant to exercise training and cardiac function in patients with type 2 diabetes (T2D).

Chapter 1 addressed the global burden of T2D, the high prevalence of cardiovascular disease risk (cardiometabolic risk), and the increased risk of heart failure in the T2D population in general. In particular, diabetic cardiomyopathy was addressed by discussing proposed pathogenesis of this entity. Furthermore, the high prevalence of diastolic dysfunction in asymptomatic T2D individuals was identified through previous studies. Few studies have investigated the effect of aerobic exercise on cardiac function in general, and on diastolic function in particular, in patients with T2D, but trials performed on other groups with cardiometabolic disease (i.e. heart failure) clearly indicate that aerobic exercise training can induce cardiac remodeling. However, no randomized controlled trial (RCT) had investigated the effects of exercise intensity, particularly HIIE, on cardiac function in T2D individuals with pre-identified reductions in diastolic function. Moreover, Chapter 1 addressed the reduced cardiorespiratory fitness observed in trials of T2D patients and patients with diastolic dysfunction and discussed the increased cardiovascular risk and the risk of overall mortality or premature death that low cardiorespiratory fitness represents. Exploring previous reviews, meta-analysis, and exercise trials in T2D patients identified that aerobic exercise training has the potential to significantly improve cardiorespiratory fitness in this patient group. Two different exercise intensities and the potential effects of these intensities on cardiac function and cardiorespiratory fitness were discussed. Based on positive findings in studies reviewed, it seems likely that aerobic exercise training, and high intensity exercise (HIIE) in particular as compared to traditional, moderate intensity exercise (MIE; as the recommended approach in global guidelines), play an important role when addressing improved cardiac function as a treatment target in patients with T2D and diastolic dysfunction. However, a lack of information in regard to the effect of exercise intensities on individuals with T2D was identified and it was noted that further research is required to investigate the effect of intensity on diastolic function in T2D individuals. Finally, Chapter 1 (section 1.2) introduced the studies comprised in this thesis; Study 1 investigated the effect of HIIE and MIE training (12 weeks and one year follow up) on diastolic function and cardiorespiratory fitness and Study 2 explored the effect of acute HIIE and MIE in the postprandial state after fast food ingestion on cardiac workload. Study 1 is presented in Chapters 3-4 and forms the basis for Papers I-II, while Study 2 is presented in

Chapter 5 and forms the basis for Paper III. Chapter 2 discusses the methods used in Chapters 3-5.

The main study of this thesis (Study 1) was an RCT, of which the results are reported in Chapter 3. This was the first study to our knowledge to assess the effect of HIIE versus MIE on a group of T2D individuals with pre-identified diastolic dysfunction. This study contributes significantly to the area of exercise training in patients with T2D; in particular by finding that exercise intensity is important to improving diastolic function when diastolic dysfunction is present in T2D individuals (Chapter 3). This study also conformed to previous studies showing that HIIE is highly effective compared to lower intensities in improving cardiorespiratory fitness in T2D (see Chapter 1). Finally, the one-year follow up described in Chapter 3 indicates that the HIIE group managed to sustain positive findings (improved diastolic function and cardiorespiratory fitness) more than the MIE group. Overall, the findings from this study (Chapter 3) provide insight into the potential for more targeted exercise guidelines for T2D patients with reduced diastolic function. Furthermore, this study provides support for the newer global exercise guidelines (published after commencing Study 1), implicating that high intensity exercise can compensate for reduced use of time to improve cardiometabolic risk factors, such as reduced cardiorespiratory fitness and glycemic control as well as central obesity.

The sub-study generated from Study 1 (described in Chapter 4) investigated the effect of exercise on cardiac untwist and twist parameters. This pilot study compared the T2D individuals described in Chapter 3 with healthy individuals, which had previously participated in the Echocardiography in Nord-Trøndelag Health study (HUNT3) (Dalen et al., 2010). Chapter 4 conforms to previous studies showing that patients with diastolic dysfunction and normal ejection fraction have similar LV twist parameters (i.e. LV rotation, twist, untwist rate [UTR], and peak UTR) to healthy subjects. Furthermore, this study confirms that early diastolic untwisting, which is considered imperative for optimal suction in the early stages of diastole, is delayed when LV relaxation is altered. Chapter 4 indicated that delayed time to peak UTR is common in well-controlled, asymptomatic T2D individuals with diastolic dysfunction as compared with healthy counterparts (Chapter 4) and confirms previous studies observing delayed peak UTR in a number of cardiometabolic diseases, such as heart failure; coronary artery disease; and hypertension (Mor-Avi et al., 2011). Second, this pilot study indicated that exercise training (both HIIE and MIE) can normalize the timing of untwist in T2D individuals with diastolic dysfunction, a novel observation that highlights the potential usefulness of

exercise training as an effective tool in secondary prevention in T2D individuals. Overall, time to peak UTR was the only diastolic variable measured in this thesis that normalized after 12 weeks of exercise training in T2D patients with diastolic dysfunction. This indicates that time to peak UTR might be a more sensitive parameter to evaluate early diastolic changes than LV UTR, not only for identification, but also for follow-up after exercise intervention. This study thus indicated that time to peak UTR is a useful marker of early diastolic function, which potentially can serve as a therapeutic target for improving diastolic function. However, the clinical value of this observation is not known and needs future exploration. Chapter 4 thus provided implications for further research to investigate the role of time to peak UTR in diastolic dysfunction.

Finally, Study 2 (Chapter 5) assessed the effect of acute exercise (HIIE and MIE) as opposed to NE (sedate behavior) on postprandial cardiac function after ingesting fast food in T2D individuals and healthy counterparts. No study has to our knowledge previously investigated the effect of exercise on the postprandial alterations in cardiac function. This pilot study confirms previous studies showing that cardiac workload is altered in the postprandial phase in T2D as well as in healthy individuals. Furthermore, the findings of this study indicated that patients with T2D have a tendency towards greater cardiac workload in the postprandial phase (after fast food) than matched counterparts without T2D. However, this study is too small to draw conclusions. Indeed, the clinical importance of a potential increased cardiac workload due to food, in the context of increased cardiovascular risk due to postprandial dysmetabolism, needs to be further investigated in the T2D population. Nonetheless, it is necessary for exercise physiologists to define exercise prescriptions that will produce the desired effect on individuals with postprandial dysmetabolism (i.e. to reduce oscillating excessive elevations in circulation glucose throughout the day) for preventing the development of cardiovascular disease in individuals with T2D. Even though this study indicated that pre-exercise cannot modify postprandial changes in cardiac function, heart rate (HR), blood pressure, or biochemical measures in T2D, this study implicates grounds for further research in this field.

6.1.1 Key thesis discoveries

6.1.1.1 Chapter 3 (Paper I)

- In individuals without traditional signs of CVD and T2D, ~57% had diastolic dysfunction (measured as e' < 8 cm/s).

In individuals with T2D and diastolic dysfunction:

Twelve weeks exercise intervention:

- Both HIIE and MIE improved diastolic function (e') at rest, but HIIE was superior to MIE.
- Only HIIE improved other diastolic variables (E, E/e', E/A) at rest.
- Only HIIE improved early filling velocity (E) during stress echocardiography.
- Only HIIE improved systolic function (S', global strain, global strain rate) at rest. HIIE was superior to MIE in improving S' and global strain rate.
- Only HIIE improved systolic function (global strain rate) during stress echocardiography.
- Both HIIE and MIE improved cardiorespiratory fitness (VO_{2peak}), but HIIE was superior to MIE.
- Only HIIE improved endothelial function (FMD), glycosylated hemoglobin (HbA_{1c}), and body mass index (BMI).
- Both HIIE and MIE reduced waist circumference (WC).

At one year follow up:

- Only the HIIE group sustained improved diastolic function (e') at rest compared to baseline.
- Only the HIIE group sustained improvement in VO_{2peak}, despite a significant decrease in cardiorespiratory fitness from 12 weeks to one year in both the HIIE and MIE groups.
- The HIIE group sustained reduced WC, and the MIE-group increased WC from 12 weeks to one year.
- Body fat percentage was reduced over one year in the HIIE group only.
- HbA_{1c}-levels and FMD returned to baseline in the HIIE group at one-year post-test.
- No significant changes from the 12-week post-test to one year were seen for other variables.

6.1.1.2 Chapter 4 (Paper II)

In patients with T2D and diastolic dysfunction (measured as e' < 8 cm/s):

- LV peak UTR, peak twist rate, and peak twist were similar to healthy counterparts.
- Time to peak UTR was delayed at baseline compared to healthy counterparts.
- Twelve weeks of exercise training significantly shortened the time to peak UTR. At the 12-week post-test, the time to peak UTR was normalized as compared to healthy counterparts, independent of exercise intensity.

6.1.1.3 Chapter 5 (Paper III)

In patients with T2D compared to BMI and age-matched individuals (controls):

- Diastolic and systolic workload (e' and E, global strain rate, and S') increased after fast food ingestion in both groups.
    - The diastolic workload was subsequently reduced within four hours post-meal, but an indication of sustained increase in diastolic workload, as measured by e', was observed in the T2D group four hours after the meal.
    - Systolic workload (global strain rate and S') remained elevated (compared to baseline) within four hours post-meal in both groups.
- Both groups increased resting HR within 30 minutes after the meal.
    - HR subsequently decreased in both groups, but only the controls regained baseline resting HR four hours after the meal.
- In contrast to the controls, the mean systolic blood pressure in the T2D group dropped within two hours after fast food and reversed within four hours post-meal in the T2D group.
- Neither exercise program (HIIE or MIE) 16-18 hours pre-meal influenced cardiac function, HR, blood pressure response, or biochemical measures in the postprandial phase.

6.2 FUTURE RECOMMENDATIONS

Future studies need to provide optimal lifestyle prescriptions to both treat and prevent the development of diabetic cardiomyopathy in particular and CMR in general. Indisputably, further studies are necessary to extend the findings of the present thesis and previous research. Only a small number of studies have been conducted in the area of exercise training and cardiac function in T2D and important questions still remain. Arguably, there are several important future areas to be investigated.

6.2.1 Future exercise approaches for improved cardiac function and cardiometabolic risk reduction in type 2 diabetes

6.2.1.1 Individualized lifestyle (i.e. exercise and dietary) interventions

Future exercise intervention studies should include individual analysis of the risk profile of the patient, as one size does not fit all. Study 1 in this thesis performed exercise intervention on a clearly defined T2D group by selecting individuals with diastolic dysfunction, whereas Study 2 included a less defined group of T2D individuals. This might have affected the results and the interpretation of results.

As for the general population, more and more evidence is accumulated in the field of cardiometabolic disease (included T2D) that engaging in physical activity and exercise training are important components in reducing the severity of CMR factors, such as low cardiorespiratory fitness, poor glycemic control, obesity and central obesity. Nonetheless, it is still not entirely clear what kind of physical exercise/exercise training (aerobic exercise training; resistance exercise training; combined resistance and aerobic endurance training) or what intensity, frequency, duration, time and volume that can yield the optimal benefit for each separate risk factor.

Indeed, the T2D population is a heterogenic group presenting with a variety of micro- and macrovascular complications in addition to CMR-factors such as obesity, poor glycemic control, poor cardiorespiratory fitness and diastolic dysfunction; or a more severe presence of cardiomyopathy. More research is warranted to develop methods to categorize T2D subgroups; to make sure the researchers actually compare individuals that can be compared. Moreover, future research should strive to develop exercise intervention programs designed for optimal health benefits in each and every T2D subgroup. For example, it can be expected that an obese

person with T2D and poor glycemic control have a somehow different exercise (or lifestyle) intervention requirement to enhance health than a lean person with T2D and relatively well controlled glycemic control. Nonetheless, it is indisputable that both individuals would benefit from regular exercise.

Consequently, future research should also strive to better understand the heterogeneity previously observed in the adaptive response to exercise in individuals with T2D (Bouchard et al., 2012; Osler et al., 2015; Solomon et al., 2013). Identifying the predictors of these individual responses and how to modify them will provide the foundation for personalized exercise prescription. This will ultimately allow the design of optimal exercise programs that target skeletal muscles and the nervous system to improve cardiac function and cardiorespiratory fitness in particular, and CMR in general on the individual level in patients with T2D. In this context, an implementation of exercise-nutrient protocols in future research that considers the timing and type (i.e. carbohydrate, protein, or fat) of nutrients the study participant ingests either before or after acute exercise is also needed to maximize health benefits (Zierath and Wallberg-Henriksson, 2015).

6.2.1.2 Adherence to exercise

Indeed, the primary goal in T2D management should be sustained lifestyle changes in terms of increased physical activity (i.e. exercise training) and nutritional changes, together with the promotion of knowledge and awareness of the benefits of lifestyle changes in regard to health (Eriksson and Lindgarde, 1991; Knowler et al., 2002; Pan et al., 1997; Tuomilehto et al., 2001). Future research should investigate which exercise method or strategy is best for achieving adherence to exercise training in the long-term in T2D population. The importance of an exercise intervention and its long-term benefit is dependent on the adherence to the exercise training program. Indeed, it appears as if whatever endurance exercise program is carried out, it is the exercise training that is carried out throughout life (consistently several times a week) that makes a difference to cardiac health (Bhella et al., 2014; Carrick-Ranson et al., 2014).

One obstacle, which often is suggested as a barrier to exercise training when health professionals are asked, is low motivation (Vanhees et al., 2012), which experience from administrating the projects of this thesis confirms. To carry out exercise training throughout life, a central factor is enjoyment in regard to the activity that is prescribed. Although individual preferences must be taken into consideration, an HIIE bout represents a somehow greater variation (i.e. pace and HR variation) throughout the exercise session compared to a traditional

MIE bout, and might thus be more motivating to carry out. Indeed, Bartlett et al. found that HIIE was perceived as more enjoyable than MIE (Bartlett et al., 2011).

Furthermore, lack of time is one of the most commonly cited barriers to physical activity or exercise training (Kimm et al., 2006; Stutts, 2002; Trost et al., 2002; Vanhees et al., 2012) and further research in regard to the potential of HIIE (including low-volume HIIE) as an achievable exercise strategy in daily life is important from a public health perspective. Even though a large body of evidence demonstrates the effectiveness of endurance training (and lifestyle interventions in general) to improve health markers in cardiometabolic disease, individuals fail to translate current exercise guidelines into action (Olsen et al., 2008). This thesis support previous studies in metabolic disease suggesting that HIIE is a potent, time-efficient therapeutic intervention that is more effective in improving cardiac function and cardiorespiratory fitness than traditional MIE in the short term as well as over a one-year perspective (Hordern et al., 2009; Weston et al., 2014). This thesis thus supports previous studies showing that incorporating HIIE into rehabilitation programs, both in the short- and long-term, may be more achievable for individuals with T2D to reach exercise levels that can yield health benefits.

Another important area concerning adherence to exercise is the contrast between supervised versus unsupervised exercise programs. Lack of help from professionals and lack of education/knowledge about physical activity have also been listed as barriers to physical activity (Vanhees et al., 2012). Indeed, previous studies state that supervised exercise training are associated with greater improvements in CMR than less strict strategies, such as exercise counselling alone (Balducci et al., 2010; Umpierre et al., 2011; Vanhees et al., 2012). According to Study 1 in this thesis, it seems reasonable to suggest that HIIE is the most effective exercise regimen to improve diastolic function in T2D individuals, however it is not yet known whether it is the most applicable to home-based exercise prescription. Previous research suggests that HIIE, as performed in this thesis, is a feasible activity to be performed independently (i.e. home-based). For instance, participants in a study by Wisloff et al. adequately performed HIIE (as performed in the present study) without HR monitors and still managed to reach required HRs according to the HIIE protocol (Wisloff et al., 2007). This indicated that HIIE can be translated into real world situations after familiarization with an exercise physiologist present. Furthermore, Moholdt et al. confirmed these findings by demonstrating adherence to HIIE during home-based exercise training in individuals with coronary artery disease (Moholdt et al., 2012). Nonetheless, future research should determine

whether HIIE is sustainable in a non-supervised setting (i.e. home-based). Moreover, future studies should consider the potential difference in effect between supervised versus unsupervised exercise programs when planning exercise interventions comparing HIIE and MIE. Furthermore, in light of the apparent difficulty to achieve adherence to exercise in the long term in individuals with T2D, it is imperative that studies apply exercise programs that can be applied to real-life settings in the future. In the context of exercise interventions and supervision, telecommunication, such as mobile applications for diabetes self-management, should be considered as long-term options (El-Gayar et al., 2013).

Future studies should also investigate whether varying between training modalities is central to exercise adherence. It would also be interesting to include cognitive behavioral therapy into future long-term exercise and/or diet interventions (over 24 months) to investigate whether this additional intervention could promote a greater adherence to exercise in the long-term in individuals with T2D. However, the efficacy of behavioral counseling is uncertain (U. S. Preventive Services Task Force, 2002).

6.2.1.3 Long term effects of HIIE versus MIE

The findings of this thesis emphasize the importance of exercise guidelines recommending vigorous activity several days a week. However, much more work is needed to determine whether the recommendations indicated by this thesis provide the optimal exercise approach to prevent or slow down the development of diastolic dysfunction in individuals with T2D in the long term.

Furthermore, it remains to be determined whether HIIE protocols (either high-volume or low-volume) can confer all benefits associated with prolonged exercise training regimens. Studies that comprised HIIE regimens have been of relatively low volume and short duration and future research must establish whether the early adaptations observed after these interventions are similar to interventions of lower intensity when sustained over a longer time period (i.e. years). The time course of exercise adaptations is different between exercise training protocols and the HIIE regimen can yield rapid response, whereas traditional, moderate, continuous intensity exercise can occur more slowly. However, which exercise regimen yields the greatest clinical improvements over time has yet to be determined and requires further investigation. Comparisons between current recommendations for exercise prescription and HIIE are still warranted in this setting. Indeed, the prescription of HIIE represents a practical strategy with the potential to be incorporated into daily living. However, future work involving long-term

interventions in a variety of clinical cohorts is required to better understand how manipulating the exercise stimulus impacts cardiac remodeling, cardiorespiratory fitness, and other health markers in people with T2D.

6.2.1.4 The optimal exercise training protocol

Further studies are needed to establish the optimal HIIE protocol for the T2D population. Based on the findings of Weston et al., it appears that high-volume HIIE is required to achieve greater health outcomes (i.e. cardiac remodeling, VO_{2peak}) than MIE for individuals with cardiometabolic disease (Gibala et al., 2012; Gibala and McGee, 2008; Weston et al., 2014). However, less is known about the effect of low-volume HIIE and more research is needed to compare low-volume HIIE with high-volume HIIE and traditional MIE, and the combination of these in regard to cardiac remodeling in the long-term. Future research should strive to establish whether low-volume HIIE could yield similar cardiac remodeling in T2D as observed for high-volume HIIE or MIE in the present thesis. The prescription of HIIE (low-volume and high-volume) represents a practical and time-efficient strategy that appears to be easily incorporated into daily life and that can subsequently reduce the economic burden associated with physical inactivity at both an individual and population level. As noted previously, the primary goal in T2D management should be sustained lifestyle changes in terms of increased physical activity (i.e. exercise training) and nutritional changes, together with the promotion of knowledge and awareness of the benefits of these lifestyle changes in regard to health. The potential of HIIE to promote important health benefits in T2D, as demonstrated in this thesis and previous research, indicates that future exercise guidelines should include HIIE strategies and different timings of exercise in concordance with meals that allow T2D individuals to choose an exercise strategy that is optimal to their medical needs; as well as to their lifestyle, habits, daily time restraints, and personal goals.

Indeed, HIIE and traditional endurance-based exercise are not mutually exclusive, and perhaps instead of comparing them directly, the incorporation of both types of exercise intensities could be an effective solution. Optimal ratios of combined HIIE and MIE, and also high-volume HIIE and low-volume HIIE, are also relevant matters to investigate as it is realistic to assume that most people will combine exercise intensities and work-bout durations (when performing interval training) over longer periods of time. Moreover, few studies have investigated combination programs with HIIE and resistance training. Further studies should strive to establish whether there is an interaction between these two and which ratios of these yields optimal benefits in regard to diabetic cardiomyopathy and CMR. Moreover, further research

should establish the importance of dietary interventions in concord with exercise training interventions in regard to cardiac function, considering the fact that it is the metabolic disturbances that indirectly affect the cardiac function in patients with T2D (Hafstad et al., 2015).

6.2.2 The effect of exercise on cardiac function in T2D

A substantial amount of research remains to better understand the relationships between metabolic disturbances and diabetic cardiomyopathy. Indeed, the changes observed in cardiac function due to exercise training are suggested to be due to both indirect effects through systemic changes as well as through direct effects due to the increased contractile activity during endurance training (Hafstad et al., 2015). Future studies should investigate the molecular mechanisms resulting in the beneficial cardiac effects of exercise training to achieve new and evidence-based treatment strategies. In regard to the effect of exercise due to increased contractile activity during endurance training, large scale trials are required to better understand the role of the timing of cardiac untwist in the context of improved diastolic function induced by endurance exercise in general, and whether intensity plays a role in improving the timing of cardiac untwist in particular.

6.2.3 The clinical importance of diastolic dysfunction and the effect of exercise intensity on cardiovascular outcomes.

The effect of long-term HIIE interventions versus MIE on the development of diabetic cardiomyopathy and HF mortality is an important area of investigation. Although diastolic function might be an early marker of future CVD in patients with T2D, it is not known whether improvement in this parameter can contribute to reduce cardiovascular outcomes. However, mortality benefits have been observed in individuals with improved diastolic function compared to those whose diastolic function remained the same or worsened (Halley et al., 2011). On the other hand, recent large-scale trials investigating HIIE versus MIE have failed to find a difference between these exercise intensities in CVD and HF populations (Conraads et al., 2015; Stoylen et al., 2012), so the role of intensity in HF has not yet been established. Furthermore, even though enhanced cardiorespiratory fitness can improve CMR (i.e. by reduced hyperglycemia) in T2D (Jakicic et al., 2013), there are no randomized controlled trial data on the effects of change in aerobic capacity on mortality in the T2D population. Moreover, the Look AHEAD study found that reduction of traditional cardiovascular risk factors after lifestyle intervention (minimum 175 minutes of MIE per week) did not reduce the risk of

cardiac events in T2D subjects (Wing et al., 2013). Thus, more research of a variety of exercise strategies are warranted in this area; future exercise recommendations for the T2D population should be based on large scale randomized controlled trials investigating the effect of different exercise approaches on cardiac function, cardiorespiratory fitness, and traditional cardiovascular risk factors and definite health outcomes over the long-term (i.e. years). Furthermore, due to the heterogeneity of the T2D population and the diverse response to exercise observed in exercise intervention studies; it is required that future studies are more critical in regard to selecting T2D individuals into subgroups by grading level of disease and/or organ system that is most affected making sure that the exercise intervention is targeted. Of course, this requires that researchers become better at identifying subgroups and targeting the right exercise prescription.

6.2.3.1 Future assessment of diastolic dysfunction

If the diagnosed diastolic dysfunction should be considered important for T2D patients, we need a less complex grading system for the clinician to use and for follow-up. The guidelines have recently changed (Nagueh et al., 2016), but are probably still too concise to be used in "real life". Certainly, future research should target individuals at risk of developing heart disease using early markers of myocardial dysfunction; one potential early marker in the future could be time to UTR, if the methods are improved, automated and all limitations overcome. Furthermore, future studies should include cardiac function during exercise and stress tests, which can reveal cardiac dysfunction and should be included in a risk analysis of T2D patients.

UTR occurs during the diastolic isovolumic period before blood flow or wall motion begins, and measurement of untwists and UTR could constitute novel early markers for the assessment of LV relaxation. Whether untwist and UTR can be more sensitive than other diastolic function parameters require more investigation. Further research is warranted to understand the role of time to peak UTR in diastolic dysfunction. Further studies are necessary to establish the most practical and reliable parameters to identify the early stage of diastolic dysfunction.

STE has facilitated the measurement of LV twist and has provided important knowledge about cardiac mechanics both in experimental settings and during some cardiac diseases. However, several issues concerning the technique must be further developed such as the lack of standardization of imaging planes as well as the use of different speckle-tracking algorithms. The development of 3D STE can track motion of speckles within the scan volume, irrespective

of its direction, and is likely to allow standardization of LV planes. However, 3D STE is limited by relatively low temporal and spatial resolution.

In future guidelines, it might also be important to consider adding recommendations in regard to the timing of the echocardiographic measurement as the effect of a meal may influence the echocardiographic results and interpretation, according to Chapter 5.

6.2.4 Postprandial dysmetabolism and diabetic cardiomyopathy

Finally, future studies should determine the role of postprandial dysmetabolism in the context of diabetic cardiomyopathy. Although cardiac workload is increased in the postprandial state, it is not known whether this is clinically relevant in the context of diabetic cardiomyopathy or whether targeting postprandial dysmetabolism in exercise programs can contribute to preventing or slowing down the development of diabetic cardiomyopathy or reduce cardiovascular outcomes in T2D. However, postprandial hyperglycemia and dyslipidemia have been associated with increased cardiovascular risk, and improvements have shown to reduce cardiovascular risk (see Chapter 5). The effect of strategic exercise timing in concordance to meals (i.e. to reduce fluctuating postprandial hyperglycemia throughout the day) versus traditional exercise training (according to exercise guidelines; i.e. exercise at least every second day) on the development of diabetic cardiomyopathy and cardiometabolic risk factors in T2D individuals should thus be further investigated.

Further studies are also warranted to determine whether food in general, and fast food in particular, in fact induces greater stress to the myocardium in patients with T2D compared to healthy counterparts. If this is confirmed, this may have implications for food choices in T2D. In addition, future investigations should address potential differences between metabolically fit individuals versus individuals with metabolic risk factor clustering when addressing postprandial cardiac function in this context. Moreover, future studies should investigate the effect of acute exercise of various intensities and volumes, as well as timing in concordance to meals in this setting. Even though it was out of the scope for this thesis, the hypoglycemic spikes on end-organ function using continuous blood sugar measurements should also be considered.

6.3 MAIN CONCLUSIONS

The results of this thesis demonstrate that exercise intensity is important to improving subclinical LV diastolic function and cardiorespiratory fitness in patients with T2D and diastolic dysfunction in both the short- (three months) and long-term (one year). The results emphasize the importance of targeting exercise guidelines towards higher intensities particularly when aiming to improve diastolic and systolic function, cardiorespiratory fitness (VO_{2peak}), glycemic control (HbA_{1c}), and body composition (BMI, waist circumference, and body fat percentage) in T2D individuals with diastolic dysfunction. However, after one year, several positive adaptations to HIIE and MIE were reduced or returned to baseline levels, which possibly reflects a lack of adherence to exercise over time (i.e. months and years) in this patient group. This points to the potential need for a multifactorial and more individualized approach in future research to target optimal exercise training to prevent or delay the development of CMR in general and diabetic cardiomyopathy in particular.

This thesis also demonstrates that patients with T2D and diastolic dysfunction have a delayed timing of untwist (time to peak UTR) compared to healthy counterparts. Furthermore, this thesis provides new insights by demonstrating that the timing of LV UTR, which is imperative to the magnitude of cardiac suction in early diastole, might be sensitive to endurance exercise in patients with T2D, independent of intensity. Although this positive finding indicates that the timing of cardiac untwist represents a suitable pathophysiological target for the treatment of diastolic dysfunction, this is not yet clear and needs further investigation.

This thesis also demonstrates that fast food induces increased cardiac workload in both T2D individuals and their BMI- and age-matched healthy counterparts, which cannot be modified by acute pre-exercise. Furthermore, this thesis indicates that fast food interacts with cardiac workload to a greater extent in T2D individuals than in healthy counterparts. However, this needs to be confirmed in future RCTs before conclusion. Future research in this field should include echocardiography to better understand the effects of food to the heart in T2D; and strive to identify optimal time, model, intensity, volume, and frequency for exercise prescription to reduce direct and indirect effects of fluctuating metabolic disturbances in individuals with T2D. Thus, exercise-nutrient protocols in future research should consider a diversity of timing and type of acute exercise either before or after ingesting food (i.e. carbohydrate, protein, or fat) to optimize future exercise programs to achieve health benefits.

REFERENCES

Balducci, S., Zanuso, S., Nicolucci, A., De Feo, P., Cavallo, S., Cardelli, P., . . . Italian Diabetes Exercise Study, I. (2010). Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in subjects with type 2 diabetes mellitus: a randomized controlled trial: the Italian Diabetes and Exercise Study (IDES). *Arch. Intern. Med., 170*(20), 1794-1803.

Bartlett, J. D., Close, G. L., MacLaren, D. P., Gregson, W., Drust, B., & Morton, J. P. (2011). High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. *J. Sports Sci., 29*(6), 547-553.

Bhella, P. S., Hastings, J. L., Fujimoto, N., Shibata, S., Carrick-Ranson, G., Palmer, M. D., . . . Levine, B. D. (2014). Impact of lifelong exercise "dose" on left ventricular compliance and distensibility. *J. Am. Coll. Cardiol., 64*(12), 1257-1266.

Bouchard, C., Blair, S. N., Church, T. S., Earnest, C. P., Hagberg, J. M., Hakkinen, K., . . . Rankinen, T. (2012). Adverse metabolic response to regular exercise: is it a rare or common occurrence? *PLoS One, 7*(5), e37887.

Carrick-Ranson, G., Hastings, J. L., Bhella, P. S., Fujimoto, N., Shibata, S., Palmer, M. D., . . . Levine, B. D. (2014). The effect of lifelong exercise dose on cardiovascular function during exercise. *J Appl Physiol (1985), 116*(7), 736-745.

Conraads, V. M., Pattyn, N., De Maeyer, C., Beckers, P. J., Coeckelberghs, E., Cornelissen, V. A., . . . Vanhees, L. (2015). Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study. *Int. J. Cardiol., 179*, 203-210.

Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A., & Stoylen, A. (2010). Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ. Cardiovasc. Imaging, 3*(5), 614-622.

El-Gayar, O., Timsina, P., Nawar, N., & Eid, W. (2013). Mobile applications for diabetes self-management: status and potential. *J. Diabetes Sci. Technol., 7*(1), 247-262.

Eriksson, K. F., & Lindgarde, F. (1991). Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmo feasibility study. *Diabetologia, 34*(12), 891-898.

Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol., 590*(Pt 5), 1077-1084.

Gibala, M. J., & McGee, S. L. (2008). Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? *Exerc. Sport Sci. Rev., 36*(2), 58-63.

Hafstad, A. D., Boardman, N., & Aasum, E. (2015). How Exercise May Amend Metabolic Disturbances in Diabetic Cardiomyopathy. *Antioxid Redox Signal*.

Halley, C. M., Houghtaling, P. L., Khalil, M. K., Thomas, J. D., & Jaber, W. A. (2011). Mortality rate in patients with diastolic dysfunction and normal systolic function. *Arch. Intern. Med., 171*(12), 1082-1087.

Hordern, M. D., Coombes, J. S., Cooney, L. M., Jeffriess, L., Prins, J. B., & Marwick, T. H. (2009). Effects of exercise intervention on myocardial function in type 2 diabetes. *Heart, 95*(16), 1343-1349.

Jakicic, J. M., Egan, C. M., Fabricatore, A. N., Gaussoin, S. A., Glasser, S. P., Hesson, L. A., . . . Look, A. R. G. (2013). Four-year change in cardiorespiratory fitness and influence on glycemic control in adults with type 2 diabetes in a randomized trial: the Look AHEAD Trial. *Diabetes Care, 36*(5), 1297-1303.

Kimm, S. Y., Glynn, N. W., McMahon, R. P., Voorhees, C. C., Striegel-Moore, R. H., & Daniels, S. R. (2006). Self-perceived barriers to activity participation among sedentary adolescent girls. *Med. Sci. Sports Exerc., 38*(3), 534-540.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., . . . Diabetes Prevention Program Research, G. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med., 346*(6), 393-403.

Moholdt, T., Bekken Vold, M., Grimsmo, J., Slordahl, S. A., & Wisloff, U. (2012). Home-based aerobic interval training improves peak oxygen uptake equal to residential cardiac rehabilitation: a randomized, controlled trial. *PLoS One, 7*(7), e41199.

Mor-Avi, V., Lang, R. M., Badano, L. P., Belohlavek, M., Cardim, N. M., Derumeaux, G., . . . Zamorano, J. L. (2011). Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. *Eur. J. Echocardiogr., 12*(3), 167-205.

Nagueh, S. F., Smiseth, O. A., Appleton, C. P., Byrd, B. F., 3rd, Dokainish, H., Edvardsen, T., . . . Waggoner, A. D. (2016). Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr., 29*(4), 277-314.

Olsen, R. H., Krogh-Madsen, R., Thomsen, C., Booth, F. W., & Pedersen, B. K. (2008). Metabolic responses to reduced daily steps in healthy nonexercising men. *JAMA, 299*(11), 1261-1263.

Osler, M. E., Fritz, T., Caidahl, K., Krook, A., Zierath, J. R., & Wallberg-Henriksson, H. (2015). Changes in gene expression in responders and nonresponders to a low-intensity walking intervention. *Diabetes Care, 38*(6), 1154-1160.

Pan, X. R., Li, G. W., Hu, Y. H., Wang, J. X., Yang, W. Y., An, Z. X., . . . Howard, B. V. (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care, 20*(4), 537-544.

Solomon, T. P., Malin, S. K., Karstoft, K., Kashyap, S. R., Haus, J. M., & Kirwan, J. P. (2013). Pancreatic beta-cell function is a stronger predictor of changes in glycemic control after an aerobic exercise intervention than insulin sensitivity. *J. Clin. Endocrinol. Metab., 98*(10), 4176-4186.

Stoylen, A., Conraads, V., Halle, M., Linke, A., Prescott, E., & Ellingsen, O. (2012). Controlled study of myocardial recovery after interval training in heart failure: SMARTEX-HF--rationale and design. *Eur J Prev Cardiol, 19*(4), 813-821.

Stutts, W. C. (2002). Physical activity determinants in adults. Perceived benefits, barriers, and self efficacy. *AAOHN J., 50*(11), 499-507.

Trost, S. G., Owen, N., Bauman, A. E., Sallis, J. F., & Brown, W. (2002). Correlates of adults' participation in physical activity: review and update. *Med. Sci. Sports Exerc., 34*(12), 1996-2001.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., . . . Finnish Diabetes Prevention Study, G. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med., 344*(18), 1343-1350.

U. S. Preventive Services Task Force. (2002). Behavioral counseling in primary care to promote physical activity: recommendation and rationale. *Ann. Intern. Med., 137*(3), 205-207.

Umpierre, D., Ribeiro, P. A., Kramer, C. K., Leitao, C. B., Zucatti, A. T., Azevedo, M. J., . . . Schaan, B. D. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA, 305*(17), 1790-1799.

Vanhees, L., Geladas, N., Hansen, D., Kouidi, E., Niebauer, J., Reiner, Z., . . . Vanuzzo, D. (2012). Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II. *Eur J Prev Cardiol, 19*(5), 1005-1033.

Weston, K. S., Wisloff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med., 48*(16), 1227-1234.

Wing, R. R., Bolin, P., Brancati, F. L., Bray, G. A., Clark, J. M., Coday, M., . . . Yanovski, S. Z. (2013). Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N. Engl. J. Med., 369*(2), 145-154.

Wisloff, U., Stoylen, A., Loennechen, J. P., Bruvold, M., Rognmo, O., Haram, P. M., . . . Skjaerpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation, 115*(24), 3086-3094.

Zierath, J. R., & Wallberg-Henriksson, H. (2015). Looking Ahead Perspective: Where Will the Future of Exercise Biology Take Us? *Cell Metab., 22*(1), 25-30.

Paper I

JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
VOL. 64, NO. 16, 2014

ISSN 0735-1097/$36.00
PUBLISHED BY ELSEVIER INC.

Letters

High-Intensity Interval Exercise Effectively Improves Cardiac Function in Patients With Type 2 Diabetes Mellitus and Diastolic Dysfunction

A Randomized Controlled Trial

Left ventricular diastolic dysfunction (DD) may lead to heart failure and is found in approximately 50% of asymptomatic patients with type 2 diabetes mellitus (T2DM). Little is known about the effect of exercise on DD in T2DM (1), but moderate-intensity exercise (MIE) seems insufficient to improve myocardial function. Studies indicate that high-intensity interval exercise (HIIE) is more effective than MIE in reducing cardiovascular risk factors in T2DM and in reversing left ventricular remodeling in patients with post-infarction heart failure. The aim of this study was to compare the effect of HIIE (4 × 4-min interval, 90% to 95% maximal heart rate, 40 min/bout, 3/week) and MIE according to current guidelines (≥10 min/bout, 210 min/week) on DD, defined as peak early diastolic tissue Doppler velocity (e′) <8 cm/s (2), and other cardiovascular risk factors in patients with T2DM and DD. Our hypothesis was that HIIE, more than MIE, would improve these measures.

We prescreened 83 patients for DD who had T2DM for <10 years and no known cardiovascular disease. A total of 47 patients (55.9 ± 6.0 years; 36% female; duration of T2DM: 3.6 ± 2.5 years) met the inclusion criteria (e′ <8 cm/s). The subjects were randomized to home-based MIE (n = 23) and supervised HIIE (n = 24) and tested at baseline, 12 weeks (MIE, n = 17; HIIE, n = 20), and 1 year (MIE, n = 16; HIIE, n = 16). The patients in the MIE group were younger than those in the HIIE group (mean 54.7 ± 5.3 vs. 58.6 ± 5.0 years) but did not differ by sex (35.3% vs. 40.0% female) or duration of T2DM (3.0 ± 2.6 vs. 4.2 ± 2.3 years). After 12 weeks, exercise was home based in both groups.

Repeated-measures analysis of variance models (generalized linear model, linear mixed model) were applied to compare intervention groups with respect to mean change in outcome variables. Results from baseline to 12 weeks are shown in Table 1.

Both groups showed improved diastolic function (e′) at rest, but HIIE showed more improvement than MIE. Only HIIE improved transmitral peak early diastolic velocity (E), diastolic filling pressure (E/e′), and E/A ratio. A higher proportion of patients in the HIIE group had improved diastolic function to e′ >8 cm/s during the 12-week period (80.0% vs. 41.2%; p = 0.02, chi-square test). During exercise, only HIIE improved diastolic function (E). Lack of improvement in e′ during exercise may be explained by the use of different echocardiographic methods at rest and during exercise.

A nonsignificant decrease in e′ at rest was seen from 12 weeks to 1 year (−0.45 and −0.24 cm/s for HIIE and MIE, respectively). However, in contrast to the MIE group, the HIIE group still had improved diastolic function (e′) compared with baseline. Improvement in E was sustained in the HIIE group after 1 year.

After 12 weeks, HIIE but not MIE improved systolic function at rest (peak systolic tissue Doppler velocity, S′; global strain and global strain rate) and during exercise (global strain rate).

At baseline, mean peak oxygen consumption (Vo_{2peak}) (n = 37) was approximately 16% lower compared with a healthy population (the Nord-Trøndelag Health Study [HUNT] study). Both intervention groups increased Vo_{2peak}, but HIIE did so more than MIE. The improvement in Vo_{2peak} was sustained at 1 year in the HIIE group, but not in the MIE group, despite a significant decrease from 12 weeks to 1 year (−1.68 vs. −0.19 ml/kg/min, respectively).

After 12 weeks, waist circumference was reduced in both groups, whereas only HIIE reduced body mass index. From 12 weeks to 1 year, the MIE group, but not the HIIE group, had increased waist circumference (2.01 vs. −0.12 cm) and tended toward increased body mass index (0.58 vs. −0.12 kg/m^2). Body fat percent did not improve after 12 weeks but was reduced in the HIIE group after 1 year (mean change −1.31%). Twelve weeks of HIIE improved flow-mediated dilation (incomplete data due to impaired ultrasound image quality), hemoglobin A_{1c}, and high-sensitivity C-reactive protein level. Improved flow-mediated dilation and hemoglobin A_{1c} were not sustained

TABLE 1 Baseline and 12-Week Post-Test Results for Diastolic Echocardiographic Variables at Rest

	MIE (n = 17)					HIIE (n = 20)					MIE vs. HIIE
	N	Baseline	12 Weeks	Difference	p Value*	n	Baseline	12 Weeks	Difference	p Value*	p Interaction†
DD classification											
Mild (grade I)	17	5 (29.4)				20	1 (5.0)				
Moderate (grade II)	17	12 (70.6)				20	18 (90)				
Severe (grade III)	17	0 (0.0)				20	1 (5.0)				
Diastolic function, supine											
E, cm/s	14	63.4 ± 9.5	64.4 ± 13.4	1.0 ± 14.0	0.78	20	64.8 ± 10.7	75.1 ± 15.8	10.3 ± 12.7	0.002	0.05
e′, cm/s	17	7.1 ± 0.7	7.6 ± 1.1	0.5 ± 0.7	0.02	20	7.0 ± 0.7	8.8 ± 1.2	1.8 ± 1.1	<0.001	<0.001
E/e′	14	9.1 ± 1.8	8.6 ± 1.9	−0.5 ± 1.6	0.28	20	9.3 ± 1.7	8.6 ± 1.9	−0.7 ± 1.5	0.05	0.67
E/A, ratio	14	0.92 ± 0.18	0.99 ± 0.21	0.07 ± 0.23	0.28	20	0.93 ± 0.21	1.07 ± 0.26	0.13 ± 0.18	0.003	0.36
Systolic function, supine											
S′, cm/s	14	7.7 ± 1.2‡	7.6 ± 1.0	−0.1 ± 1.1	0.76	20	6.8 ± 0.8‡	7.8 ± 1.5	0.9 ± 1.4	0.007	0.03
Global strain, %	15	−16.7 ± 2.2	−16.4 ± 2.1	0.3 ± 1.6	0.51	20	−17.2 ± 1.9	−18.2 ± 2.2	−1.0 ± 1.9	0.03	0.05
Global strain rate, s^{-1}	16	−1.00 ± 0.15‡	−0.95 ± 0.10	0.06 ± 0.23	0.14	20	−0.87 ± 0.11‡	−0.97 ± 0.13	−0.10 ± 0.17	0.03	0.009
Vo_{2peak}											
ml/kg/min	16	33.2 ± 7.4	34.4 ± 7.7	1.2 ± 2.2	0.04	20	31.5 ± 6.1	35.6 ± 6.3	4.1 ± 2.9	<0.001	0.002
$ml/kg^{0.75}$/min	16	102.0 ± 23.8	105.2 ± 23.9	3.2 ± 6.1	0.06	20	98.6 ± 17.7	110.4 ± 20.0	11.8 ± 9.5	<0.001	0.003
l/min	16	2.96 ± 0.81	3.0 ± 0.79	0.06 ± 0.17	0.17	20	2.96 ± 0.57	3.29 ± 0.68	0.33 ± 0.29	<0.001	0.003
FMD											
FMD, %	10	13.0 ± 9.8	13.0 ± 9.9	0.0 ± 6.2	0.99	17	9.2 ± 9.6	18.5 ± 9.6	9.2 ± 11.2	0.004	0.03
FMD_{norm}, %	10	13.7 ± 12.6	14.6 ± 19.3	1.0 ± 10.0	0.76	16	8.9 ± 11.4	22.8 ± 15.1	13.9 ± 18.5	0.009	0.05
Heart rate, beats/min											
Rest	16	74 ± 10‡	72 ± 8	−2 ± 8	0.39	19	68 ± 8‡	66 ± 9	−1 ± 8	0.48	0.84
Maximal	16	168 ± 17	170 ± 14	2 ± 4	0.10	20	167 ± 10	169 ± 10	2 ± 4	0.08	0.85
Blood pressure, mm Hg											
Systolic	17	135.4 ± 11.9	134.9 ± 14.8	−0.5 ± 11.9	0.87	20	142.1 ± 18.3	142.5 ± 20.6	−0.5 ± 15.8	0.90	0.85
Diastolic	17	80.9 ± 7.1	80.8 ± 6.6	−0.1 ± 5.8	0.97	20	81.7 ± 6.9	78.3 ± 9.0	−3.4 ± 9.3	0.12	0.21
Biochemical values											
HbA_{1c}, %	16	6.7 ± 0.7	6.5 ± 0.6	−0.2 ± 0.7	0.30	20	7.0 ± 1.2	6.6 ± 0.9	−0.4 ± 0.5	0.007	0.36
HbA_{1c}, mmol/mol	16	50.0 ± 5.2	48.0 ± 4.4	−2.0 ± 7.0	0.30	20	53.0 ± 9.1	49.0 ± 6.7	−4.0 ± 5.0	0.007	0.36
HOMA-IR	15	2.6 ± 1.0	2.5 ± 0.9	−0.1 ± 0.5	0.32	20	2.7 ± 0.7	2.7 ± 1.0	0.0 ± 0.8	1.0	0.61
hs-CRP, mg/l	16	1.6 ± 1.2‡	1.6 ± 1.2	−0.1 ± 1.3	0.86	19	3.7 ± 2.8‡	2.1 ± 1.3	−1.6 ± 2.6	0.02	0.04
Anthropometric values											
BMI, m/kg^2	17	29.7 ± 3.7	29.4 ± 3.8	−0.3 ± 0.8	0.13	20	30.2 ± 2.8	29.7 ± 2.4	−0.5 ± 0.7	0.009	0.52
WC, cm	17	106.5 ± 8.7	104.5 ± 7.3	−2.0 ± 2.5	0.005	20	108.6 ± 7.7	106.0 ± 6.8	−2.6 ± 2.9	0.001	0.55
Body fat, %	15	27.5 ± 7.3	27.2 ± 6.7	−0.3 ± 2.1	0.58	19	27.9 ± 7.7	27.6 ± 8.5	−0.3 ± 1.3	0.36	0.97

Values are n (%) or mean ± SD. *p value: within-group difference. †p value: difference in mean change between groups. ‡Difference between groups at baseline, $p < 0.05$.

A = transmitral late diastolic velocity; BMI = body mass index; DD = diastolic dysfunction; E = transmitral peak early diastolic velocity; e′ = peak early diastolic tissue Doppler velocity; FMD = flow-mediated dilation of the brachial artery; FMD_{norm} = FMD normalized, FMD/shear rate (mean flow/mean diameter); HbA_{1c} = glycosylated hemoglobin; HIIE = high-intensity interval exercise; HOMA-IR = homeostatic model assessment of insulin resistance; hs-CRP = high-sensitive C-reactive protein; MIE = moderate-intensity exercise; S′ = peak systolic tissue Doppler velocity; Vo_{2peak} = peak oxygen consumption; WC = waist circumference.

after 1 year. No significant changes were seen for other variables.

Limitations of this study include the small size, significant dropout rate (albeit similar to other studies in this arena), lack of a control group without exercise, and supervised exercise in only the HIIE group, which could introduce better compliance.

This pilot evaluation, one of the first randomized studies to assess the effect of exercise intensity on DD in patients with T2DM, shows that HIIE may modify the natural history of diabetic cardiac dysfunction. In patients with T2DM and DD, HIIE was more effective than MIE in improving diastolic and systolic function as well as Vo_{2peak}. This indicates that exercise intensity is an important factor in improving cardiac function in early stages of T2DM and DD. Larger studies in the future should explore whether this is an effective and low-cost intervention in these patients with few other good therapeutic options.

Siri M. Hollekim-Strand, MSc
Marit R. Bjørgaas, MD, PhD
Grethe Albrektsen, PhD
Arnt E. Tjønna, PhD
Ulrik Wisløff, PhD
***Charlotte B. Ingul, MD, PhD**

*Department of Circulation and Medical Imaging
Norwegian University of Science and Technology
Olav Kyrres gate 3
7491 Trondheim
Norway
E-mail: charlotte.b.ingul@ntnu.no

http://dx.doi.org/10.1016/j.jacc.2014.07.971

Please note: This study was funded by the Liaison Committee between the Central Norway Regional Health Authority (Stjørdal, Norway) and the Norwegian University of Science and Technology (Trondheim, Norway) and K. G. Jebsen Foundation for Medical Research, Center for Exercise in Medicine, at Norwegian University of Science and Technology (Trondheim, Norway). The funders had no role in the design and conduct of the study. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

REFERENCES

1. Marwick TH, Hordern MD, Miller T, et al. Exercise training for type 2 diabetes mellitus: impact on cardiovascular risk: a scientific statement from the American Heart Association. Circulation 2009;119:3244–62.

2. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr 2009;10:165–93.

Paper II

Exercise Training Normalizes Timing of Left Ventricular Untwist Rate, but Not Peak Untwist Rate, in Individuals with Type 2 Diabetes and Diastolic Dysfunction: A Pilot Study

Siri Marte Hollekim-Strand, MSc, Sigve Fredrik Høydahl, MD, Turid Follestad, PhD, Håvard Dalen, MD, PhD, Marit Rokne Bjørgaas, MD, PhD, Ulrik Wisløff, PhD, and Charlotte Björk Ingul, MD, PhD,
Trondheim and Levanger, Norway

Background: There is limited information regarding the role of left ventricular (LV) twist and the effect of exercise in type 2 diabetes (T2D). The aim of this study was to compare LV twist parameters in patients with T2D versus healthy control subjects and the effects of high-intensity interval exercise (HIIE) and moderate-intensity exercise (MIE) on LV twist in patients with T2D with diastolic dysfunction.

Methods: This study, which included both prospective and retrospective components, included 47 patients with T2D and diastolic dysfunction and 37 healthy individuals. Patients with T2D were randomized to HIIE (4 × 4 min at 90%–95% of maximal heart rate, three times a week, 120 min/wk; n = 24) or MIE (210 min/wk; n = 23) for 12 weeks and examined with echocardiography (LV twist by speckle-tracking method) at baseline and posttest. The control subjects received no intervention and were matched according to age, gender, and body mass index to those completing the intervention.

Results: In total, 37 subjects completed 12 weeks of MIE (n = 17) or HIIE (n = 20). LV peak untwist rate (UTR) was similar in patients with T2D and control subjects (P > .05). At baseline, LV peak UTR, relative to total diastolic period, occurred 5.8 percentage points later in patients with T2D compared with control subjects (P = .004). Time to peak UTR was shortened by 6.5 percentage points (P = .002) and 7.7 percentage points (P < .001) after MIE and HIIE, respectively. Time to peak UTR was similar to that in control subjects after exercise interventions.

Conclusions: In patients with T2D and diastolic dysfunction, LV peak UTR was similar, but time to peak LV UTR was delayed compared with control subjects. Twelve weeks of endurance exercise normalized the timing of UTR. (J Am Soc Echocardiogr 2016;29:421-30.)

Keywords: Speckle-tracking echocardiography, Type 2 diabetes, Diastolic dysfunction, Left ventricular twist, Exercise training

Type 2 diabetes (T2D) is associated with cardiomyopathy, which may lead to heart failure.[1] Early diabetic cardiomyopathy is often characterized by diastolic dysfunction (DD), which has been detected in about 50% of asymptomatic and normotensive subjects with well-controlled T2D.[2,3]

Although not commonly evaluated, a fundamental component in normal diastolic function is left ventricular (LV) untwist, which contributes to LV relaxation and suction.[4,5] LV twist and untwist are the wringing and unwringing motion of the left ventricle around the long axis of the ventricle. The sequence of twisting and untwisting events establishes the peak early diastolic blood flow velocity across the mitral valve.[4] Twist during systole contributes with storage of energy, and the subsequent recoil releases the restoring forces, which contributes to LV diastolic relaxation and early filling.[6] Assessment of LV untwisting has thus been introduced as a promising index in the evaluation of early diastolic function.[7,8] However, there is limited information regarding the role of LV twist in T2D.

Exercise training is a cornerstone in T2D management to reduce cardiovascular disease and mortality,[9-12] and several studies have shown that high-intensity interval exercise (HIIE) is superior to moderate-intensity exercise (MIE) according to current exercise guidelines in reducing cardiovascular risk factors.[13-17] We recently reported that HIIE is superior to MIE in improving diastolic function measured by peak mitral annular early tissue Doppler

From the K. G. Jebsen Centre of Exercise in Medicine at the Department of Circulation and Medical Imaging (S.M.H-S., S.F.H., U.W., C.B.I.), the Department of Public Health and General Practice (T.F.), the Department of Circulation and Medical Imaging (H.D.), and the Department of Cancer Research and Molecular Medicine (R.B.), Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway; the Department of Medicine, Levanger Hospital, Nord-Trøndelag Health Trust, Levanger, Norway (H.D.); and the Department of Endocrinology, St Olav's Hospital, Trondheim, Norway (R.B.).

This study was funded by the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology, and the K. G. Jebsen Foundation for Medical Research.

Reprint requests: Charlotte Björk Ingul, MD, PhD, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Postbox 8905, NO-7491 Trondheim, Norway (E-mail: *charlotte.b.ingul@ntnu.no*).

0894-7317/$36.00

http://dx.doi.org/10.1016/j.echo.2016.01.005

Abbreviations

AVC = Aortic valve closure

BMI = Body mass index

DD = Diastolic dysfunction

EF = Ejection fraction

HIIE = High-intensity interval exercise

IVRT = Isovolumic relaxation time

LV = Left ventricular

MIE = Moderate-intensity exercise

pp = Percentage points

T2D = Type 2 diabetes

UTR = Untwist rate

velocity (e′) in patients with T2D and DD.[17] However, to our knowledge no study has investigated the effects of exercise on LV untwist and untwist rate (UTR) in patients with T2D and DD. Thus, the aims of this study were to (1) determine the effects of HIIE and MIE on LV untwist and UTR in patients with T2D (duration < 10 years) and DD and (2) compare these with healthy individuals with no exercise intervention.

METHODS

Study Population

A total of 84 subjects, 47 patients with T2D and DD and 37 healthy individuals, were enrolled in the study. The T2D group was recruited prospectively, and the control subjects were recruited retrospectively.

The T2D study population studied has been described previously[17] and was recruited through a local newspaper and from the outpatient population at St Olav's Hospital, Trondheim, Norway (August 2010 to March 2013). Inclusion criteria for the individuals with T2D included age 20 to 65 years, diagnosis with T2D within 10 years, no use of insulin, and reduced diastolic function, defined as e′ < 8 cm/sec,[18] at baseline testing. Exclusion criteria included overt cardiovascular disease, atrial fibrillation or other significant cardiac arrhythmia, untreated hypertension, diabetic retinopathy or neuropathy, albuminuria, LV ejection fraction (EF) < 40%, body mass index (BMI) > 35 kg/m^2, ischemia on exercise stress echocardiography on a stationary bicycle, pregnancy, inability to exercise, drug or alcohol abuse, and physical activity level above minimum guidelines for T2D.[9]

The T2D study participants were stratified according to gender and randomized to either HIIE ($n = 24$) or MIE ($n = 23$) (Figure 1). The Unit of Applied Clinical Research at the Norwegian University of Science and Technology performed the randomization procedures. The trial (ClinicalTrials.gov identifier NCT01206725) was approved by the Regional Committee for Medical and Health Research Ethics of Central Norway in September 2010.

The healthy control subjects ($n = 37$) were recruited from the Nord-Trøndelag Health Study database, consisting of 1,266 complete echocardiograms from healthy individuals free of known cardiac disease, diabetes, and hypertension.[19] They were matched, to the extent possible, according to age, gender, and BMI with the final 37 participants with T2D performing posttest measurements. Age and BMI differences between the T2D group and control group of 5 years and 5 kg/m^2, respectively, were accepted. The control group had no exercise intervention. Informed consent was obtained from all participants, and all were insured.

Outcome Measures

The primary outcome measure was LV peak UTR. Secondary outcome measures were time to peak LV UTR, peak LV twist rate, and peak LV twist.

Intervention and Exercise Training Protocols

T2D Group. The MIE group performed home-based exercise training for 12 weeks in accordance with guidelines from the Norwegian Diabetes Association (2005), recommending MIE for 210 min/wk (exercise bouts ≥10 min in duration),[20] similar to the guidelines of the International Diabetes Federation.[9] At 4 and 8 weeks of the intervention period, the MIE group was contacted by phone or e-mail to motivate to continue with MIE as prescribed.

The HIIE group performed supervised exercise training by walking or jogging on an inclined treadmill three times a week for 12 weeks (120 min/wk). Following 10 min of warmup at 70% of maximal heart rate, the HIIE group performed 4-min work bouts four times at 90% to 95% of maximal heart rate with 3-min recovery periods between work bouts at 70% of maximal heart rate and 5 min of cool-down (altogether 40 min). During the HIIE sessions, heart rate monitors (Polar RS 400; Polar Electro, Kempele, Finland) were used to ensure that the required exercise intensity was achieved and maintained. The individuals in the MIE and HIIE groups recorded daily physical activity.

Echocardiographic Recordings

Echocardiographic examinations (Vivid 7 scanner, phased-array [M4S and M3S transducers]; GE Vingmed Ultrasound AS, Horten, Norway) were performed at baseline and 12 weeks posttest for the MIE and HIIE groups.[17] The control subjects were examined only once.[19] A detailed description of the echocardiographic recordings has previously been published.[21,22]

The same operator (C.B.I.) performed the echocardiographic recordings at both time points for the T2D population and the same operator (H.D.) for the healthy control group. The images were obtained in parasternal and three standard apical views (four chamber, two chamber, and apical long-axis), both in B-mode and with color tissue Doppler imaging, and three loops were acquired for each image. The images were digitally stored on a hard disk for offline analysis. All recordings were performed with the individuals in the left lateral supine position during breath holds at end-expiration. Basal and apical LV short-axis levels were recorded for the analysis of twist and standardized to two anatomic landmarks. The basal level was recorded from the parasternal position, using the mitral valve leaflet location in the middle of the LV cavity as the landmark. The apical level was recorded more distally toward the apex, just proximal to the level with luminal closure at end-systole. All loops were recorded with the LV cross-sections as circular as possible. To optimize image quality, the sector depth and width were adjusted in each subject, resulting in a mean frame rate of 69 ± 13 frames/sec (range, 51–113 frames/sec). To determine the timing of cardiac events, tissue Doppler velocity curves with the sample volume at the basal septum were conducted immediately before the acquisition of the short-axis images, to minimize differences in heart rate. Aortic valve closure (AVC) was set at the end of the negative spike after ejection.[23]

Echocardiographic Analyses

Offline data analyses were performed using commercially available two-dimensional strain software (EchoPAC PC version 112; GE Medical Systems, Milwaukee, WI). All analyses were performed blinded to exercise group allocation as well as baseline and posttest. Analyses of conventional echocardiographic diastolic measures were performed as previously described.[21,22]

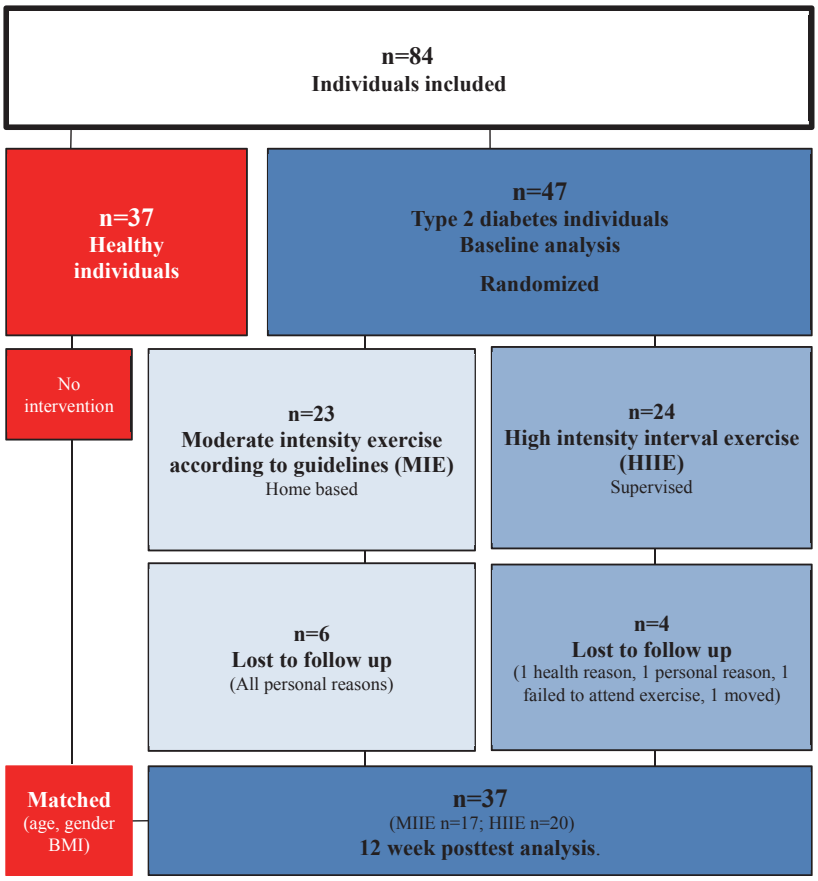


Figure 1 Flowchart of the study.

The analyses of the twist parameters have been described previously.[24] To analyze twist parameters, one cardiac cycle from the basal and apical short-axis data set with a well-defined endocardial border during late systole was selected for analyses. Regions of interest of the left ventricle were adjusted to include most of the myocardium, but not the pericardium. The endocardial borders of the apical and basal short-axis plane at end-systole were manually traced and subsequently tracked by the software.[24] When the software or the observer signaled poor tracking quality, the observer readjusted the region of interest until acceptable tracking was obtained. If nonadjustable, segments with poor tracking were discarded. For the twist analysis, default spatial and temporal smoothing was used.

The twist variables are presented in Tables 1 to 3. In addition, the timing of AVC and the length of the cardiac cycle (i.e., the time interval between the R waves on the electrocardiogram) were measured. Curves of basal and apical LV rotation, twist, and twist rates were automatically generated by the software. LV twist was calculated (Excel; Microsoft Corporation, Redmond, WA) as apical LV rotation relative to the base. Counterclockwise rotation as viewed from the LV apex was expressed as a positive value and clockwise rotation as a negative value (Figure 2). Peak UTR was defined as the first negative peak after AVC.

To adjust for intra- and intersubject differences in heart rate, the time sequences from AVC to peak UTR were normalized to the percentage of total diastolic duration. Diastole was expressed as a percentage of the total duration of diastole, and the diastolic period was defined from 0% (AVC) to 100% (end-diastole) (Figure 2).[25] Time to peak UTR was measured from time of AVC (0%) to peak UTR as a percentage of the length of diastole (100%) (Figure 2).

Resting Heart Rate and Blood Pressure

Resting heart rate was measured by electrocardiography at the end of the resting echocardiographic measurement in both groups. Blood pressure was measured by oscillometry using a SunTech Tango M2 Stress BP Monitor (SunTech Medical, Morrisville, NC) and a Dinamap 845XT (GE Medical Systems) in the T2D group and control subjects, respectively, as previously described.[17,26]

Variability of Echocardiographic Measurements

In total, 15 individuals were used for measurements of intra- and interobserver variability, and five for each group were randomly selected (five patients with T2D at baseline, five with T2D posttest, and five subjects without diabetes) for LV twist parameters.

Table 1 Summary of the LV twist and untwist data for the healthy control subjects and intervention groups

Variable	Control		T2D, baseline		MIE, baseline		MIE, posttest		HIIE, baseline		HIIE, posttest	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
LV base												
Heart rate basal (beats/min)	37	66 ± 11.3	36	71.2 ± 9.8	17	72.9 ± 10.3	15	72.1 ± 9.1	19	69.8 ± 9.3	19	65.2 ± 9.3
Peak basal rotation	37	−5.2 ± 2.7	36	−5.5 ± 3.2	17	−5.3 ± 3.8	15	−4.3 ± 2.5	19	−5.6 ± 2.7	19	−4.2 ± 2.2
Basal TR (deg/sec)	37	−42.1 ± 17.2	36	−54.2 ± 26.1	17	−54.4 ± 24.8	15	−54 ± 23.1	19	−54.1 ± 27.9	19	−45.4 ± 17.8
Basal UTR (deg/sec)	37	52.3 ± 16.4	36	57.2 ± 25.6	17	60.9 ± 29.6	15	52.3 ± 28.4	19	54 ± 21.8	19	47.4 ± 23.6
Time to peak basal UTR (% of diastole)	37	8 ± 4.7	35	13.9 ± 7.2	16	14.4 ± 6	15	9.4 ± 4.8	19	13.5 ± 8.3	19	8.4 ± 4.6
LV apex												
Heart rate apical (beats/min)	37	64.8 ± 10.5	36	70.2 ± 9.6	17	73.2 ± 10.2	15	71.7 ± 9.7	19	67.5 ± 8.4	19	65.7 ± 9.1
Peak apical rotation	37	8.5 ± 3.5	36	8.5 ± 3.6	17	8.9 ± 3.7	15	8.2 ± 4.2	19	8 ± 3.6	19	9.2 ± 4
Apical TR (deg/sec)	37	40.8 ± 11.9	36	49.3 ± 24.1	17	52 ± 29.6	15	52.9 ± 24.3	19	46.9 ± 18.3	19	45.7 ± 23.4
Apical UTR (deg/sec)	37	−61.4 ± 18.5	36	−59.8 ± 25.3	17	−56.9 ± 28.1	15	−76 ± 27.1	19	−62.5 ± 23	19	−61.9 ± 23.4
Time to peak apical UTR (% of diastole)	37	8.8 ± 6.3	35	19.4 ± 9.4	16	21 ± 9.2	16	9.7 ± 8.5	19	18.1 ± 9.6	19	10.2 ± 5
LV twist												
Peak twist	37	13.1 ± 3.6	36	12.3 ± 5.1	17	12.5 ± 5.7	15	10.5 ± 4.7	19	12.2 ± 4.7	19	11.3 ± 4.2
Peak TR (deg/sec)	37	65.4 ± 20.9	36	72.7 ± 31.8	17	72 ± 33.8	15	67.9 ± 26.2	19	73.3 ± 30.9	19	70.1 ± 29.4
Peak UTR (deg/sec)	37	−92.8 ± 23.4	36	−83.2 ± 33.4	17	−86.5 ± 34.9	15	−89.4 ± 33.8	19	−80.2 ± 32.6	19	−77.5 ± 25.1
Time to peak UTR (% of diastole)	37	10.5 ± 7.4	35	16.3 ± 8.9	16	16.1 ± 9	15	10.2 ± 7.7	19	16.5 ± 9	19	8.8 ± 4.1

TR, Twist rate.
Data are expressed as mean ± SD.

Table 2 Results for comparison of the T2D groups with the healthy control subjects for LV twist and untwist variables

Variable	T2D vs control, baseline				MIE vs control, posttest				HIIE vs control, posttest			
		95% CI				95% CI				95% CI		
	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*
LV base												
Heart rate basal (beats/min)	5.2	0.3	10.2	.04	6.1	0.0	12.2	.05	−0.8	−6.5	4.9	.78
Peak basal rotation	−0.2	−1.6	1.2	.74	0.9	−0.6	2.5	.23	1.0	−0.3	2.3	.13
Basal TR (deg/sec)	−12.1	−22.5	−1.7	.02	−11.9	−25.6	1.9	.09	−3.3	−13.4	6.7	.51
Basal UTR (deg/sec)	5.0	−5.1	15.1	.33	0.0	−16.4	16.4	.99	−4.9	−17.3	7.5	.43
Time to peak basal UTR (% of diastole)	5.9	3.0	8.9	<.001	1.5	−1.6	4.5	.33	0.5	−2.2	3.1	.72
LV apex												
Heart rate apical (beats/min)	5.4	0.7	10.1	.03	6.9	0.7	13.2	.03	1.0	−4.5	6.4	.73
Peak apical rotation	−0.1	−1.7	1.6	.94	−0.3	−2.9	2.2	.79	0.7	−1.5	2.9	.52
Apical TR (deg/sec)	8.5	−0.5	17.4	.06	12.1	−1.8	25.9	.08	4.9	−6.9	16.7	.40
Apical UTR (deg/sec)	1.6	−8.8	12.0	.76	−14.6	−30.5	1.4	.07	−0.6	−13.2	12.1	.93
Time to peak apical UTR (% of diastole)	10.6	6.8	14.4	<.001	0.9	−4.0	5.7	.72	1.4	−1.7	4.5	.38
LV twist												
Peak twist	−0.7	−2.8	1.3	.47	−2.6	−5.4	0.2	.07	−1.8	−4.1	0.5	.12
Peak TR(deg/sec)	7.3	−5.3	20.0	.25	2.5	−13.3	18.3	.74	4.8	−10.8	20.3	.53
Peak UTR (deg/sec)	9.6	−3.9	23.1	.16	3.4	−16.5	23.3	.73	15.2	1.2	29.3	.03
Time to peak UTR (% of diastole)	5.8	1.9	9.7	.004	−0.3	−5.1	4.5	.91	−1.7	−4.8	1.4	.27

Estimate, Estimated mean difference; *TR*, twist rate.
The table shows results from two-sample *t* tests for comparing the T2D group and control subjects at baseline and the HIIE and MIE groups after the intervention to controls for resting heart rate and the variables describing diastolic and systolic function.

Table 3 Estimated exercise intervention effects on twist and untwist variables

	MIE, posttest vs baseline				HIIE, posttest vs baseline				HIIE vs MIE			
		95% CI				95% CI				95% CI		
Variable	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*
LV base												
Heart rate basal (beats/min)	−0.1	−4.8	4.6	.96	−5.3	−9.5	−1.1	.02	−5.2	−11.1	0.6	.08
Peak basal rotation	1.2	−0.6	2.9	.19	1.2	−0.4	2.9	.13	0.1	−1.9	2.1	.94
Basal TR (deg/sec)	0.2	−14.3	14.7	.98	8.8	−4.6	22.1	.19	8.6	−8.0	25.1	.30
Basal UTR (deg/sec)	−5.9	−20.6	8.8	.42	−9.0	−22.4	4.3	.18	−3.2	−20.7	14.4	.72
Time to peak basal UTR (% of diastole)	−4.6	−8.4	−0.8	.02	−5.5	−9.0	−2.0	.003	−0.9	−5.2	3.4	.67
LV apex												
Heart rate apical (beats/min)	−0.8	−4.6	2.9	.65	−2.5	−5.9	0.8	.13	−1.7	−6.5	3.1	.48
Peak apical rotation	−0.4	−2.3	1.4	.64	1.0	−0.7	2.7	.25	1.4	−0.9	3.8	.23
Apical TR (deg/sec)	2.8	−11.1	16.8	.68	−3.1	−15.9	9.7	.63	−5.9	−22.5	10.6	.47
Apical UTR (deg/sec)	−16.8	−31.5	−2.0	.03	−1.6	−15.1	11.9	.81	15.1	−2.3	32.6	.09
Time to peak apical UTR (% of diastole)	−10.0	−14.5	−5.6	<.001	−8.8	−12.9	−4.7	<.001	1.2	−4.1	6.6	.64
LV twist												
Peak twist (deg)	−1.9	−4.1	0.4	.10	−1.0	−2.9	1.0	.33	0.9	−1.9	3.7	.52
Peak TR (deg/sec)	−4.5	−22.3	13.2	.61	−2.7	−18.9	13.6	.74	1.8	−19.0	22.7	.86
Peak UTR (deg/sec)	−4.1	−19.7	11.4	.59	4.3	−9.8	18.3	.54	8.4	−11.0	27.8	.39
Time to peak UTR (% of diastole)	−6.5	−10.6	−2.5	.002	−7.7	−11.3	−4.1	<.001	−1.2	−6.1	3.8	.64

Estimate, Estimated mean difference; *TR*, twist rate.
The table shows estimated effects of the HIIE and MIE interventions on the basis of linear mixed models.

Intraobserver variability was performed by a single investigator by blinded assessment on two separate occasions. Interobserver variability was assessed by two investigators who were unaware of each other's measurements and of the study time point.

The percentage of diastole was used in the analysis of reproducibility. Reproducibility data are expressed as coefficients of variation and as the mean percentage error (mean error). The coefficient of variation was calculated as the ratio of the SD of the repeated measurements for each individual to the mean. Mean error was derived as the absolute difference between the two sets of observations, divided by the mean of the observations.[27] The average values of the 15 individuals are presented.

Statistical Analyses

The measurements are presented as mean ± SD unless otherwise stated. Differences between the HIIE and MIE intervention groups and within-group effects of intervention were analyzed using linear mixed models. Within-subject correlations were taken into account by using a random intercept in the linear mixed model. The baseline means were restricted to be equal for the two interventions, because of randomization to intervention group. Tests for between-group and within-group differences were performed as post hoc *t* tests for the appropriate pairs of model parameters. Differences between the T2D group and the healthy control subjects were analyzed using two-sample *t* tests, for the full T2D group at baseline and for the MIE and HIIE subgroups separately after the intervention. Given the limited population available for matching, the matching of the control group aimed for no significant differences between groups with respect to age, gender, and BMI. Therefore, the patients with T2D and healthy control subjects were treated as independent groups, not paired, in the statistical analysis.

For all tests, a significance level of .05 were used. No formal adjustments for multiple testing because of the number of variables considered were made. However, the risk for incidental findings was kept in mind when interpreting the results.

The analyses were carried out using SPSS version 21.0 (IBM, Armonk, NY) and the R statistical package.[28]

RESULTS

Data from 37 participants with T2D were available for posttests, 17 in the MIE group and 20 in the HIIE group (Figure 1). In total, 8.4% of the segments in basal and apical short-axis views were discarded because of inadequate tracking.

Before intervention, six of the participants with T2D were classified as having DD grade 1, 30 were classified as having DD grade 2, and one participant had DD grade 3.[17] After exercise intervention, 18 participants with T2D had DD grade 2 and the remaining had DD grade 1.

Clinical Characteristics

The participants in the T2D group were older than control subjects (56.5 vs 51.0 years, $P = .004$) and had higher BMIs (29.9 vs 27.0 kg/m^2, $P < .001$). There was no difference in gender distribution between the T2D group and control subjects.

There were no significant differences in age, gender distribution, or BMI between the MIE and HIIE groups.[17] The use of medications was similar in the MIE and HIIE groups (Table 4).

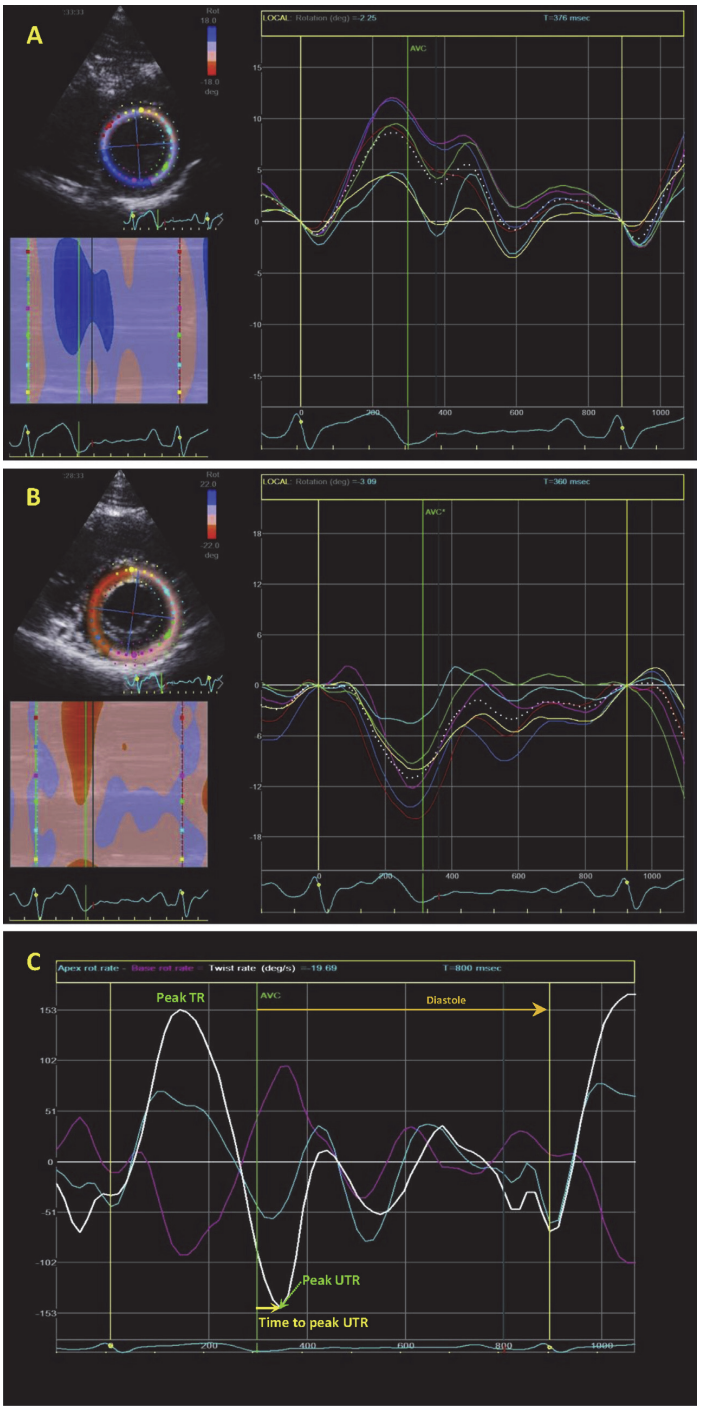


Figure 2 LV basal and apical rotation, twist, and UTR. **(A)** Apical counterclockwise rotation expressed as a positive value. Six curves in different colors represent different myocardial segments. The white dashed line is the average value of the six segments. **(B)** Basal rotation expressed as a negative value. **(C)** Curves showing peak twist rate (TR) and UTR. Time to peak UTR is measured from time of AVC to peak UTR (*yellow arrow*) as a percentage of the length of diastole (*orange arrow*).

Table 4 Medication use in the control group, MIE group, and HIIE group

Medication	Control group (*n* = 37)	T2D MIE (*n* = 17)	T2D HIIE (*n* = 20)
Antidiabetic	0	12 (71%)	18 (85%)
Antihypertensive	0	6 (35%)	7 (35%)
Antiplatelet	0	3 (18%)	4 (20%)
Statins	0	7 (41%)	4 (20%)

Exercise Compliance

The HIIE group performed 94% of the scheduled training sessions, and 94% of the MIE subjects reported physical activity to be ≥210 min/wk.[17]

LV Twist Parameters

LV twist results at baseline and 12-week posttest are described in Tables 1 and 2. The effects of HIIE and MIE are shown in Table 3. Comparison between the exercise intervention groups (at baseline and 12-week posttest) and the healthy control subjects are given in Table 2.

There were no differences between patients with T2D and control subjects in LV peak UTR, peak twist rate, or peak twist, except for basal twist rate ($P = .02$) (Table 2).

At baseline, time to peak LV UTR relative to total diastolic period occurred 5.8 percentage points (pp) later in patients with T2D compared with control subjects ($P = .004$) (Table 2, Figures 3A–3C). When normalized for resting heart rate, time to peak UTR from AVC in the T2D group was significantly delayed compared with control subjects at baseline (mean difference, 0.53 msec/beat; 95% CI, 0.20–0.86 msec/beat; $P = .002$).

Twelve weeks of HIIE and MIE, respectively, reduced time to peak UTR by 7.7 pp ($P < .001$) and 6.5 pp ($P = .002$) (Table 3, Figure 3B). When normalized for resting heart rate, HIIE and MIE reduced time to peak UTR by 0.41 msec/beat (95% CI, 0.13–0.69 msec/beat; $P = .006$) and 0.46 msec/beat (95% CI, 0.16–0.77 msec/beat; $P = .004$), respectively.

Time to peak apical UTR was reduced on average by 10.0 pp after MIE ($P < .001$) and 8.8 pp after HIIE ($P < .001$) (Table 3). Time to peak basal UTR was reduced by 4.6 pp after MIE ($P = .02$) and 5.5 pp after HIIE ($P = .003$), and time to peak UTR was reduced by 6.5 pp after MIE ($P = .002$) and 7.7 pp after HIIE ($P < .001$) (Table 3). There were no significant changes after MIE or HIIE for other twist variables (Table 3). At follow-up after 12 weeks, there were no significant differences between the exercise groups and control subjects for any twist variables (Table 2, Figure 3C). At baseline, peak UTR occurred during isovolumic relaxation time (IVRT) in 27% of the participants with T2D, whereas it occurred after IVRT in 73% of the participants with T2D. After intervention, peak UTR occurred during IVRT in 85.5% and after IVRT in 13.5% of the patients with T2D. Peak UTR occurred during IVRT in all participants in the control group.

A summary of the comparison between the T2D group and control subjects on other echocardiographic variables (peak early diastolic transmitral flow velocity [E], peak late diastolic transmitral flow velocity [A], E/A ratio, e′, E/e′ ratio, IVRT, peak systolic tissue Doppler velocity [S′]), LV dimensions and volumes, resting heart rate, and blood pressure is available in the online supplement (see Supplemental Material). Traditional echocardiographic variables for the T2D group only have recently been published.[17]

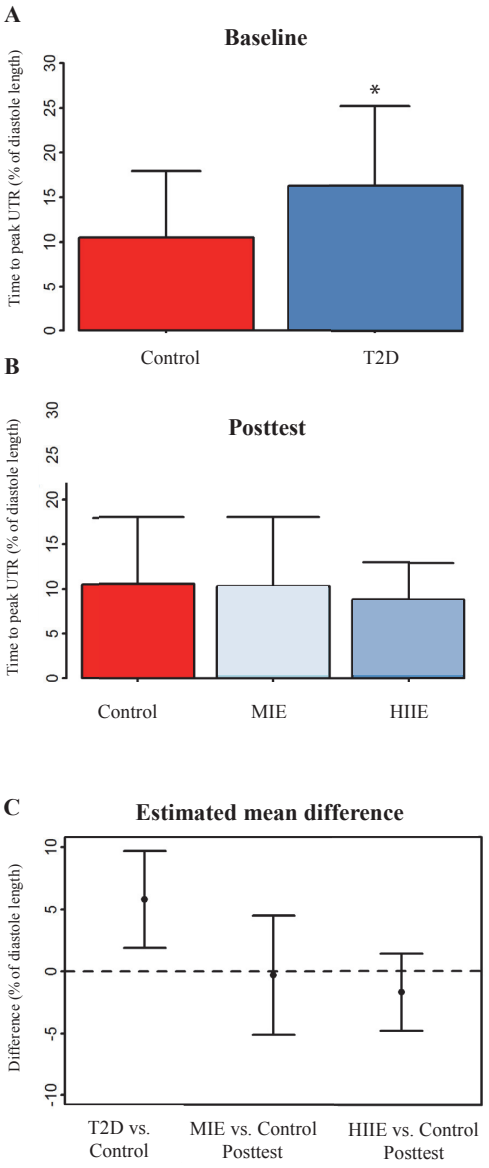


Figure 3 Effect of endurance exercise training on time to LV peak UTR. **(A)** Mean and SD for time to peak UTR for the healthy control group and the T2D group at baseline. *$P < .01$. **(B)** Mean and SD for the control subjects, MIE and HIIE group at posttest. **(C)** Estimated mean difference between patients with T2D and control subjects at baseline and between the MIE and HIIE group and control subjects at posttest and 95% CI for the differences.

Intraobserver and Interobserver Variability for Echocardiographic Measurements

Intra- and interobserver variability was low for most twist variables, especially for time to apical UTR (Table 5).

Table 5 Intraobserver and interobserver variability of LV twist variables

Variable	Mean COV (%)	Mean error (%)	Bias	Lower LoA (−2 SDs)	Upper LoA (+2 SDs)
Intraobserver					
Time to apical UTR	2.45	3.46	−2.27	−60.11	55.47
Time to basal UTR	0.89	1.26	1.27	−17.82	20.35
Time to peak UTR	2.21	3.13	4.00	−38.72	46.72
Peak twist	7.50	10.61	0.77	−2.28	3.82
Peak UTR	11.62	16.43	0.80	−33.15	34.76
Interobserver					
Time to apical UTR	1.45	2.05	−1.33	−26.77	24.11
Time to basal UTR	6.82	9.65	26.73	−72.13	125.60
Time to peak UTR	2.36	3.34	−0.93	−42.28	40.41
Peak twist	7.38	10.44	0.97	−2.04	3.99
Peak UTR	6.41	9.07	0.36	−19.48	20.19

Bias, Mean of the differences measured between the two repeated measurements (intra- and interobserver); *COV*, coefficient of variation; *LoA*, 95% limit of agreement; *mean error*, mean of the errors in each patient.
The percentage of diastole was used in the analysis of reproducibility for time to peak UTR.

DISCUSSION

The main findings of the present study were that patients with T2D and DD had normal LV peak UTR, twist rate, and peak twist, but delayed time to peak UTR at rest, compared with healthy individuals. Furthermore, we found that 12 weeks of MIE and HIIE normalized time to peak UTR in subjects with T2D. This is to our knowledge the first study to evaluate the effect of exercise training on LV twist parameters in patients with T2D and DD.

Untwist and Twist Rate

The lack of difference between the control and T2D groups with regard to LV rotation, twist, and UTR is in accordance with the results of Wang *et al.*[29] who found reduced LV twist and UTR in patients with LV systolic dysfunction and depressed EFs, but not in those with DD and normal EFs. The onset of LV untwisting and the magnitude of peak untwisting velocities either remained normal or were reduced and were significantly delayed.[29] Our results also support those of Fonseca *et al.*,[30] who compared healthy subjects and patients with T2D (with abnormal filling patterns) using magnetic resonance imaging and echocardiography and found no difference in peak UTR between groups.

In our T2D study population, the majority of participants had moderate DD.[17] Our findings of apparently normal LV twist and UTR in T2D support the findings of Park *et al.*,[31] who found that diastolic untwisting is increased in mild DD but normalized or reduced in patients with more severe DD.

Time to Peak UTR

Our finding of reduced time to peak UTR is consistent with the results of several previous studies observing delayed early diastolic untwisting in states associated with worsened LV relaxation, such as DD with normal EF,[29] DD due to aortic stenosis,[32] severe LV hypertrophy,[33] and age.[25] Thus, the present study also supports the suggestion of delayed untwist reflecting an ineffective relaxation of the ventricle.[4] However, because of different assessment modes and point of time at which timing is measured (i.e., onset of untwist or time to peak UTR), and different patient groups, results are difficult to compare.

Nevertheless, timing seems to be important in DD as delayed diastolic untwisting may be caused by less radial displacement during early diastolic period.[25] Wang *et al.*[29] also found that a subgroup of patients with DD, preserved EFs, and delayed onset of untwisting also had delayed peak untwisting. This indicates that time to peak UTR also reflects the onset of untwisting, which coincides with the onset of relaxation, preceding suction due to elastic recoil.[4] Furthermore, in patients with DD, preserved EFs, and delayed peak untwisting, peak untwist happened almost simultaneously with early transmitral filling (E).[29] This indicates that time to peak UTR is a useful marker of early diastolic function, which potentially can serve as a therapeutic target for improving diastolic function.

The observation that exercise normalizes time to peak UTR is novel and highlights the usefulness of exercise training as an effective tool in secondary prevention in patients with T2D. The fact that time to peak UTR was the only diastolic variable measured (see Supplemental Table 1), which became normalized after exercise training in patients with T2D and DD, also indicates that time to peak UTR might be a more sensitive parameter to evaluate early diastolic changes compared with LV UTR. The magnitude of UTR depends on many factors, such as changes in preload, afterload, and contractility,[6] making it difficult to compare studies. For example, the magnitude of twist and untwist can substantially increase after short-term exercise[34] but can be reduced at rest in endurance-trained individuals after long-term exercise.[35] End-diastolic volumes were larger in the diabetes group compared with control subjects, but there was no difference in end-systolic volume. There was no change in end-diastolic or end-systolic volume after intervention (see Supplemental Material). However, both global strain rate and S′ increased significantly after intervention, indicating increased contractility.[17] Increased contractility would contribute to increases in recoil and suction and therefore an increased relaxation rate that would contribute to an earlier onset of untwist. However, the effect of load is unknown.

Time to peak UTR has not previously been extensively used in the evaluation of diastolic function, and other indices, such as negative torsion acceleration incorporating UTR, have been proposed to be useful and might be more reliable than time to peak UTR.[36] However, this needs to be tested in future studies. Furthermore, our results indicate that time to apical UTR has higher reproducibility compared with basal UTR. The differences in heart rate or frame rate between apical and basal short-axis images could result in an incorrect value of LV twist, which could be avoided by using only the apical short-axis images. Apical rotation by speckle-tracking echocardiography has been suggested as a simplified index of LV twist, because apical rotation is the dominant contributor to LV twist.[37]

Reduced diastolic function can be reversible, but early identification and treatment intervention are essential to prevent the development of heart failure. The time to peak apical UTR appears to be sensitive not only for identification but also for follow-up after exercise training intervention. Further research is warranted to understand the role of time to peak UTR in DD.

Study Limitations and Strengths

Limitations include the relatively small sample size and information bias due to self-reporting of exercise in the MIE group. Supervised

exercise only in the HIIE group could have resulted in better compliance in this group. However, the improvement of diastolic function in the MIE group is in line with previous MIE studies.[38]

The control group had lower BMIs than the exercise groups and was significantly younger than the HIIE group. However, there was no difference between groups in gender distribution, and the difference in BMI was relatively small between groups (2.9 kg/m^2). According to Aljaroudi *et al.*,[39] the severity of DD increases more with BMI $>$ 30 kg/m^2 compared with 25 to 29.9 kg/m^2. Thus, the relatively small difference in BMI between groups in the present study can be expected to have only a minor impact on our results. Nevertheless, the matching between the T2D group and control subjects is considered suboptimal in relation to age. When adjusting for age for diastolic variables (e′, E, A, IVRT, UTR, and time to peak UTR) and S′, using control subjects and baseline data for T2D, a significant increase with age was found for A ($P < .001$) and decreases with age for e′ ($P < .001$) and S′ ($P = .001$). However, after adjusting for age, significant differences between groups (patients with T2D vs control subjects) were still found (mean differences of -3.3 and -1.1 for e′ and S′, respectively [$P < .001$] and 0.1 for A [$P = .01$]).Thus, suboptimal age matching does not explain all variability between groups for these variables.

Furthermore, a previous study showed no significant change with age in LV twist in healthy individuals between 31 and 60 years of age.[40]

Two-dimensional speckle-tracking echocardiography has some methodologic limitations, mostly related to image quality and through-plane motion of the myocardium. To ensure accuracy and compatibility of the echocardiographic recordings, the same operator conducted recordings within each group, the levels of the basal and apical short-axis views were well defined, and equal frame rates were used within each patient. However, to ensure high reproducibility, interobserver variability in imaging acquisition (test-retest) should have been assessed.

Temporal normalization and interpolation of the raw data were not performed and could have influenced the calculation of twist and twist rates, because basal and apical images are acquired at different cardiac cycles. However, time to apical UTR would not have been affected.

In four subjects, there was a difference in heart rate of more than five beats between the apical and basal recordings, which may have led to inaccuracy in the final results.

Strengths of this study are the prospective study design of the intervention, high compliance to exercise, blinded data analysis, and high reproducibility for major LV twist variables.

CONCLUSIONS

In individuals with T2D and DD, LV twist parameters were similar to those in healthy subjects except for time to peak UTR, which was delayed at baseline. Both MIE and HIIE normalized time to peak UTR after 12 weeks in patients with T2D and DD.

REFERENCES

1. Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB. The incidence of congestive heart failure in type 2 diabetes: an update. Diabetes Care 2004;27:1879-84.
2. Zabalgoitia M, Ismaeil MF, Anderson L, Maklady FA. Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. Am J Cardiol 2001;87:320-3.
3. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG. Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 2001;24:5-10.
4. Notomi Y, Popovic ZB, Yamada H, Wallick DW, Martin MG, Oryszak SJ, et al. Ventricular untwisting: a temporal link between left ventricular relaxation and suction. Am J Physiol Heart Circ Physiol 2007;294:H505-13.
5. Notomi Y, Martin-Miklovic MG, Oryszak SJ, Shiota T, Deserranno D, Popovic ZB, et al. Enhanced ventricular untwisting during exercise: a mechanistic manifestation of elastic recoil described by Doppler tissue imaging. Circulation 2006;113:2524-33.
6. Sengupta PP, Tajik AJ, Chandrasekaran K, Khandheria BK. Twist mechanics of the left ventricle: principles and application. JACC Cardiovasc Imaging 2008;1:366-76.
7. Burns AT, La Gerche A, Prior DL, Macisaac AI. Left ventricular untwisting is an important determinant of early diastolic function. JACC Cardiovasc Imaging 2009;2:709-16.
8. Opdahl A, Remme EW, Helle-Valle T, Edvardsen T, Smiseth OA. Myocardial relaxation, restoring forces, and early-diastolic load are independent determinants of left ventricular untwisting rate. Circulation 2012;126:1441-51.
9. International Diabetes Federation. Global Guideline for Type 2 Diabetes Available at: http://www.idf.org/sites/default/files/IDF%20T2DM%20Guideline.pdf Accessed October, 20152012.
10. International Diabetes Federation. IDF Diabetes Atlas. 6th ed.. Available at: https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf; 2013. Accessed September, 2015.
11. Mitka M. Study: exercise may match medication in reducing mortality associated with cardiovascular disease, diabetes. JAMA 2013;310:2026-7.
12. Hordern MD, Coombes JS, Cooney LM, Jeffriess L, Prins JB, Marwick TH. Effects of exercise intervention on myocardial function in type 2 diabetes. Heart 2009;95:1343-9.
13. Tjonna AE, Lee SJ, Rognmo O, Stolen TO, Bye A, Haram PM, et al. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. Circulation 2008;118:346-54.
14. Rognmo O, Hetland E, Helgerud J, Hoff J, Slordahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. Eur J Cardiovasc Prev Rehabil 2004;11:216-22.
15. Boule NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus. Diabetologia 2003;46:1071-81.
16. Rognmo O, Moholdt T, Bakken H, Hole T, Molstad P, Myhr NE, et al. Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. Circulation 2012;126:1436-40.
17. Hollekim-Strand SM, Bjorgaas MR, Albrektsen G, Tjonna AE, Wisloff U, Ingul CB. High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: a randomized controlled trial. J Am Coll Cardiol 2014;64:1758-60.
18. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009;22:107-33.
19. Dalen H, Thorstensen A, Vatten LJ, Aase SA, Stoylen A. Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. Circ Cardiovasc Imaging 2010;3:614-22.
20. The Norwegian Diabetes Association. Type 2-diabetes og fysisk aktivitet. Available at: http://www.diabetes.no/Type+2-diabetes+og+fysisk+aktivitet.9UFRzQXz.ips Last Update Date: September, 2011. Accessed September, 2015.
21. Ingul CB, Tjonna AE, Stolen TO, Stoylen A, Wisloff U. Impaired cardiac function among obese adolescents: effect of aerobic interval training. Arch Pediatr Adolesc Med 2010;164:852-9.

22. Molmen HE, Wisloff U, Aamot IL, Stoylen A, Ingul CB. Aerobic interval training compensates age related decline in cardiac function. Scand Cardiovasc J 2012;46:163-71.
23. Aase SA, Torp H, Stoylen A. Aortic valve closure: relation to tissue velocities by Doppler and speckle tracking in normal subjects. Eur J Echocardiogr 2008;9:555-9.
24. Notomi Y, Lysyansky P, Setser RM, Shiota T, Popovic ZB, Martin-Miklovic MG, et al. Measurement of ventricular torsion by two-dimensional ultrasound speckle tracking imaging. J Am Coll Cardiol 2005;45:2034-41.
25. Nakai H, Takeuchi M, Nishikage T, Kokumai M, Otani S, Lang RM. Effect of aging on twist-displacement loop by 2-dimensional speckle tracking imaging. J Am Soc Echocardiogr 2006;19:880-5.
26. Dalen H, Thorstensen A, Romundstad PR, Aase SA, Stoylen A, Vatten LJ. Cardiovascular risk factors and systolic and diastolic cardiac function: a tissue Doppler and speckle tracking echocardiographic study. J Am Soc Echocardiogr 2011;24:322-32.
27. Thorstensen A, Dalen H, Amundsen BH, Aase SA, Stoylen A. Reproducibility in echocardiographic assessment of the left ventricular global and regional function, the HUNT study. Eur J Echocardiogr 2010;11:149-56.
28. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org; 2014.
29. Wang J, Khoury DS, Yue Y, Torre-Amione G, Nagueh SF. Left ventricular untwisting rate by speckle tracking echocardiography. Circulation 2007;116:2580-6.
30. Fonseca CG, Dissanayake AM, Doughty RN, Whalley GA, Gamble GD, Cowan BR, et al. Three-dimensional assessment of left ventricular systolic strain in patients with type 2 diabetes mellitus, diastolic dysfunction, and normal ejection fraction. Am J Cardiol 2004;94:1391-5.
31. Park SJ, Miyazaki C, Bruce CJ, Ommen S, Miller FA, Oh JK. Left ventricular torsion by two-dimensional speckle tracking echocardiography in patients with diastolic dysfunction and normal ejection fraction. J Am Soc Echocardiogr 2008;21:1129-37.
32. Stuber M, Scheidegger MB, Fischer SE, Nagel E, Steinemann F, Hess OM, et al. Alterations in the local myocardial motion pattern in patients suffering from pressure overload due to aortic stenosis. Circulation 1999;100:361-8.
33. Takeuchi M, Borden WB, Nakai H, Nishikage T, Kokumai M, Nagakura T, et al. Reduced and delayed untwisting of the left ventricle in patients with hypertension and left ventricular hypertrophy: a study using two-dimensional speckle tracking imaging. Eur Heart J 2007;28:2756-62.
34. Neilan TG, Ton-Nu TT, Jassal DS, Popovic ZB, Douglas PS, Halpern EF, et al. Myocardial adaptation to short-term high-intensity exercise in highly trained athletes. J Am Soc Echocardiogr 2006;19:1280-5.
35. Zocalo Y, Bia D, Armentano RL, Arias L, Lopez C, Etchart C, et al. Assessment of training-dependent changes in the left ventricle torsion dynamics of professional soccer players using speckle-tracking echocardiography. Conf Proc IEEE Eng Med Biol Soc 2007;2007:2709-12.
36. Burns AT, La Gerche A, MacIsaac AI, Prior DL. Augmentation of left ventricular torsion with exercise is attenuated with age. J Am Soc Echocardiogr 2008;21:315-20.
37. Opdahl A, Helle-Valle T, Remme EW, Vartdal T, Pettersen E, Lunde K, et al. Apical rotation by speckle tracking echocardiography: a simplified bedside index of left ventricular twist. J Am Soc Echocardiogr 2008;21:1121-8.
38. Brassard P, Legault S, Garneau C, Bogaty P, Dumesnil JG, Poirier P. Normalization of diastolic dysfunction in type 2 diabetics after exercise training. Med Sci Sports Exerc 2007;39:1896-901.
39. Aljaroudi W, Halley C, Houghtaling P, Agarwal S, Menon V, Rodriguez L, et al. Impact of body mass index on diastolic function in patients with normal left ventricular ejection fraction. Nutr Diabetes 2012;2:e39.
40. Kim HK, Sohn DW, Lee SE, Choi SY, Park JS, Kim YJ, et al. Assessment of left ventricular rotation and torsion with two-dimensional speckle tracking echocardiography. J Am Soc Echocardiogr 2007;20:45-53.

SUPPLEMENTARY MATERIAL

Comparison between the T2D group and the control group in other LV echocardiographic variables, LV dimensions and volumes, resting heart rate, and blood pressure.

At baseline, e′, peak early diastolic mitral inflow (E), and the E/A ratio were lower, and filling pressure (E/e′ ratio) and late diastolic mitral inflow (A) were higher in the T2D group compared with the control subjects ($P < .001$ for all) (Table 1). There was no significant difference in IVRT between the T2D group and the control group at baseline ($P = .11$) (Supplemental Table 1).

IVRT was reduced from baseline to posttest in the HIIE group (−9.5 msec; 95% CI, −18.2 to −0.9 msec; $P = .03$) but not in the MIE group (−4.7 msec; 95% CI, −14.3 to 5.0 msec; $P = .33$), but there was no difference in change between groups (MIE vs HIIE: −4.9 msec; 95% CI, −16.1 to 6.2 msec; $P = .37$).

At 12-week posttest, there were still significant differences in diastolic function between the MIE group and the control subjects (e′, E, E/e′ ratio, A, and E/A ratio) and the HIIE group and the control subjects (e′, E/e′ ratio, A, E/A ratio, and IVRT), but not between the HIIE group and the control subjects for E (Supplemental Table 1).

At baseline, peak systolic tissue Doppler velocity (S′) was significantly lower in the T2D group than in the control group ($P < .001$) and was still reduced compared with control subjects in the MIE group ($P = .005$) and HIIE group ($P = .04$) after intervention (Supplemental Table 1).

LV Dimensions and Volumes

The left interventricular septum and posterior wall were significantly thicker in both diastole and systole in patients with T2D compared with control subjects at baseline (interventricular septal thickness in diastole 1.07 ± 0.17 vs 0.89 ± 0.15 cm [$P = .001$] and in systole 1.62 ± 0.31 vs 1.29 ± 0.21 cm [$P < .001$]; posterior wall thickness in diastole 1.10 ± 0.15 vs 0.88 ± 0.16 cm [$P < .001$] and in systole 1.79 ± 0.28 vs1.52 ± 0.22 cm [$P = .001$]). There was no difference in LV internal dimension between the control subjects and patients with T2D. Intervention did not change LV dimensions. Left atrial volume index was 35.1 ± 9.9 mL/m^2 in patients with T2D at baseline and remained similar after intervention ($P = .09$).

At baseline, end-diastolic volume was lower in the control group compared with the T2D group (122.7 vs 155.6 mL, respectively, $P < .001$), whereas there was no significant difference in end-systolic volume between groups (67.3 vs 68.8 mL, respectively). End-diastolic and end-systolic volumes did not change after exercise in the T2D group (155.6 vs 153.1 mL and 68.9 vs 68.8 mL, respectively). Left atrial volume index decreased from baseline to posttest in the T2D group by 4.3 mL/m^2 ($P < .001$).

Resting Heart Rate and Blood Pressure

At baseline, mean resting heart rate was significantly higher in the T2D group than in the control group ($P = .008$; Supplemental Table 1). Diastolic blood pressure was higher in patients with T2D than control subjects at baseline (4.6 mm Hg; 95% CI, 0.7 to 8.5 mm Hg; $P = .02$), but not systolic blood pressure (4.8 mm Hg; 95% CI, −1.7 to 11.4 mm Hg; $P = .15$).

At 12-week posttest, there was no significant difference in resting heart rate between the HIIE group and control subjects, whereas heart rate was still higher in the MIE group ($P = .007$; Supplemental Table 1). There was no significant difference between the intervention groups and control subjects in systolic or diastolic blood pressure at posttest (Supplemental Table 1).

Supplemental Table 1 Results for comparison between the T2D groups and control subjects for LV longitudinal diastolic and systolic function as well as resting heart rate and blood pressure

	T2D vs control, baseline				MIE vs control, posttest				HIIE vs control, posttest			
		95% CI				95% CI				95% CI		
Variable	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*	Estimate	Lower	Upper	*P*
Resting HR (beats/min)	6.4	1.7	11.1	.008	8.8	2.6	15.1	.007	1.3	−3.9	6.4	.62
Systolic BP (mm Hg)	4.8	−1.7	11.4	.15	2.9	−5.8	11.7	.50	10.5	−0.0	21.1	.05
Diastolic BP (mm Hg)	4.6	0.7	8.5	.02	4.6	−0.1	9.3	.06	2.0	−3.2	7.3	.44
E (cm/sec)	−11.7	−17.8	−5.6	<.001	−9.6	−18.3	−0.9	.03	−0.6	−9.4	8.2	.89
A (cm/sec)	0.1	0.1	0.2	<.001	0.1	0.1	0.2	.003	0.2	0.1	0.3	.001
E/A ratio	−0.4	−0.6	−0.3	<.001	−0.4	−0.6	−0.2	<.001	−0.3	−0.5	−0.1	.005
e′ (cm/sec)	−4.0	−4.8	−3.1	<.001	−3.4	−4.4	−2.4	<.001	−2.2	−3.1	−1.2	<.001
E/e′ ratio	2.6	1.7	3.5	<.001	2.2	1.0	3.4	.001	2.0	0.9	3.1	.001
IVRT (msec)	−8.2	−18.2	1.8	.11	−13.2	−23.8	−2.7	.02	−18.1	−30.1	−6.1	.004
S′ (cm/sec)	−1.4	−1.9	−0.8	<.001	−1.0	−1.6	−0.3	.005	−0.8	−1.6	−0.1	.04

BP, Blood pressure; *Estimate*, estimated mean difference; IVRT, isovolumic relaxation time; *HR*, heart rate.
The table shows results from two-sample *t* tests for comparing the T2D group and controls at baseline and the HIIE and MIE groups after the intervention to control subjects for the variables describing diastolic and systolic function and resting HR.

Paper III

Hollekim-Strand et al. Nutrition Journal (2015) 14:79
DOI 10.1186/s12937-015-0069-1

NUTRITION JOURNAL

RESEARCH Open Access

Fast food increases postprandial cardiac workload in type 2 diabetes independent of pre-exercise: A pilot study

Siri Marte Hollekim-Strand[1], Vegard Malmo[1,2], Turid Follestad[3], Ulrik Wisløff[1] and Charlotte Björk Ingul[1*]

Abstract

Background: Type 2 diabetes aggravates the postprandial metabolic effects of food, which increase cardiovascular risk. We investigated the acute effects of fast food on postprandial left ventricular (LV) function and the potential effects of pre-exercise in type 2 diabetes individuals.

Methods: We used a cross-over study including 10 type 2 diabetes individuals (7 male and 3 females; 53.4 ± 8.1 years; 28.3 ± 3.8 kg/m^2; type 2 diabetes duration 3.1 ± 1.8 years) and 10 controls (7 male and 3 females; 52.8 ± 10.1 years; 28.5 ± 4.2 kg/m^2) performing high intensity interval exercise (HIIE; 40 min, 4 × 4min intervals, 90–95 % HR_{max}), moderate intensity exercise (MIE; 47 min, 70 % HR_{max}) and no exercise (NE) in a random order 16–18 hours prior to fast-food ingestion. Baseline echocardiography, blood pressure and biochemical measurements were recorded prior to and 16–18 hours after exercise, and 30 minutes, 2 hours and 4 hours after fast food ingestion.

Results: LV diastolic (peak early diastolic tissue velocity, peak early diastolic filling velocity), and systolic workload (global strain rate, peak systolic tissue velocity, rate pressure product) increased after consumption of fast food in both groups. In contrast to controls, the type 2 diabetes group had prolonged elevations in resting heart rate and indications of prolonged elevations in diastolic workload (peak early diastolic tissue velocity) as well as reduced systolic blood pressure after fast food consumption. No significant modifications due to exercise in the postprandial phase were seen in any group.

Conclusions: Our findings indicate that fast-food induces greater and sustained overall cardiac workload in type 2 diabetes individuals versus body mass index and age matched controls; exercise 16–18 hours pre-meal has no acute effects to the postprandial phase.

Trial registration: ClinicalTrials.gov: NCT01991769.

Background

Most of the day is spent in the postprandial state and frequent ingestion of energy dense food, rich in processed carbohydrates, saturated fats and salt (fast-food), increases the risk of cardiometabolic diseases [1–3].

Type 2 diabetes aggravates the postprandial metabolic effects of food because it impairs the transport, delivery and/or storage of carbohydrates and fats [4]. Although type 2 diabetes is an accepted cause of heart failure [5] and approximately 50 % of asymptomatic individuals with well-controlled type 2 diabetes show signs of diastolic dysfunction [6], little is known about the acute effects of food in the view of cardiac function in this population.

Endothelial function is impaired after fast food ingestion, which is related to increases in circulating glucose, triglycerides and/or elevated oxidative stress [7, 8]. However little is known about the acute consequence of excessive elevations in circulating glucose and/or triglycerides after a meal on cardiac function in type 2 diabetes.

Although few studies have investigated the acute effects of fast food on cardiac function, it is observed that food-induced elevation of circulating glucose and oxidative stress reduce diastolic function in insulin-treated type 2 diabetes patients [9], and that acute elevations in circulating triglycerides yield compensatory increases in systolic function of the left ventricle in healthy individuals [10].

* Correspondence: charlotte.b.ingul@ntnu.no
[1]K.G. Jebsen Centre of Exercise in Medicine at Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, PO Box 8905 7491 Trondheim, Norway
Full list of author information is available at the end of the article

Chronic exercise improves cardiac function [6] and even single bouts of exercise can improve endothelial function, triglycerides and oxidative stress in healthy and reduce postprandial glucose elevations in type 2 diabetes individuals [8, 11, 12]. Exercise 16–18 hours pre-meal has previously shown to induce improvements in total antioxidant status (TAS) and endothelial function, rather than reduce the circulating glucose or triglycerides [8]. In this setting, high intensity interval exercise (HIIE) was more effective in improving postprandial endothelial function compared to moderate intensity exercise (MIE) [8].

No study has investigated whether fast food ingestion induces an acute increase in cardiac workload, or whether this may be modified by pre-exercise as observed for endothelial function [8].

The purpose of this study was thus to explore whether a single fast food meal affects left ventricular (LV) diastolic and systolic function in the four hour postprandial phase, and whether exercise (HIIE or MIE) 16–18 hours prior to a single fast food meal could affect LV function, resting heart rate, blood pressure and/or other biochemical measures in type 2 diabetes patients.

Methods

Study participants

Type 2 diabetes individuals and healthy controls were recruited through a local newspaper and from advertisement at St. Olav's University hospital, Trondheim, Norway. The study was performed from February to June 2012.

The inclusion criteria included: age 40 to 65 years and type 2 diabetes within the past 10 years with no use of insulin. Exclusion criteria included: known cardiovascular or lung disease, uncontrolled hypertension, orthopaedic or neurological restrictions, body mass index (BMI) >35 kg/m^2, pregnancy, inability to exercise, smoking, drug or alcohol abuse, planned surgery during the trial period, serious eating- and/or personality disorders, reluctance to sign informed consent form, or more physical activity than today's recommendations [13].

Ten type 2 diabetes individuals and 10 healthy age and BMI matched controls were included. The protocol was approved by the Regional Committee for Medical and Health Research Ethics and registered in the Clinical Trials Registry (ClinicalTrials.gov identifier: NCT01991769). Informed consent was obtained from all participants.

Design

A minimum of one week before the first trial, peak oxygen uptake (VO_{2peak}) was assessed (Jaeger LE2000CE, Hochberg, Germany) as previously described [14]. Each of the 20 subjects participated in all trials (HIIE, MIE and no exercise (NE)), in a randomized order with a minimum of one week between trials. The timeline representing each trial is illustrated in Figs. 1, 2, 3 and 4. Baseline-1 measurements were performed on the day prior to fast-food ingestion in a resting (sedate behavior/refrained from exercise, ≥48 hours) and fasting (≥12 hours) state that abstained from caffeine, citrus and alcohol (16–18 hours). During the 16–18 hour period after HIIE, MIE or NE, the subjects were instructed to abstain from exercise, caffeine, citrus and alcohol and to report for baseline-2 in a fasting (≥12 hours) state the following morning. Before overnight fasting commenced, prior to all baseline-1 and baseline-2 measurements, subjects ingested a mixed meal at the same time and of the same content (same manufacturer, same amount) as they did before baseline-1 in their first trial. This meal was typically a standard Norwegian supper (i.e., whole grain sandwich with butter/margarine and meat/egg/fish/cheese and milk or water). Following baseline-2, fast food was ingested and measurements performed 30 minutes, 2 hours and 4 hours after the meal. The participants remained inactive in the laboratory after fast-food ingestion until end of the protocols.

Outcome measures

The primary outcome measure was LV diastolic function measured as peak early diastolic tissue velocity (e'). Secondary outcome measures were LV late diastolic tissue velocity (a'), LV early diastolic filling velocity (E), LV late diastolic filling velocity (A), LV filling pressure (E/e'), E/A-ratio, deceleration time (DT), isovolumic relaxation time (IVRT), LV global strain and strain rate, LV peak systolic tissue velocity (S') as well as resting heart rate, blood pressure, circulating glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, C-peptide, total antioxidant status (TAS) and high sensitive c-reactive protein (hs-CRP).

Exercise training protocols

All exercise trials were supervised. HIIE was performed by walking or running on an inclined treadmill. Following 10 min warm up at approximately 70 % of maximal heart rate obtained at exercise testing (HR_{max}), the HIIE-group performed four intervals at 90-95 % of HR_{max} with 3 minutes recovery periods between intervals at 70 % of HR_{max} and 5 minutes cool down; 40 min altogether. The MIE protocol consisted of 47 minutes exercise at 70 % of HR_{max} to achieve approximately similar total energy expenditure as for HIIE.

Fast food

The fast food consisted of a vegetarian mozzarella pizza (Dr. Oetker); 335 g (874 kcal/ 3655 kJ), 83.4 g carbohydrates, 44.2 g fat, and 34.8 g protein. A previous study indicated that this pizza induces a marked postprandial increase in circulating glucose, triglycerides, C-peptide and

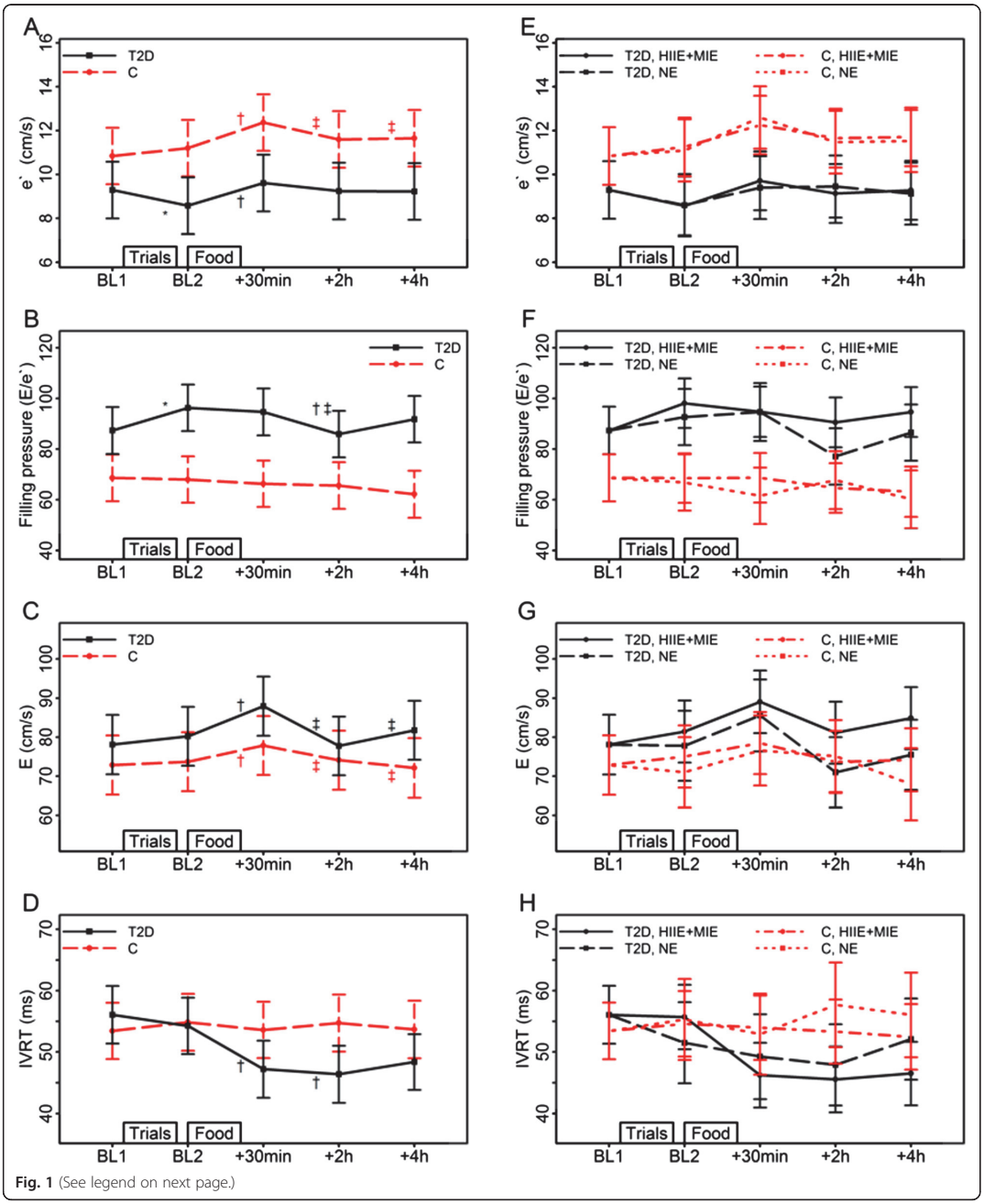


Fig. 1 (See legend on next page.)

(See figure on previous page.)
Fig. 1 Effects of fast food (*left* panel; all trials combined) and exercise (*right* panel; high intensity interval exercise +moderate intensity exercise vs. no exercise) on left ventricular diastolic function. Abbreviations: BL, baseline; C, control group; e', peak early diastolic tissue Doppler velocity; E/e' , filling pressure; E, peak early filling velocity; HIIE, high intensity interval exercise; HIIE+MIE, exercise combined; IVRT, isovolumic relaxation time; MIE, moderate intensity exercise; NE, no exercise; T2D, type 2 diabetes group. Estimated means and 95 % CIs from LMMs with the factors time, group and their interaction (left panel, figures **a**-**d**), and with the factors time, group, trial and their interactions (right panel, figures E-H). In the left panel significant ($p < 0.01$) time differences are indicated by *(from BL1), † (from BL2), ‡ (from food +30 min) and § (from food +2 h). For peak early filling velocity (**e**) there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups

a decrease in TAS as well as a transient impairment in endothelial function in healthy individuals [8].

Clinical and laboratory examinations

Resting echocardiography

Three consecutive cycles in B-mode acquisitions (mean frame rate 53/sec) and color tissue Doppler imaging (TDI) (mean frame rate 159.9/sec) were recorded from the 3 apical views (four-chamber, two-chamber, and long- axis) and B-mode from the parasternal view. Measurements included E, A, IVRT and DT. Pulsed wave tissue Doppler velocities were measured at the four mitral annular sites in the four-chamber and two-chamber views. The mean of these points was used for S', e' and a' [15]. The ratio E/e' was calculated as an estimate of LV filling pressure [16]. Global strain and strain rate were calculated from 2-dimensional strain echocardiography [17]. Measurements obtained in this study are in accordance with standard procedures recommended by the American Society of Echocardiography [18], and no subjects were excluded because of impaired echocardiographic image quality. Images were analyzed off line using EchoPAC version BT12 (GE Vingmed Ultrasound, Horten, Norway). The observer was blinded to group participation, trial and point in time during ultrasound analysis.

Resting heart rate and blood pressure

The lowest heart rate observed during echocardiography was defined as the resting heart rate. Upright blood pressure measurements were performed using Philips SureSigns V52 (Andover, Massachusetts, US). Before baseline-1 and baseline-2, participants rested in a sitting position for at least 10 minutes before measurements. Blood pressure at these time points was noted as the median of three recordings. At the remaining time points, upright blood pressure was measured only once. Rate pressure product (RPP; heart rate x systolic blood pressure) was calculated to determine myocardial workload.

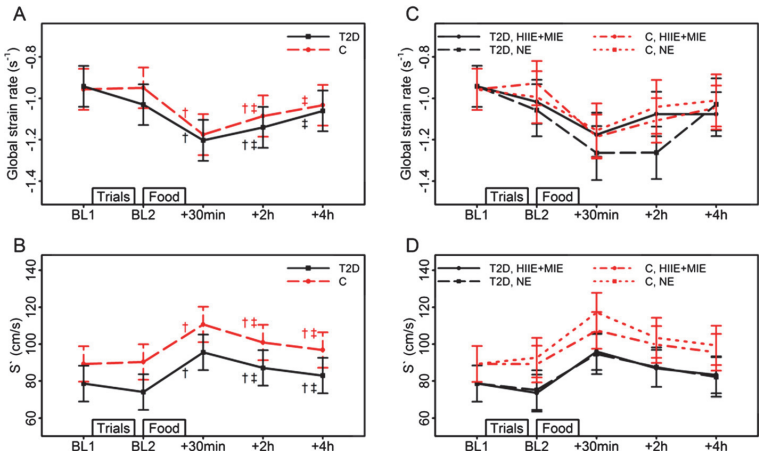


Fig. 2 Effects of fast food (*left* panel; all trials combined) and exercise (*right* panel; high intensity interval exercise +moderate intensity exercise vs. no exercise) on left ventricular systolic function. Abbreviations: BL, baseline; C, control group; HIIE, high intensity exercise; HIIE+MIE, exercise combined; MIE, moderate intensity exercise; NE, no exercise; S' , peak systolic tissue Doppler velocity; T2D, type 2 diabetes group. Estimated means and 95 % CIs from LMMs with the factors time, group and their interaction (*left* panel, figures **a**-**b**), and with the factors time, group, trial and their interactions (right panel, figures **c**-**d**). In the left panel significant ($p < 0.01$) time differences are indicated by *(from BL1), † (from BL2), ‡ (from food +30 min) and § (from food +2 h). For S' and global strain rate there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups

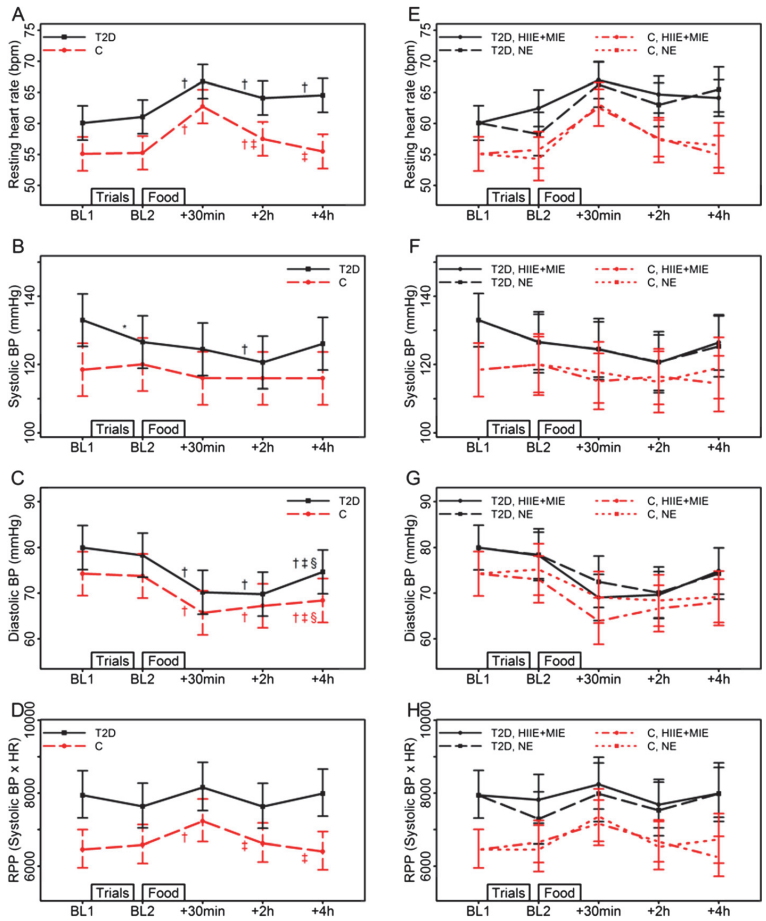


Fig. 3 Effects of fast food (*left* panel; all trials combined) and exercise (right panel; high intensity interval exercise+moderate intensity exercise vs. no exercise) on resting heart rate and blood pressure. Abbreviations: BL, baseline; BP, blood pressure; C, control group; HIIE, high intensity exercise; HIIE+MIE, exercise combined; HR, resting heart rate; MIE; moderate intensity exercise; NE, no exercise; RPP, rate pressure product; T2D, type 2 diabetes group. Estimated means and 95 % CIs from LMMs with the factors time, group and their interaction (*left* panel, figures **a**-**d**), and with the factors time, group, trial and their interactions (*right* panel, figures **e**-**h**). In the left panel significant ($p < 0.01$) time differences are indicated by *(from BL1), † (from BL2), ‡ (from food +30 min) and § (from food +2 h). For diastolic BP there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups. For RPP, the means and CIs are shown as back-transformed values, computed by direct exponentiation of the means and CIs from the LMM based on log-transformed data

Biochemical analysis

Blood was collected after blood pressure measurements and before echocardiography. Blood glucose, C-peptide, plasma triglycerides, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) and high-sensitive C-reactive protein (hs-CRP) were analyzed according to standard procedures at the St. Olavs Hospital (Trondheim) at all time-points; HbA_{1c} was measured at baseline-1. Blood glucose was measured using photometric hexokinase UV method (Roche Modular, Roche Diagnostics, Germany) and C-peptide was measured using chemiluminescence method (Immulite 2000, Siemens Medical Solutions, New Jersey, US). The blood lipids were measured using photometric, enzymatic colorimetric method (Roche Modular, Roche Diagnostics Germany). Hs-CRP was measured using Tina-quant CRPHS immunoturbidimetric assay (Roche Modular, Roche Diagnostics, Germany). HbA_{1c} was measured using TINIA (Turbidimetric Inhibition immunoassay) (Roche Cobas Integra 400 plus, Roche Diagnostics, Germany). Insulin sensitivity was calculated using

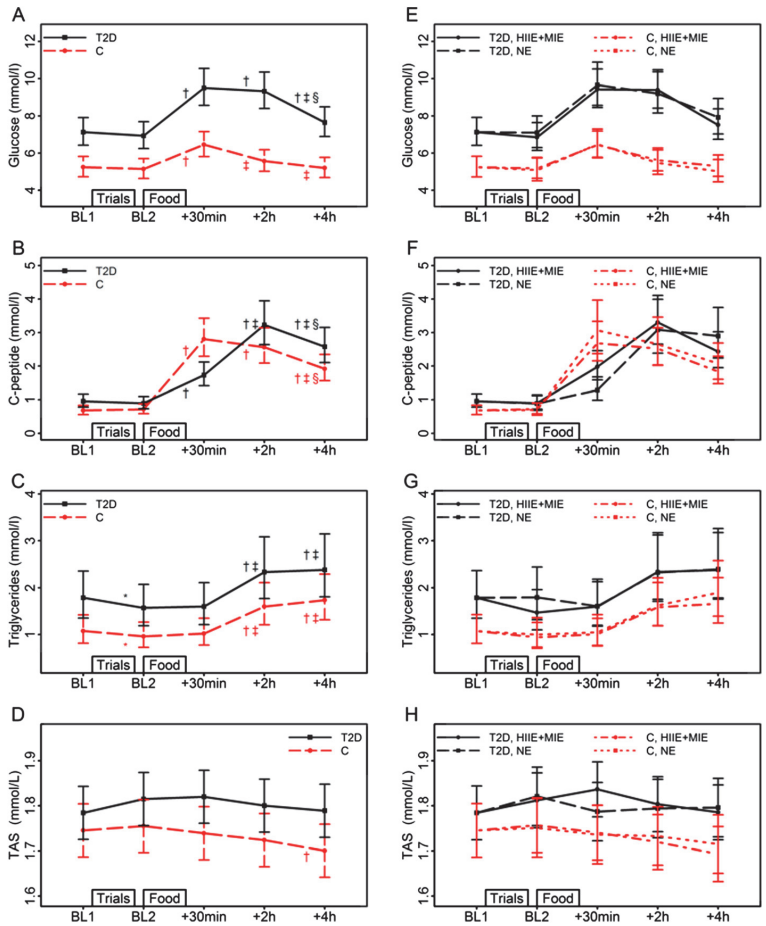


Fig. 4 Effects of fast food (*left* panel, trials combined) and exercise (*right* panel; high intensity interval exercise+moderate intensity exercise vs. no exercise) on blood glucose, C-peptide, triglycerides and total antioxidant status. Abbreviations: BL, baseline; C, control group; HIIE, high intensity exercise; HIIE+MIE, exercise combined; MIE, moderate intensity exercise; NE, no exercise; TAS, total antioxidant status; T2D, type 2 diabetes group. Estimated means and 95 % CIs from LMMs with the factors time, group and their interaction (*left* panel, figures **a**-**d**), and with the factors time, group, trial and their interactions (*right* panel, figures **e**-**h**). In the left panel significant ($p < 0.01$) time differences are indicated by *(from BL1), † (from BL2), ‡ (from food +30 min) and § (from food +2 h). For triglycerides there is no significant time and group interaction, and the indicated significant time differences refer to the main effect of time for both groups. Except for TAS, the means and CIs are shown as back-transformed values, computed by direct exponentiation of the means and CIs from the LMMs based on log-transformed data

the HOMA2 calculator (The Homeostasis Assessment Model, University of Oxford, UK). Total antioxidant status (TAS) was analyzed as previously described [19].

Statistical methods

The statistical analysis was performed by linear mixed models (LMMs). Within-subject correlations were considered using a random intercept in the LMM. A full model included group (type 2 diabetes or controls), trial (HIIE, MIE, or NE), and time (baseline-1, baseline-2, and 30 minutes, 2 hours and 4 hours post-meal). Models with two levels of the trial factor (HIIE and MIE combined or NE) were also considered. Tests for overall effects of factors and factor interactions were done by likelihood ratio tests using significance level 0.05. Post hoc comparisons specified and tested appropriate linear combinations (contrasts) of the estimated model parameters for the selected models. In all models, the baseline means (baseline-1) for each group were restricted to be equal for the three exercise trials due to randomization to trials within each group

[20]. Outcome variables not meeting the normal assumptions of the LMM were log transformed prior to the statistical analysis in cases where this transformation improved the approximation to the normal distribution. The analyses were performed in the R statistical package [21].

This study is explorative rather than confirmative, and thus we did not perform any formal adjustment for multiple testing. However, if nothing else is explicitly stated, the results presented and discussed here are statistically significant at p<0.01.

Results

Subject characteristics

Subject characteristics are reported in Table 1. Twenty participants completed all trials (HIIE, MIE, NE), and exercised according to prescribed exercise heart rates and followed instructions of sedentary behavior during the NE trial. No adverse events were reported. Since no statistically significant effects of pre-exercise were found, the results discussed below are from LMMs including the factors group and time.

Effect of pre-exercise

Since no statistically significant effects of pre-exercise were found, the results discussed below are from LMMs including the factors group and time.

Cardiac function

The LV diastolic responses to fast food are illustrated in Fig. 1a-d.

Table 1 Subject characteristics

	Type 2 diabetes	Control	*p*
n	10	10	
Male/Female, n (%)	7/3 (70/30)	7/3 (70/30)	
Diabetes duration, years	3.1 ± 1.8	-	
Age, years	53.4 ± 8.1	52.8 ± 10.1	0.89
Body mass index, kg/m^2	28.3 ± 3.8	28.5 ± 4.2	0.91
Waist circumference, cm	107.1 ± 27.5	104.4 ± 13.8	0.78
HbA_{1c}, % (mmol/mol)	6.4 ± 1.0 (46.0 ± 7.0)	5.5 ± 0.2 (36.0 ± 1.5)	0.01
HOMA-ir	2.2 ± 0.7	1.7 ± 0.7	0.10
VO_{2peak}, ml/kg/min	38.8 ± 7.8	36.2 ± 8.8	0.51
VO_{2peak}, L/min	3.39 ± 0.84	3.36 ± 0.92	0.93
Medical agents, n (%)			
Anti-diabetic	6 (60)	0 (0)	
Statins	1 (10)	1 (10)	
Anti-hypertension	6 (60)	1 (10)	

Data are means ± SD unless otherwise indicated
Abbreviations: *HbA1c* glycosylated hemoglobin, *HOMA-ir* homeostatic assessment model- insulin resistance, VO_{2peak} peak oxygen uptake
T-tests were used to test for differences between groups at baseline

The type 2 diabetes group had an overall poorer diastolic function (e') and higher filling pressure (E/e') versus controls (Fig. 1a-b).

In general, diastolic workload increased (higher e', a', E and A) within 30 minutes after the meal in both groups. Subsequently, diastolic workload reversed towards baseline-2 levels, but in contrast to controls ($p = 0.10$), the type 2 diabetes group showed an indication of increased diastolic workload as measured by e' that persisted 4 hours after the meal ($p = 0.02$). Late diastolic filling (a') remained elevated 4 hours after fast food in both groups.

Filling pressure (E/e') and isovolumic relaxation time (IVRT) were reduced within 2 hours after the meal in the type 2 diabetes group; this was not significantly different after fast food in the controls. The difference between groups in change from baseline-2 to 2 hours post-meal was almost significant for IVRT ($p = 0.03$) but not for filling pressure ($p = 0.08$). No effect of time was observed for E/A ratio because both E and A increased. Supernormal filling is associated with vigorous recoil of the ventricle during diastole with an increase of negative pressure in the ventricle and evacuation of blood from the atrium. This causes a high E wave, shortening of the IVRT and normal deceleration time. We found that the shortened IVRT and lack of changes to deceleration time might be due to the same mechanism.

Pre-exercise did not influence postprandial early diastolic velocity (e') (Fig. 1e) or any other diastolic echocardiographic variables (Fig. 1f-h).

The LV systolic response to fast food are illustrated in Fig. 2a-b. In general, systolic workload (global strain rate and S') increased 30 min after the meal in both groups. Systolic workload was subsequently reversed, but remained significantly elevated (global strain rate and S') compared to baseline-2 after 4 hours in both groups (Fig. 2a-b).

Pre-exercise did not affect systolic function (Fig. 2c-d).

Hemodynamic measurements

The heart rate, blood pressure and RPP responses to fast food are illustrated in Fig. 3a-d.

The type 2 diabetes group had an increased heart rate versus controls ($p < 0.01$ or $p < 0.05$) at all time-points except 30 minutes after fast food ($p = 0.06$). Resting heart rate increased within 30 minutes after the meal and subsequently decreased in both groups; it decreased to a larger extent in controls than in type 2 diabetes. Only the controls re-gained baseline resting heart rate after 4 hours (Fig. 3a).

From baseline-1 to baseline-2, systolic blood pressure decreased in type 2 diabetes, but not in controls. Within 2 hours after fast food, the mean systolic blood pressure in the type 2 diabetes group decreased and subsequently

reversed within 4 hours post-meal. In contrast, systolic blood pressure in the controls did not change after ingestion of fast food ingestion (Fig. 3b).

Overall, RPP was higher in the type 2 diabetes group versus to controls (baseline-1 and 4 hours, $p < 0.01$; baseline 2 and 2 hours: $p < 0.05$; 30 minutes, $p = 0.05$). The RPP was increased post-meal in both controls and type 2 diabetes ($p < 0.01$ and $p < 0.05$, respectively). It subsequently reduced within 2 hours ($p < 0.01$ and $p < 0.05$, respectively), but was changed back to baseline 2 levels after 4 hours only in the controls (Fig. 3d).

Pre- exercise did not influence heart rate, blood pressure response or RPP (Fig. 3e-h).

Biochemistry

The response of circulating glucose, C-peptide, triglycerides and TAS to fast food is illustrated in Fig. 4a-d, respectively. Fast food increased the glucose levels within 30 minutes after the meal in both groups. The subsequent drop in mean glucose levels was delayed in the type 2 diabetes group versus the control group. This was indicated by the fact that the difference 2 hours versus 30 minutes post-meal is significant for controls ($p < 0.001$) but not for type 2 diabetes group ($p = 0.5$)— only the controls returned to baseline glucose levels 4 hours post-meal (Fig. 4a). Concurrently, C-peptide levels peaked at 30 minutes post-meal in controls versus at 2 hours post-meal in the type 2 diabetes group (Fig. 4b).

The type 2 diabetes group had indications of an overall higher triglyceride level than the controls ($p < 0.05$, Fig. 4c). The effect of fast food on triglyceride levels was similar in both groups.

The TAS response to fast food was similar for the two groups—TAS decreased within 4 hours in both controls and type 2 diabetes ($p < 0.01$ and $p < 0.05$, respectively; Fig. 4d). Hs-CRP, HDL and LDL did not change in the postprandial phase in either group.

Pre-exercise did not influence any biochemical variables measured at any time-point (Fig. 4e-h).

Discussion

Our findings indicate that fast-food induces greater overall cardiac workload in type 2 diabetes individuals than in BMI and age matched controls. Pre-exercise did not modify fast food induced changes in LV function, resting heart rate, blood pressure, blood glucose, triglycerides or total antioxidant status.

The observed postprandial increase in diastolic workload in both type 2 diabetes and healthy overweight individuals is novel. Our data contrast the few previous studies that investigated the effects of lipid infusions or a carbohydrate rich meal on cardiac function [9, 10]. Further study is needed to determine whether this is a result of the "combined meal" used here. The other studies [9, 10] might also have missed an initial increase in diastolic workload due to measuring postprandial response 1 or 2 hours after infusion or ingestion, respectively. In contrast to our finding of increased diastolic workload (e') after the meal, von Bibra et al. [9] observed a significantly reduced diastolic workload (e') 2 hours after ingesting a pure carbohydrate meal (48 g). Nielsen et al. [22] found no changes in diastolic function after short-term hyperglycemia by insulin discontinuation in insulin dependent type 2 diabetes individuals. However, these participants [9, 22] had longer history of type 2 diabetes and were insulin dependent.

The present study indicates that fast food interacts with LV diastolic function to a greater extent in type 2 diabetes individuals compared to controls. This could be explained by the prolonged postprandial increase in heart rate in the type 2 diabetes group: An increased heart rate increases diastolic function (E, e') and shortens IVRT. The decrease in filling pressure in the type 2 diabetes group within 2 h post-meal, could be explained by the indication of sustained increase in e' at this time point in this group while E is reduced.

We could speculate whether the postprandial diastolic compensations observed in the type 2 diabetes group is an early sign of diastolic dysfunction. However, further research is needed to investigate the progress and interaction of food ingestion and diastolic compensations in type 2 diabetes across different disease stages.

The increased LV systolic workload after fast food ingestion is in line with Holland et al. [10] who demonstrated increased systolic workload (LV global strain rate) induced by increased circulating triglycerides after intra venous administration of a fat emulsion in healthy individuals, and Nielsen et al. [22] who observed increased systolic workload (S' and strain rate) due to hyperglycemia in type 2 diabetes individuals with and without heart failure. However, our data is in contrast to von Bibra et al. [9] who observed no postprandial change in systolic function (S') in insulin dependent type 2 diabetes individuals with longer duration after ingesting carbohydrates. The diverse findings may be due to different measurement times as well as differences in methods used to increase circulating glucose and/or triglycerides.

The RPP differences seen here between groups suggest that the type 2 diabetes group had greater cardiac workload compared to controls.

The higher heart rate at rest and prolonged increases in heart rate after fast food consumption by type 2 diabetes individuals relative to controls may be due to several factors including cardiovascular autonomic neuropathy (CAN) that can cause abnormalities in heart rate control by reduced vagal activity and/or high sympathetic activity [23]. Furthermore, both glucose ingestion [24] and elevated plasma fatty acid concentrations [25] may stimulate

the cardiac autonomic nervous system with a possible increase in catecholamines. Thus, the effects of fast food seen here may be due to catecholamine induced increases in inotropy that result in increased contractility of the cardiac muscle as well as increased dromotropic and chronotropic effects that increases the heart rate.

Although increased heart rate is commonly observed during euglycemic clamp in this patient group as well as those with metabolic syndrome [23], von Bibra et al. [9] observed no particular increase in resting heart rate 2 hours after a carbohydrate-rich meal in insulin dependent type 2 diabetes individuals. This may be due to the long standing type 2 diabetes [9], which increase the possibility of depressed sympathetic activity [23]. Nielsen et al. [26] observed a tendency towards increased heart rate ($p = 0.08$) due to high levels of lipid infusion versus low lipid infusion controls—this indicates that fast food may increase heart rate more than healthy foods.

The postprandial reduction in systolic blood pressure in the type 2 diabetes group might be an early stage of postprandial systolic hypotension, which is a common hemodynamic condition in diabetes [27] and is associated with an increased risk of cerebrovascular disease [28]. The mechanisms mediating postprandial reductions in blood pressure are not fully understood, but food-induced neurohormonal changes leading to reduced vascular resistance in the splanchnic vasculature as well as CAN, resulting in impaired sympathetic nervous activity has been suggested [29–31].

Gudmundsdottir et al. [32] investigated the postprandial changes after a healthy meal versus a fast food meal and found small changes in blood lipids and hs-CRP with no differences between meals. Our study supports these findings with no changes in cholesterol and hs-CRP.

In the present study, pre-exercise did not modify TAS in type 2 diabetes or controls. This is in contrast to Tyldum et al. [8] who observed a significant exercise-induced improvement in TAS, associated with improvements in endothelial function in healthy normal weight men (42 ± 4 years) using the same protocol as described here. This indicates that our participants had a poorer response to exercise versus lean and healthy individuals [8]. This may possibly be due to central obesity and poorer metabolic control in our study participants versus normal weight individuals.

The lack of exercise-induced improvements in postprandial TAS in the present study may be explained by a lack of exercise induced postprandial changes in circulating glucose- and triglyceride levels. Although the lack of exercise-induced TAS changes contrast with the findings of Tyldum et al. [8], the lack of exercise induced changes in glucose- and triglyceride levels did concur. Nevertheless, exercise-induced changes have previously been observed due to acute exercise on postprandial triglyceride levels and hyperglycemia [33–35].

However, studies are difficult to compare due to different measurement methods, meal composition and size, timing of exercise, exercise mode, intensity and duration. Inadequate energy expenditure [36] and/or inadequate exercise timing relative to the meal may explain the lack of exercise induced reduction in postprandial glucose [37] - and/or triglyceride excursion [38], etc. The acute effect of different exercise modes and timing of these on the postprandial response of the LV certainly needs to be further investigated in type 2 diabetes as the time course of adaptation may be different in the heart/endothelium than normal body weight persons.

Conclusions

Our findings indicate that fast food induces greater and sustained overall cardiac workload in the postprandial phase in type 2 diabetes individuals compared to BMI and age matched controls. Pre-exercise had no acute effects to the postprandial phase. The acute interaction of food on cardiac function in type 2 diabetes needs further study. More research is also needed on the effects of other exercise methods and exercise timing on postprandial cardiac function and other cardiovascular risk markers in this patient group.

Limitations

The limitations of this study include a small sample size and similarity (BMI and WC) between groups. We evaluated the effect of fast-food on LV function, and therefore the individual effects of carbohydrates, fat and salt cannot be evaluated. A recent study demonstrated no effect on diastolic function in normotensive healthy men after one week of high dietary sodium intake [39]. However a previous study found that one week of high dietary sodium intake impair myocardial relaxation [40]. The strengths of this study include the strictly controlled study environment, supervised exercise interventions as well as the similarity in BMI and WC between groups as it excludes potentially confounding effects of adiposity.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

SMH designed the protocol, retrieved funding, administrated the project, collected- and interpreted data and wrote the manuscript; VM collected data, contributed to the discussion and reviewed the manuscript; TF performed the statistical analyses, contributed to the discussion and reviewed the manuscript; UW supervised the project, contributed to the discussion and interpretation of data and edited the manuscript; CBI supervised the project, retrieved funding, designed the protocol, collected and interpreted data, reviewed and edited the manuscript and collected data. All authors read and approved the final manuscript.

Statement of assistance
The authors acknowledge the echocardiographic assistance of colleagues at the St. Olavs Hospital and Norwegian University of Science and Technology, Trondheim, Norway: Torfinn Eriksen, Bjørn Olav Haugen, Ola Kleveland, Erik Madsen, Tomas Renhult Skaug and Asbjørn Støylen, as well as the research assistance of Bjarne Nes and Arnt Erik Tjønna, Nina Backlund and Gøril Bakken Rønning.

Guarantors
C.B.I and S.M.H are equal guarantors taking responsibility of the contents of the article.

Funding
The study was funded by the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU), K.G. Jebsen Foundation for Medical Research and the Norwegian Diabetes Association.

Author details
[1]K.G. Jebsen Centre of Exercise in Medicine at Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, PO Box 8905 7491 Trondheim, Norway. [2]Department of Cardiology, St. Olavs Hospital, Trondheim, Norway. [3]Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

Received: 8 April 2015 Accepted: 31 July 2015
Published online: 14 August 2015

References

1. Cavalot F, Pagliarino A, Valle M, Di Martino L, Bonomo K, Massucco P, et al. Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. Diabetes Care. 2011;34:2237–43.
2. Pereira MA, Kartashov AI, Ebbeling CB, Van Horn L, Slattery ML, Jacobs Jr DR, et al. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. 2005;365:36–42.
3. Eberly LE, Stamler J, Neaton JD, Multiple Risk Factor Intervention Trial Research G. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. Arch Intern Med. 2003;163:1077–83.
4. Stephenson EJ, Smiles W, Hawley JA. The Relationship between Exercise, Nutrition and Type 2 Diabetes. Med Sport Sci. 2014;60:1–10.
5. de Simone G, Devereux RB, Chinali M, Lee ET, Galloway JM, Barac A, et al. Diabetes and incident heart failure in hypertensive and normotensive participants of the Strong Heart Study. J Hypertens. 2010;28:353–60.
6. Hollekim-Strand SM, Bjorgaas MR, Albrektsen G, Tjonna AE, Wisloff U, Ingul CB. High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: a randomized controlled trial. J Am Coll Cardiol. 2014;64:1758–60.
7. Ceriello A, Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, et al. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes. 2004;53:701–10.
8. Tyldum GA, Schjerve IE, Tjonna AE, Kirkeby-Garstad I, Stolen TO, Richardson RS, et al. Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. J Am Coll Cardiol. 2009;53:200–6.
9. von Bibra H, St John Sutton M, Schuster T, Ceriello A, Siegmund T, Schumm-Draeger PM. Oxidative stress after a carbohydrate meal contributes to the deterioration of diastolic cardiac function in nonhypertensive insulin-treated patients with moderately well controlled type 2 diabetes. Horm Metab Res. 2013;45:449–55.
10. Holland DJ, Erne D, Kostner K, Leano R, Haluska BA, Marwick TH, et al. Acute elevation of triglycerides increases left ventricular contractility and alters ventricular-vascular interaction. Am J Physiol Heart Circ Physiol. 2011;301:H123–128.
11. Gillen JB, Little JP, Punthakee Z, Tarnopolsky MA, Riddell MC, Gibala MJ. Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. Diabetes Obes Metab. 2012;14:575–7.
12. Karstoft K, Christensen CS, Pedersen BK, Solomon TP. The acute effects of interval- Vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a crossover, controlled study. J Clin Endocrinol Metab. 2014;99:3334–42.
13. International Diabetes Federation: Global Guideline for Type 2 Diabetes Available at: http://www.idf.org/sites/default/files/IDF%20T2DM%20Guideline.pdf Accessed March 28, 20142012.
14. Rognmo O, Hetland E, Helgerud J, Hoff J, Slordahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. Eur J Cardiovasc Prev Rehabil. 2004;11:216–22.
15. Thorstensen A, Dalen H, Amundsen BH, Aase SA, Stoylen A. Reproducibility in echocardiographic assessment of the left ventricular global and regional function, the HUNT study. Eur J Echocardiogr. 2010;11:149–56.
16. Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. Circulation. 2000;102:1788–94.
17. Reisner SA, Lysyansky P, Agmon Y, Mutlak D, Lessick J, Friedman Z. Global longitudinal strain: a novel index of left ventricular systolic function. J Am Soc Echocardiogr. 2004;17:630–3.
18. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18:1440–63.
19. Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. Circulation. 2007;115:3086–94.
20. Fitzmaurice GM, Laird NM, Ware JH. Applied Longitudinal Analysis. New York: Wiley; 2004. p. 126–32.
21. Team RC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. http://www.R-project.org.
22. Nielsen R, Norrelund H, Kampmann U, Botker HE, Moller N, Wiggers H. Effect of acute hyperglycemia on left ventricular contractile function in diabetic patients with and without heart failure: two randomized cross-over studies. PLoS One. 2013;8:e53247.
23. Valensi P, Chiheb S, Fysekidis M. Insulin- and glucagon-like peptide-1-induced changes in heart rate and vagosympathetic activity: why they matter. Diabetologia. 2013;56:1196–200.
24. Paolisso G, Manzella D, Rizzo MR, Barbieri M, Gambardella A, Varricchio M. Effects of glucose ingestion on cardiac autonomic nervous system in healthy centenarians: differences with aged subjects. Eur J Clin Invest. 2000;30:277–84.
25. Paolisso G, Manzella D, Rizzo MR, Ragno E, Barbieri M, Varricchio G, et al. Elevated plasma fatty acid concentrations stimulate the cardiac autonomic nervous system in healthy subjects. Am J Clin Nutr. 2000;72:723–30.
26. Nielsen R, Norrelund H, Kampmann U, Kim WY, Ringgaard S, Schar M, et al. Failing heart of patients with type 2 diabetes mellitus can adapt to extreme short-term increases in circulating lipids and does not display features of acute myocardial lipotoxicity. Circ Heart Fail. 2013;6:845–52.
27. Jansen RW, Lipsitz LA. Postprandial hypotension: epidemiology, pathophysiology, and clinical management. Ann Intern Med. 1995;122:286–95.
28. Tabara Y, Okada Y, Uetani E, Nagai T, Igase M, Kido T, et al. Postprandial hypotension as a risk marker for asymptomatic lacunar infarction. J Hypertens. 2014;32:1084–90. discussion 1090.
29. Lipsitz LA, Ryan SM, Parker JA, Freeman R, Wei JY, Goldberger AL. Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. Circulation. 1993;87:391–400.
30. Luciano GL, Brennan MJ, Rothberg MB. Postprandial hypotension. Am J Med. 2010;123:281 e281–286.
31. Parati G, Bilo G. Postprandial blood pressure fall: another dangerous face of blood pressure variability. J Hypertens. 2014;32:983–5.
32. Gudmundsdottir FD: Effects of two different types of fast food on postprandial metabolism in normal- and overweight subjects. *MSc thesis*. University of Iceland Faculty of Food Science and Nutrition, School of Health Sciences 2012.
33. Zhang JQ, Ji LL, Nunez G, Feathers S, Hart CL, Yao WX. Effect of exercise timing on postprandial lipemia in hypertriglyceridemic men. Can J Appl Physiol. 2004;29:590–603.

34. Gill JM, Al-Mamari A, Ferrell WR, Cleland SJ, Packard CJ, Sattar N, et al. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. J Am Coll Cardiol. 2004;44:2375–82.
35. van Dijk JW, Manders RJ, Tummers K, Bonomi AG, Stehouwer CD, Hartgens F, et al. Both resistance- and endurance-type exercise reduce the prevalence of hyperglycaemia in individuals with impaired glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic patients. Diabetologia. 2012;55:1273–82.
36. Peddie MC, Rehrer NJ, Perry TL. Physical activity and postprandial lipidemia: are energy expenditure and lipoprotein lipase activity the real modulators of the positive effect? Prog Lipid Res. 2012;51:11–22.
37. Poirier P, Tremblay A, Catellier C, Tancrede G, Garneau C, Nadeau A. Impact of time interval from the last meal on glucose response to exercise in subjects with type 2 diabetes. J Clin Endocrinol Metab. 2000;85:2860–4.
38. Zhang JQ, Thomas TR, Ball SD. Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. J Appl Physiol (1985). 1998;85:1516–22.
39. Mak GS, Sawaya H, Khan AM, Arora P, Martinez A, Ryan A, et al. Effects of subacute dietary salt intake and acute volume expansion on diastolic function in young normotensive individuals. Eur Heart J Cardiovasc Imaging. 2013;14:1092–8.
40. Tzemos N, Lim PO, Wong S, Struthers AD, MacDonald TM. Adverse cardiovascular effects of acute salt loading in young normotensive individuals. Hypertension. 2008;51:1525–30.