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Cardiac Function at Rest and During Exercise in Patients with Healed Myocardial Infarction and in Heart Transplant Recipients

– Effects of a high-intensity interval exercise program

Thesis for the degree of Philosophiae Doctor

Trondheim, February 2014

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Hjertefunksjon målt med ekkokardiografi etter hjerteinfarkt og hjertetransplantasjon. Endringer under arbeidsbelastning og etter trening.

Ekkokardiografi er en sikker, brukervennlig og lett tilgjengelig undersøkelse for å vurdere hjertefunksjonen. Ved bruk av ulike teknikker, kan man kvantitere blodstrømshastigheter samt hastigheter og deformasjon i selve hjertemuskelen. Informasjon om hjertets pumpefunksjon, både i hvile og under arbeidsbelastning, har behandlingsmessig og prognostisk betydning.

I denne doktorgradsavhandlingen har vi ved bruk av ekkokardiografi undersøkt hjertefunksjonen hos pasienter med tidligere hjerteinfarkt og pasienter med gjennomgått hjertetransplantasjon. Vi har undersøkt fysiologiske endringer i hjertefunksjonen under fysisk belastning hos begge pasientgrupper og effekten av langvarig trening hos hjertetransplanterte. I tillegg har vi undersøkt hvordan tiden donorhjertet er utenfor kroppen (ischemi-tiden) og donors alder påvirker hjertefunksjonen hos transplanterte pasienter.

Siden mange pasienter med gjennomgått hjerteinfarkt har redusert arbeidskapasitet, ønsket vi å undersøke venstre hjertekammer under sykkelbelastning hos denne pasientgruppen. Vi fant at venstre hjertekammers kontraksjon økte med økende belastning (systolisk reserve), mens hjertets evne til hurtigere å slappe av, og dermed øke fylling (diastolisk reserve), var redusert. Dette kan forklare noe av årsaken til den reduserte arbeidskapasiteten hos disse pasientene.

Hjertetransplantasjon øker overlevelsen hos pasienter med alvorlig hjertesvikt, og er i dag et akseptert behandlingsalternativ. Selv om arbeidskapasiteten øker etter transplantasjon, er den fortsatt lavere enn aldersforventet. Vi ønsket derfor å undersøke i en randomisert, kontrollert studie om høy-intensitets intervalltrening kan bedre arbeidskapasiteten hos disse pasientene. Vi fant at et år med intervalltrening økte arbeidskapasiteten og utholdende muskelstyrke, mens derimot hjertefunksjonen og blodtrykket var uendret. Ved ekkoundersøkelse under sykkelbelasting fant vi både en systolisk og en diastolisk reservekapasitet, men at denne ikke ble bedret etter treningsintervensjon. Videre fant vi at vevshastigheter i høyre hovedkammer var lett redusert ved lenger ischemitid, mens hjerter fra eldre donorer hadde reduserte diastoliske vevshastigheter i venstre hovedkammer.

Samlet sett har vi vist at ekkokardiografi er et godt og viktig verktøy til å vurdere hjertets pumpefunksjon hos to ulike pasientgrupper, også under arbeidsbelastning.

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Overnevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i medisinsk teknologi.
Disputas finner sted i auditoriet på Medisinsk teknisk forskningssenter,
torsdag 13. februar 2014, kl 12.15.

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I.   Rustad LA, Amundsen BH, Slørdahl SA, Støylen A. **Upright bicycle exercise echocardiography in patients with myocardial infraction shows lack of diastolic, but not systolic, reserve: a tissue Doppler study.** Eur J Echocardiogr. 2009;10:503-8

II.   Rustad LA, Nytrøen K, Amundsen BH, Gullestad L, Aakhus S. **One year of high-intensity interval training improves exercise capacity, but not left ventricular function in stable heart transplant recipients: A randomised controlled trial.** Eur J Prev Cardiol. 2012 Nov 26 [Epub ahead of print]

III. Nytrøen K, Rustad LA, Aukrust P, Ueland T, Hallén J, Holm I, Rolid K, Lekva T, Fiane AE, Amlie JP, Aakhus S, Gullestad L. **High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients.** Am J Transplant. 2012;12:3134-42.

IV.   Rustad LA, Nytrøen K, Andreassen A, Geiran O, Endresen K, Gullestad L, Aakhus S, Amundsen BH. **Heart transplant systolic and diastolic function is impaired by prolonged pre-transplant graft ischemic time and high donor age: an echocardiographic study.** Eur J of Cardio-Thoracic Surg. 2013 (44) e97–e104.

3   Abbreviations

a'	-	late (atrial) diastolic mitral inflow velocity
BMI	-	body mass index
BP	-	blood pressure
CAD	-	coronary artery disease
CAV	-	cardiac allograft vasculopathy
CO	-	cardiac output
CPET	-	cardiopulmonary exercise testing
DT	-	deceleration time of early diastolic mitral inflow velocity
E	-	early diastolic mitral inflow velocity
e'	-	early diastolic mitral annular velocity
ECG	-	electrocardiogram
EF	-	ejection fraction
GIT	-	graft ischemic time
HF	-	heart failure
HIIT	-	high-intensity interval training
HR	-	heart rate
HR_{peak}	-	peak heart rate
HTx	-	heart transplantation
IVRT	-	isovolumic relaxation time
LA	-	left atrium
LV	-	left ventricle
MAE	-	mitral annular excursion
MI	-	myocardial infarction
PCWP	-	pulmonary capillary wedge pressure
RER	-	respiratory exchange ratio
RV	-	right ventricle
s'	-	systolic mitral annular velocity
SR	-	strain rate
TDI	-	tissue Doppler imaging
VO_{2max}	-	maximal oxygen uptake
VO_{2peak}	-	peak oxygen uptake
W	-	watt

4 Background

4.1 Echocardiography

Echocardiography is a safe, user-friendly and cost-effective method to evaluate cardiac size and function. It has a unique combination of detailed real-time information, high spatial and temporal resolution in addition to comprehensive information on cardiac structure and function; hence it has become an important tool in cardiology during the last decades.

4.1.1 Tissue Doppler Imaging

Blood flow velocities, myocardial velocities and deformation in the myocardial tissue itself can be quantified by measuring the Doppler shift of the reflected ultrasound from moving scatters. As only velocities parallel to the ultrasound beam is measured, caution should be made when there is misalignment between the direction of motion of the object and the direction of the ultrasound wave. The error is proportional to the cosine of the angle. The Doppler shift for reflected ultrasound ($\Delta f = f - f_0$) and subsequently the velocity (v) of the moving target (blood cells or myocardium) are given by the Doppler-equation:

$$\Delta f = (2 \cdot f_0 \cdot v \cdot cos\,\theta\,)\,/\,c \quad \rightarrow \quad v = (\Delta f \cdot c)\,/\,(2 \cdot f_0 \cdot cos\,\theta)$$

where Δf = Doppler shift, f_0 = transmitted frequency, v = target velocity (blood or myocardium), θ = insonation angle (between ultrasound beam and velocity vector) and c = velocity of the ultrasound in tissue ($\approx$1540 m/s).

Blood flow has high velocity but lower intensity, whereas the reflected signal from tissue has high intensity but low velocity. Thus, by using either high pass or low pass filters and reject either low or high velocities, respectively, blood flow or tissue velocities can be displayed separately. Tissue Doppler Imaging (TDI) has the benefit that both systolic and diastolic myocardial velocities can be measured with the same

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method within the same heart cycle.[1] TDI-derived myocardial velocities give robust information about the ventricular function, and can be obtained either by pulsed wave (PW) tissue Doppler or by velocities extracted from colour TDI. In PW tissue Doppler the whole frequency spectrum of the Doppler shift is displayed, whereas in colour TDI only the mean frequency of the Doppler shift is displayed, causing lower absolute velocities compared with the PW TDI-method.[2-5] In colour TDI, each pixel in the ultrasound image is colour coded according to the velocity and displayed superposed to the grey-scale images (B-mode). Both methods have certain advantages and disadvantages. The PW TDI is conventionally measured at the outer edge of the band-shaped spectrum, and is therefore sensitive to gain-settings and localization of the peak velocity in the Doppler spectrum. Further, only velocities where the sample volume is placed are measured. The colour tissue Doppler signal consists of fewer samples per time unit than PW TDI, is more prone to acoustic artifacts (e.g. reverberations) and requires off-line post-processing. However, even though the off-line post-processing might be time-consuming, colour TDI has the benefit that corresponding velocity, displacement (motion) and strain rate data of the same region of interest (ROI) are presented in time trace.

4.1.2 Strain and strain rate

Based on the velocity difference between two neighbouring points in the tissue Doppler image, deformation in the myocardium itself can be calculated.[6] Strain means deformation, and is defined as fractional change of tissue length. Strain is expressed in a dimensionless unit, conventionally as percentage shortening (negative values) when measurements are done in the ventricular systole in the longitudinal direction. Linear strain can be expressed by the Lagrangian formula:

$$\acute{\varepsilon} = (L-L_0) / L_0 = \Delta L / L_0$$

where $\acute{\varepsilon}$ is longitudinal strain, ΔL is absolute change in length, and L_0 is baseline length.

Strain rate (SR) is strain by time unit (s^{-1}), which equals the velocity difference (Δv) per length (L).

$$SR = \acute{\varepsilon}/\Delta t \quad \rightarrow \quad SR = (\Delta v)/L_0 \quad \rightarrow \quad SR = (v_1 - v_2)/L_0$$

The strain rate is negative during shortening (systole) and positive during elongation (diastole). Thus, two objects can possible have the same amount of strain but different strain rates if one object performs the same deformation (strain) faster than the other.

4.1.3 Speckle tracking imaging

An ultrasound grey-scale image (B-mode) consists of an irregular, random speckle pattern, originating from reflection and interference effects between overlapping echoes. The speckle pattern is characteristic for a specific region of the myocardium, and follows the motion of the myocardium when the myocardium moves from frame to frame. Thus, if a region is defined in one frame, a software algorithm identifies the area with the most similar speckle pattern in the next frame within a defined search area. Myocardial deformation (e.g. strain) is assessed based on the deformational changes of the speckles.[7] Speckles can be tracked in all directions, and compared to the TDI-method, speckle tracking has the benefit that it is angle-independent.

4.2 Measuring cardiac function and dysfunction

4.2.1 Systolic function

Several echocardiographic techniques and methods can be used for evaluating systolic function. However, they all have in common that they give information about ventricular contraction which depend on the contractility, but is not equivalent. Left ventricular (LV) contractility is the result of the intrinsic contractile properties of the myocytes. Increased contractility results in a greater velocity of the contraction when other influencing factors such as preload and afterload are kept constant. Factors that increase contractility include adrenergic stimulation, inotropic agents (e.g. dobutamin) and heart rate (HR). The latter is called the force-frequency effect (e.g. the Bowditch

staircase effect/the treppe effect),[8] where increasing HR increases the contractility and thereby also the myocardial oxygen demand.

True contractility of the myocardium cannot be measured by presently available imaging techniques. However, some echocardiographic measurements are more closely linked to contractility than others, as the myocyte contraction most often occurs on the early 1/3 of systole. Thus, echocardiographic peak systolic velocity indices such as mitral annular tissue velocity (s') and SR better reflect alterations in contractility and force development, than variables measured in end-systole (strain, displacement, ejection fraction (EF) and fractional shortening), which reflects deformation (shortening of the myocytes), stroke volume and volume ejection.[9-11]

4.2.2 Diastolic function

The two major determinants of the diastolic function are ventricular relaxation and compliance, where relaxation primarily influence the early phase of diastole, and compliance the later phases of diastases and atrial contraction. In addition, several factors such as pericardial constraint, loading conditions, left atrium (LA) pressure and HR influence on the diastolic filling and function.[12,13]

The ventricular relaxation phase is caused by a decrease in the cytosolic calcium level, a complex ATP-consuming process, where calcium is re-uptaken into the sarcoplacmatic reticulum. The contractile elements are deactivated by phosphorylation, and returned to their original length.[8,14] Invasively measured, relaxation is defined as the peak negative change in LV pressure over time (-dP/dt). Tau, the time constant of relaxation, describes the time rate of LV pressure during isovolumic relaxation. Since relaxation is an energy depending process, conditions with reduced ATP supply have impaired relaxation. Impaired relaxation is an early event in angina pectoris, as the reduced oxygen-supply impairs the energy generation and diminishes the supply of ATP required for calcium level decrease.[8] During catecholamine stimulation, the relaxation rate may increase and thereby enhancing the LA-LV pressure gradient, and thereby increasing early diastolic mitral flow velocity. This phenomenon is also known as 'suction'. Relaxation rate may also be increased by elastic recoil, since potential energy is stored in the cytoskeletal

proteins (e.g. titin) during contraction. This potential energy is disengaged during diastole, and thereby increases relaxation rate.

In addition to relaxation, ventricular compliance is a major determinant of diastolic function. The non-linear end-diastolic pressure-volume relation defines the passive properties of the chamber when it is fully relaxed. By invasive measurements compliance is defined as the change in volume over the change in pressure; $C = dV/dP$.[8] The modest rise in pressure, despite a doubling of ventricular volume during diastole, reflects the high compliance of the ventricular wall. Pathological loss of compliance is usually due to abnormalities of the myocardium, such as fibrosis. A less compliant LV demands a higher LA pressure for maintaining the same early diastolic filling.[8]

4.2.3 Diastolic dysfunction

In young healthy subjects the time of pressure-equilibration between the LV and LA is short, due to a fast relaxation and elastic recoil. Hence early diastolic mitral inflow velocity (E), the ratio of early diastolic (E) to atrial (A) mitral inflow velocity (E/A) and mitral annular early diastolic velocity (e') are higher, and isovolumic relaxation time (IVRT) and deceleration time of E (DT) shorter than in older subjects where the relaxation might be impaired.[15] Beyond ageing, heart disease is the main cause of impaired diastolic function. Abnormal relaxation results in slower pressure-decline of the LV, so the IVRT and DT are prolonged, and both E and e' are decreased. This shifts the burden of maintaining an adequate end-diastolic volume from the early diastolic filling to the atrial contraction, thus the E/A is decreased. If the relaxation is further reduced, LV filling pressure increases in order to compensate for the impaired LV relaxation and maintain the SV. Increased LV filling pressure shortens the time to pressure-equilibration and reduces both IVRT and DT, while early mitral flow velocity and E/A will increase. e' will however be diminished, resulting in an increased E/e' ratio, which reflect increased LV filling pressure.[16,17] The echocardiographic classification of diastolic dysfunction is divided into four stages, where the two situations described above are named impaired relaxation (stage I) and restrictive filling pattern (stage III or IV), respectively.[12,14,18]

4.2.4 Left ventricular filling pressure and E/e'

Since E primarily reflects the pressure gradient between LA and LV during early diastole and is affected by preload and alterations in LV relaxation[19] and e' provides information about LV relaxation (tau),[20] dividing E by e' can theoretically estimate LV filling pressure. Nagueh et al[16] and Ommen et al[17] have both shown than LV filling pressure can be estimated non-invasively by calculating the ratio the E/e' ratio. This ratio has shown good correlation with invasively measured LV filling pressure in various conditions including sinus tachycardia,[21] severe aortic valve stenosis,[22] HTx recipients,[23] hypertrophic cardiomyopathy,[24] and HF with normal EF.[25] An E/e' ratio <8 predicts normal LV filling pressure, while a ratio >15 has a very high accuracy for identifying elevated LV filling pressure at rest.[17] Furthermore, studies have also shown that the E/e' ratio shows good correlation with LV filling pressure during supine exercise in patients with dyspnea and EF > 50% and in patients referred to left heart catetherization.[26,27] This is based on the assumption that patients with impaired relaxation will have an increase in e' with exercise of lesser magnitude than the increase in mitral E velocity. Consequently, the E/e' ratio will increase as opposed to patients with normal relaxation where E and e' will increase in parallel.

4.2.5 Exercise intolerance and diastolic dysfunction

Kitzman et al showed in 1991 that an excessive rise in pulmonary capillary wedge pressure (PCWP) during exercise is an important cardiac cause of exertion dyspnoea in patients with HF and preserved systolic function.[28] The patients were unable to increase CO during exercise without an abnormal elevation in LA pressure, since the LV was unable to enhance its diastolic filling. Also the E/e' ratio has shown a good correlation with exercise intolerance in patients with suspected CAD,[29] systolic HF[30,31] and hypertrophic cardiomyopathy.[32] In a large cross-sectional study consisting of non-ischemic patients with an EF >50%, LV diastolic dysfunction and a E/e' ratio >15, measured both at rest and post-exercise, was independently associated with reduced exercise capacity.[33] Moreover, Podolec et al found that the E/e' ratio at peak exercise had better correlation with exercise capacity than E/e' measured at rest in patients with ischemic HF.[34]

4.2.6 Exercise stress echocardiography

Several methods can be used for cardiac stress testing; pharmacological (e.g. dobutamine) and exercise stress testing are the most widely used. The former is a highly validated and widely used method for detecting CAD by assessment of regional wall motion or deformation. However, it is an unphysiological way to stress the heart, as HR increases without increasing venous return, and hence end-diastolic volume and SV decreases. The American Society of Echocardiography guidelines recommend exercise stress testing in patients who are capable of performing exercise rather than pharmacological stress testing for detecting of ischemic heart disease and assessment of valvular heart disease.[35] Exercise stress echocardiography provides valuable information concerning patient symptoms, exercise capacity, cardiovascular function and the hemodynamic response during usual forms of activity, and can be performed on either a treadmill or on a bicycle in the supine, semi-supine or the upright position.[35]

During the last decade, exercise stress echocardiography (also called ergometric stress echocardiography and diastolic stress test) has been increasingly applied, as several studies have used it for detecting diastolic dysfunction and cardiac reserve capacity in a variety of diseases.[36-39] Holland *et al* showed that patients with elevation in E/e' during exercise had worse outcome than those with normal exercise E/e', independent of ischemia, and moreover that exercise echocardiography was most valuable in patients with normal resting E/e'.[40] Thus, exercise stress echocardiography is a valuable tool in "unmasking" diastolic dysfunction, differentiating cardiac from non-cardiac causes of exertional dyspnoea, in addition to being a tool with prognostic importance.

4.3 *Exercise capacity and exercise training*

In general, the individual exercise capacity is limited by four factors: 1) pulmonary diffusion capacity, 2) cardiac output (CO) (e.g. stroke volume (SV) x heart rate (HR)) 3) oxygen carrying capacity (haemoglobin) and 4) skeletal muscle function (peripheral oxygen diffusion gradient, mitochondrial function, capillary density).[41]

The Fick-equation defines the relation of CO and arteriovenous oxygen difference (AVO_2) to VO_2 during exercise:

$$VO_2 = CO \times (O_{2Art} - O_{2Ven}) = SV \times HR \times (O_{2Art} - O_{2Ven})$$

In healthy individuals, the capacity of large skeletal muscle-groups exceeds the capacity of the central circulation for supplying it with oxygen,[42] and the prevailing view is that the maximal exercise capacity is limited primarily by the rate of oxygen delivery, not the ability of the muscles to take up oxygen from the blood.[41] Thus, CO is the main factor limiting exercise capacity, and thus the most important determinant of VO_{2max}. HR increases linearly with increasing exercise-intensity in normal subjects. Whether SV increases linearly as well, is somewhat unsure and has been a subject for debate. The traditional view is that SV increases up to approximately 50% of VO_{2max}, and then plateaus.[43] However, studies have also shown that SV increases up to VO_{2max} in both athletes[44,45] and healthy non-athletes.[46,47] SV increases mainly due to three reasons during exercise: 1) increased filling (preload) due to altered blood flow distribution during exercise and increased venous return caused by the muscle pump, 2) increased contractility, due to increased sympathetic activity and HR (called force-frequency relationship, e.g. the Bowditch effect) and 3) decreased afterload and peripheral resistance (vasodilatation in exercising muscles).

4.3.1 Cardiac adaptation to endurance training

As maximal HR (HR_{max}) is mainly unaffected by exercise training in non-transplant hearts,[48-51] enhanced SV is the most important way to increase CO, and thereby VO_{2peak} in healthy individuals. SV is determined by two inter-related factors; ventricular size and function. Physical training induces cardiac remodelling by increasing LV volume and mass.[52,53] In well-trained athletes, in fact all four cardiac chambers are enlarged.[53] In addition, exercise training improve LV compliance and LV diastolic and systolic function during exercise, thus enabling greater filling and emptying of the left ventricle.[52,54-57] Increased plasma volume, which is a frequent finding after exercise

training, might contribute to the increase of SV by improving LV filling and preload.[58,59] Also improved endothelium-mediated vasodilatation in peripheral vasculature and thereby reduced peripheral resistance and afterload, might contribute to an increased SV after exercise training. Altogether, these improvements increase the oxygen supply to the performing muscle.

4.3.2 High-intensity interval training

Accumulating evidence suggest that the magnitude of the benefits from exercise training increases proportionally with the intensity of the individual exercise training sessions. A study by Helgerud *et al* showed that in healthy young male subjects, improvements in VO_{2peak} and SV were intensity dependent. Those who trained with the highest exercise intensity (90-95% of HR_{max}), also had the highest exercise response, when comparing with an isocaloric exercise program at lower exercise intensity but longer duration.[60] Also in patients with chronic HF and CAD, improvements in VO_{2peak} are intensity dependent, with high-intensity interval training (HIIT) being superior to moderate intensity training.[50,61]

The increased exercise capacity by high-intensity training may be explained by the opinion that there is a close relation of SV and intensity; SV increases in concurrence with higher intensities, and the largest SV is reached near VO_{2max}. Hence, high-intensity training is an effective training-method to improve SV. Also peripheral factors, such as endothelial function and muscular mitochondrial function, improve significantly by high-intensity training compared to training programs performed at lower intensities in HF patients.[50]

The basic principle of interval exercise training is that periods of high-intensity exercise training are interspersed by periods of lower intensities that allow for recovery, which then allow the subject to continue in high-intensity exercise intervals. It is the accumulated time in the high-intervals that determine the outcome of the exercise training. However, the recovery periods between the intervals are needed, as the intensity is usually above the anaerobe threshold in non-athletes, and accumulation of

lactate and fatigue occurs within a few minutes at continuous exercise at high-intensity.[62]

4.4   *Myocardial infarction and diastolic dysfunction*

An acute MI is characterized by regional necrosis and fibrosis in the myocardium, and thus reduced contractile forces. Increased accumulation of interstitial collagen might cause increased LV chamber stiffness, and residual ischemia may also impair the active part of the relaxation.[63] The myocardial injury may therefore lead to both systolic and diastolic dysfunction.[1,64] As already mentioned, diastolic dysfunction might cause elevated LV filling pressure and exercise intolerance. An exercise capacity about 60% of age-predicted is reported in MI patients.[65] Elevated LV filling pressure after MI adds an increased likelihood of remodelling causing HF.[66] Thus, diastolic dysfunction and consequently elevated filling pressure, is an important predictor of morbidity and mortality after MI.[67,68] It is therefore important to identify diastolic dysfunction and limited exercise capacity at an early stage in patients with sustained MI, in order counteract remodelling and thus prevent HF.[69]

4.5   *Heart transplantation*

The first HTx on human was performed in December 1967 by Christian Barnard in Cape Town, South Africa. Due to insufficient immunosuppressive therapy, the long-term survival of HTx recipients in the -60's and -70's was poor. When cyclosporine was introduced as an immunosuppressive drug in HTx in 1980, survival increased. The first HTx in Norway was performed at Rikshospitalet in November 1983.[70] HTx has now become an accepted therapy for selected candidates with severe congestive HF and NYHA class IV.

HTx recipients are exposed to various risk factors for cardiovascular disease and may suffer from severe complications over time; e.g. cardiac allograft vasculopathy (CAV), renal failure and hypertension.[71] The prevalence of hypertension is high; approximately 90% 5 years after HTx. In addition, there is increased risk of malignancy.[71] Thus,

average survival rate after HTx is still reduced compared to healthy individuals, with a median survival of 12 years in Norway.[70]

Even though HTx is an accepted therapy, only about 30 HTx are performed on average in Norway each year[70] and 5000 worldwide[71], due to few potential donors. Thus, to counteract this shortage, both older grafts and prolonged graft ischemic time are accepted. Guidelines recommend pre-transplant GIT less than 4 hours, particularly when donor age is >45 years.[72] Whether prolonged GIT influence on recipient long-term survival, is inconsistently described in the literature. While some studies have reported that prolonged GIT does not influence recipient survival after HTx,[73,74] larger studies indicate that extended GIT impairs patient survival, particularly when donor is of older age.[71,75] To what extend prolonged GIT and high donor age affect cardiac function is unknown.

4.5.1 Limitations to exercise capacity in HTx recipient

Even though exercise capacity improves after HTx, it still remains lower than age- and gender predicted values.[76] A VO_{2peak} between 50 and 75% of age-predicted values long-term after transplantation are reported in most studies.[77-80] The underlying mechanisms for the reduced exercise capacity is not completely understood, but is due to a combination of both central and peripheral physiological abnormalities.[76,81,82]
A denervated heart, leading to chronotropic incompetence during exercise, has long been recognized as a major limiting central factor. HR increase during exercise depends on circulating catecholamine rather than on intact autonomic regulation, and thus resting HR is higher and HR_{max} is lower than compared to non-transplant subjects. Even though this improves towards normalization during the first year and the recipients reaches a close to normal HR_{peak},[83] no studies have so far shown complete re-innervation of both sympaticus and parasympaticus.

Some of the limited exercise capacity might be related to diastolic dysfunction and increased myocardial stiffness, which is present even long-term following transplantation.[81,84-86] These abnormalities might be due to repeated episodes of mild

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rejection, accelerated CAV or side-effects of immunosuppressive medications.[81,84-87] Important peripheral limitations are abnormalities in muscular function, oxygen delivery and utilization.[76,81] Long-term use of cyclosporine causes muscle atrophy and a shift towards a larger amount of fast-twitch (type II, glycolytic) muscle fibers,[88] and corticosteroids results in mitochondrial dysfunction, causing elevated serum lactate levels during exercise and oxidative damage of skeletal muscles.[89] Most often the HTx recipient has also suffered from long-term chronic HF before transplantation, resulting in skeletal muscle dysfunction, decreased mitochondrial content and decreased capillary density.[76,90,91] Even though resting EF increases remarkably after HTx[92] and a contractile reserve is present during exercise,[82,93] exercise capacity is still reduced and VO_{2peak} values are lower than age- and gender- predicted. This highly underscores the importance of improving peripheral factors and specifically muscular function, in order to increase exercise capacity in HTx recipients.

4.5.2 Exercise training programs in HTx recipient

Due to denervation and a slow HR response, the general opinion regarding exercise training has been a long warm up and moderate-intensity in order to keep HR on a steady state. Interval training, with fast increases and decreases in HR have been seen as "unphysiologic" and avoided.

There are a few randomized controlled trials on exercise training in HTx, most containing moderate-intensity training (Table 1). However, as the HR_{peak} improves towards normalization during the first year, [83] some studies have investigated the feasibility and the efficiency of high-intensity interval training in HTx recipients. A study by Hermann *et al* consisted of high-intensity interval training (HIIT),[94] whereas Haykowsky *et al* partly used HIIT.[95] However, no prior studies consisting of exercise training have lasted longer than 6 months, and none have examined the cardiac effect of HIIT with comprehensive echocardiographic techniques. Bearing in mind the high prevalence of cardiovascular risk factors in HTx recipients, improving general healthy and secondary prevention is of great importance and highly relevant in this patient population.

Table 1.

Summary of *randomized controlled trials (RCT's) on exercise training in HTx recipients.*

Study	n / mean age (years)	Mean time after HTx	Exercise intervention	Mean change in VO_{2peak} (mL/kg/min) within group	Mean change in VO_{2peak} (mL/kg/min) between groups	Other findings
Kobashigawa *et al* 1999 [96]	n=27 52 yrs	1 month	Strength and endurance, moderate intensity, 6 months	Ex: 9.2→13.6 Ctr: 10.4 → 12.3	2.5 p=0.001	EF (echo) unchanged
Bernardi *et al* 2007 [97]	n=24 52 yrs	6 months	Endurance. Moderate intensity. Home-based. 6 months	Ex: 14.9→19.6 Ctr: 14.3 → 15.6	3.4 p<0.05	Reduced systolic and diastolic BP
Karapolat *et al* 2008 [98]	n=28 42 yrs	1.5 years	Strength and endurance. Moderate intensity. 8-weeks	Ex: 16.7→19.5 Ctr: 20.1 → 19.5	3.4 p=0.001	
Wu *et al* 2008 [99]	n=37 56 yrs	2 years	Strength and endurance. Moderate intensity 8 weeks. Home-based,	Ex: 12.1→13.2 Ctr: 13.7 → 13.2	1.6 non-significant	Improved muscular endurance
Haykowsky *et al* 2009 [95]	n=43 59 yrs	5 years	Strength and endurance, moderate intensity first 8 weeks, and high-intensity 4 weeks	Ex: 21.2→24.7 Ctr: 18.2 → 18.2	3.5 p=0.003	Unchanged cardiac systolic function (rest and submax echo) and endothelial function
Hermann *et al* 2011 [94]	n=27 50 yrs	7 years	Endurance., high-intensity 85% of HR_{peak} 8 weeks,	Ex: 23.9→28.3 Ctr: 24.6 → 23.4	5.6 p<0.001	Reduced systolic BP improved endothelial function

5 Aims of study

Main objective

The main objective of this thesis was to use newer echocardiographic techniques to investigate the cardiac function during exercise and after exercise training.

The specific hypotheses of the papers are as follow:

Paper I

Upright bicycle exercise stress echocardiography can be used to detect exercise-induced cardiac systolic and diastolic dysfunction in a group of unselected patients with prior MI.

Paper II

HIIT improves exercise capacity and cardiac systolic and diastolic function in stable HTx recipients

Paper III

HIIT improves exercise capacity, and thereby central and peripheral factors in stable HTx recipients.

Paper IV

Prolonged graft ischemic time and high donor age impairs cardiac systolic and diastolic function in stable HTx recipients.

6   Patients and Methods

6.1   Study subjects

Study I

The patients in this study were recruited among the population remitted to St. Olav's Hospital, Trondheim, between 2004 and 2006. Twenty-one patients with ST-elevation MI at least three months earlier were included. Patients with significant arrhythmias, valve disease or recurrent ischemia or patients unable to bicycle were excluded. One patient was excluded due to poor image quality in upright position and two patients due to valve disease, thus 18 MI patients were included. All patients had a coronary angiogram at the time of the MI. At the time of the study, four patients had significant stenoses (>50%) in one of the non-culprit major epicardial arteries, found at the time of the initial angiography, and still untreated by PCI. The findings in this group were compared to those in an age-and sex-matched control group. Participants in the control group were recruited among university staff and by advertisements at public places, and 21 healthy subjects meeting the inclusion criteria were included (no history of heart disease and no risk factors (smoking, hypertension, diabetes mellitus, hypercholesterolemia or sudden death in first degree relative <60 years)). One participant was excluded due to a previously undiscovered severe aortic insufficiency and two because of poor image quality in upright position, thus 18 controls were included.

Study II, III and IV

Study II, III and IV consisted of HTx recipients scheduled for an annual follow-up at Oslo University Hospital HF, Rikshospitalet, between 2009 and 2010. The inclusion criteria were assessment 1-8 years after HTx, optimal medical treatment, stable clinical condition, ability to perform maximal exercise testing and willingness to fulfill one year of exercise training. Exclusion criteria were HF, clinical signs of rejection, atrial fibrillation, need for revascularization or other intervention.
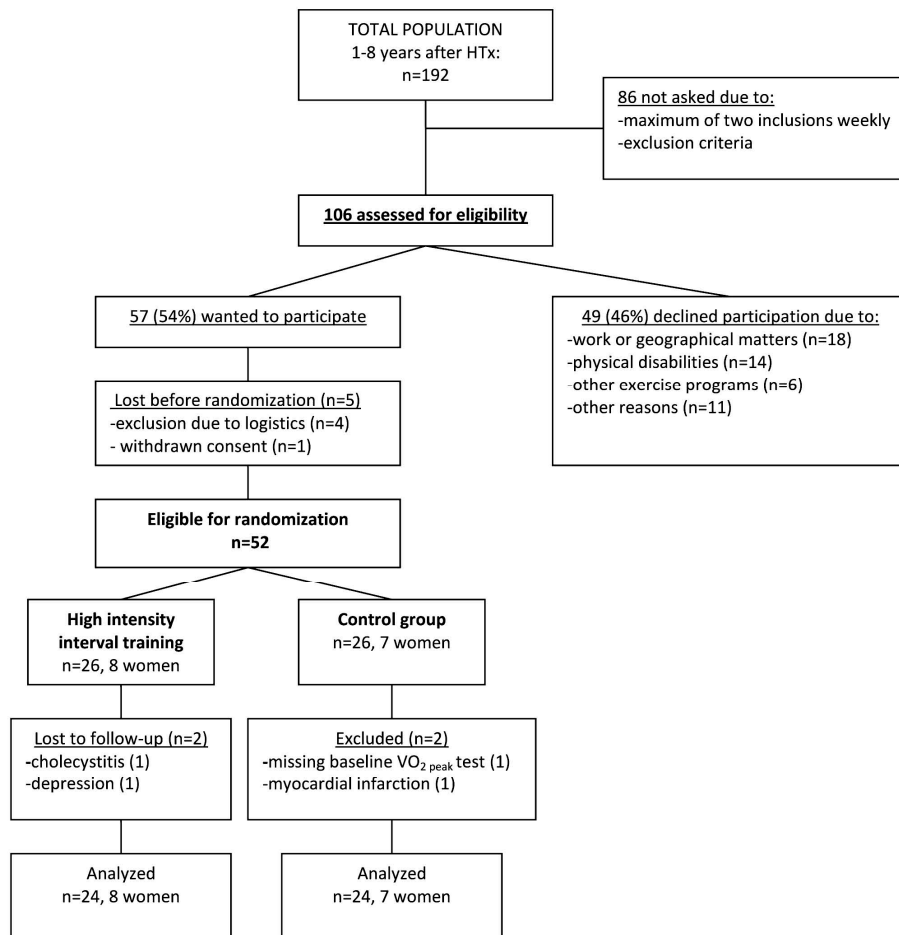
Figure 1. *Flow chart of patient inclusion study II-IV.*

From a cohort of 192 HTx recipients scheduled for an annual follow-up 1-8 years after HTx, 106 HTx recipients were asked to participate (Figure 1). Thus, 86 recipients were not asked to participate due to either: a) known not meeting inclusion criteria (e.g. disabilities or severe co morbidities) and b) only capacity to include two recipients per week. If several potential participants were scheduled for an annual control the same week, we randomly asked only two HTx recipients.

Fifty-seven recipients gave their informed consent. Four recipients were excluded due to logistics and one acceptance was withdrawn, thus 52 HTx recipients were finally included and eligible for randomization. Study IV consisted of baseline data from these 52 HTx recipients.

The HTx recipients were randomly assigned in a 1:1 ratio to either HIIT or control group, stratified by time after heart transplant surgery (1-2 years post transplantation and 3-8 years post transplantation). Consecutively numbered, sealed envelopes were provided by an independent statistician before inclusion started. After lost to follow-up and exclusions, statistical analyses in study II and III comprised in total 48 recipients, 24 in each group.

6.2 Echocardiography

6.2.1 Echocardiography examination

Echocardiography was performed with Vivid 7 in study I and either Vivid 7 or E9 in study II-IV and a phased array sector transducer (GE Vingmed Ultrasound, Horten, Norway). In all studies a complete echocardiography examination was recorded at supine rest with the patient in the left decubitus position. Two-dimensional grey-scale echocardiographic recordings (B-mode) and colour tissue Doppler imaging of standard apical projections (four-chamber, two-chamber and long axis) comprising three consecutive heart cycles each were obtained.[100] Frame rate during colour TDI was about 100 frames/s and during B-mode >60 frames/s. Blood flow velocities in the LV outflow tract and mitral inflow were obtained from the apical position using pulsed wave (PW) Doppler with the sample volume at the aortic annulus and tip of mitral leaflets, respectively.

Echocardiography examinations were performed by BHA in study I and by LAR in study II-IV.

6.2.2 Exercise stress echocardiography

Study I

In study I the exercise stress echocardiography examination was performed on a multistage upright bicycle ergometer. Subjects were examined at sitting rest, then during upright bicycling, starting at a workload of 25 watt (W), with an increment to 50W and 75W after five minutes. Higher loads were not performed due to unsatisfactory image quality with increasing upper-body motion and respiration frequency. The pedal-frequency was 60-70 rounds per minute. On each stage both B-mode and colour TDI were recorded in the 4- and 2-chamber views two minutes after each change in workload, in addition to the mitral inflow velocity profile by PW Doppler. Three of 42 subjects were excluded from analysis in this study due to poor image quality in the upright position.

Study II

In study II exercise stress echocardiography was performed on a bicycle ergometer in the semi-supine position (Ergoline, Germany). Patients pedalled at constant frequency (50-65 rounds per minute), beginning at a workload of 25W, with an increment of 25W every 2 minutes. Images were obtained at rest in the semi-supine position and during bicycling at workloads of 50W, 100W and at submaximal load defined by muscular fatigue. Images at submaximal load were performed 1-2 minutes/25w before termination of the test. Echocardiographic imaging of the apical four- and two-chamber views with tissue Doppler overlay and transmitral pulsed wave Doppler flow were obtained at each stage immediately after increments in load. Submaximal load was defined as the highest load each individual managed (100W or above).

Exercise echocardiography was performed in 44 recipients at baseline. Four examinations were not performed, two due to logistics and two due to poor image quality at rest. At follow-up, exercise echocardiography was performed in 39 recipients; nine examinations were not performed, one due to logistics, four due to missing examination at baseline and four due to poor image quality during baseline exercise echocardiography. Examinations with angle-deviation >20$^\mathrm{o}$ in the tissue Doppler images or reduced image quality during

exercise (lung shadow) were excluded from analysis. Thus, 29 recipients were included in the analyses of exercise echocardiography, 15 in the exercise group and 14 in the control group.

6.2.3 Echocardiographic analysis

The echocardiographic data were stored digitally and analyzed off-line in dedicated software (EchoPac, GE Vingmed Ultrasound, Horten, Norway). In study I mitral annular velocities, SR and end-systolic strain were analyzed in a customized in-house software (GcMat, GE Vingmed Ultrasound) running under Matlab (MathWorks, Natic, Mass), while all other variables were analysed in EchoPac.

The average of at least three cardiac cycles was used in analysis of Doppler recordings. LV mitral annular velocities in systole (LVs'), early (LVe') and late diastole (LVa') were measured at septal, lateral, anterior and posterior mitral annular regions by colour TDI (Figure 2). In study I, custom-made software (GcMat) was used, which has the benefit that one can follow the atrio-ventricular plane throughout the heart cycle.

LV systolic mitral annular displacement (distance) was calculated by integration of tissue Doppler velocity signal (distance per time) at the same sites. In study I the term "mitral annular excursion" (MAE) was used instead of mitral annular displacement. The average of the four points was used in study I and II, while only septal and lateral velocities were used in study IV. Right ventricular (RV) systolic and early diastolic annular velocities (RVs' and RVe'), as well as RV displacement, were measured in the lateral tricuspid annular region in the apical 4-chamber view by colour TDI.[101]

LV E, A, DT, IVRT and ejection time (ET) was measured from the PW Doppler signal. The E/e' ratio was calculated. When fusion of E and A mitral filling waves occurred, it was analyzed as an E wave. Even with complete merging of the E and A velocities, E/e' can be used to estimate PCWP.[21] Recordings with fused diastolic mitral annular velocities (e' and a') were excluded in study I.
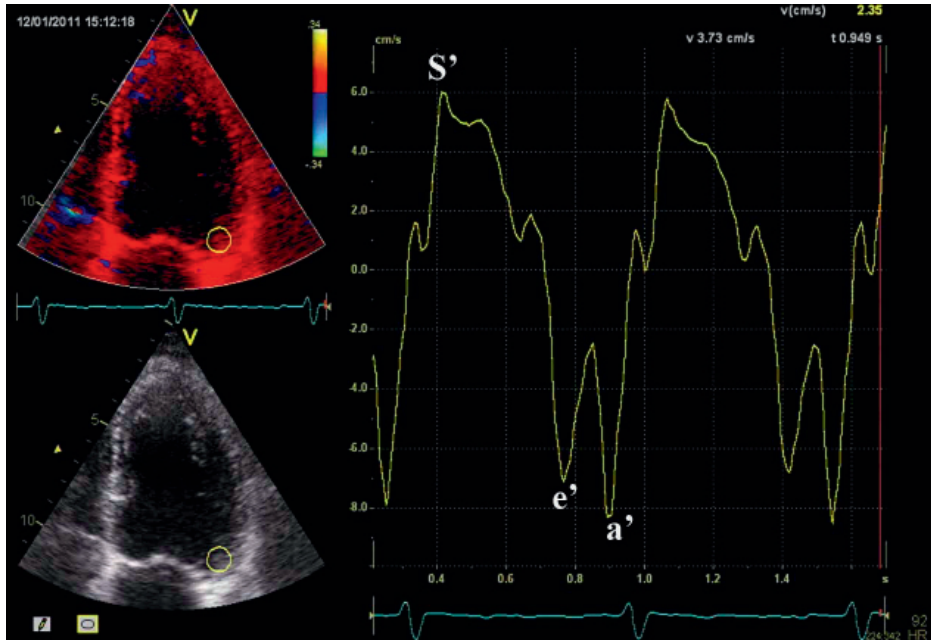
Figure 2. *Mitral annular velocity on the lateral side of the left ventricle.*

LV dimensions were measured from parasternal M-mode registrations (average of short and long axis recording). LV EF was measured by the modified Simpson's method using the four- and two-chamber views. LA area was measured in the four-chamber view. SV was calculated from pulsed Doppler flow measurement in LV outflow tract assuming a circular aortic annulus. CO was calculated from SV multiplied by HR. Systemic arterial compliance was obtained as the ratio of SV/pulse pressure (mL/mmHg) in study IV.

LV peak systolic longitudinal strain was measured using speckle-tracking echocardiography (2D strain method, EchoPac, GE Vingmed, BT 10) in an 18-segment model, converted to a 16-segment model in study II and IV.[100] LV global longitudinal strain was obtained by averaging the peak systolic shortening of the 16-segment model. Subjects with <8 of 16 (50%) segments evaluable were not included in the analyses in study II and IV. In study I, peak systolic SR and end-systolic strain were measured in tissue Doppler images by a previously described method.[102] The method tracked the

two-dimensional motion of the segment borders by combining tissue Doppler and speckle-tracking methods, and calculated angle-independent strain and SR values from the measured change of segment length through the cardiac cycle. The mean value of the analysable segments in each patient was used in statistical analysis. In study I, subjects were excluded from statistical analysis if less than six of 12 (50%) segments could be analysed.

All echocardiographic recordings were analyzed by one investigator (LAR), and all echocardiographic analyses were performed blinded to group assignment (study II-IV), clinical and donor data.

6.3 Exercise intervention

The exercise intervention in study II and III consisted of HIIT performed on a treadmill. Each participants randomized to training intervention was assigned to a local, cooperating physiotherapist for individual supervision of every HIIT-session. The intervention was divided into three 8-week periods of exercise, with three sessions every week (in all 72 sessions) throughout 12 months. The last 8-weeks-period was finished 1-2 weeks before follow-up test. Additionally, participants in the exercise group were also encouraged to exercise on their own between the 8-weeks-periods. All participants were provided with their own HR monitor (Polar Electro, Kempele, Finland) to obtain the assigned exercise intensity. Both the supervised sessions and their solo training were monitored and logged.

HIIT was performed as 10-minutes warm-up before exercising 4x4 minute intervals at 85-95% of peak heart rate (HR_{peak}), with a 3-minute active recovery, corresponding to Borg scale 11-13, between intervals (Figure 3). HR_{max}, recorded during CPET at baseline, was used to determine each participant's training zone. Speed and inclination of the treadmill were adjusted continuously to ensure that every training session was carried out at the assigned HR throughout the training period. No intervention was given to the control group other than basic, general care given to all HTx recipients. Thus, the control group was requested to continue exercise as before inclusion.

Warm up	Interval	Active pause	Interval	Active pause	Interval	Active pause	Interval	Cool down
60-70% of HRmax	85-95%	60-70% Borg 11-13	85-95%	60-70% Borg 11-13	85-95%	60-70% Borg 11-13	85-95%	60-70%
10 minutes	4 min	3 min	4 min	3min	4 min	3 min	4 min	3-5 min

Figure 3. *Principles of high-intensity interval training*

6.4 Cardiopulmonary exercise testing

The maximal exercise capacity was tested using a modified treadmill walking test following the recommendations for CPET in chronic HF patients from the European Society of Cardiology's working group on cardiac rehabilitation and exercise physiology and working group on HF.[103] Patients started with a warm-up period of 10 minutes in order to find a proper, individualized walking speed on the treadmill (Technogym Runrace). After 10 minutes we increased the inclination by 2% every 2 minutes until exhaustion. A Borg score >18 and/or respiratory exchange ratio (RER) $\geq$ 1.05 were used as criteria for an adequate maximal exercise test.

Lung function and breath-by-breath gas exchange was measured using the Sensormedics Vmax, software Vmax/Spectra version 07-2B (Yorba Linda, CA). Electrocardiogram (ECG), HR, O_2-uptake, CO_2-production, ventilation (VE) and RER were monitored continuously before, during and after exercise. Blood pressure (BP) was measured automatically (Tango, Sun Tech Medical Instruments, NC) before, every second minutes during exercise and in the recovery phase. After termination of the test, the treadmill was stopped and the patients rested in an upright position for a recovery period of 2 minutes. Oxygen uptake, carbon dioxide production, minute ventilation and RER were calculated on line, and $VO_{2peak,}$ VE/VCO_2 slope and O_2 pulse were recorded.

VO_{2peak} was defined as mean of the three highest 10-seconds measurements. Predicted values were based on the American College of Sports Medicine 2009 guidelines.[104]

O_2-pulse, which is strongly correlated with CO and a surrogate of stroke volume,[78,105] was calculated as the ratio of VO_2 (mL/min) to HR. Anaerobic threshold (AT) was defined using the V-slope method and was identified in 60% of the patients.[105] HR_{peak} was the peak HR during exercise. Age-predicted maximum HR ($\%HR_{max}$) was calculated as: $HR_{peak}/220-age \times 100$, with <85% considered pathologically low.[106] HR reserve was measured using the difference between HR_{peak} and HR_{rest} (resting HR measured during echocardiography). Age-predicted HR reserve, or chronotropic response index (CRI), was calculated as $(HR_{peak} - HR_{rest})/(220-age- HR_{rest})$, where a ratio <0.80 was considered abnormal.[106] The follow-up test was performed with the same individual speed as the baseline test. Effect of exercise at sub-maximal levels is presented as the difference in HR and RER between the baseline and follow-up test, measured at 60% and 80% of peak exercise.

6.5   Coronary angiography and endomyocardial biopsies

Coronary angiography was performed in study II and IV. We classified vasculopathy as follows: a) severe vasculopathy: significant stenosis, i.e. ≥ 50% of lumen diameter in ≥1 major epicardial graft vessel(s) and/or >70% in ≥2 distal branches; b) moderate vasculopathy: non-significant stenosis, i.e. 33-50% of lumen diameter in ≥1 major epicardial graft vessel(s) and/or >70% in one distal branch; c) mild vasculopathy: luminal irregulations, i.e. stenosis <33% of lumen diameter in ≥1 major epicardial graft vessel(s). All angiograms were reviewed by experienced invasive cardiologists, and compared with the previous years' angiograms to detect the presence of luminal irregularities or obstructions.

Endomyocardial biopsies from the right ventricle were performed using standard procedures in all HTx recipients on their 1st or 2nd annular medical follow-up visit and when indicated (e.g. prior rejections) on their 3rd annular visit.

6.6   Isokinetic muscle strength

Muscular strength and muscular exercise capacity was tested isokinetically, using a dynamometer (Cybex 6000, Lumex Inc, Ronkonkoma, NY, USA). The quadriceps (extension) and the hamstrings (flexion) muscles were used. The test was performed in a sitting position, testing one leg at a time, after 10 minutes warm up on an ergometer bicycle. Muscle strength was tested at an angular velocity of 60°/sec. Five repetitions were performed, and the mean peak value in Newton meter (Nm) was calculated. Muscular exercise capacity was measured in joule (J) as the sum of the total work of 30 repetitions with an angular velocity of 240°/sec. Bilateral strength in quadriceps and bilateral strength in hamstrings was calculated [(right + left) /2].

6.7   Bioelectrical impedance analysis

Body composition data were collected using Tanita 418MA and BC-558 (Tanita Corporation, Arlington Heights, IL). Compared with the gold-standard in body composition measurement, dual-emission X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) is considered a reliable and more accessible method of body composition screening.[107] The recorded variables included body mass index (BMI), body fat, total body water, muscle mass, visceral fat, bone mass, metabolic age and basal metabolic rate. All participants were screened at the same time of day, with means of three measurements calculated for all patients.

6.8   Biochemistry

Regular blood screening was performed in the morning, in fasting site in the participants in study II, III and IV. Hemoglobin (Hb), white blood cells, creatinine, urea, estimated glomerular filtration rate (eGFR), uric acid, liver and thyroid function, cyclosporine concentration, lipid status and HbA_{1C} were measured.

For measurements of inflammatory and myocardial markers, peripheral venous blood was drawn into pyrogen-free tubes with EDTA as anticoagulant. The tubes were

immediately immersed in melting ice and centrifuged within 30 minutes at 2000g for 20 minutes to obtain platelet-poor plasma. All samples were stored at -80ºC until analysis. N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP) was determined by an electrohemiluminescence immunoassay on a modular platform (Roche Diagnostics, Basel, Switzerland), C-reactive protein (CRP) by a high-sensitivity particle-enhanced immunoturbidimetric assay (Tina-quant CRP [Latex HS], Roche Diagnostics), and interleukin (IL)-6, IL-8 and IL-10 by immunoassays, obtained from R&D Systems (Minneapolis, MN). The intra and inter-coefficients of variation were<10% for all assays.

6.9 Health-related quality of life

In study III health-related quality of life (HRQoL) was measured with the generic questionnaire Short Form 36 (SF-36) version 2. SF-36 contains 36 items which measure HRQoL on eight scales, which were aggregated into two sum-scores: Physical Component Summary (PCS) and Mental Health Summary (MCS). The results were aggregated into to sum-scores, Physical Component Summary and Mental Component Summary, which was reported on a standardized scale with a mean of 50 and a SD of 10, based on the 1998 US general population. In addition to the questionnaires, patients were asked to subjectively rate on a visual analogue scale (VAS-scale) how much participation in this study had improved their HRQoL: "0" indicating nothing and "100" indicating extremely much.

6.10 Statistics

Continuous data are presented as mean ± standard deviation (SD) or as median (range) (interquartile range in study III) in the case of non-symmetrically data sample distribution, and categorical data are presented as counts/percentages. Within-group changes over time were assessed by paired samples t-test or Wilcoxon non-parametric signed rank rest. Between-group comparisons were performed using two-sided independent t-test or Mann-Whitney U test. In study II, comparisons at follow-up were done using analysis of covariance (ANCOVA) with the baseline values as covariates as

recommended.[108] Categorical variables were compared using the Chi-Square test or the Fisher's exact test. For investigating hemodynamic changes during exercise stress echocardiography in study I and II, we used a general linear model for repeated measurements ANOVA (Greenhouse-Geisser) with exercise intensity as within-subjects factor and group as between-subjects factor. Bonferroni post-hoc test were applied. Pearson sample correlations, univariate and multiple linear regression analysis (hierarchical, forced entry (enter) method) were used to evaluate the association between continuous variables of cardiac function or VO_{2peak} and potential predictors (study III and IV). The multiple linear regression analysis are presented with a model summary R^2 (i.e. coefficient of determination), B (i.e. unstandardized regression coefficient) and a p-value (i.e. testing whether B=0). Differences were considered significant for p-values < 0.05. Analyses were performed using SPSS 13.0 in study I and version 18.0 in study II-IV (SPSS Inc., Chicago, IL, USA).

6.11 Ethical considerations

All participants were > 18 years of age, and all gave written informed consent. Participants in study II and III were thoroughly informed about the exercise intervention program and potential risk factors. Study I was approved by the central Regional Ethics Committee in Norway, while study II, III and IV were approved by the South-East Regional Ethics Committee (Clinicaltrials.gov identifier: NCT 01091194). All studies were conducted according to the declaration of Helsinki. No adverse events occurred during the studies.

7 Results and summery of papers

7.1 Paper I

In this study we compared systolic and diastolic LV function during upright bicycle exercise in patients with previous MI, and compared the results to those of a healthy age- and sex- matched control group. The patients had relatively well preserved LV function, although LV EF was lower in the MI-group (46 vs. 54%, p<0.01). LVs' was lower in the MI patients at all stages, but increased during exercise in both groups. LVe' was similar at rest, but increased in the control group only from 50 to 75W. E increased during exercise in both groups, thus the E/e'-ratio increased during exercise in the MI group only. The control group had higher absolute value of end-systolic strain and MAE than the MI group. Both strain and MAE increased during exercise, but levelled off from 50 to 75W in both groups. HR was similar in both groups at rest and during exercise. No new or worsening wall motion abnormalities were detected during exercise in any of the patients. When changing from supine to upright position we observed that LVs', MAE (displacement), LVe', E, EDV and end-systolic strain decreased in both groups, whereas E/e' increased.

7.2 Paper II

In this randomized controlled trial we investigated the effect of HIIT on exercise capacity and cardiac systolic and diastolic function in a group of stable HTx recipients. The participants were examined both at rest and during semi-supine bicycle exercise at baseline and follow-up with echocardiography.

One year of HIIT increased VO_{2peak} from 27.7 ± 5.5 at baseline to 30.9 ± 5.0 ml/kg/min at follow-up, while the control group remained unchanged (28.5 ± 7.0 vs. 28.0 ± 6.7 ml/kg/min, p<0.001 for difference between the groups). LV systolic and diastolic functions at rest and during exercise were generally unchanged by HIIT. Despite some small, although significant changes, the clinical importance of these differences is limited.

During exercise stress echocardiography we found both a systolic and a diastolic reserve capacity; however this reserve did not improve after HIIT. At baseline-testing, all LVs', LVe', E and displacement indicies increased with increasing exercise intensity from semi-supine rest to submaximal load (all P-ANOVA<0.001 for both groups). Since both E and LVe' increased, E/e' was unchanged during exercise in both groups. These exercise-induced hemodynamic changes at baseline-testing were present at follow-up in both groups, with no significant differences between groups for any of the parameters at any stage.

7.3 Paper III

In this paper we examined the effect of HIIT in stable HTx recipients, with focus on exercise capacity and several central and peripheral factors that influence on exercise capacity.

We found that a long-term, partly supervised and community-based HIIT-program is an applicable, effective and safe way to improve exercise capacity, muscular endurance in HTx recipients. VO_{2peak} increased in the exercise group, while there was no significant change in the control group, resulting in a significant difference of 3.6 [95% CI 2.0, 5.2] mL/kg/min between groups at follow-up (p<0.001). HR_{peak} increased toward normal values in the exercise group (%HR_{max} 96±10%) while remaining unchanged in the control group, resulting in a higher HR reserve in the exercise group. Systolic BP at peak exercise was higher in the exercise group than in the controls, however diastolic BP during peak exercise and systolic and diastolic BP at rest were similar. Muscular endurance (total work) in quadriceps and hamstrings increased significantly by 15% and 19%, respectively, in the exercise group, while remaining unchanged in the control group. Regarding HRQoL, there were no significant changes in any of the sum-scores in SF-36. However, the exercise group had significantly higher scores on the SF-36 General Health subscale at follow-up; 54 vs. 49, respectively (p<0.005). As for subjectively improved health, the exercise group reported 65 on the VAS scale vs. 26 in the control group (p<0.001). Numerically, the exercise group had positive changes in

their body composition, but there were no significant differences in changes between the study groups at follow-up. No differences were found in biochemical parameters between groups at follow-up.

7.4   Paper IV

In this study we investigated the effect of graft ischemic time (GIT) and donor age on cardiac function by use of echocardiography. In general, we found that prolonged GIT impaired both systolic and diastolic function at interventricular septum and RV free wall, whereas donor age impairs LV diastolic function.

Recipients with GIT ≥ median (200 min) had significantly lower septal LVs' (15%), RVs' (22%), septal LVe' (22%), RVe' (23%) and LV EF than recipients with GIT <200 min. Non-significant differences were found in lateral LVs', lateral LVe' and annular displacement in both the LV and RV. E was equal in both groups and septal E/e´ trended higher in the group with GIT ≥200 min (p=0.062). Peak VO$_2$ was slightly lower (10%) among patients with GIT ≥200min (p=0.098).

Recipients with donor age ≥37 years had significantly lower LVe' velocities, both septal and lateral, than recipients with younger donors. Furthermore, E/e´ was significantly higher and DT was longer in recipients with older donor hearts. However, no significant differences were found between groups in systolic parameters or exercise capacity.

There was a linear negative association between GIT and septal LVs' and LVe', RVs' and RVe', and between donor age and LVe', both septal and lateral.

8   Discussion

8.1   Methodological considerations of echocardiography

In this thesis we have evaluated systolic and diastolic cardiac function, both at rest and during exercise in patients with healed MI and transplanted hearts by use of different echocardiographic methods, particularly tissue Doppler imaging. Further, we have shown that factors related to the heart transplantation and the donor is detectable with echocardiography.

8.1.1 Feasibility

An echocardiography examination was feasible in all participants in all studies at rest. None participants were excluded due to reduced image quality at rest, but three of 42 subjects were excluded from analysis in study I due to poor image quality in the upright position.

Since there is often a size mismatch between the donor heart and the recipient pericardial sac, the transplanted heart is often positioned more lateral than normal. Thus, it was challenging to obtain satisfactory image quality in some HTx recipients. Further, obesity and lung shadow also reduced the image quality in some participants. Thus, in the speckle tracking based strain analysis, only 73% of all segments were analysed at baseline in study II. However, obtaining good quality images without angle-misalignment were even more challenging during exercise stress echocardiography. When disregarding the three participants who did not perform exercise stress echocardiography due to logistics, satisfactory image-quality without angle-deviation was obtained in about 2/3 of the HTx recipients.

8.1.2 Tissue Doppler Imaging

In all studies we have used annular tissue Doppler for quantifying annular systolic and diastolic function. This is an attractive method in everyday clinical practise as it is easy

and fast to obtain and gives robust measurements of global ventricular function. We used colour TDI in our studies, as this allows for post-processing and thus also information about systolic displacement by integrating the velocities. Furthermore, obtaining two colour tissue Doppler images (4- and 2-chamber view) during the exercise stress echocardiography examinations were less time-consuming than four spectral PW Doppler images. Even though the guidelines prefer PW TDI as more validation studies have been performed with this technique,[18] the two methods seem equal with respect to reproducibility and identification of contraction changes.[5,10] Recent studies of healthy individuals have used the colour TDI method, and shown good correlation against mortaility.[68,109]

Since colour tissue Doppler signals consists of fewer samples per time unit than PW TDI, it is important to ascertain that the sampling rate is high enough for obtaining peak myocardial velocities. If the sampling rate is much lower than the rate of change of peak values, the probability of hitting the peak is low and the measurement will underestimate peak values. In our studies we used frame rate with about 100 frames per second, which is usually sufficient for measuring mitral annular velocities. Since frame rate is inversely related to line density (number of lines in the scanner sector), higher frame rates will decrease the lateral resolution if the sector width is kept constant.

A limitation to the tissue Doppler method is that small shifts in probe position or heart position (respiration) might influence measurements of velocities and deformation. As the tissue Doppler sample volume is stationary whereas the heart moves, different parts of the myocardium will be imaged during the cardiac cycle. In study I we used customized software which has the benefit that one can follow the atrio-ventricular plane throughout the heart cycle, to avoid this 'out of plane'-problem. However, in study II-IV, and particularly during the exercise stress echocardiography in study II, this might have influenced the displacement and velocity values.

8.1.3 Methodological considerations of exercise stress echocardiography

In both study I and II we used a protocol with fixed watt-stages, and not stages based on HR_{peak} or VO_{2peak}. In study I all participants, except for two in the MI group, bicycled until 75W, which was set as the 'final load' in this study. Thus, the relative exercise-intensity was lower in the healthy controls, than in the MI group. However, HR was similar between groups at all exercise stages, likely due to medical treatment with beta-blockers in the MI patients. In study II, the participants bicycled until muscular fatigue occurred, and we tried to obtain images 1-2 min before termination of the test. Since the test was repeated after one year, the fixed watt-stages made it easy to compare stages at follow-up with the same stages at baseline test. The highest load/watt each participant achieved was used as 'submaximal load' in the analyses. Due to upper-body movement and heavy breathing, it was challenging to obtain images on max load in both studies. However, obtaining images post-exercise does not solve this problem. Peteiro *et al* have previously shown that peak treadmill imaging is more sensitive and of higher prognostic value than post-exercise imaging for the diagnosis of CAD, and that quality of peak images in the apical views are similar to those acquired post-exercise.[110] Further, studies have shown that the time-course of post-exercise PCWP is highly variable.[111,112] Borlaug *et al* showed that PCWP returned to baseline values within one minute post-exercise in both patients with HF with normal EF and healthy individuals.[112] Thus, in order to examine systolic and diastolic dysfunction and CAD, obtaining images at the highest achievable load is of importance.

In study I we used upright bicycle exercise stress echocardiography, whereas in study II we used a semi-supine bicycle. Upright bicycle tests are more similar to the patients' everyday situation, whereas a semi-supine bicycle such as the one used in study II, has the benefit that it can be tilted into the lateral position, and thus improve image quality. Invasive studies have shown that filling pressure is higher during supine exercise in healthy subjects and in patients with CAD, compared to upright exercise due to increased preload.[113,114] The test position is therefore of relevance when comparing results from different studies. This was also illustrated in study I when the study

subjects changed position from supine to upright rest, thereby decreasing venous return and preload. Systolic parameters reflecting SV (end-systolic strain and MAE) and diastolic parameters reflecting LV filling and preload (EDV, E) decreased, in addition to LVs' and LVe'. The preload-dependency of mitral inflow velocities is well known.[115] For tissue Doppler velocities, the effects of load are more complex. In the presence of impaired LV relaxation, preload and LV filling pressures have a minimal effect on e'. On the other hand, with normal or enhanced LV relaxation, preload increases e'.[20]

8.2 *Exercise-induced cardiac changes - study I and II*

In both study I and II we aimed to investigate a cardiac reserve capacity. In study I we found a limited diastolic reserve, while a systolic reserve was present despite reduced systolic function at rest, compared to healthy controls. In study II we found that both a diastolic and a systolic reserve was present in HTx recipients, but we did not compare with healthy controls in this study, thus it is unsure whether the reserves were supra- or subnormal. However, similar exercise echocardiography protocols have been performed by Ha *et al* although they used PW tissue Doppler in the septal region only. Compared with their result at 50W, the HTx recipients in study II had lower septal LVs' and LVe' velocities than the healthy controls in the studies by Ha, but higher LVe' and similar LVs' compared to patients with hypertrophic cardiomyopathy or diabetes.[37,38]

8.2.1 Systolic function during exercise testing

The exercise-induced systolic reserve seen in both MI and the HTx patients were in agreement with hemodynamic responses to exercise seen in healthy individuals.[116,117] The LVs' increased with increasing intensity in both MI and HTx patients, and in addition the increased peak systolic SR in the MI patients indicates a systolic reserve capacity. Since these parameters are measured in early systole, they probably reflect alterations in contractile force better than end-systolic parameters.[9,10] Increased sympathetic tone and increased HR (force-frequency effect) might have contributed to the increased contractile force during exercise. The systolic reserve seen in the HTx recipients is in agreement with a study by Borow *et al* who found normal contractility

and contractile reserve in transplanted hearts, by measuring the end-systolic wall stress' response to altered afterload and inotropi.[93]

Both the MI and HTx patients increased their SV, as judged by increased peak systolic mitral displacement during exercise. Exercise-induced increases in SV and CO have also been found measured invasively by thermodilution in both HTx recipients[118] and MI-patients.[119] In this latter study by Andersen *et al*, they found increased PCWP during exercise,[119] which is in line with our increased E/e' in study I. This indicates that the MI patients, in order to maintain a sufficient SV during exercise, must increase their LV filling pressure. The HTx recipients on the other hand, increased their LV filling, and thus SV, without increasing LV filling pressure (e.g. E/e'), and the increase in SV was more likely due to increased relaxation during exercise, which is similar to the healthy controls in study I. See discussion below.

8.2.2 Diastolic function during exercise testing

8.2.2.1 HTx recipients

In contrast to previous studies reporting diastolic dysfunction and increased PCWP in HTx recipients,[81,118,120] we discovered a diastolic reserve and an unchanged E/e' during exercise in our study. However, to our knowledge, diastolic function has not previously been investigated with exercise stress echocardiography in HTx recipients. Rudas *et al* found an abnormal increase in PCWP during exercise in the supine position, and also increased PCWP in the upright position in HTx recipients.[118] They concluded that the marked increase in LV filling pressures during exercise and the difference between supine and upright exercise, imply an abnormal left-shifted and steep diastolic pressure-volume relation in the transplanted heart. No studies have so far validated the E/e' ratio with invasively measured PCWP or LV filling pressure during exercise in HTx recipients, but Sundereswaran *et al* found a good correlation between PCWP and E/e' at rest in these patients.[23] Thus, it is questionable whether the E/e' have limitations in reflecting LV filling pressure in HTx recipients during exercise, or whether the HTx cohort in our study had better diastolic function and more compliant ventricles than in

previous studies. In fact, the HTx cohort in our study had much higher exercise capacity compared to other studies.[76-80]

8.2.2.2 Patients with myocardial infarction

There might be several reasons for the limited diastolic reserve in the MI group. Ischemia might be one contributing factor to the limited diastolic reserve in the MI patients. Four of 18 MI patients in our study had significant stenoses still untreated at the time of the study. All these stenoses were in one of the non-culprit epicardial arteries, but their hemodynamic significance was not tested during angiography. Even though no new or worsening wall motion abnormalities were detected during exercise, the ischemic effect of diffuse, peripheral arteriosclerosis could affect relaxation. In the setting of ischemia and reduction in blood perfusion to the myocardium, one of the first abnormalities is a reduction in active relaxation and further diastolic dysfunction.[8]

Also the reduced systolic function might have impaired the diastolic relaxation. During LV systolic contraction, potential energy is normally stored within the cytoskeletal protein (e.g. titin), which is disengaged during diastole and increases relaxation rate. Thus, the reduced systolic contraction might have caused less effective elastic recoil in the MI patients. Further, in HF patients with high diastolic stiffness, an isoform switching in titin and phosphorylation deficit have been seen,[121] and in elderly patients, increased turnover of interstitial collagen is associated with both diastolic and systolic HF.[122] These findings might also influence the diastolic function in MI patients and contribute to increased LV chamber stiffness and elevated LV filling pressure during exercise.

The increased E/e' in the MI patients is in agreement with invasive studies, which have found increased PCWP and LV end-diastolic pressure during exercise in patients with CAD[114] and MI.[119] Andersen *et al* have recently investigated the exercise hemodynamics invasively in patients with and without diastolic dysfunction and preserved EF after MI.[119] They found that post-MI patients with preserved EF and diastolic dysfunction at rest (defined as E/e' >8 and LA volume index >34mL/m^2) had a

very rapid and abnormal rise in PCWP and right atrial pressure, and at max exercise 94% of the subjects had PCWP >25mmHg. Also the MI patients without diastolic dysfunction at rest showed an abnormal increase in PCWP at max exercise compared to the healthy controls, although less extensive than the patients with diastolic dysfunction at rest. This underscores the need for exercise tests to unmask diastolic dysfunction not seen at rest.

It should be noted that neither this study by Andersen *et al*[119] nor our study have investigated the presence of diastolic dysfunction before the MI occurred. Patients with pre-infarction hypertension have reduced improvement of diastolic function after an infarction[123] suggesting that pre-infarct myocardial function is also important for myocardial outcome.

8.2.3   Clinical perspective of the diastolic dysfunction in MI patients

The diastolic dysfunction and the increased LV filling pressure in the MI patients during exercise, likely explain the limited exercise capacity in this patient population. In a similar study to our, Lele *et al* found a strong correlation between exercise capacity and indices of diastolic function during exercise, using radionuclide ventriculography.[65] The non-dilated LA area in the MI patients indicates that the LV filling pressure was not chronically elevated in our cohort. Still, the abnormal diastolic dysfunction and LV filling as seen during exercise adds prognostic information, as it might identify patients at increased risk of developing heart failure. Elevated LV filling pressure at rest is a strong predictor for LV remodeling after acute MI.[66]

The loss of diastolic reserve with exercise stress likely represent an early key step in the progression from asymptomatic (NYHA I) to symptomatic HF (NYHA II/III). Thus, the diastolic dysfunction in MI patients may represent an important window for initiation of novel therapies to prevent HF progression, and thereby increase survival.

8.2.4 Does the E/e' ratio estimate LV filling pressure?

The ability of the E/e' ratio to predict filling pressure at rest has been demonstrated in patients with various heart diseases.[16,21-24] Ommen *et al* showed that E/e' < 8 predicts normal LV filling pressure and E/e' > 15 identifies increased LV filling pressure (>12mmHg), whereas in the range of 8 to 15 there is an uncertainty in the prediction of filling pressure in a group of unselected patients referred for left heart catheterization.[17] These cut-off values have been used in the guidelines for evaluating LV diastolic function.[18] Burgess *et al* showed that an E/e' >13 during exercise, had a sensitivity of 73% and a specificity of 96% for identifying of an elevated exercise LV filling pressure.[26] They also demonstrated that an abnormal exercise E/e' occurred in over a third of patients with normal filling and delayed relaxation at rest.[26]

However, recently studies with contrary results regarding E/e' and LV filling pressure have been presented. Andersen *et al* investigated patients with recent MI, LVEF >45% and E/e' in the 'uncertain range' between 8 and 15 at rest with simultaneous right heart catheterization and symptom-limited semi-supine bicycle stress echocardiography.[124] Interestingly, they found that both E and e' increased with increasing intensity, whereas E/e' slightly decreased and PCWP increased from 13 ± 4 to 33 ± 8 mmHg. There were neither correlations between PCWP and E/e' at rest, submaximal load, peak exercise nor post-exercise. At peak exercise 87% of the patients had PCWP > 25 mmHg, whereas only one had E/e' >15. Further, Dalsgaard *et al* found a correlation between E/e' and PCWP at rest, but not during exercise in patients with severe aortic valve stenosis.[22] Also Mullens *et al* failed to detect a relation between E/e' at rest and PCWP, or changes in E/e' and PCWP in patients with advanced, decompensated HF.[125]

These studies imply that an estimation of LV filling pressure by the E/e' ratio might be unreliable in some patients populations and in some settings. Thus, caution should be made when diastolic function and LV filling pressure are evaluated based on the E/e' only. However, evaluating several diastolic parameters together and using the E/e' as a supplement, most likely give reliable information about the LV filling properties. In addition, Doppler variables should be viewed in the context of ventricular size and

function, as well as LA size.[126] Thus, the E/e' is more likely an additional tool, than an independent predictor for estimating LV filling pressure.

8.3 Effects of exercise training in HTx recipients - study II and III

In study II and III we investigated the feasibility and the effect of HIIT on central and peripheral factors in a group of stable HTx recipients. HIIT has traditionally been avoided in HTx recipients, mainly due to concerns over chronotropic insufficiency and safety. In study II and III we demonstrated that high-intensity training is feasible and safe also in HTx recipients, and significantly improved VO_{2peak} and muscular strength. Renal function, regular blood parameters and cyclosporine concentration were similar between the groups both at baseline and follow-up, and there were no rejections during the year of follow-up.

8.3.1 The exercise intensity and exercise intervention

Despite the cardiac denervation in the HTx recipients, HR_{peak} in the participants in study II and III were surprisingly high; between 70 to 116% of age predicted HR_{peak}, on average 93 ± 11 %. Consequently, the exercise intensity during the intervals (which were supposed to be between 85-95% of HR_{peak}) was comparable to the exercise intensity used in studies on non-transplant subjects.

The exercise intervention programs were performed in the participants' home-town supervised 1:1 with a cooperating physiotherapist. This might have been a huge motivation for the participants, thus there was a very good compliance during intervention and 96% of the 72 planned HIIT-training sessions were completed. Even though the participants in the control group were requested to continue exercise training as before inclusion, we cannot elude the possibility that the control group increased their exercise training in the intervention period, as all participants were highly motivated for exercise training before randomization. We asked the control group at follow-up how often they had exercised with an exercise duration >30 minutes and a Borg scale >14

the last 3 months before follow-up testing; 37.5% in the control group had exercised once or less per week, 37.5% 2-3 times per week and 25% 4 times or more per week. However, as the control group did not increase their exercise capacity at follow-up, we assume that a potential effect of this exercise was sparse.

8.3.2 Effect of HIIT on VO_{2peak}

The increase in VO_{2peak} [ml/kg/min] by 12.7% in our study is greater than found in most rehabilitation programs consisting of moderate-intensity training among HTx patients (Table 1). However, compared to other patient populations who completed similar high-intensity training, the increase VO_{2peak} in study II and III was moderate. Congestive congenital HF patients increased VO_{2peak} by 46%,[50] CAD patients by 17.9%,[61] hypertensive subjects by 15%,[127] while subjects with metabolic syndrome increased VO_{2peak} by 35%.[51] However, the increase in exercise capacity was higher than what has been seen in after exercise training in recipients with other solid organ transplantation. In a systematic review and meta-analysis on exercise training in solid organ transplant recipients, Didsbury et al recently have shown that HTx recipients was the only group with significant improvement in maximal oxygen uptake.[128] Regimens that were of at least 12 weeks in duration, included supervision and commenced within 1 year after the transplant surgery appeared to be the most effective. Among other solid organ transplant recipients (kidney and liver), no significant improvements after exercise training were observed. However, it should be noted that none of the RCT's in kidney, liver or lung recipients consisted of supervised training programs with durations greater than 8 weeks.

The improvement in VO_{2peak} of 3.6 mL/min/kg between groups in our study is considered clinically significant as 3.5 mL/min/kg equals 1 metabolic equivalent (MET). In a large prospective study consisting of men referred for exercise testing for various clinical reasons, Myers et al found that a 1 MET increase in exercise capacity, was equal to a 12% increase in survival.[129] However, in an other study by Myers et al, they showed that PCWP >12mmHg, right atrial pressure >25mmHg and creatinin >118 umol/L rather than exercise capacity, were predictors for mortality in HTx recipients.[130]

Thus, further research is needed to see whether an increase in VO_{2peak} due to exercise training, improve survival in HTx recipients.

8.3.3 Effects of HIIT on central and peripheral factors

8.3.3.1 Cardiac function

In study II and III we saw that the significant increase in VO_{2peak} in the exercise group was not accompanied by improvements of clinical importance in cardiac dimensions, volumes, systolic or diastolic function, neither at rest nor during exercise echocardiography. To our knowledge, cardiac systolic function has been investigated after exercise training in HTx recipients in previous studies, but none have demonstrated a positive exercise-induced effect on systolic cardiac function. Haykowsky *et al* performed echocardiography both at rest and during submaximal cycle exercise, and calculated stroke area and EF by measuring LV systolic and diastolic cavity area in the parasternal short-axis view. They did not find any improvement in systolic function after 12 weeks of supervised aerobic and strength training in HTx recipients.[95] Neither Geny *et al*, who calculated SV and CO by the Fick method,[131] nor Kobashigawa *et al*, who used echocardiography to estimate EF,[96] found any cardiac improvement after exercise interventions. Thus, the sparse improvement in cardiac function is in agreement with prior studies in HTx recipient. However, none of the former mentioned studies have investigated the effect of exercise training on cardiac function with comprehensive, newer echocardiographic techniques.

8.3.3.2 Cardiac function after exercise training in other patient populations

In contrast to our findings in study II, other patients groups have shown great improvements in cardiac function after similar exercise programs (Table 2). Wisløff *et al* found that systolic function and diastolic relaxation, as well as LV reverse remodelling, improved significantly after 12 weeks of HIIT in patients with congestive HF due to MI.[50] LVs' increased by 22%, systolic mitral annular displacement by 30%, SV by 17% and EF by 10 percentage points, and LVe' increased by 49% and E/e'

decreased by 26%, all significant changes. Further LV systolic and diastolic internal diameters declined.

Study	EF %	EDV mL	SV mL	Peak systolic displacement mm	LV S' cm/s	LVe' cm/s	E/e'
Study II **3x8 weeks HIIT**	52→53	97→100	67 → 66	9.5→10.2	5.9→5.7	8.1→8.1	8.6→9.7
Mølmen-Hansen et al[127] **Hypertensives** **3 months HIIT**	58→64	110→122	72→80	na	6.9→7.4	8.0→9.3	na
Wisløff et al[50] **Heart failure (post-infarction)** **12 weeks HIIT**	28→38	248→203	57→67	7.4→9.6	4.8→5.8	4.5→6.7	16→12

Table 2. *Cardiac function after HIIT in other patient population. Tissue Doppler velocities are mean of four mitral points. Mølmen-Hansen and Wisløff used PW TDI.*

Also Mølmen-Hansen *et al* performed a similar study on subjects with hypertension, and found that 12 weeks of HIIT had a beneficial effect on BP and cardiac function; EF, SV, LVS' (by 15%) and LVe' (by 14%) all increased significantly and both systolic and diastolic BP decreased.[127] However, in a study by Amundsen *et al* consisting of CAD patients, systolic function was unchanged after 10 weeks of HIIT, although diastolic function, measured by LV early diastolic SR increased.[132] Compared to the HTx-recipients, the HF patients had more severely reduced cardiac function, and in that regard not directly comparable with the HTx-recipients. The hypertensive individuals on the other hand had comparable echocardiographic parameters at baseline, and might be indicative of what we could have expected of improvement in cardiac function.

There are very few studies with exercise stress echocardiography before and after exercise training intervention. In a study with sedentary, healthy seniors, Mølmen *et al* found that 12 weeks of HIIT increased LVe' at both rest and during exercise, whereas an increase in LVs' was only seen during submaximal exercise.[133] Also a study in obese adolescent, Ingul *et al* found an increase in mitral annular excursion, LVs', global strain, and LVe' at both rest and during exercise after 3 months of HIIT.[134] These studies show that it is feasible to detect effects of exercise interventions during exercise stress echocardiography on a submaximal level. Hence, the interesting question is why HTx recipients did not have similar cardiovascular response to HIIT. Further research is needed to clarify the mechanisms behind this lack of cardiovascular improvements.

8.3.3.3 Chronotropic response

In study II and III we found that HR_{peak} increased toward normal values in the exercise group only, which is in agreement with other exercise intervention studies on HTx recipients.[92,135] Chronotropic incompetence due to denervation is repeatedly regarded as one of the most important factors limiting VO_{2peak} in HTx recipients.[76,81] The increased HR_{peak} and thus increased CO, might therefore explain some of the increased VO_{2peak} seen in the exercise group.

8.3.3.4 Arterial blood pressure

HIIT has previously shown a significant effect on BP in hypertensive patients, a reduction in systolic BP by 12 mmHg (p<0.001) and diastolic BP by 8 mmHg (p<0.001) have been reported.[127] Thus, we were surprised that HIIT did not reduce BP in our study, as there is a high prevalence of hypertension among HTx recipients.[71] Even though the finding of unchanged BP after exercise training is accordance with some previous studies,[95,131] other have shown a positive effect of exercise training on resting arterial BP on HTx recipients.[94,97,135] Hermann *et al* found that 8 weeks of high intensity aerobic exercise was associated with a significant reduction in systolic BP (from 142 ± 17 to 127 ± 13 mmHg, p= 0.02) and improved endothelial function in the exercise group.[94] Also Bernardi *et al* found similar decrease in resting systolic BP and diastolic BP after an exercise training program.[97] However, the exercise intervention in our study was even more comprehensive, lasted longer and had even more participants than in these studies by Hermann and Bernardi. Immunosuppressive drugs, such as cyclosporine A and corticosteroids have negative effects on the endothelium and arterial function,[87,136] but the HTx recipients in our studies did not differ from the subjects in the former mentioned studies in that regard. Thus, there is no simple answer to the missing effect on resting arterial BP seen in our work. However, it should be noted that we did not perform a 24-h ambulatory BP monitoring, thereby possible effects of the "white coat phenomenon" can have influenced the result.

8.3.3.5 Muscular function

In study III we demonstrated that quadriceps and hamstrings muscular exercise capacity improved significantly in HTx recipients, and that this increase had a high correlation with the change in VO_{2peak}. The increased muscular exercise capacity is in accordance with other studies on HTx recipients. Haykowsky *et al* found that 12 weeks of aerobic training increased total lean tissue mass and maximal strength,[95] whereas Wu *et al* showed that home-based exercise improved muscular endurance.[99] Further, Lampert *et al* found that endurance training increased the volume density of total mitochondria in skeletal muscle in HTx recipients, although capillary density did not increase.[137] In chronic HF, Esposito *et al* showed that isolated quadriceps training increased maximal

exercise capacity without increasing CO by improvements in muscle structure and peripheral O_2 transport and O_2 utilization.[138] Also Wisløff *et al* showed that 3 months of HIIT increased the skeletal muscles mitochondrial function and increased the maximal rate of Ca^{2+} reuptake into sarcoplasmatic reticulum in patients with HF.[50]

When looking at all the data from study II and III together, the increased VO_{2peak} was predominantly due to improved muscular exercise capacity and not improved cardiac function. Unfortunately we did not perform skeletal muscular biopsies, and cannot fully explain why muscular exercise capacity increased. Though, based on knowledge from previous research, it seems reasonable to assume that the improved muscular exercise capacity was due to improved muscle structure and improved mitochondrial density and function.[50,137,138]

Unfortunately, we did not include a group with moderate exercise intensity in our work, thus we cannot conclude that high-intensity interval training is better than moderate intensity training, but we can conclude that HIIT is effective in increasing exercise capacity and muscular strength, and that interval training on high intensities is feasible in HTx recipients.

8.4 The impact of graft ischemic time and donor age on cardiac function- study IV

8.4.1 Ischemia and cardiac function

In study IV we showed that tissue Doppler echocardiography could detect effects of prolonged GIT and high donor age years after transplantation, as judged by reduced mitral annular velocities of the interventricular septum (IVS) and the RV. Also in a recent study by Ahlgren *et al*, a relation between GIT and RV function, measured as tricuspid annular planar systolic excursion (TAPSE) was found.[139] Interestingly, we found the highest impact of GIT in the same myocardial segments as where the changes after coronary by-pass surgery most often occur.[140-144] Further, these segments are also

the one were HTx recipients have reduced velocities compared to healthy individuals.[145,146]

Although the occurrence of RV and septal dysfunction after cardiac surgery is a well-known phenomenon, no clear explanation is widely accepted. Hypotheses relating the degree of myocardial preservation (ischemia) during cardiopulmonary bypass and pericardial changes have been proposed.[142,143] A study by Diller *et al* consisting of patients undergoing either on-pump or off-pump coronary bypass surgery found RV dysfunction even 18 months after surgery in both groups. Thus, they concluded that the cardiac dysfunction seen after surgery was unlikely due to hypothermia or immune-inflammatory activation.[144] These hypothesis and explanations are, however, somewhat unlikely in our cohort of HTx recipients. In our study, all recipients underwent the same surgical procedure and medical treatment, and theories related to surgery, such as pericardiotomy, are not plausible explanations for the differences observed between the GIT-groups. Thus, we assume that a transplanted heart demonstrates similar cardiac abnormalities as observed in native hearts following conventional cardiac surgery with cardiopulmonary bypass, but that the postoperative impairment is accentuated by a prolonged GIT in HTx recipients.

D'Andrea have recently investigated the RV function in HTx recipients with both 2D and 3D echocardiography.[146] They found a reduced RV performance in the long axis, as shown by significantly lower TAPSE and longitudinal RVs', whereas RV diameter was increased and 3D RV EF preserved compared to healthy controls. Thus, they emphasised that these findings were rather due to geometrical than functional changes in the RV after HTx. We did not perform 3D echocardiography in our study, and therefore not able to measure RV EF. However, it is questionable whether prolonged GIT solely enhances geometrical changes without altering RV performance as well.

8.4.2 Donor age, diastolic function and aging

In study IV we found that increasing donor age was associated with decreasing LVe'. Furthermore, we saw that DT was longer and E/e' significantly higher in HTx recipients

with older donor hearts. These findings were not accompanied by findings in systolic parameters or exercise capacity. It is well known that diastolic function impairs with increasing age, thus it was reasonable that this was evident in the recipients after transplantation. Aging is associated with myocardial fibrosis, leading to increased stiffness of the myocardium,[147] and also decreased active relaxation.[148]

In line with our findings, both Dalen *et al*[5] and Nikitin *et al*[101] have fond that LVs' and RVe' decreased with increasing age in a population free from cardiovascular disease, and Dalen found decreased RVs' as well. We did not find any associations between donor age and systolic parameters or RV function. This might be due to the fact that Nikitin used subjects as old as 89 years, while in the present study no donors were older than 58 years.

8.4.3 Prognostic and clinical importance

Several studies have shown that the degree of cardiac LV systolic and/or diastolic dysfunction measured by tissue Doppler is a prognostic marker in the general population[109,149] and in patients with cardiac disease, such as atrial fibrillation,[150] acute MI[151] and LV systolic HF.[152,153] Also RV systolic and diastolic tissue Doppler parameters have shown prognostic importance in patients with LV systolic HF.[154] This raises the question of the prognostic importance of the findings in study IV. To what degree does the reduced LV and RV systolic and diastolic velocity, due to prolonged GIT or high donor age, affect mortality? Further research is needed to answer that question.

Considering the shortage of available donor hearts, the results in study IV do not support refusal of donors with prolonged GIT or older age. However, the findings support the practice of eager to match younger donor grafts to younger recipients.

8.5   Limitations

Data in study I were not blinded. A fully blinded analysis was not realistic as HR and regional wall motion abnormalities were visible in the images. In study I four patients with residual stenoses in major (non-culprit) epicardial arteries were included. The MI group was in this way representative of the CAD population. VO_{2peak} should have been included in study I for more accurate evaluation of the relation between the different variables and exercise capacity.

An important limitation in both study II and III is the open design, but it is impossible to perform an exercise intervention study blinded. However, the echocardiographic analyses were done blinded regarding the participants' group assignment. The minimal change in VO_{2peak} in the control group, as well as the fact that each patient served as his/hers own control, indicates that there was a true effect of HIIT.

The inclusion criteria and type of intervention may have led to a selection bias in study II, III and IV. Participants were defined as stable and healthy, and they possibly had a higher-than-average motivation for exercise. Although baseline characteristics of the participants were mainly similar with the entire HTx cohort, they were quite fit with an average VO_{2peak} of 80% of predicted, which is higher than in most other HTx cohorts. At the same time, the participants were very heterogeneous in BMI and VO_{2peak}, and the results are most likely representative for stable HTx recipients in general.

In study II LV systolic and diastolic function was not assessed during maximal bicycle exercise due to low image quality and upper body movement, thus the effect of HIIT on cardiac function during peak exercise is therefore unknown. Due to reduced image quality, angle mis-alignment and logistics, 19 participants were not included in the exercise stress echocardiographic analyses. This obviously decreased the power to detect significant changes.

9   Main conclusions

Paper I

Upright bicycle exercise echocardiography is feasible for detecting exercise induced changes in cardiac systolic and diastolic function in a group of unselected patients with prior small MI. Despite reduced systolic function at rest in the patients, a systolic reserve was seen during exercise. In contrast, early diastolic annular velocities were similar with those in the control group, but levelled off during exercise, and thus there was lack of a diastolic reserve. The estimate of filling pressure (E/e') increased during exercise. Thus, upright exercise echocardiography unmasks diastolic dysfunction not seen at rest in this population.

Paper II

High-intensity interval training in HTx recipients improves exercise capacity. However, cardiac systolic and diastolic function investigated both at rest and during submaximal exercise echocardiography, was predominantly unchanged after the exercise intervention. Both a systolic and a diastolic reserve capacity were present during exercise echocardiography at baseline, but did not increase in the exercise group at follow-up. A transplanted heart responds differently to aerobic exercise training than a non-transplanted heart.

Paper III

A long-term, partly supervised and community-based HIIT-program is an applicable, effective and safe way to improve exercise capacity in HTx patients. Muscular endurance increased, general health improved and HR_{peak} increased toward normal values. This suggests that such form of exercise program should be applied to a broader population of HTx recipients.

Paper IV

Prolonged GIT impairs both systolic and diastolic function measured at the interventricular septum and RV free wall in stable HTx recipients, whereas increasing donor age impairs LV diastolic function only. Although the impairment is moderate and does not influence exercise capacity, the duration of graft ischemia and donor age should be taken into account when evaluating for cardiac dysfunction in HTx recipients.

10 Future perspectives

Through the course of this thesis, new and interesting questions have arisen that deserve further attention.

The fact that the comprehensive exercise program only had minimal effects on a transplanted heart raises several hypotheses. We included HTx recipients 1-8 years after transplantation, and possibly this was too late, as long-term effects (and side-effects) of immunosuppressive and immunological reactions to the graft might have influenced the results. Thus, it would be interesting to investigate the cardiac effects of introducing HIIT in newly transplanted patients, and to determine in a prospective study whether this intervention translates into a better prognosis in HTx recipients. Further, myocardial biopsies and thereby information about the myocardial function on a cellular level such as contractility and calcium-handling, might give additional basal information about the cardiac adaptation to exercise training in HTx recipients.

In our work we only used echocardiography for assessment of cardiac function. However, La Gerche *et al* have recently shown that LV mass measured by cardiac magnetic resonance imaging (MRI) was the strongest predictor for VO_{2peak} in healthy trained and untrained individuals, and superior to LV mass, LV volumes and parameters reflecting LV function (strain, SR, s', e' and EF measured both at rest and during exercise) measured by echocardiography.[117] Thus, investigating the effects of exercise training with cardiac MRI could give further information about potential cardiac adaptation to exercise training in HTx recipients.

In our work we have mainly measured cardiac function and deformation in the longitudinal direction. However, LV rotation also gives valuable information about early, active LV relaxation and suction,[155,156] LV rotation can be measured accurately by speckle tracking echocardiography.[157] Esch *et al* have recently investigated the LV torsion and untwisting in HTx recipients during exercise, and found that HTx recipients had blunted peak LV torsion and untwisting during exercise compared to donor age-

matched individuals. Thus, it could be of interest to investigate whether LV rotation improves by HIIT in HTx recipients, and further whether prolonged GIT impairs LV rotation.

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Paper I

Upright bicycle exercise echocardiography in patients with myocardial infarction shows lack of diastolic, but not systolic, reserve: a tissue Doppler study

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KEYWORDS
Echocardiography;
Exercise;
Myocardial infarction;
Diastole;
Tissue Doppler imaging

Aims The aim of this feasibility study was to compare systolic and diastolic left ventricular (LV) function during upright bicycle exercise in patients with chronic myocardial infarction (MI).
Methods and results Eighteen patients with first-time MI and no signs of heart failure at rest underwent upright bicycle exercise at 25, 50, and 75 W, and were compared with 18 age-matched controls. Systolic (S′) and early (E′) mitral annular velocities and early mitral filling velocity (E) were measured at each stage. LV ejection fraction was lower in the MI group (46 vs. 54%, $P < 0.01$), while end-diastolic volumes were similar. S′ was lower in the MI patients, but increased during exercise in both groups. E′ was similar at rest, but increased in the control group only. Early mitral filling (E) increased in both groups, thus the E/E′ ratio increased during exercise in the MI group only. Heart rate was similar in both groups.
Conclusions Upright exercise echocardiography is feasible and can unmask early diastolic dysfunction and increased LV filling pressures in patients with small prior MIs.

Introduction

Diastolic function is determined by the rate of myocardial relaxation, a combination of an active ATP-consuming process and myocardial tissue properties (recoil and compliance). After an acute myocardial infarction (MI), accumulation of interstitial collagen in the myocardium due to necrosis and fibrosis leads to a stiff myocardium.[1] Residual ischaemia may also impair the active part of relaxation. The myocardial injury therefore leads to both systolic and diastolic dysfunction.[1,2] Previous studies have shown that diastolic dysfunction is an important predictor of morbidity and mortality after MI.[3,4] Diastolic dysfunction can cause elevated left ventricular (LV) filling pressure, which is associated with reduced exercise capacity[5] and an increased likelihood of remodelling.[6]

Mitral annular early diastolic velocity, measured by tissue Doppler (E′), quantifies the diastolic function of the LV.[7] The ratio of early transmitral flow (E) to early diastolic mitral annular velocity (E/E′ ratio) has been shown to be a non-invasive marker of LV filling pressure both at rest[8,9]

and during supine exercise.[10,11] The E/E′ ratio also predicts elevated LV filling pressure in the upright position.[12] Importantly, tissue Doppler mitral annular velocities allow systolic and diastolic functions to be compared with the same method.[13]

In patients with sustained MI, limited exercise capacity during physical activity is an important symptom and target for treatment. In normal subjects LV contraction and relaxation velocities increase during incremental exercise, and this systolic and diastolic reserve is necessary to increase cardiac output and supply oxygen to exercising muscles.[14] In patients with MI, studies using radionuclide techniques have shown that diastolic function during exercise seems more closely linked to exercise capacity than systolic function.[15] Using tissue Doppler echocardiography, myocardial function can be studied non-invasively during upright exercise, which is more relevant for daily activity than supine measurements. Therefore, the aim of the present study was to see whether upright bicycle exercise echocardiography is a feasible method for studying changes in systolic and diastolic function during moderate-intensity exercise in a group of unselected patients with prior MI.

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Methods

Population

Twenty-one patients with first-time ST-elevation MI at least 3 months earlier were asked to participate. Patients with significant arrhythmias or valve disease, recurrent ischaemia, or unable to use bicycle were excluded. LV ejection fraction (EF) was not used as a selection criterion. One patient was excluded due to poor image quality in upright position and two patients due to valve disease. Thus, 18 patients were included in the study (11 anterior and 7 posterior wall infarctions, EF range 25–63%). Twenty-one healthy age-matched volunteers were asked to participate as controls. They had no history of heart disease and no risk factors (smoking, hypertension, diabetes mellitus, hypercholesterolaemia, or sudden death in first degree relative <60 years). One was excluded because of a previously undiscovered severe aortic insufficiency and two because of poor image quality in upright position. Thus, 18 controls were included. For patient characteristics see *Table 1*. All patients had a coronary angiogram at the time of the MI. The study was approved by the regional ethical committee, and conducted according to the declaration of Helsinki. All patients gave written informed consent.

Examinations

Echocardiography was performed with Vivid 7 and a 2.5 MHz phased array sector transducer (GE Vingmed Ultrasound, Horten, Norway) by a single experienced operator. The recordings at rest were made with the subjects in the left lateral position and in end-expiratory apnea. The exercise echo examination was performed on a multistage upright bicycle ergometer. Subjects were examined at rest (supine and upright) and during upright bicycling at workloads of 25, 50, and 75 W, each stage lasting 5 min. Higher

Table 1 Demographics, drug therapy, and basic echocardiographic characteristics

	Infarction (n = 18)	Controls (n = 18)	P-value
Demographics			
Age (years)	59 ± 9	63 ± 11	0.246
Male/women	14/4	13/5	
BMI (kg/m^2)	25 ± 3	24 ± 3	0.712
BSA (m^2)	1.9 ± 0.2	1.9 ± 0.1	0.788
Diabetes	1	0	
Hypertension	1	0	
Systolic BP rest	131 ± 16	128 ± 9	0.583
Diastolic BP rest	82 ± 6	82 ± 8	0.933
Drug therapy			
Beta-blocker	18 (100%)	0	
ACE/ATII	4 (22%)	0	
Diuretics	2 (11%)	0	
Echocardiography, rest			
EF (%)	46 ± 7	53 ± 8	0.005
WMSI	1.2 ± 0.2		
EDV (mL)	130 ± 31	138 ± 41	0.504
EDVi (mL/m^2)	68 ± 12	73 ± 16	0.288
LA (cm^2)	21 ± 3	21 ± 6	0.977
LAi (cm^2/m^2)	11 ± 2	11 ± 2	0.931
IVRT (ms)	85 ± 15	87 ± 14	0.680

Values are mean ± SD.

ACE, angiotensin converting enzyme inhibitor; ATII, angiotensin II receptor blocker; BMI, body mass index; BSA, body surface area; EDV, end-diastolic volume; EDVi, EDV indexed to BSA; EF, ejection fraction; IVRT, isovolumetric relaxation time; LA, left atrial area; Lai, LA area indexed to BSA; WMSI, wall motion score index.

loads were not performed due to unsatisfactory image quality with increasing upper body motion and respiration frequency. The pedal-frequency was 60–70 rounds per minute. Two MI patients did not reach 75 W, one because of dyspnea (this patient had chronic obstructive pulmonary disease as well) and one because of muscular fatigue. No patients reported chest pain. Four- and two-chamber views with tissue Doppler overlay (framerate 100–160 frames/s) and transmitral PW Doppler flow were obtained at each stage, 2 min after each change in workload.

Analysis

All data were stored digitally and analysed offline. Mitral annular velocities, strain rate, and end-diastolic strain were analysed in a customized software (GcMat, GE Vingmed Ultrasound) running under Matlab (MathWorks, Natic, MA, USA). Other measurements were made in Echopac PC (GE Vingmed Ultrasound). Mitral annular velocities in systole (S′) and early (E′) and late (A′) diastole were measured by colour tissue Doppler images in the septal, lateral, anterior, and inferior points and averaged. Recordings with fused diastolic mitral annular velocities (E′ and A′) were excluded. Systolic strain rate and end-systolic strain were measured in tissue Doppler images, by a previously described method. The method tracked the two-dimensional motion of the segment borders by combining tissue Doppler and speckle tracking methods, and calculated angle-independent strain and strain rate values from the measured change of segment length through the cardiac cycle.[16] The average of the six segments analysed in both the four- and two-chamber views was used. Recordings where less than 6 of the 12 segments could be analysed were excluded. Complete datasets were obtained in 14 subjects in each group.

When fusion of the early (E) and late (A) mitral filling waves occurred, it was analysed as an E wave. Even with complete merging of the E and A velocities, E/E′ can be used to estimate pulmonary capillary wedge pressure.[17] Left ventricular ejection fraction was measured in the supine position by the modified Simpson's method using the four- and two-chamber views. Left atrial area was measured in the four-chamber view. Systolic mitral annular excursion (MAE) was measured from the integrated tissue Doppler velocity signal in the same four points as the mitral annular velocities, and averaged. Isovolumetric relaxation time (IVRT) and ejection time (ET) were measured in the upright position at rest, 25 and 75 W, by averaging three cycles.

Statistics

Values are presented as mean ± SD. Independent samples *t*-tests were used to compare the MI and control group at each stage. Paired samples *t*-tests were used to compare measurements in supine and upright position at rest, and a general linear model for repeated measurements, with exercise intensity (25, 50, and 75 W) as within-subjects and MI-/control group as between-subjects factors, was used to evaluate the change during exercise (Greenhouse–Geisser). The trend during increasing exercise intensity in each group was compared by testing for a significant linear effect in the intensity–group interaction in within-subjects contrasts. Isovolumetric relaxation time, ET, and deceleration time (DT) were compared using paired samples *t*-test with *post hoc* Bonferroni correction of *P*-values ($n = 2$). Differences were considered significant for *P*-values less than 0.05. Analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

There were no significant differences in demographics between the two groups (*Table 1*). Of the MI patients, 16 were successfully treated with reperfusion in the acute phase, 15 with percutaneous coronary intervention (PCI) and one with thrombolysis. The median time between

Table 2 Exercise echocardiography

	Supine	Sitting	25 W	50 W	75 W
Diastolic function					
E (cm/s)					
Infarction	$58 \pm 9^{\dagger}$	$43 \pm 12^{*}$	59 ± 14	$68 \pm 16^{\dagger}$	$79 \pm 19^{\S}$
Control	49 ± 12	$39 \pm 7^{*}$	50 ± 8	57 ± 11	$66 \pm 13^{\S}$
A (cm/s)					
Infarction	54 ± 12	$46 \pm 12^{*}$	57 ± 17	62 ± 16	$72 \pm 17^{\S}$
Control	52 ± 16	$42 \pm 15^{*}$	49 ± 10	54 ± 10	$61 \pm 13^{\S}$
E′ (cm/s)					
Infarction	6.4 ± 1.4	$3.6 \pm 1.2^{*}$	6.0 ± 1.8	6.6 ± 2.0	$6.5 \pm 1.9^{\dagger\dagger}$
Control	6.8 ± 2.4	$4.4 \pm 1.3^{*}$	6.8 ± 1.4	8.0 ± 1.9	$8.7 \pm 1.7^{\S}$
E/E′					
Infarction	9.4 ± 2.9	$12.7 \pm 4.0^{*\dagger}$	10.6 ± 4.1	$11.4 \pm 5.0^{\dagger}$	$13.6 \pm 6.1^{\ddagger\S\dagger}$
Control	8.0 ± 2.7	$9.9 \pm 3.2^{*}$	7.6 ± 1.4	7.4 ± 1.4	7.8 ± 1.6
EDV (mL)					
Infarction	130 ± 31	$103 \pm 29^{*}$	126 ± 27	131 ± 29	$134 \pm 29^{\ddagger}$
Control	138 ± 41	$106 \pm 34^{*}$	129 ± 43	127 ± 39	125 ± 34
Systolic function					
S′ (cm/s)					
Infarction	$5.6 \pm 1.1^{\dagger}$	$5.1 \pm 1.0^{*\dagger}$	$6.3 \pm 1.2^{\dagger}$	$7.2 \pm 1.7^{\dagger}$	$7.7 \pm 1.7^{\S\dagger}$
Control	6.5 ± 1.1	$5.8 \pm 0.8^{*}$	7.5 ± 1.6	8.3 ± 1.4	$9.4 \pm 1.7^{\S}$
SRs (s^{-1})					
Infarction	-0.81 ± 0.10	-0.82 ± 0.12	$-0.94 \pm 0.20^{\dagger}$	-1.11 ± 0.25	$-1.24 \pm 0.4^{\S}$
Control	-0.86 ± 0.11	-0.91 ± 0.17	-1.1 ± 0.15	-1.19 ± 0.17	$-1.37 \pm 0.18^{\S}$
MAE (mm)					
Infarction	$11.2 \pm 2.2^{\dagger}$	$7.9 \pm 2.5^{*}$	$10.7 \pm 2.0^{\dagger}$	$11.7 \pm 2.3^{\dagger}$	$11.9 \pm 2.7^{\S}$
Control	12.6 ± 1.9	$9.3 \pm 2.0^{*}$	12.1 ± 2.2	13.3 ± 2.4	$13.1 \pm 2.1^{\S}$
ϵ_{ES} (%)					
Infarction	$-14 \pm 3^{\dagger}$	$-11 \pm 3^{*\dagger}$	$-12 \pm 3^{\dagger}$	$-13 \pm 3^{\dagger}$	$-13 \pm 4^{\S\dagger}$
Control	-15 ± 2	$-13 \pm 2^{*}$	-15 ± 3	-17 ± 3	$-17 \pm 2^{\S}$
HR					
HR (bpm)					
Infarction	59 ± 9	$66 \pm 10^{*}$	75 ± 11	86 ± 14	$99 \pm 16^{\S}$
Control	58 ± 10	$64 \pm 8^{*}$	73 ± 14	85 ± 17	$98 \pm 23^{\S}$

Echo measurements during rest in supine and upright position, and during the exercise stages. Values are mean $\pm$ SD.

A, late diastolic mitral flow velocity; E, early diastolic mitral flow velocity; E′,early diastolic mitral annular velocity; EDV, left ventricular end-diastolic volume; HR, heart rate; MAE, mitral annular excursion; S′, systolic mitral annular velocity, SRs, peak systolic strain rate; ϵ_{ES}, end-systolic strain.

$^{*}P < 0.05$ compared with supine position, $^{\dagger}P < 0.05$ compared with same stage in controls, ‡significant difference between groups for trend during exercise ($P < 0.05$), §significant increase during exercise ($P < 0.01$).

infarction and examination was 6 months [range 4–9 months (one patient 10 years)]. At the time of the study, five patients had significant stenoses ($>50\%$) in one of the non-culprit major epicardial arteries, found at the time of the initial angiography, and still untreated by PCI. All patients in the MI group were on beta-blockers. EF was lower in the MI group than in the control group ($P = 0.005$), while left atrial area was similar (*Table 1*). Heart rate (HR) increased significantly from supine to sitting, with a further increase at each stage for both groups, with no differences between the groups (*Table 2*).

Diastole during exercise

E′ showed no difference between the two groups at rest, while E was higher in the MI group in supine ($P < 0.04$), but not upright position at rest. E′ did not increase during exercise in the MI group, in opposite to the control group, where E′ increased significantly with increasing intensity ($P = 0.029$ between groups) (*Figure 1*). Accordingly, E′ was significantly different between the two groups at 75 W ($P = 0.003$). E increased during exercise within both groups, with higher values in the MI group at 25, 50, and 75 W ($0.047 < P < 0.057$ for all). Consequently, E/E′ increased during exercise in the MI group ($P = 0.022$ between groups), and E/E′ was higher in the MI group than in the control group at 50 and 75 W ($P = 0.03$ and $P = 0.004$, respectively). A′ increased with increasing intensity in both groups ($P < 0.001$). The E/A ratio did not change between stages, nor were there any differences between the groups. DT decreased during exercise in both groups, with a significantly lower value in the MI group compared with the control group at 25 W ($P = 0.016$). Isovolumetric relaxation time decreased from rest to 25 W and further during exercise in both groups. During exercise EDV tended to increase in the MI group, and decrease in the control group ($P < 0.01$ for difference in trends).

Systole during exercise

S′ increased with increasing exercise intensity for both groups ($P < 0.001$), and was lower in the MI group than in the control group at all stages (*Figure 1*). The absolute value of peak systolic strain rate increased significantly
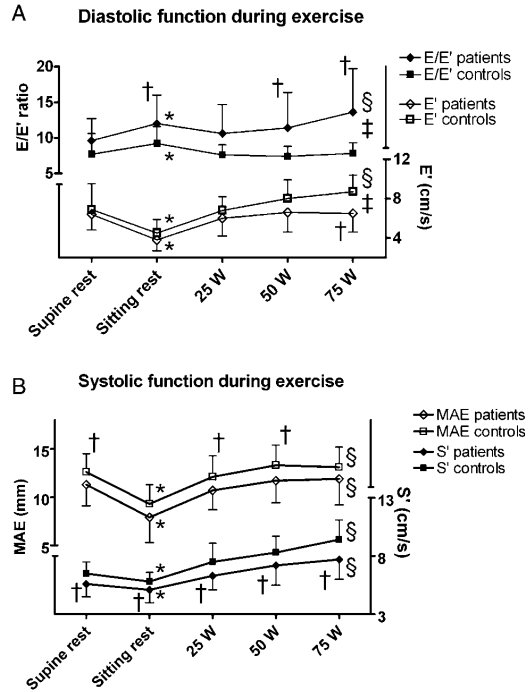
A   Diastolic function during exercise

B   Systolic function during exercise

Figure 1 (*A*) Diastolic function during exercise. (*B*) Systolic function during exercise. (*A*) Changes in the ratio of early diastolic mitral filling velocity to early diastolic mitral annular velocity (E/E′) (the two upper lines) and E′ (the two lower lines) between the stages in patients with myocardial infarction and controls. (*B*) Corresponding changes in mitral annular excursion (MAE) and systolic mitral annular velocity (S′). *$P < 0.05$ compared to supine position, †$P < 0.05$ compared to same stage in controls, ‡significant difference between groups for trend during exercise ($P < 0.05$), §significant increase during exercise ($P < 0.01$). Markers indicate mean and 1 SD.

with increasing intensity in both groups ($P < 0.001$). In contrast to S′, the difference between the groups was significant only at 25 W ($P = 0.043$). Mitral annular excursion increased during exercise in both groups ($P = 0.001$), and was lower in the MI group at 50 and 75 W. The absolute value of end-systolic strain increased during exercise in both groups ($P < 0.01$), but this was mostly due to the change from 25 to 50 W, in line with the MAE data. The control group had significantly higher absolute value of end-systolic strain than the MI group at all stages. ET decreased from 25 to 75 W in both groups (*Table 3*).

In the MI patients there was a weak correlation between EF at rest and the systolic reserve (difference between S′ at 75 W and S′ at rest) ($r = 0.43$, $P = 0.094$). Systolic reserve was not related to wall motion score index at rest ($r = -0.23$, $P = 0.39$). Diastolic reserve (E′ at 75 W and E′ at rest) was not related to neither EF nor WMSI at rest ($r = 0.052$, $P = 0.86$ and $r = 0.10$, $P = 0.74$, respectively).

Only five patients had untreated residual significant stenoses in a non-culprit coronary artery at the time of the study. Comparing the patients with and without residual stenoses, there were no significant differences neither for

Table 3 Systolic and diastolic time intervals at rest and during exercise

	Sitting	25 W	75 W
ET (ms)			
Infarction	326 ± 24	328 ± 44	282 ± 57*
Control	304 ± 45	322 ± 22	277 ± 31*
IVRT (ms)			
Infarction	118 ± 22	83 ± 24*	60 ± 18*
Control	105 ± 18	75 ± 13*	54 ± 12*
DT (ms)			
Infarction	240 ± 56	175 ± 46*†	148 ± 66
Control	254 ± 44	218 ± 53*	161 ± 48*

Values are mean ± SD.
*$P < 0.05$ compared with previous stage, †$P < 0.05$ compared with same stage in controls.
DT, deceleration time of the early diastolic mitral velocity; ET, ejection time of the systole; IVRT, isovolumteric relaxation time.

S′, E′, nor E/E′. At 75 W, the S′ values were 6.8 ± 1.9 vs. 7.9 ± 1.6 ($P = 0.239$), E′ 6.4 ± 2.7 vs. 6.5 ± 1.6 ($P = 0.94$), and E/E′ 16 ± 7.7 vs. 13 ± 5.7 ($P = 0.44$) for the patients with and without residual stenoses, respectively. No new or worsening wall motion abnormalities were detected during exercise in any of the patients. Three patients showed improved wall motion in hypo-/akinetic segments during exercise.

Changes from supine to upright position

From supine to upright position, E, E′, S′, MAE, EDV, and end-diastolic strain decreased significantly for both the MI and the control group (*Table 2, Figure 1*A and *B*). The E/E′ ratio increased from supine to upright position in both groups (both $P < 0.02$). Peak systolic strain rate and E/A were unchanged.

Discussion

Diastolic function and left ventricular filling pressure during exercise

As contractility increases during exercise in healthy subjects, early diastolic relaxation rate increases as well.[14,18,19] The increase is caused by the positive relaxation-frequency relationship (Bowditch effect), increased recoil, and increased sympathetic tone and levels of catecholamines, giving a lower early diastolic LV pressure during isovolumic relaxation.[20,21] This is consistent with the increase in E′ in the control group. In the MI group there was no increase in E′ with increasing exercise intensity, thus the MI patients seem to have a blunted relaxation reserve. E increased with increasing intensity in both groups, partly due to increased venous return and increased effective circulating blood volume. In addition, improved relaxation (E′) contributed to the increase in the healthy subjects, while an increased filling pressure was a likely contributor in the MI patients, as E′ was unchanged from 25 to 75 W. Consequently, E/E′ increased during exercise in the MI group, but not in the controls. An increased filling pressure in the MI group is consistent with the increase in EDV.

Previous invasive studies of upright exercise have shown moderately increased or unchanged LV end-diastolic pressure in normal subjects,[22] and increased LV filling pressure in patients with coronary artery disease.[23] Studies using the E/E' ratio have also found an increased ratio during exercise, although these studies were done in supine position on patients with various kinds of heart disease.[10,11]

Isovolumetric relaxation time and DT decreased during exercise in both groups. As E' increased in the control group only, we suggest that the change in IVRT and DT was due to a more rapid relaxation in the controls, and an increased filling pressure in the patients.

Systolic function

As expected, systolic function was reduced both at rest and during exercise in the MI patients. However, indices of LV contractility (S' and SR$_s$) increased in both groups, likely due to increased sympathetic tone and the force-frequency relationship.[24] Reduced afterload during exercise may also have contributed.[25] The increased EDV and filling pressure-indices in the MI group suggest that this contractile reserve was partly a Frank-Starling effect. The lack of change in EDV or E/E' in the control group suggests that the Frank–Starling mechanism is not used by healthy individuals, at least during moderate-intensity exercise.[14,26]

Although there was no difference in systolic reserve during exercise between the two groups, the weak relationship (trend) between EF at rest and the systolic reserve in the MI group suggests that this might be due to small infarctions in the included patients. As only 3 of 18 MI patients showed improvement in wall motion score at peak exercise, we were not able to determine the contribution of stunned or hibernated myocardium to the measured systolic reserve during exercise.

Changes from supine to upright position

In accordance with previous findings[14,27] we found evidence of a decreased venous return and LV filling (EDV and E), a decreased stroke volume (MAE, strain, and S'), and a compensatory increase in HR during the transition from supine to upright position. E' decreased as well, suggesting that it is preload-dependent in an acute setting.[14] Invasive studies on normal subjects and patients with coronary artery disease conclude that LV filling pressure is similar or lower in the upright than in the supine position.[22,23] Thus, the observation that E/E' increased from supine to upright position and then tended to decreased to 25 W confirms previous studies,[28,29] and identifies a limitation of E/E' as a marker of LV filling pressure. The E/E' ratio has only been validated in the supine or semi-supine position.[9,11,30]

Possible mechanisms

The findings in the present study might be explained by an ability of the remaining viable myocardium to compensate for the loss of contractile force, but not the loss of actively relaxing and recoiling tissue. Furthermore, a reduced stroke volume (MAE) in the MI patients probably reduced the recoiling forces and thereby E'. Alternatively, our findings could be caused by general rather than regional myocardial impairment, possibly linked to the atherosclerotic process.

This is supported by a previous study where no relation was observed between the E/E' ratio and infarct size.[6] Related to this, pre-infarction hypertension reduces the regain of diastolic function after an infarction.[31,32] Better endothelial function in the control group, and thus lower afterload, may also explain some of the differences between the groups. The normal left atrial area in the MI group indicates that LV filling pressure was not chronically elevated.

Tissue edema secondary to acute reperfusion can affect both systolic and diastolic function, but disappears earlier than 3 months, which was the lower time limit for inclusion. Previous studies have shown that increased ventricular and arterial stiffness leads to increased energy demand at higher stroke volumes, impaired relaxation, and reduced diastolic reserve during exercise.[33,34] LV and arterial stiffness was not assessed in the present study, but was probably higher in the MI group.

Ischaemia could also have caused the differences in the present study, but the study was not powered to study this.

Clinical implications

The present study demonstrates that moderate-intensity exercise echocardiography is feasible, and gives additional information about LV diastolic function in patients with relatively small MIs. It remains to be determined if this information can give incremental prognostic or diagnostic power compared with diastolic function and estimates of LV filling pressure obtained at rest.[4,6,35]

Limitations

Angiotensin-converting enzyme-inhibitors, angiotensin II-antagonists, and diuretics reduce both pre- and afterload, and would be expected to normalize findings in the MI group. Thus, it is unlikely that our findings were due to these drugs. The effect of beta-blockers on LV relaxation is not straightforward to predict.[36,37] Maximum work capacity or HR were not measured, so we could not calculate the individual relative exercise intensity. We chose to use fixed workloads, as this is more attractive in clinical practice. The similar HRs in MI patients and controls probably reflect a tendency to underestimate the needed beta-blocker dose. The low signal-to-noise ratio in strain rate-measurements can probably explain why strain, but not SR, was significantly different between the two groups.

Conclusion

Despite reduced systolic function at rest, patients with small prior MIs were able to increase their systolic annular velocities during exercise, probably due to utilization of the Frank–Starling mechanism. In contrast, the early diastolic annular velocities leveled off, and the estimate of filling pressure (E/E') increased during exercise. Thus, we conclude that upright exercise echocardiography is feasible and unmasks diastolic dysfunction not seen at rest in this population. Future studies should evaluate the prognostic significance of this finding.

Funding

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Paper II

European Journal of

Preventive Cardiology

One year of high-intensity interval training improves exercise capacity, but not left ventricular function in stable heart transplant recipients: A randomised controlled trial

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Abstract

Background: Heart transplant recipients have lower exercise capacity and impaired cardiac function compared with the normal population. High-intensity interval training (HIIT) improves exercise capacity and cardiac function in patients with heart failure and hypertension, but the effect on cardiac function in stable heart transplant recipients is not known. Thus, we investigated whether HIIT improved cardiac function and exercise capacity in stable heart transplant recipients by use of comprehensive rest- and exercise-echocardiography and cardiopulmonary exercise testing.

Design and methods: Fifty-two clinically stable heart transplant recipients were randomised either to HIIT (4×4 minutes at 85–95% of peak heart rate three times per week for eight weeks) or to control. Three such eight-week periods were distributed throughout one year. Echocardiography (rest and submaximal exercise) and cardiopulmonary exercise testing were performed at baseline and follow-up.

Results: One year of HIIT increased VO_{2peak} from 27.7 ± 5.5 at baseline to 30.9 ± 5.0 ml/kg/min at follow-up, while the control group remained unchanged (28.5 ± 7.0 *vs.* 28.0 ± 6.7 ml/kg per min, $p < 0.001$ for difference between the groups). Systolic and diastolic left ventricular functions at rest and during exercise were generally unchanged by HIIT.

Conclusions: Whereas HIIT is feasible in heart transplant recipients and effectively improves exercise capacity, it does not alter cardiac systolic and diastolic function significantly. Thus, the observed augmentation in exercise capacity is best explained by extra-cardiac adaptive mechanisms.

Introduction

Heart transplant (HTx) recipients are exposed to various risk factors for cardiovascular disease and may suffer from severe complications over time, for example cardiac allograft vasculopathy (CAV) and hypertension.[1] Even though exercise capacity improves after HTx, it still remains subnormal compared with healthy individuals.[2] The underlying mechanisms for the reduced exercise capacity are not completely understood, but it is probably due to a combination of central and peripheral physiological abnormalities.[2,3] Both chronotropic incompetence due to denervation as well

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as reduced stroke volume with reduced cardiac output (CO) has been proposed as an explanation for the reduced exercise capacity, but peripheral factors such as reduced muscle strength, reduced oxidative capacity, reduced capillary density and endothelial dysfunction[2,3] also contribute.

High-intensity interval training (HIIT) has demonstrated superior effects on exercise capacity and cardiac function when compared with moderate intensity exercise in patients with heart failure[4] and hypertension.[5] Several randomised-controlled-trials on HTx recipients have shown a positive effect on exercise capacity after HTx,[6–9] but the effect is modest and the mechanisms of effect are unclear. Only one study has involved exercise training with high intensity[9] and few have investigated the cardiac effects of the exercise-intervention in HTx recipients by echocardiography.[8] None have investigated how exercise training influences cardiac systolic and diastolic function by comprehensive echocardiography techniques utilising tissue Doppler measurements and regional myocardial deformation analysis. By echocardiography it is possible to quantify changes in myocardial function both at rest and during exercise. Tissue Doppler imaging (TDI) enables quantification of both systolic and diastolic myocardial function on a beat-to-beat basis,[10] while 2D speckle tracking enables regional deformation (e.g. strain) to be quantified.[11]

Thus, we hypothesised that a well organised HIIT will improve exercise capacity and cardiac function, measured by CPET and echocardiography at rest and during exercise in HTx recipients.

Methods

Study population and randomisation

We prospectively recruited 57 clinically stable recipients during their annual follow-up between 2009 and 2010 (Figure 1; flow chart). The inclusion criteria were assessment 1–8 years after HTx, optimal medical treatment, stable clinical condition, ability to perform maximal exercise testing and willingness to fulfil one year of exercise training. Exclusion criteria were heart failure, clinical signs of rejection, atrial fibrillation, need for revascularisation or other intervention. Of the initially 57 recipients, 52 were eligible for randomisation (Figure 1). Participants were randomly assigned in a 1:1 ratio to either HIIT (exercise group (EG)) or control group (CG) stratified by time after heart transplant surgery (1–2 years after HTx and 3–8 years after HTx). Consecutively numbered, sealed envelopes were provided by an independent statistician before inclusion started.

All recipients were treated according to our immunosuppressive protocol (Table 1), and all

recipients had bi-caval right atrial anastomosis. All participants were >18 years of age, and all gave written informed consent. The study was approved by the Regional Ethics Committee in Norway, and conducted according to the declaration of Helsinki. (ClinicalTrial.gov Identifier: NCT 01091194.)

Exercise training intervention

Recipients randomised to HIIT met for supervised training by a local physiotherapist. The intervention was divided into three eight-week periods of exercise with three sessions every week. The last eight-week period was finished 1–2 weeks before follow-up. All training consisted of walking or running uphill on a treadmill, with a 10-minute warm-up and 4×4-minute intervals at 85–95% of peak heart rate (HR_{peak}), separated by three-minute active pauses, corresponding to Borg scale 11–13. Subjects in the EG had a heart rate (HR) monitor (Polar Electro, Kempele, Finland) to obtain the assigned exercise intensity. Speed and inclination of the treadmill were adjusted continually to ensure that every training session was carried out at the assigned HR throughout the training period. Subjects in the EG were encouraged to exercise on their own between the eight-week periods. The CG was requested to continue exercise as before inclusion.

Cardiopulmonary exercise testing

The maximal exercise capacity was tested using a modified treadmill walking test following the European Society of Cardiology's recommendations for CPET in chronic heart failure patients.[12] The treadmill protocol was carried out as previously described.[13] A Borg score >18 and/or respiratory exchange ratio (RER) ≥ 1.05 were used as criteria for an adequate maximal exercise test. Predicted values were based on the American College of Sports Medicine 2009 guidelines.[14]

Echocardiography

Echocardiography was performed with GE Vivid 7 or E9 with a phased array sector transducer (GE Vingmed Ultrasound, Horten, Norway). The echocardiographic data were stored digitally and analysed off-line in dedicated software (EchoPAC, GE Vingmed Ultrasound). Two-dimensional grey-scale echocardiographic recordings and additional colour tissue Doppler imaging (cTDI) of standard apical projections (four-chamber, two-chamber and long axis) comprising three consecutive heart cycles each were obtained according to recommendations.[15] Blood flow velocities in the left ventricular (LV) outflow tract and mitral inflow were
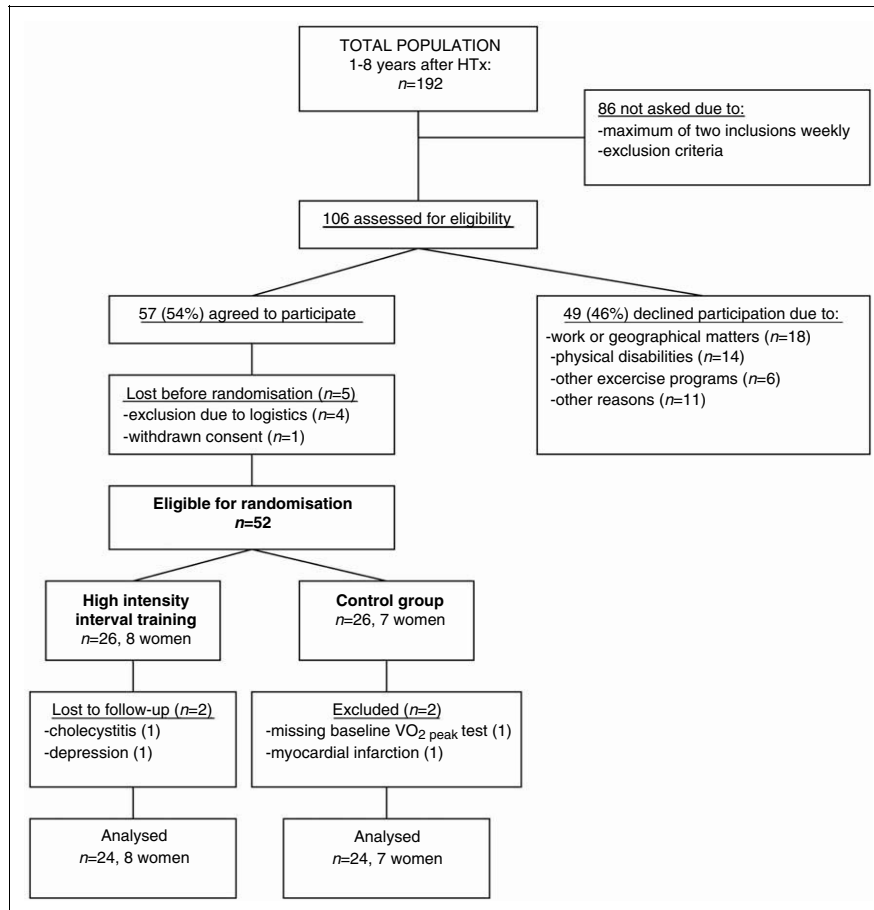
Figure 1. Patient flow of the study population.

obtained from the apical position using pulsed wave Doppler with the sample volume at the aortic annulus and tip of mitral leaflets, respectively.

The average of at least three cardiac cycles was used in analysis of Doppler recordings. LV early (E) and late (A) mitral inflow velocity, deceleration time of the E-wave (DT) and isovolumic relaxation time (IVRT) were measured from the pulsed wave Doppler signal. LV mitral annular velocities in systole (LVs′), early (LVe′) and late diastole (LVa′) were measured in the septal, lateral, anterior and posterior mitral annular regions by cTDI and averaged. LV systolic mitral annular displacement (distance) was calculated by integration of tissue Doppler velocity signal (distance per time)

at the same four regions, and averaged. The ratio of early diastolic mitral flow velocity (E) to early diastolic mitral annular velocity (LVe′) was calculated. The E/e′ reflects left ventricular filling pressure,[16] and has also been validated in HTx recipients.[17] Right ventricular (RV) systolic and early diastolic annular velocities (RVs′ and RVe′), as well as RV displacement, were measured in the lateral tricuspid annular region in the apical four-chamber view by cTDI. LV peak-systolic longitudinal strain was measured using speckle-tracking echocardiography (2D strain method) in an 18-segment model converted to a 16-segment model by exclusion of the apical cap.[15] LV global longitudinal strain was obtained by averaging the peak systolic

Table 1. Patient characteristics at baseline

Variable	EG ($n = 24$)	CG ($n = 24$)	p-value
demographics			
Recipient age, years	56 (20–72)	58 (19–71)	0.445
Recipient male gender, %	67	71	1.000
Graft ischaemic time, minutes	211 (50–291)	184 (44–301)	0.734
Years since HTx	5 (1–8)	4 (1–7)	0.505
BMI, kg/m^2	27.2 ± 5	26.3 ± 4	0.481
Primary diagnosis, % (n)			
Cardiomyopathy	58 (14)	50 (12)	0.934
Ischemic heart disease	29 (7)	29 (7)	
Congenital heart disease	4 (1)	8 (2)	
Other	8 (2)	13 (3)	
Prior rejections, % (n)			
2R[a]	25 (6)	4 (1)	0.087
AMR-1[a]	4 (1)	4 (1)	
2R and AMR-1[a]	0	8 (2)	
Medications, %			
Cyclosporine/tacrolimus/everolimus	92/8/13	80/13/21	ns
Mycophenolate mofetil/azathioprine	92/0	96/4	ns
Corticosteroids	96	88	0.609
Statin	100	100	–
Beta blocker	17	25	0.724
Calcium-channel blocker	17	33	0.559
ARB/ACE-inhibitor	29	42	0.547
Biochemical data			
Haemoglobin, g/dl	13.9 ± 1.3	13.9 ± 1.2	1.000
eGFR, ml/min/1.73m^2	60 (34–60)	60 (41–60)	0.025
NT-proBNP, pmol/L	35.0 (2.9–182.0)	29.5 (5.1–159.0)	0.831

Values are expressed as mean ± standard deviation, median (range) or as percentage where appropriate; 2R: moderate rejection; ACE: angiotensin-converting enzyme; AMR-1: antibody-mediated rejection; ARB: angiotensin II receptor antagonist; BMI: body mass index; CG: control group; EG: exercise group; eGFR: estimated glomerular filtration rate; HTx: heart transplant; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; [a]The International Society for Heart and Lung Transplantation (ISHLT) grading system for heart rejections.[19]

shortening in each of the 16 segments. A global average was not calculated if >8 of 16 segments were excluded due to poor tracking ($n = 2$ in EG and $n = 4$ in CG). LV ejection fraction (EF) and LV volumes were measured by the modified Simpson's method using the four- and two-chamber views. Stroke volume (SV) was calculated from pulsed Doppler flow measurement in LV outflow tract assuming a circular aortic annulus, and cardiac output (CO) was obtained by multiplying HR by SV.

Exercise echocardiography

Exercise echocardiography was performed on a bicycle ergometer in the semi-supine position (Ergoline, Germany). Patients pedalled at constant frequency (50–65 rounds per minute), beginning at a workload of 25 watt (W), with an increment of 25W every 2 min. Images were obtained at rest in the semi-supine position and during bicycling at workloads of 50W, 100W and at submaximal load defined by muscular fatigue which usually appeared at 1–2 min/25W before peak termination of the test. Echocardiographic imaging of the apical four- and two-chamber views with tissue Doppler overlay (frame rate 100–160 frames/s) and transmitral pulsed wave Doppler flow were obtained at each stage immediately after increments in load. Blood pressure and ECG were recorded at each workload. The highest load each individual managed (100W or above) was used as the highest submaximal level in the analysis.

Exercise echocardiography was performed in 44 recipients at baseline. Four examinations were not

performed, two due to logistics and two due to poor image quality at rest. At follow-up, exercise echocardiography was performed in 39 recipients; nine examinations were not performed, one due to logistics, four due to missing examination at baseline and four due to poor image quality during baseline exercise echocardiography. Examinations with angle-deviation $>20°$ in the tissue Doppler images or reduced image quality during exercise (lung shadow) were excluded from analysis. Thus, 29 recipients were included in the analyses of exercise echocardiography, 15 in the EG and 14 in the CG.

From the exercise echocardiography test we measured mitral flow, LVs', LVe' and displacement in the four mitral annular regions at rest, 50W, 100W and at submaximal level.

Coronary angiography and endomyocardial biopsies

All but two HTx recipients underwent coronary angiography at both time-points to evaluate for cardiac allograft vasculopathy. We classified vasculopathy as follows: a) severe vasculopathy: significant stenosis, i.e. $\geq50\%$ of lumen diameter in ≥1 major epicardial graft vessel(s) and/or $>70\%$ in ≥2 distal branches; b) moderate vasculopathy: non-significant stenosis, i.e. 33–50% of lumen diameter in ≥1 major epicardial graft vessel(s) and/or $>70\%$ in one distal branch; c) mild vasculopathy: luminal irregulations, i.e. stenosis $<33\%$ of lumen diameter in ≥1 major epicardial graft vessel(s). All angiograms were reviewed by experienced invasive cardiologists, and compared with the previous years angiograms to detect the presence of luminal irregularities or obstructions. Endomyocardial biopsies were performed using standard procedures in all HTx recipients on their first ($n = 9$) or second ($n = 5$) annual medical follow-up visit and in three out of seven recipients on their third annual visit at baseline. Regular blood screening and blood pressure measurements were performed.

All echocardiographic and angiographic analyses were performed blinded to recipients' group assignment.

Statistics

Continuous variables are presented as mean ± SD or as median (range) where appropriate. Within-group changes over time were assessed by paired samples t-test or Wilcoxon non-parametric signed rank rest.

Between-group comparisons at baseline were performed using two-sided independent t-test or Mann–Whitney U test. Comparisons at follow-up were done using analysis of covariance (ANCOVA) with the baseline values as covariates as recommended.[18] However, between-group comparisons at follow-up of the biochemical parameters were done using independent t-test of delta values. For investigating haemodynamic changes during exercise echocardiography, we used a general linear model for repeated measurements ANOVA (Greenhouse–Geisser) with exercise intensity (rest, 50W and submaximal level) as within-subjects factor and group as between-subjects factor, and post-hoc Bonferroni correction was used when comparing two intensity-levels. Categorical variables were compared using the chi-square test or Fischer's exact test. Differences were considered significant for p-levels <0.05. Statistical analyses were performed using standard software (SPSS version 18.0, SPSS Inc., Chicago, Illinois, USA).

Primary outcome was VO_{2peak} and secondary outcomes were left and right ventricular systolic and diastolic function measured with echocardiography. The study was powered to detect a difference in VO_{2peak} of 4 ml/kg/min after exercise-intervention. With an alpha of 5% and 80% power, 14 participates were needed in each group. In order to compensate for dropouts and have greater power for analysis of secondary end-points, we included in total 52 HTx recipients.

Results

HTx recipients and donor characteristics at baseline

After lost to follow-up and exclusions, statistical analyses comprised 24 recipients in each group (Figure 1). Baseline characteristics are given in Table 1, with no significant differences between the EG and the CG, except for estimated glomerular filtration rate (eGFR) ($p = 0.025$), which was lower in the EG.

Exercise intervention

Of the 72 planned HIIT-training sessions, 69 ± 6 sessions were performed with an average intensity of $91.5 \pm 2.5\%$ of HR_{peak} during the intervals. In addition, the EG performed 66 ± 20 solo training sessions of various activities, with a mean intensity of $76 \pm 6\%$ of their HR_{peak}. In the last three months before follow-up testing, 37.5% in the CG had exercised once or less per week, 37.5% two to three times per week and 25% four times or more per week, with an exercise duration ≥30 min length and a Borg scale >14. There were no adverse events during testing, or during the intervention period in either group.

Exercise capacity

HIIT resulted in a significant increase in VO_{2peak} of 3.2 ± 3.1 ml/kg/min (12.7%), while VO_{2peak} remained

Table 2. Effects of high-intensity interval training on exercise capacity

Variable	EG ($n = 24$)		CG ($n = 24$)		Between-groups p-ANCOVA
	Baseline	Follow-up	Baseline	Follow-up	
Exercise capacity					
VO_{2peak}, ml/kg/min	27.7 ± 5.5	$30.9 \pm 5.3^{**}$	28.5 ± 7.0	28.0 ± 6.7	<0.001
% of predicted VO_{2peak}	80 ± 20	$89 \pm 18^{**}$	82 ± 20	82 ± 19	0.255
VO_{2peak}, L/min	2.3 ± 0.5	$2.6 \pm 0.5^{**}$	2.3 ± 0.6	2.3 ± 0.5	<0.001
RER	1.07 ± 0.06	1.08 ± 0.04	1.06 ± 0.05	1.07 ± 0.05	0.336
HR_{peak}, bpm	159 ± 14	$163 \pm 13^{*}$	154 ± 15	154 ± 17	0.016
% of predicted HR_{peak}	93 ± 12	$96 \pm 10^{*}$	93 ± 11	92 ± 10	0.007

Values are expressed as mean $\pm$ standard deviation; CG: control group; EG: exercise group; HR: heart rate; RER: respiratory exchange ratio; $^{**}p \leq 0.001$ and $^{*}p < 0.05$ within groups using paired t-test.

unchanged in the CG (-0.4 ± 2.4 ml/kg/min, -1.1%), resulting in a significant difference at follow-up (Table 2). HR_{peak} increased by 4 bpm ± 8 ($p = 0.012$) in the EG only, while HR_{rest} was unchanged in both groups.

Echocardiographic findings at rest

Over all, no differences were found between the EG and the CG at baseline in LV and RV systolic and diastolic parameters at rest, while only minor differences were found at follow-up (Table 3). There were minor increases in LV annular displacement ($p = 0.018$), LV E ($p = 0.003$), LV DT ($p = 0.017$) and LV E/e$'$ ($p = 0.014$) within the EG from baseline to follow-up. In the CG LVs$'$ ($p = 0.003$) and LVa$'$ ($p = 0.025$) increased from baseline to follow-up and LV E/A ($p = 0.013$) decreased. There were no significant differences within either group in the other cardiac parameters measured.

Echocardiographic findings during exercise

There were no differences between baseline and follow-up data within or between groups in peak Borg score, maximal workload or duration of exercise. Eight recipients in the EG and seven in the CG did not achieve more than 100W on either time-point.

At baseline-testing, all LVs$'$, LVe$'$, E and displacement increased with increasing exercise intensity from semi-supine rest to submaximal load (all p-ANOVA<0.001 for both EG and CG) (Table 4 and Figure 2). Since both E and LVe$'$ increased, E/e$'$ was unchanged during exercise ($p = 0.564$ and $p = 0.129$ for EG and CG, respectively). These exercise-induced haemodynamic changes at baseline testing were present at follow-up in both groups, with no significant differences between groups for any of the parameters at any stage (Table 4 and Figure 2).

Coronary angiography, endomyocardial biopsies and biochemistry

Five recipients in the EG had mild vasculopathy at both time-points. In the CG eight recipients had mild vasculopathy at baseline and seven at follow-up, two had moderate vasculopathy at baseline, while two had severe vasculopathy at follow-up, of whom one recipient was treatable with percutaneous coronary intervention. One asymptomatic recipient in the CG had a rejection of grade 1R[19] at baseline detected by routine myocardial biopsy, considered insignificant and not specifically treated. No rejections were discovered at follow-up.

No differences were found in biochemical parameters between groups at follow-up.

Discussion

The main findings of the present study are that HIIT is feasible in HTx recipients and effectively improves exercise capacity. However, no improvements in cardiac systolic and diastolic function of clinical importance were measured using comprehensive echocardiographic investigations. The observed increase in exercise capacity is therefore likely due to extra-cardiac adaptations.

Exercise capacity

The significant increase in VO_{2peak} after HIIT in HTx recipients is in agreement with Hermann et al.[9] However, they found a larger increase in VO_{2peak} (4.4 ml/kg/min) after only one eight-week period of HIIT, which could be due to less fit patients at baseline in their study. It is noteworthy that the relative increase in VO_{2peak} by 12.7% in the current study was moderate compared with patient groups with other cardiac diseases undergoing a similar exercise intervention

Table 3. Effects of high-intensity interval training on echocardiographic parameters of cardiac function

Variable	EG ($n = 24$) Baseline	EG ($n = 24$) Follow-up	CG ($n = 24$) Baseline	CG ($n = 24$) Follow-up	Between-groups p-ANCOVA
Haemodynamic parameters					
HR rest, bpm	85 ± 11	83 ± 11	79 ± 11	81 ± 13	0.102
Systolic BP, mmHg	136 ± 13	137 ± 20	131 ± 14	127 ± 14	0.269
Diastolic BP, mmHg	79 ± 11	80 ± 10	78 ± 10	75 ± 10	0.087
LV CO, L/min	5.6 ± 1.2	5.5 ± 1.2	5.0 ± 1.0	5.3 ± 1.2	0.374
LV CI, L/min per m^2	2.8 ± 0.5	2.7 ± 0.6	2.6 ± 0.5	2.7 ± 0.5	0.446
Dimensions					
IVSd, mm	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	0.670
LVIDd, mm	4.8 ± 0.5	4.9 ± 0.5	5.0 ± 0.5	5.0 ± 0.4	0.814
LVPWd, mm	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.685
Systolic parameters					
LV EF biplan, %	52 ± 6	53 ± 5	55 ± 7	56 ± 7	0.404
LV EDV, ml	97 ± 20	100 ± 24	107 ± 25	100 ± 24	0.101
LV ESV, ml	46 ± 12	48 ± 15	49 ± 16	44 ± 14	0.056
LVs′ mean, cm/s	5.9 ± 1.4	5.7 ± 1.4	5.5 ± 1.1	6.0 ± 1.0*	0.029
LV annular displacement, mm	9.5 ± 1.9	10.2 ± 2.1*	9.6 ± 1.8	10.2 ± 2.0	0.779
LV global peak systolic strain, %	−15.7 ± 1.8	−16.1 ± 2.4	−16.3 ± 2.3	−16.3 ± 2.0	0.600
RVs′, cm/s	7.2 ± 1.8	7.4 ± 2.5	6.9 ± 2.5	7.0 ± 2.1	0.847
RV annular displacement, mm	10.9 ± 3.4	11.0 ± 4.2	11.0 ± 3.7	10.7 ± 4.1	0.559
Diastolic parameters					
LV E, cm/s	0.67 ± 0.10	0.74 ± 0.13*	0.69 ± 0.13	0.68 ± 0.14	0.017
LV A, cm/s	0.36 ± 0.09	0.37 ± 0.08	0.32 0.11	0.35 ± 0.09	0.549
LV E/A	1.9 ± 0.7	2.1 ± 0.6	2.4 ± 0.8	2.1 ± 0.6*	0.036
LV DT, ms	159 ± 21	173 ± 30*	155 ± 37	167 ± 36	0.743
LV IVRT, ms	74 ± 14	75 ± 10	78 ± 13	79 ± 16	0.656
LVe′ mean, cm/s	8.1 ± 1.7	8.1 ± 2.0	8.1 ± 1.7	7.7 ± 2.0	0.439
LVa′ mean, cm/s	4.0 ± 0.9	4.3 ± 1.0	3.9 ± 1.3	4.4 ± 1.3*	0.593
LV E/e′ mean	8.6 ± 1.8	9.7 ± 2.9*	8.9 ± 2.7	9.3 ± 2.7	0.329
RV e′, cm/s	7.1 ± 2.2	7.1 ± 2.2	6.3 ± 2.3	5.7 ± 2.2	0.099

Values are expressed as mean ± standard deviation; a′: late diastolic annular velocity; BP: blood pressure; CG: control group; CI: cardiac index; CO: cardiac output; DT: deceleration time of early diastolic mitral flow velocity; E: early diastolic mitral flow velocity; e′: early diastolic annular velocity; E/A: ratio early to late mitral flow velocity; EDV: end-diastolic volume; EF: ejection fraction; EG: exercise group; ESV: end-systolic volume; HR: heart rate; IVRT: isovolumic relaxation time; IVSd: interventricular septal thickness in diastole; LV: left ventricle; LVIDd: left ventricular internal dimension in diastole; LVPWd: left ventricular posterior wall thickness in diastole; RV: right ventricle; s′: systolic annular velocity; *$p < 0.05$ within groups using paired t-test.

(i.e. congestive heart failure: 46%[4] increase in VO$_{2peak}$ (ml/kg/min), coronary artery disease: 18%,[20] hypertension: 15%,[5] metabolic syndrome: 35%[21]).

Cardiac systolic function

HIIT improved LV annular systolic displacement, both at rest and during submaximal exercise, but there was no significant improvement in the end-systolic parameters peak systolic strain and EF at rest, which are related to SV.[22] We found no change in LVs′, which is more closely related to LV contractility.[22] Neither RVs′ nor RV annular systolic displacement increased. Altogether, these data support the lack of improvement in contractile function in the EG, in both the right and the left ventricle. This is in accordance with previous work investigating LV systolic function after exercise training in HTx recipients.[8]

Cardiac diastolic function

To our knowledge, this is the first study to investigate diastolic function with echocardiography after exercise training in HTx recipients. The increase in E and E/A in

Table 4. Left ventricular function during exercise stress echocardiography at baseline and one year follow-up in the exercise group and control group

Variable	EG (n = 15) Baseline	Follow-up	CG (n = 14) Baseline	Follow-up	Between-groups p-ANCOVA
Borg score	16 ± 2	16 ± 1	16 ± 1	16 ± 1	0.455
Peak load, watt	123 ± 24	128 ± 27	130 ± 24	123 ± 23	0.080
Hemodynamics					
HR rest, bpm	93 ± 12	89 ± 12	83 ± 13	81 ± 12	0.894
HR submax, bpm[a]	134 ± 12	132 ± 10	132 ± 15	132 ± 16	0.751
Systolic BP rest, mmHg	132 ± 15	129 ± 20	131 ± 14	129 ± 10	0.953
Systolic BP submax, mmHg[a]	196 ± 28	192 ± 33	204 ± 15	195 ± 13	0.708
Diastolic BP rest, mmHg	85 ± 7	86 ± 11	87 ± 9	80 ± 9*	0.121
Diastolic BP submax, mmHg[a]	84 ± 11	87 ± 12	91 ± 16	90 ± 16	0.909
Systolic parameters					
LVs′ rest, cm/s	6.9 ± 1.9	6.8 ± 1.2	5.9 ± 1.0	6.0 ± 0.7	0.185
LVs′ submax, cm/s[a]	9.7 ± 2.0	10.1 ± 1.5	9.0 ± 1.5	8.8 ± 1.9	0.112
LV displacement rest, mm	8.8 ± 1.8	9.5 ± 1.4	9.4 ± 1.3	9.7 ± 1.2	0.794
LV displacement submax, mm[a]	11.8 ± 1.3	12.4 ± 1.5*	11.9 ± 1.7	11.7 ± 2.2	0.093
Diastolic parameters					
LVe′ rest, cm/s	7.1 ± 1.4	7.4 ± 1.6	8.0 ± 1.4	7.6 ± 1.1	0.403
LVe′ submax, cm/s[a]	12.6 ± 2.6	12.5 ± 2.6	14.0 ± 2.3	12.2 ± 2.2*	0.137
LV E rest, m/s	0.63 ± 0.13	0.65 ± 0.11	0.75 ± 0.13	0.78 ± 0.14	0.092
LV E submax, m/s[a]	1.16 ± 0.16	1.25 ± 0.19	1.21 ± 0.20	1.24 ± 0.16	0.566
LV E/e′ rest	9.2 ± 2.3	9.3 ± 2.4	9.7 ± 2.3	10.4 ± 2.3	0.248
LV E/e′ submax[a]	9.6 ± 2.6	10.4 ± 2.8	8.7 ± 1.9	10.4 ± 2.6*	0.270

Values are expressed as mean ± standard deviation; BP: blood pressure; E: early diastolic mitral flow velocity; e′: early diastolic annular velocity; HR: heart rate from echocardiography; LV: left ventricle; s′: systolic annular velocity; *$p < 0.05$ within groups using paired t-test; [a]Submax defined as the highest load each individual managed (100 W or above).

the EG only is most likely due to an increase in preload, which is normally seen after regular exercise in healthy subjects due to exercise-induced plasma-volume expansion.[23] Since LVe′ and IVRT, both indexes of LV relaxation,[16] and LV DT did not change after HIIT, the observed increase in E/e′ was probably due to the increased plasma volume. Elevated preload will raise E without much influencing e′. There were no significant differences in E/e′ between groups, and neither group had increasing E/e′-ratios with increasing exercise intensity, indicating that filling pressures were unchanged during exercise. We measured e′ from colour tissue Doppler, which yields a slightly lower value than by pulsed wave tissue Doppler,[10] thus the E/e′ ratios in the present study are slightly higher than those reported elsewhere.

Also, the unchanged IVRT and RVe′ support that HIIT did not improve diastolic relaxation. NT-proBNP values, known to correlate with LV filling pressure and diastolic dysfunction,[24] were also similar between the groups at baseline and at follow-up. Altogether, these data demonstrate that HIIT did not alter LV filling and relaxation in stable HTx recipients, neither at rest nor during exercise.

Cardiac response to exercise training in other patient groups

Similar interventions in patients with congestive heart failure or hypertension have both reported significantly increased LV systolic contractile function and diastolic relaxation after 12 weeks of HIIT.[4,5] Whereas the heart failure patients had reduced LV function when starting on HIIT, the hypertensive patients had baseline cardiac function comparable to the recipients in the present study. This underscores the deviating cardiac response to HIIT in HTx recipients.

Cardiac response during exercise echocardiography

A systolic and an early diastolic reserve capacity were present in both groups at both time-points, which is in
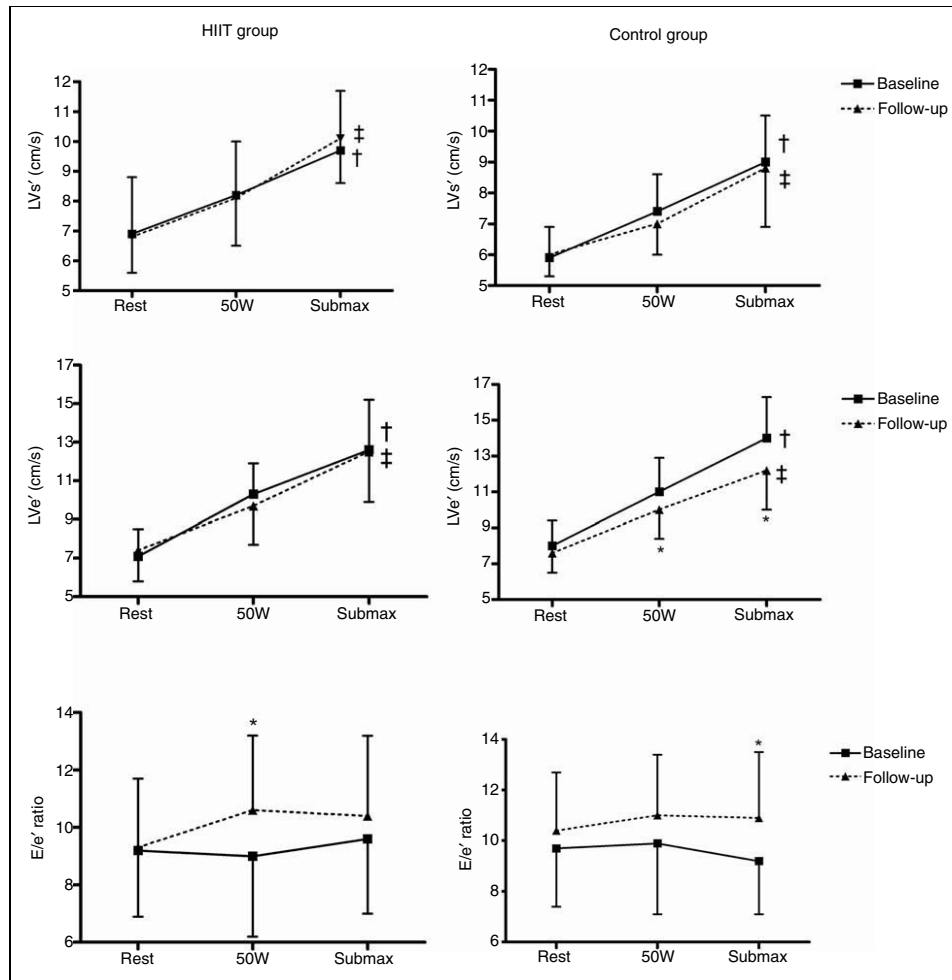
Figure 2. Left ventricle function during exercise stress echocardiography.
Markers indicate mean + 1 standard deviation.
*p-ANCOVA <0.05 compared with same stage at baseline.
†p-ANOVA <0.001 during exercise at baseline (from rest to 50 W and submax).
‡p-ANOVA <0.001 during exercise at follow-up (from rest to 50 W and submax).

agreement with findings in healthy individuals and HTx recipients. Paulus et al.[25] found a LV relaxation acceleration reserve during exercise, even though deficient compared with the CG, whereas Borow et al.[26] found normal contractility and contractile reserve during dobutamine infusion, also in HTx recipients. Ha et al.[27,28] performed studies with similar exercise echocardiography protocol as in our study, but utilised pulsed wave tissue Doppler in the septal region. Compared with their results at 50W, the HTx recipients in the present study had lower septal LVs′ and LVe′ velocities than the healthy individuals, but higher LVe′ and similar LVs′ to patients with hypertrophic cardiomyopathy or diabetes.

Why do HTx recipients respond differently to high-intensity exercise training?

In contrast to healthy individuals, who mainly have cardiac limitations to exercise capacity, HTx recipients have both cardiac and peripheral vascular limitations, which explains the reduced exercise capacity.[2,3] An important difference between a transplanted and a non-transplanted heart is denervation, where HR increase in HTx depends on circulating catecholamines as opposed to the intact autonomic regulation of HR in the normal person. Even though this improves towards normalisation during the first year and the recipient reaches a near to normal HR_{peak},[2] no studies have so far shown complete re-innervation. This raises the question of whether a normal cardiac innervation is mandatory to improve cardiac function after HIIT. However, this notion is somewhat contradicted by the finding that HR_{peak} in the present study was only slightly below age-predicted maximum, and that all in the EG managed to perform exercise intervals with an intensity of 85–95% of HR_{peak}.

The transplanted heart is also characteristic for impaired cardiac function.[29] Despite near to normal EF, HTx recipients have impaired systolic cardiac function, as evidenced by reduced LVs′, strain and strain rate[29] LV diastolic function and relaxation is also impaired at rest and during exercise[25,30] and the amount of myocardial fibrosis is found to increase during the first 12 months.[31] However, these impairments do not solely explain the lack of cardiac improvement after HIIT. In addition, all HTx recipients receive, in contrast to most other patient-groups, immunosuppressive therapy, for example, cyclosporine and corticosteroids, which have several negative effects such as hypertension, dyslipidaemia, impaired endothelial function and muscle atrophy.[2,32]

Clinical implications and study limitations

The present study demonstrates that HIIT is applicable and safe in HTx recipients despite the denervation, and improves exercise capacity without altering cardiac function significantly. There were no significant differences in pre-transplant diagnosis, age, incidence of diabetes, gender or body mass index (BMI) between those who attended and the 135 recipients who were not asked or did not want to participate in the present study. Thus, the results of the present study are most likely representative for stable HTx recipients in general.

LV systolic and diastolic function was not assessed during maximal bicycle exercise due to low image quality, and the effect of HIIT on peak LV function is therefore unknown.

Conclusion

HIIT is feasible in HTx recipients and improves exercise capacity without significant alterations of cardiac systolic and diastolic function. This suggests that the increased exercise capacity is due to extra-cardiac adaptations. A transplanted heart responds differently to aerobic exercise training from a non-transplanted heart. Further research is needed to clarify the basal mechanisms for this observation.

Conflict of interest

None declared.

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Paper III

Brief Communication

High-Intensity Interval Training Improves Peak Oxygen Uptake and Muscular Exercise Capacity in Heart Transplant Recipients

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Heart transplant (HTx) recipients usually have reduced exercise capacity with reported VO_{2peak} levels of 50–70% predicted value. Our hypothesis was that high-intensity interval training (HIIT) is an applicable and safe form of exercise in HTx recipients and that it would markedly improve VO_{2peak}.

Secondarily, we wanted to evaluate central and peripheral mechanisms behind a potential VO_{2peak} increase. Forty-eight clinically stable HTx recipients >18 years old and 1–8 years after HTx underwent maximal exercise testing on a treadmill and were randomized to either exercise group (a 1-year HIIT-program) or control group (usual care). The mean ± SD age was 51 ± 16 years, 71% were male and time from HTx was 4.1 ± 2.2 years. The mean VO_{2peak} difference between groups at follow-up was 3.6 [2.0, 5.2] mL/kg/min (p < 0.001). The exercise group had 89.0 ± 17.5% of predicted VO_{2peak} versus 82.5 ± 20.0 in the control group (p < 0.001). There were no changes in cardiac function measured by echocardiography. We have demonstrated that a long-term, partly supervised and community-based HIIT-program is an applicable, effective and safe way to improve VO_{2peak}, muscular exercise capacity and general health in HTx recipients. The results indicate that HIIT should be more frequently used among stable HTx recipients in the future.

Key words: Aerobic exercise, chronotropic response, heart transplantation, maximum oxygen uptake, muscle strength, VO_{2peak}

Introduction

Exercise capacity improves after a heart transplant (HTx), but continues to be subnormal compared with healthy individuals (1,2). Among factors considered to explain these abnormalities are reduced cardiac output due to chronotropic incompetence or reduced stroke volume, myocardial diastolic dysfunction and peripheral abnormalities (e.g. reduced muscle strength and oxidative capacity, abnormal blood supply because of impaired vasodilatation/capillary density) (2,3).

Several studies demonstrate a positive effect of aerobic exercise after HTx (4–6), but almost all have used exercise with moderate intensity, and the peak oxygen uptake (VO_{2peak}) remain below normal ranging from 50% to 70% of predicted values (1,2). High-intensity interval training (HIIT) has been shown to be an efficient form of exercise to improve physical capacity in patients with coronary artery disease and heart failure (HF) (7,8). However, except for the study by Hermann et al. (9), HTx recipients have not been exposed to this type of exercise mainly because it has been considered "unphysiological" due to chronotropic incompetence. We have previously shown that the heart

rate (HR) response is not a limiting factor in HTx recipients' exercise capacity (10,11) and recent studies suggest that peripheral factors, rather than the heart, may limit exercise capacity in these patients (6). It has also been shown that VO_{2peak} in HTx recipients is independent of the exercise protocol (12). Thus, we reasoned that HIIT would improve VO_{2peak} in HTx recipients, and result in a higher percent of predicted VO_{2peak} than previously shown in most studies. Secondarily, we wanted to investigate possible mechanisms behind a potential increase in VO_{2peak}.

Materials and Methods

Patients and settings

We prospectively recruited 57 clinically stable HTx patients during their annual follow-up between 2009 and 2010 (Figure 1). The inclusion criteria were: age >18 years; 1–8 years after HTx; optimal medical treatment; stable clinical condition; ability to perform maximal exercise test on a treadmill; willingness and ability to perform a 1-year HIIT-program; and provision of written informed consent. Exclusion criteria were: unstable condition; need for revascularization or other intervention; infection; physical disability preventing participation and exercise capacity limited by other disease or illness. All participants were treated according to our immunosuppressive protocol with a calcineurin inhibitor, corticosteroids and mycophenolate mofetil or azathioprine, as well as statins (Table 1).

Of the 57 initially recruited patients, five were excluded (Figure 1), and 52 patients underwent baseline testing and were randomized, using computer generated randomization sequences, to either intervention group (HIIT) or control group (usual care). There were no significantly different baseline characteristics between the study population and the rest of the HTx cohort (n = 135), not included in the study (data not shown). The study was approved by the South-East Regional Ethics Committee in Norway (ClinicalTrial.gov identifier: NCT 01091194).

Intervention

The exercise intervention was HIIT performed on a treadmill. Each patient was assigned to a local, cooperating physiotherapist for individual supervision of every HIIT-session. The intervention was divided into three 8-week periods of exercise with three sessions every week. Additionally, the patients were encouraged to continue any physical activity on their own. All participants were provided with their own HR monitor and both the supervised sessions and their solo training were monitored and logged. The HIIT-sessions consisted of 10 min warm-up, followed by four 4 min exercise bouts at 85–95% of maximum heart rate (HR_{max}), interposed by 3 min active recovery periods (Figure 2) corresponding to ~11–13 on the Borg, 6–20 rated perceived exertion (RPE), scale. HR_{max}, recorded during the maximal exercise test at baseline, was used to determine each patient's training zone. Speed and/or increased inclination of the treadmill were adjusted individually to reach the desired HR. No intervention was given to the control group other than basic, general care given to all HTx patients.

Exercise testing

We used a modified test protocol from the European Society of Cardiology (13). The treadmill test protocol was carried out as previously described (10). Test termination criteria were respiratory exchange ratio (RER) > 1.05 and/or Borg 6–20 RPE scale > 18. Effect of exercise at submaximal levels is presented as the difference in HR and RER between the exact same time points of the baseline and follow-up test, corresponding to 60% and 80% of maximal exercise at the baseline test.

Muscle strength and muscular exercise capacity

Quadriceps (extension) and hamstrings (flexion) muscle strength and muscular exercise capacity were tested isokinetically (Cybex 6000, Lumex Inc, Ronkonkoma, NY, USA). The test was performed in a sitting position, testing one leg at a time. Muscle strength was tested at an angular velocity of 60°/sec. Five repetitions were performed, with the mean peak value in Newton meter (Nm) calculated for each patient. As a measure of muscular exercise capacity, total work during 30 isokinetic contractions at 240°/sec were measured, with total work in Joule (J) calculated as the sum of all repetitions.

Biochemistry

Regular blood screening was performed in the morning, in fasting site, for all patients by routine laboratory methods. Platelet-poor EDTA plasma for measurements of inflammatory and myocardial markers were collected and stored as previously described (10). N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and C-reactive protein (CRP) were analyzed as described elsewhere (14). Interleukin (IL)-6, IL-8 and IL-10 were analyzed by enzyme immunoassays (R&D Systems, Minneapolis, MN, USA).

Health-related quality of life

Health-related quality of life (HRQoL) was measured with the generic questionnaire Short Form 36 (SF-36), version 2. The results were aggregated into two sum-scores: Physical Component Summary (PCS) and Mental Component Summary (MCS), reported on a standardized scale with a mean of 50 and a SD of 10, based on the 1998 US general population. Patients were also asked to subjectively rate on a VAS scale how much participation in this study had improved their HRQoL.

Miscellaneous

Echocardiography and bioelectrical impedance analysis were performed as previously described (10).

Statistical analysis

Continuous data are expressed as mean ± SD or median (interquartile range), and categorical data are presented as counts/percentages. In-group comparisons were made using paired samples t or Wilcoxon signed rank tests, and between-group comparisons were made using unpaired t or Mann–Whitney U tests, as appropriate. For categorical data, χ^2 or Fischer's exact tests were used. Correlations, univariate and multiple regression analysis (hierarchical, enter method) were used to evaluate the association between the change in VO_{2peak} at follow-up and the change in different predictors. The following potential predictors were evaluated for its effect on the change in VO_{2peak}: age; sex; LVEF; CO; LVe'; change in peak HR; $\%HR_{max}$; HR reserve; CRI; BMI; body fat; muscle strength; eGFR; NT-proBNP and CRP. p-values < 0.05 (two-sided) were considered statistically significant.

The power analysis were based on an expected change in VO_{2peak} of 25% in the EG, and a SD of change without intervention of 4 mL/kg/min. With an alpha of 5% and power of 80% we would need at least 14 patients in each group. We included a total of 52 patients in order to compensate for dropouts and to be able to look into secondary end points.

Results

Four of the 52 initially included patients were lost to follow-up due to their health condition or missing data (Figure 1) leaving 48 patients eligible for further analysis. Baseline characteristics are given in Table 1 with no significant differences between the exercise group (EG) and the control group (CG).
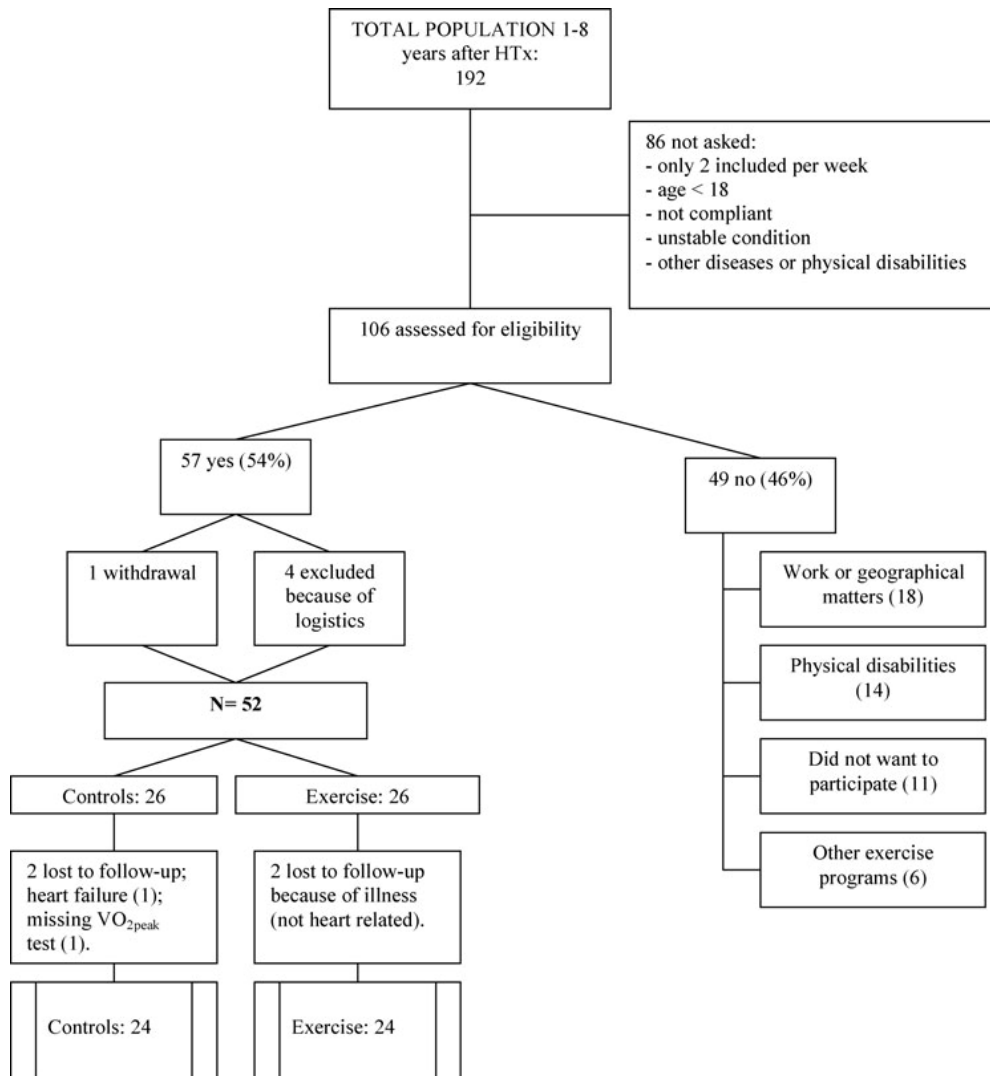
Figure 1: Flow chart of the study population. Exercise group (n = 24), Control group (n = 24).

Compliance with exercise

Of the 72 planned HIIT-sessions, 69 ± 6 sessions were performed with an intensity of 91.5 ± 2.5% of HR_{max}. Each exercise-bout lasted 3.9 ± 0.2 minutes (Figure 2). During the weeks between the supervised periods, 66 ± 20 solo training sessions of various activities were performed, with an average HR of 76 ± 6% HR_{max}.

The last 3 months before follow-up testing, 37.5% in the CG had exercised once or less per week, 37.5%—two to three times per week and 25% four times or more per week, with an exercise duration >30 minutes and a RPE >14 on the Borg 6–20 scale.

Effect of HIIT on responses during maximal exercise

VO_{2peak} increased in the EG with no significant change in the CG, resulting in a significant difference of 3.6 [95% CI 2.0, 5.2] mL/kg/min between the groups at follow-up (Table 2, Figure 3(A)). In line with this, at follow-up VO_{2peak}

Table 1: Baseline characteristics of the heart transplant (HTx) study population

	Exercise group (EC) n = 24	Control group (CG) n = 24	t-test, p-Value EG vs. CG
Sex (% men)	67	71	1.000[a]
Age (years)	48 ± 17	53 ± 14	0.306
Donor age (years)	34 ± 12	38 ± 13	0.251
Ischemic time (min)	179 ± 82	159 ± 95	0.446
Time after HTx (years)	4.3 ± 2.4	3.8 ± 2.1	0.443
Years of HF prior to HTx	4.2 ± 5.0	3.8 ± 2.6	0.766
Smoking (%)			
Yes/No/Exsmoker	4/63/33	0/71/29	0.760
Echocardiography			
EF (%)	52.3 ± 5.7	54.8 ± 7.3	0.217
LVEDD (cm)	4.8 ± 0.5	5.0 ± 0.5	0.266
CO (L/min)	5.6 ± 1.2	5.0 ± 1.0	0.060
LVe′ (cm/s)	8.1 ± 1.7	8.1 ± 1.7	0.993
Medication (%)			
Ciclosporine/Tacrolimus/Everolimus	92/8/13	80/13/21	0.416/1.000/0.701[a]
Mycophenolate/Azatioprine	92/0	96/4	1.000/1.000[a]
Prednisolone	96	88	0.609[a]
Beta blocker	17	25	0.724[a]
Calcium blocker	17	33	0.559[a]
ARB/ACE inhibitors	33	38	0.763[a]
Diuretics	29	33	0.755[a]
Statins	100	100	–
Blood samples			
Hemoglobin (g/dL)	13.9 ± 1.3	13.9 ± 1.2	0.895
eGFR (mL/min/1.73m^2)	55 ± 8	59 ± 4	0.050
NT-proBNP (pmol/L)	35.0 (24)	29.5 (54)	0.551[b]
CRP (mg/L)	0.9 (1.3)	1.8 (3.1)	0.886[b]
HbA$_{1c}$ (%)	5.7 (0.6)	5.7 (0.6)	0.864[b]

Data are expressed as mean ± SD, median (interquartile range) or percentage.
[a]X^2/Fischer exact test. [b]Mann–Whitney U test.
HTx = heart transplantation; HR = heart rate; EF = ejection fraction; LVEDD = left ventricular end diastolic diameter; CO = cardiac output; LVe′ = left ventricle early diastolic mitral annular velocity; ARB = angiotensin II receptor blocker; ACE = angiotensin converting enzyme; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; CRP = C-reactive protein; HbA$_{1c}$ = hemoglobin A$_{1c}$.

was 89.0 ± 17.5% and 82.5 ± 20.0% of predicted in the EG and CG, respectively (Table 2). Also, VE$_{max}$ increased in the EG, but not in the CG, resulting in a significant difference in changes between the groups (Table 2). After HIIT, %HR$_{max}$ and HR reserve were higher in the EG compared with the CG (Table 2).

Systolic, but not diastolic, blood pressure (BP) at peak exercise was higher in the EG than in the CG with a significant difference in changes between the groups (Table 2). Although O$_2$-pulse, which reflects stroke volume, improved significantly in the EG after HIIT, the changes between the groups were not significant (Table 2), and all variables reflecting systolic or diastolic function, including NT-proBNP values remained unchanged in both groups (data not shown).

Effect of HIIT on responses during sub-maximal exercise
HR and RER decreased significantly during submaximal exercise intensities (60% and 80% of baseline maximal exercise) in the EG, but not in the CG, resulting in sig-

nificant differences in the changes of these variables (Figure 4). AT improved from 1.39 to 1.64 L/min, occurring at 64% of the actual VO$_{2peak}$ at follow-up in the EG, while there was no change in the CG, but the difference in changes did not reach statistical significance (Table 2).

Effect of HIIT on responses during rest
During the study, resting HR decreased slightly in the EC and increased slightly in the CG, resulting in a significant difference at follow-up (Table 2). This difference was confirmed by the 24 h Holter recordings (data not shown), which showed a significant reduction of minimum HR in the EG; 69 versus 66 beats/min at baseline and follow-up, respectively (p < 0.05). Numerically, HR declined more rapidly after exercise in the EG after 30 sec (p = 0.054 comparing EG and CG), with no differences at 1 and 2 min (Table 2). Systolic and diastolic BP at rest was similar (Table 2). Echocardiographic parameters of myocardial function and pulse-wave analysis of arterial compliance were similar between the groups (data not shown).
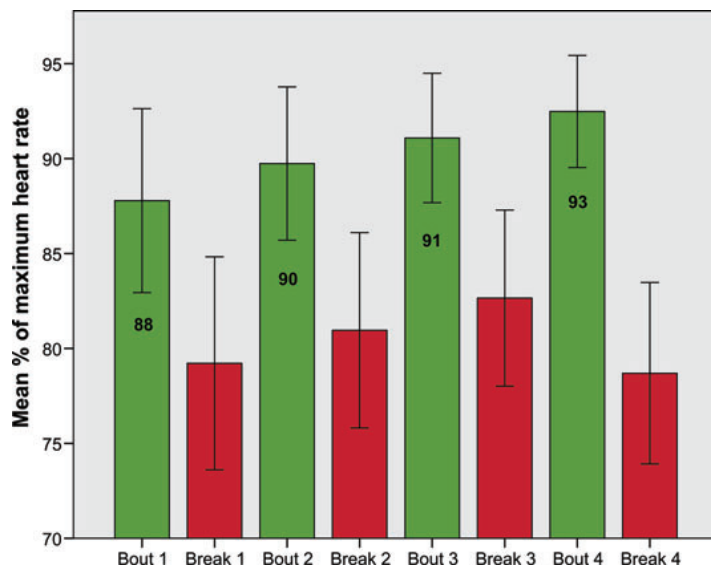
Figure 2: Level of intensity (% of maximum heart rate) during high-intensity interval training.*Error-bars represent 1 SD. Exercise group (n = 24), Control group (n = 24).

Effect of HIIT on muscle strength and muscular exercise capacity

Quadriceps maximal strength did not change in the EG, while it was reduced in the CG (Table 2). There were no changes in hamstrings maximal strength. Quadriceps and hamstrings muscular exercise capacity increased significantly by 15% and 19%, respectively, in the EG, while remaining unchanged in the CG (Table 2), resulting in a significant difference in the change in total work (J) in both quadriceps and hamstrings between the groups (Figure 3B).

Effect of HIIT on body composition, biochemistry and HRQoL

Numerically, the EG had positive changes in their body composition, but there were no significant differences in changes between the study groups at follow-up (Table 2). Lipid profile, glycemic control, NT-proBNP or plasma levels of IL-6, IL-8 and CRP were also similar (data not shown).

Both groups had high HRQoL scores and there were no significant changes in any of the sum-scores (data not shown). However, there was a significant difference between the EG and the CG on the SF-36 General Health subscale at follow-up: 54 versus 49, respectively (p < 0.05). As for subjectively improved health, the EG reported 65 on the VAS scale versus 26 in the CG (p < 0.001).

Determinants of the change in VO_{2peak}

In the multiple regression analysis (Table 3), the change in %body fat and increased muscular exercise capacity (%) together explained 48% (R^2 change = 0.48) of the variance

of the change in VO_{2peak}. HR reserve added another 5% to the explained variance (R^2 change = 0.05).

Safety parameters

One patient in the CG suffered from an MI resulting in HF and was lost to follow-up (Figure 1). There were no other serious adverse events in any of the groups during the time of follow-up and there were no incidences of musculoskeletal injuries in the EG.

Discussion

HIIT has traditionally been avoided in HTx patients mainly due to concerns over chronotropic insufficiency and safety. The present study has, however, demonstrated that such training is applicable and safe in HTx patients. More importantly, the HIIT-program significantly improved VO_{2peak}, as compared to no changes in the CG. The EG reached a predicted VO_{2peak} level of 89% that is higher than shown in most other studies. This increase in VO_{2peak} was accompanied by a significant improvement in muscular exercise capacity, a decrease in resting HR, an increase in HR reserve and increase in VE_{max}, without any changes in parameters of systolic and diastolic myocardial function or parameters of inflammation. Importantly, the improvement in peak VO_2 in the present study is considered clinically significant as 3.5 mL equals 1 metabolic equivalent, and is greater than that found in most rehabilitation programs among HTx patients (4,15) or what has been observed with an introduction of ACE-inhibitors (16), beta blockers (17) or cardiac resynchronization therapy (18), among HF patients.

Table 2: Effect of exercise in the two groups

	Exercise group (EG)		Control group (CG)		Mean difference between	t-test
	Baseline	Follow-up	Baseline	Follow-up	groups [95% CI]	p-Value
Rest						
HR rest (during echocardiography)	85 ± 11	83 ± 11	79 ± 11	81 ± 13	-5 [-9, 0]	0.040
SBP (mmHg)	130 ± 17	136 ± 16	131 ± 20	129 ± 14	8 [-3, 19]	0.155
DBP (mmHg)	80 ± 10	82 ± 9	81 ± 15	82 ± 17	1 [-7, 10]	0.763
Peak exercise (treadmill)						
VO_{2peak} (mL/kg/min)	27.7 ± 5.5	30.9 ± 5.3**	28.5 ± 7.0	28.0 ± 6.7	3.6 [2.0, 5.2]	<0.001
% of predicted VO_{2peak}	80.0 ± 20.0	89.0 ± 17.5**	82.5 ± 20.0	82.0 ± 19.0	9.4 [4.5, 14.4]	<0.001
VO_{2peak} (L/min)	2.34 ± 0.51	2.57 ± 0.51**	2.29 ± 0.56	2.25 ± 0.54	0.26 [0.15, 0.37]	<0.001
RER	1.07 ± 0.06	1.08 ± 0.04	1.06 ± 0.05	1.07 ± 0.05	0.007 [-0.019, 0.032]	0.602
Borg scale (peak RPE)	18.5 ± 0.5	18.8 ± 0.4*	18.4 ± 0.6	18.5 ± 0.9	0.2 [-0.3, 0.7]	0.385
Test duration (min)	10.6 ± 2.7	14.1 ± 3.0**	12.2 ± 4.7	13.0 ± 5.9	2.9 [1.5, 4.3]	<0.001
Peak HR	159 ± 14	163 ± 13*	154 ± 15	153 ± 17	5 [0, 10]	0.035
%HR_{max}	93 ± 12	96 ± 10*	92 ± 10	92 ± 10	3 [0, 6]	0.050
HR reserve (beats/min)	74 ± 14	81 ± 13*	75 ± 17	72 ± 17	10 [4, 16]	0.002
CRI	0.89 ± 0.23	0.95 ± 0.19*	0.88 ± 0.19	0.88 ± 0.18	0.06 [0.01, 0.12]	0.019
Peak SBP (mmHg)	181 ± 33	211 ± 66*	197 ± 22	191 ± 32	35 [3, 67]	0.034
Peak DBP (mmHg)	71 ± 15	80 ± 14*	83 ± 14	91 ± 35	0 [-14, 15]	0.930
O_2 pulse (mL/beat)	15.3 ± 3.2	16.1 ± 2.5*	14.5 ± 3.9	14.7 ± 3.0	0.6 [-0.6, 1.8]	0.290
VE_{max} (L)	88.1 ± 18.9	98.4 ± 18.0**	83.0 ± 19.3	82.4 ± 18.5	10.8 [5.3, 16.3]	<0.001
VE/VCO_2 slope	29.4 ± 3.4	28.7 ± 2.6	29.1 ± 3.2	28.8 ± 3.7	-0.9 [-1.6, 1.4]	0.906
Submaximal exercise: AT (L/min)	1.39 ± 0.27	1.64 ± 0.36*	1.45 ± 0.37	1.51 ± 0.33	0.19 [-0.07, 0.45]	0.138
Heart rate recovery						
Beats at 30 sec	-6 ± 5	-8 ± 5	-7 ± 5	-7 ± 4	-3 [-5, 0]	0.054
Beats at 1 min	-15 ± 7	-16 ± 5	-14 ± 8	-15 ± 9	0 [-3, 3]	0.918
Beats at 2 min	-24 ± 7	-27 ± 6	-25 ± 12	-26 ± 11	-2 [-6, 3]	0.401
Muscle strength (Nm) and muscular exercise capacity (J)						
Quadriceps (Nm)	129 ± 44	130 ± 42	119 ± 40	111 ± 37*	8 [0, 17]	0.063
Hamstrings (Nm)	68 ± 24	71 ± 27	71 ± 25	67 ± 28	6–2, 14]	0.117
Quadriceps + hamstrings (Nm)	394 ± 131	402 ± 135	380 ± 121	359 ± 123*	29 [1, 57]	0.043
Quadriceps (J)	2984 ± 1483	3446 ± 1231**	2887 ± 1053	3035 ± 1088	313 [-13, 639]	0.059
Hamstrings (J)	1530 ± 839	1822 ± 813**	1539 ± 590	1610 ± 738	221 [-26, 468]	0.078
Quadriceps + hamstrings (J)	4514 ± 2262	5286 ± 1979**	4426 ± 1548	4645 ± 1735	534 [39, 1029]	0.035
Body composition						
Body mass index	27.2 ± 4.5	26.5 ± 4.1	26.3 ± 4.2	26.3 ± 4.7	-0.7 [-1.6, 0.2]	0.106
Body fat (%)	26.1 ± 10.3	25.2 ± 9.5	24.6 ± 10.1	25.0 ± 9.7	-1.4 [-3.2, 0.5]	0.152
Weight (kg)	86.3 ± 16.1	84.3 ± 15.8	82.3 ± 15.2	82.2 ± 15.1	-1.9 [-4.5, 0.7]	0.143

Data are expressed as mean $\pm$ SD. *p < 0.05 and **p < 0.001 within group at follow-up.
HR = heart rate; SBP/DBP = systolic/diastolic blood pressure; RER = respiratory exchange ratio; RPE = rated perceived exertion; CRI = chronotropic response index; VE_{max} = maximum ventilation; AT = anaerobic threshold; Nm = Newton meter; J = Joule.

Due to chronotropic incompetence in the denervated heart, most centers have used exercise programs with long warm-up, followed by a gradual increase in intensity toward 50–80% of peak effort. HIIT, with repeated bouts of exercise, with a rapid increase in intensity to 85–95% of peak HR sustained for some minutes, followed by a sudden decline in intensity, has been considered unphysiological in HTx patients. However, our study supports a recent study among HTx patients that such exercise training is safe and well-tolerated, and results in a significant improvement in exercise capacity (9). In addition, we have shown that such training can be done decentralized, near the patients' home

environment, supervised by local physiotherapists. At last, while most other exercise interventions have lasted for weeks to some months, our study lasted a whole year, primarily because we wanted to see if such training could be sustained by the participant for such a long time.

In contrast to findings in HF patients where HIIT has been found to induce a significant antiremodeling effect and improved myocardial function (8), HIIT did not induce any improvement in markers of myocardial function in the present study. Although there was an increase in O_2-pulse in the EG after HIIT, without any significant changes between the
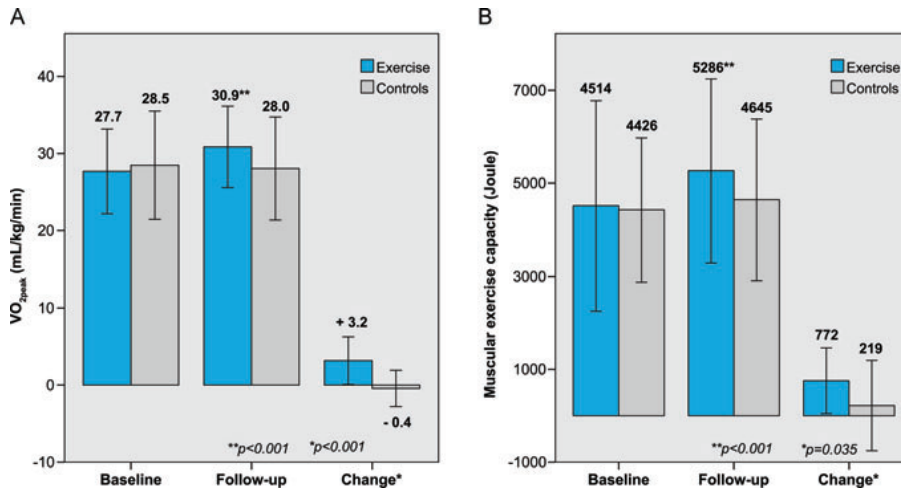
Figure 3: VO$_{2peak}$ (A) and muscular exercise capacity (B) at baseline and follow-up in the exercise and control group. *Error-bars represent 1 SD. Exercise group (n = 24), Control group (n = 24).

groups, all other variables of systolic and diastolic function were similar during follow-up. Also, there were no changes in NT-proBNP in either the EG or the CG. These findings may suggest that the effect of HIIT on the myocardium is different in HF patients as compared with HTx recipients.

Chronotropic incompetence due to denervation is repeatedly regarded as one of the most central VO$_{2peak}$ limit-

ing factors in HTx recipients (19,20). In our previous work (10,11) we found that the chronotropic responses were close to normal in two different HTx study populations and thus, potentially not a significant determinant of VO$_{2peak}$. However, in the present randomized trial, we found that HIIT significantly increased HR reserve as a result of both a higher peak HR and a lower resting HR. Thus, while HIIT had no effect on myocardial performance and remodeling in HTx recipients, it seems to have a beneficial
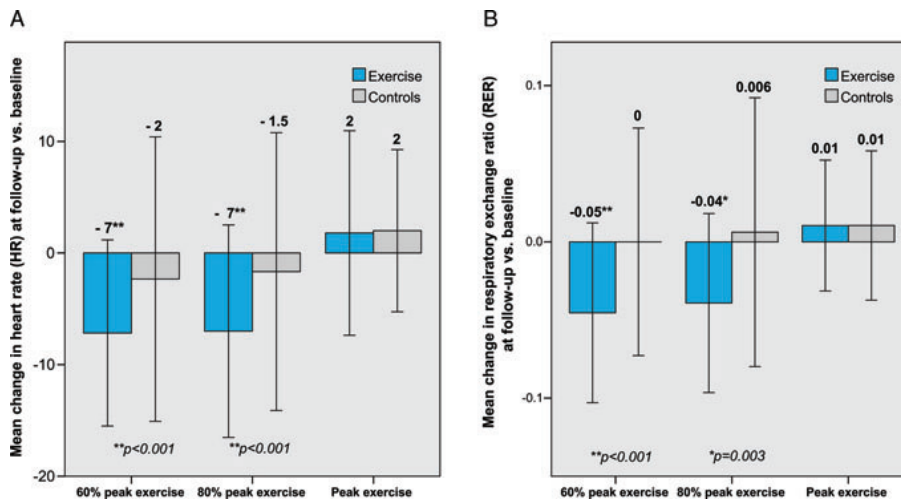

Figure 4: Change in HR (A) and RER (B) during submaximal stages at follow-up. *Error-bars represent 1 SD. Exercise group (n = 24), Control group (n = 24).

Table 3: Multiple linear regression analysis (hierarchical, enter model) of the change in VO_{2peak} (mL/kg/min) at follow-up

VO_{2peak} change at follow-up predictors (n = 48)	B [95%CI], p-Value	R^2 change, p-Value	Model summary R^2 (Adjusted R^2)	Model summary p-Value
Change in body fat (%)	−0.61 [−0.85, −0.38], <0.001	0.337, <0.001	0.529 (0.472)	<0.001
Change in muscular exercise capacity (%)	0.02 [0.01, 0.04], 0.018	0.146, <0.001		
Change in HR reserve (beats)	0.06 [−0.003, 0.13], 0.061	0.048, 0.042		
Age (years)	−0.01 [−0.06, 0.05], 0.774	0.001, 0.783		
Sex (male)	0.10 [−1.53, 1.74], 0.897	0.000, 0.897		

chronotropic effect. The reason for this is at present unclear, but might be due to improved autonomic nervous control (11,20–22).

Muscle diffusion capacity, mitochondrial enzyme levels and capillary density are potential peripheral sites for VO_{2peak} limitation (23). Although most research support cardiovascular delivery of O_2 to be the central component in VO_{2peak}, the importance of skeletal muscle function should not be underestimated, especially not in HTx recipients on immunosuppressive medication that induces skeletal muscle dysfunction. Long-term use of cyclosporine causes muscle atrophy and a shift toward a larger amount of fast-twitch muscle fibers at the expense of the slow-twitch fibers (24), while corticosteroids result in mitochondrial dysfunction (25). Both at baseline (10) and at follow-up in the current study, we found muscular exercise capacity as a strong predictor of VO_{2peak}. This is in accordance with other studies on HTx recipients with emphasis on the role of skeletal muscle function and microcirculation in physical capacity (5,6,26).

Limitations

The inclusion criteria and type of intervention may have led to a selection bias. Participants were defined as stable and healthy, and could have a higher-than-average motivation for exercise, and a baseline higher HRQoL score. In addition, the study population was relatively small and we lacked complete data on reasons for excluding patients from the initial screened population. Over 90% of the patients were still on low-dosage steroids, and based on their negative influence on muscle function, this may have affected the results. Also, the mean baseline VO_{2peak} was relatively high. However, as the values were normally distributed, this likely represents normal group heterogeneity, rather than solely well-trained subjects. Most importantly, since the CG did not undergo another exercise strategy we cannot conclude that HIIT is better than usual, moderate training, but only state that HIIT is an effective and safe form of exercise in this population.

Conclusion

In summary, we have demonstrated that a long-term, partly supervised and community-based HIIT-program is an appli-

cable, effective and safe way to improve VO_{2peak}, muscular exercise capacity and general health in HTx patients. The results suggest that HIIT should be introduced and more frequently used among stable HTx recipients. However, it remains to be determined whether this intervention translates into a better prognosis in this patient group. Forthcoming studies should also address the optimal period for HIIT intervention following transplantation.

Acknowledgments

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*

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Paper IV

Heart transplant systolic and diastolic function is impaired by prolonged pretransplant graft ischaemic time and high donor age: an echocardiographic study[†]

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Abstract

OBJECTIVES: Due to the need for suitable donors for heart transplantation (HTx), older grafts and grafts with prolonged graft ischaemic time (GIT) are accepted. The impact of GIT and donor age on post-transplant cardiac function has not been examined with either newer echocardiographic techniques (tissue Doppler imaging, TDI) or cardiopulmonary exercise testing (CPET). Thus, we studied the influence of GIT and donor age on post-transplant cardiac function and exercise capacity.

METHODS: Fifty-two stable recipients underwent echocardiography with colour TDI and CPET at a median of 4 years after HTx. Left ventricular (LV) systolic (s') and early diastolic (e') mitral annular velocities, right ventricular (RV) s', RVe' as well as LV ejection fraction (EF) and VO$_{2peak}$ were analysed.

RESULTS: HTx recipients with GIT ≥ median value (200 min) had significantly lower septal LVs' (15%, $P = 0.005$), LVEF (9%, $P = 0.015$), RVs' (21%, $P = 0.007$), septal LVe' (22%, $P = 0.001$) and RVe' velocities (23%, $P = 0.011$), and slightly lower VO$_{2peak}$ ($P = 0.098$). Recipients with grafts from donor ≥median age (37 years) had significantly lower LVe' velocities (septal LVe' $P = 0.047$ and lateral LVe' $P = 0.010$), but not LV systolic or RV parameters.

CONCLUSIONS: Prolonged GIT impairs both systolic and diastolic function at the interventricular septum and RV free wall, while increasing donor age impairs LV diastolic function. The duration of graft ischaemia and donor age should be taken into account when evaluating for cardiac dysfunction in HTx recipients.

Keywords: Heart transplantation · Graft ischaemic time · Donor age · Echocardiography · Tissue Doppler

INTRODUCTION

The shortage of cardiac allografts is a major limiting factor for heart transplantation (HTx) and, therefore, both older grafts and prolonged graft ischaemic time (GIT) are accepted. Guidelines recommend pretransplant GIT <4 h, particularly when donor age is >45 years [1]. However, the knowledge of how GIT affects post-transplant cardiac function and exercise capacity is limited. Clinical studies on GIT have focussed on long-time survival after HTx [2–5]. While some studies have reported that prolonged GIT does not influence recipient survival after HTx, others indicate that extended GIT impairs patient survival, especially when the

donor is of older age [2–5]. In healthy individuals, diastolic function is impaired with increasing age [6, 7].

The non-invasive echocardiographic technique tissue Doppler imaging (TDI), enables the measurement of both systolic and diastolic myocardial function on a beat-to-beat basis [8]. Myocardial velocities are regarded as more sensitive markers of myocardial function than traditional echocardiographic measurements, such as ejection fraction (EF) and mitral flow velocities [9, 10]. Whereas echocardiography measures cardiac morphology and function, cardiopulmonary exercise testing (CPET) measures exercise capacity, which is a valuable tool in evaluating cardiac function and prognosis. The influence of GIT and donor age on cardiac function has not been investigated with either newer echocardiographic techniques or with CPET.

Thus, our hypothesis was that prolonged GIT and increased donor age impaired cardiac systolic and diastolic function, and

that this impairment could be detected with newer echocardiographic techniques and CPET.

MATERIALS AND METHODS

Study population

Our study population consisted of HTx recipients scheduled for an annual follow-up between 2009 and 2010. The inclusion criteria were assessment 1–8 years after HTx, optimal medical treatment, stable clinical condition and the ability to perform maximal exercise testing. Exclusion criteria were heart failure, clinical signs of rejection, atrial fibrillation, need for revascularization or other intervention. All recipients had bicaval right atrial anastomosis. From a cohort of 192 HTx recipients, who were transplanted 1–8 years earlier, we asked 106 HTx recipients to participate. Forty-nine recipients declined participation in the study; 14 due to functional disabilities, 11 did not want to participate and 24 due to other reasons. From the 57 recipients who accepted participation, one acceptance was withdrawn and 4 patients were excluded due to logistics. Thus, totally, 52 HTx recipients were finally included in the study.

Donor hearts were perfused with 1000 ml St Thomas II solution at 4°C for cardioplegia, and then transported immersed in Ringers solution at 8°C between harvest and implantation. All recipients had bi-caval right atrial anastomosis, and all recipients were on conventional immunosuppressive treatment (Table 1). Previous rejections had been treated according to the guidelines [1]. All participants were >18 years of age, and all gave written informed consent. The study was approved by the South-East Regional Ethics Committee in Norway and conducted according to the declaration of Helsinki.

Echocardiography

Echocardiography was performed either with GE Vivid 7 or E9 with a phased-array sector transducer (GE Vingmed Ultrasound, Horten, Norway). The echocardiographic data were stored digitally and analysed off-line in dedicated software (EchoPAC, GE Vingmed Ultrasound). Two-dimensional grey-scale echocardiographic recordings and additional colour TDI (cTDI) of standard apical projections (four chambers, two chambers and long axis) comprising three consecutive heart cycles each were obtained according to recommendations [11]. Blood-flow velocities in the left ventricular (LV) outflow tract and mitral inflow were obtained from the apical position using pulsed-wave Doppler with the sample volume at the aortic annulus and tip of mitral leaflets, respectively. Brachial arterial blood pressure was obtained as the mean of a triplet reading by the oscillometric technique (Dinamap, ProCare, GE).

The average of at least three cardiac cycles was used in analysis of Doppler recordings. LV early (E) and late (A) mitral inflow velocities, deceleration time of the E-wave (DT) and isovolumic relaxation time (IVRT) were measured from the pulsed-wave Doppler signal. LV mitral annular velocities in systole (LVs'), early (LVe') and late diastole (LVa') were measured at both septal and lateral mitral annular regions by cTDI (Fig. 1) [8]. LV systolic mitral annular displacement (distance) was calculated by integration of tissue Doppler velocity signals (distance per time) at the same sites. LV septal and lateral values were presented separately, as velocities

and distance differ between myocardial segments [12]. The ratio of early diastolic mitral flow velocity (E) to early diastolic mitral annular velocity (LVe') was calculated. The E/e' indicates LV filling pressure non-invasively and has also been validated in HTx recipients [13, 14]. Right ventricular (RV) systolic and early diastolic annular velocities (RVs' and RVe'), as well as RV displacement, were measured in the lateral tricuspid annular region in the apical four-chamber view by cTDI [7]. LV peak-systolic longitudinal strain was measured using speckle-tracking echocardiography (two-dimensional strain method) in an 18-segment model, converted into a 16-segment model [11]. LV global longitudinal strain was obtained by averaging the peak-systolic shortening of the 16-segment model. Subjects with <8 of the 16 segments evaluable were not included in the analyses ($n = 8$). LVEF and LV volumes were measured by the modified Simpson's method using the four- and two-chamber views. The stroke volume (SV) was calculated from pulsed Doppler flow measurement in the LV outflow tract, assuming a circular aortic annulus. Systemic arterial compliance was obtained as the ratio of SV/pulse pressure (ml/mmHg).

Cardiopulmonary exercise testing

The maximal exercise capacity was tested with a modified treadmill walking test, following the European Society of Cardiology's recommendations for CPET in chronic heart failure patients [15]. During a 10-min warm-up period, the participants individually adjusted their band speed (3–6 km/h), which was constant for the rest of the test. Inclination increased by 2% every second minute ('walking uphill') until exhaustion. A Borg score >18 and/or respiratory exchange ratio ≥1.05 were used as criteria for an adequate maximal exercise test. Study participants were monitored by continuous 12-lead electrocardiogram (ECG) and blood pressure every second minute. The average of the three highest 10-seconds measurements during exercise determined VO$_{2peak}$. Predicted values were based on the American College of Sports Medicine 2009 guidelines [16].

Coronary angiography and endomyocardial biopsies

All but 3 HTx recipients underwent coronary angiography at their annular medical follow-up to evaluate for cardiac allograft vasculopathy. We classified vasculopathy as follows: (i) severe vasculopathy: significant stenosis, i.e. ≥50% of lumen diameter in ≥1 major epicardial graft vessel(s) and/or >70% in ≥2 distal branches; (ii) moderate vasculopathy: non-significant stenosis, i.e. 33–50% of lumen diameter in ≥1 major epicardial graft vessel(s) and/or >70% in one distal branch; (iii) mild vasculopathy: luminal irregularities, i.e. stenosis <33% of lumen diameter in ≥1 major epicardial graft vessel(s). All angiograms were reviewed by experienced invasive cardiologists and compared with angiograms from previous years to detect the presence of luminal irregularities or obstructions. Endomyocardial biopsies were performed using standard procedures in HTx recipients who were attending their first ($n = 9$) or second ($n = 6$) annular medical follow-up visit and in 3 of the 7 recipients on their third annular visit. Regular blood biochemistry screening was performed.

All examinations and analyses were performed blinded to information regarding donor characteristics and GIT.

Table 1:   Clinical characteristics

	GIT <200 min (*n* = 26)	GIT ≥200 min (*n* = 26)	*P*-value
Demographics			
Recipients age (years)	57 (19–72)	58 (20–71)	0.71
Recipient male gender, *n* (%)	17 (65)	20 (77)	0.36
Donor age (years)	35 ± 14	38 ± 11	0.41
Donor male gender, *n* (%)	15 (58)	14 (54)	0.78
GIT (min)	65 (44–199)	246 (201–301)	<0.001
Warm ischaemic time (min)[a]	50.8 ± 14.0	63.1 ± 13.2	0.005
Years since HTx	4 (1–7)	4 (1–8)	0.90
BMI (kg/m^2)	25.9 ± 4.0	28.0 ± 4.5	0.085
Primary diagnosis, *n* (%)			
Cardiomyopathy	15 (58)	13 (50)	0.83
Ischaemic heart disease	7 (27)	9 (35)	
Congenital heart disease	1 (4)	2 (8)	
Others	3 (12)	2 (8)	
Medications, *n* (%)			
Cyclosporine/tacrolimus/everolimus	21 (81)/4 (15)/5 (19)	24 (92)/1 (4)/3 (12)	
Mycophenolate mofetil/azathioprine	25 (96)/0	24 (92)/1 (4)	
Corticosteroids	23 (88)	25 (96)	
Statin	26 (100)	26 (100)	
Beta-blocker	6 (23)	5 (19)	
Calcium-channel blocker	8 (31)	5 (19)	
ARB/ACE inhibitor	8 (31)	11 (42)	
Prior rejections, *n* (%)[c]			
2Ra	3 (12)	4 (15)	0.77
AMR-1a	2 (8)	0	
2R and AMR-1a	1 (4)	1 (4)	
Coronary angiogram, *n* (%)[b]			
No vasculopathy	15 (60)	17 (71)	0.54
Mild vasculopathy	8 (32)	7 (29)	
Moderate vasculopathy	2 (8)	0	
Biochemical data			
Haemoglobin (g/dl)	13.9 ± 1.4	14.0 ± 1.1	0.81
HbA1c (%)	5.7 (5.1–7.2)	5.8 (5.0–11.9)	0.32
eGFR (ml/min/1.73 m^2)	60 (39–60)	60 (29–60)	0.27
NT-proBNP (pmol/l)	24 (3–105)	38 (9–660)	0.026

Values are expressed as mean ± SD, median (range) or as number and percent where appropriate.
2R: moderate rejection; ACE: angiotensin-converting enzyme; AMR-1: antibody-mediated rejection; ARB: angiotensin II receptor blocker; BMI: body mass index; eGFR: estimated glomerular filtration rate; GIT: graft ischaemic time; HbA$_{1c}$: haemoglobin A$_{1c}$; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.
[a]*n* = 22 in the GIT <200 min group and *n* = 22 in the GIT ≥200 min group.
[b]*n* = 25 in the GIT <200 min group and *n* = 24 in the GIT ≥200 min group.
[c]The International Society for Heart and Lung Transplantation (ISHLT) grading system for heart rejections (17).

Statistics

Continuous variables are presented as mean ± standard deviation (SD) or as median (range) in the case of non-symmetrically data sample distribution. The two-sided independent unpaired *t*-test or the Mann–Whitney *U*-test was performed as appropriate. Categorical variables were compared using the χ^2 test or Fisher's exact test. Pearson sample correlations were performed between continuous variables of cardiac function (myocardial annular velocities) and potential predictors (donor age, GIT, warm ischaemic time, body mass index (BMI), gender, recipient age, prior rejections, donor cause of death, donor–recipient gender mismatch and VO$_{2peak}$), and between the predictors. Multiple linear regression analyses, using the hierarchical, forced entry (enter) model, were performed in order to (i) evaluate for the effect of both GIT and donor age on cardiac function and (ii) identify a model with several predictors with cardiac function as the dependent variable. In the latter regression analysis, predictors with a significance level of the Pearson correlation coefficient <0.2 were included in the multiple linear regression analysis. Thereafter, several custom models were made until a model with significant predictors was identified. The model assumptions were thoroughly checked for multicollinearity and possible interactions. The multiple linear regression analyses are presented with a model summary R^2 (i.e. coefficient of determination), *B* (i.e. unstandardized regression coefficient) and a *P*-value (i.e. testing whether *B* = 0).

Differences were considered significant for *P*-levels <0.05. Statistical analyses were performed using the standard software (SPSS version 18.0, SPSS, Inc., Chicago, IL, USA).

RESULTS

HTx recipients and donor characteristics

The study cohort consisted of 52 HTx recipients, comprising 37 men and 15 women. Recipient age at inclusion was 52 ± 16 years and median time since HTx was 4.0 (range 1–8 years). Donor age
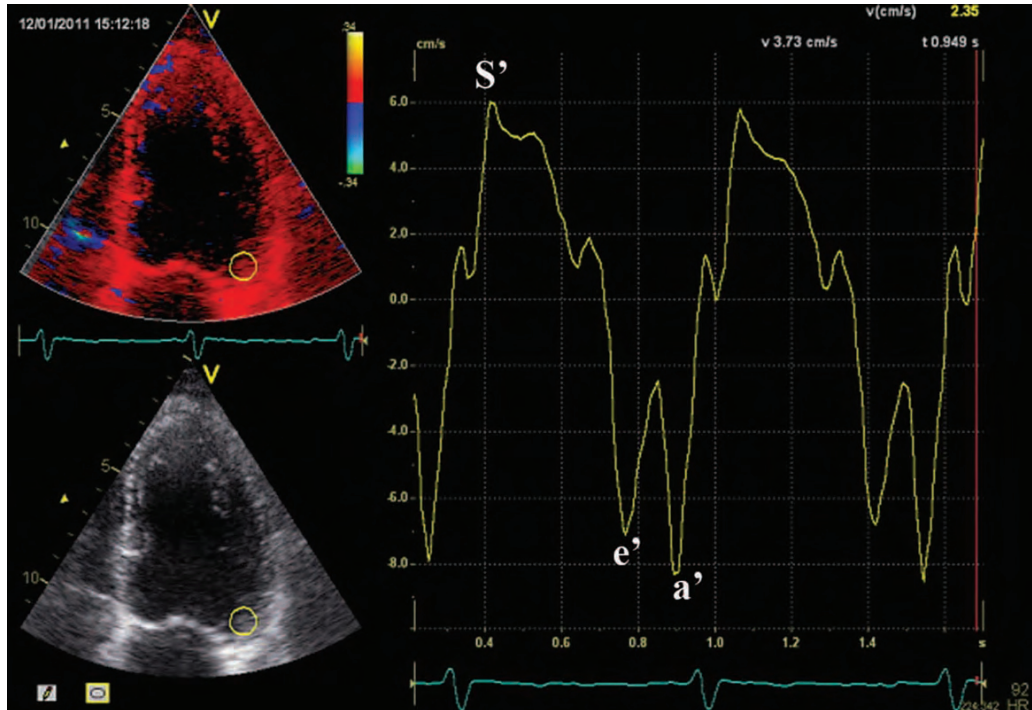
Figure 1: Colour tissue Doppler on the left ventricle. Mitral annular velocities measured on the lateral (yellow) site of the left ventricle. *a′*: late diastolic mitral annular velocity; *e′*: early diastolic mitral annular velocity; *s′*: systolic mitral annular velocity.

was 36 ± 13 (11–58 years), and median GIT was 200 (44–301 min). Nineteen recipients had GIT <2 h, 19 between 2–4 h and 14 had GIT > 4 h. The distribution of GIT reflects the geographical diversity in Norway, where donor hearts with short GIT are from the transplant centre or nearby hospitals, and donor hearts with long GIT are predominantly from distant hospitals. There were donor–recipient gender mismatch in 12 recipients; 2 female recipients had male donors and 10 male recipients had female donors. All underwent echocardiography and CPET, except 1 who declined CPET due to acute arthritis urica. None had severe vasculopathy on angiography, but 15 recipients (31%) had mild vasculopathy and 2 recipients had moderate vasculopathy. One asymptomatic recipient had a rejection of grade 1R detected by routine myocardial biopsy, considered insignificant and not requiring specific treatment [17].

Graft ischaemic time and cardiac function

For the purpose of analysis, recipients were separated into two groups with GITs above or below the median (200 min), respectively (Table 1). There were no significant differences in demographics, aetiology of heart failure, incidence of coronary vasculopathy, donor age, mechanisms of donor brain death or time since HTx between the groups. Recipients with GIT ≥200 min had significantly higher N-terminal prohormone of brain natriuretic peptide (NT-proBNP; *P* = 0.026) and longer warm ischaemic time (*P* = 0.005). There were no differences between the groups in the frequency or grade of previous heart rejections. There were no differences in medications between groups.

Recipients with GIT ≥200 min had significantly lower septal LV*s′* and RV*s′* velocities (15 and 22%, respectively) than those with GIT <200 min (Table 2 and Fig. 2). Non-significant differences were found in lateral LV*s′* and in LV and RV annular displacement. EF was significantly lower in recipients with GIT ≥200 min, while LV global longitudinal peak-systolic strain was equal in the two groups. Looking into the diastolic parameters, recipients with GIT ≥200 min had significantly lower septal LV*e′* and RV*e′* velocities (22 and 23%, respectively) than those with GIT <200 min (Table 2). Non-significant differences were found in lateral LV*e′*. *E* was equal in both groups and septal *E/e′* tended to be higher in the group with GIT ≥200 min (*P* = 0.062). No significant differences were found in LV *a′*, *E/A*, DT, IVRT or estimated systemic arterial compliance.

Peak VO$_2$ was slightly lower (10%) among patients with GIT ≥200 min (*P* = 0.098).

We also separated the recipients into groups with GITs above or below 100 min and found principally the same result as separating them at the median: septal LV*s′*, septal LV*e′*, RV*s′* and EF were significantly lower in those with GIT <100 min, whereas RV*e′* reached borderline significance (*P* = 0.056). Also, when separating the recipients into groups with GITs above or below 4 h, we found significantly lower septal LV*e′*, RV*s′* and RV*e′*, while septal LV*s′* (*P* = 0.067) were lower in those with GIT <4 h.

Table 2: Cardiac function, exercise capacity and haemodynamics

	GIT <200 min (n = 26)	GIT ≥200 min (n = 26)	P-value
Systolic parameters			
LVs' septal (cm/s)	5.9 ± 1.2	5.0 ± 1.0	0.005
LVs' lateral (cm/s)	6.4 ± 2.1	5.8 ± 1.5	0.26
RVs' (cm/s)	7.7 ± 1.9	6.0 ± 2.2	0.007
LV septal displacement (mm)	8.6 ± 1.8	7.9 ± 2.5	0.27
RV displacement (mm)	11.5 ± 3.3	9.9 ± 3.8	0.12
LV EF biplan (%)	56 ± 6	51 ± 6	0.015
LV peak-systolic strain (%)[a]	−16.0 ± 2.2	−15.6 ± 2.4	0.55
LV SV (ml)	64 ± 16	66 ± 15	0.59
LV CO (l/min)	5.2 ± 1.1	5.4 ± 1.0	0.42
LV CI (l/min/m^2)	2.7 ± 0.5	2.7 ± 0.5	0.73
Diastolic parameters			
LVE (cm/s)	69 ± 12	68 ± 11	0.70
LV E/A	2.2 ± 0.8	1.9 ± 0.6	0.25
LV DT (ms)	154 ± 32	159 ± 27	0.61
LV IVRT (ms)	78 ± 13	73 ± 14	0.17
LVe' septal (cm/s)	7.4 ± 1.6	5.8 ± 1.5	0.001
LVe' lateral (cm/s)	9.6 ± 1.9	8.7 ± 2.7	0.15
LV E/e' septal	9.8 ± 2.9	12.4 ± 6.4	0.062
LV E/e' lateral	7.4 ± 1.7	8.1 ± 3.2	0.31
RVe' (cm/s)	7.3 ± 2.3	5.6 ± 2.2	0.011
Exercise capacity[b]			
VO$_{2peak}$ (ml/kg/min)	29.2 ± 6.2	26.3 ± 6.1	0.098
% of predicted VO$_{2peak}$	90.0 ± 21.3	78.9 ± 21.0	0.066
VO$_{2peak}$ (l/min)	2.32 ± 0.57	2.25 ± 0.50	0.65
Haemodynamics			
HR rest (beats/min)	81 ± 9	84 ± 14	0.52
Systolic BP rest (mmHg)	133 ± 16	137 ± 14	0.54
Diastolic BP rest (mmHg)	80 ± 11	79 ± 9	0.86
Arterial compliance (ml/mmHg)	1.15 ± 0.28	1.20 ± 0.31	0.56

Values are expressed as mean ± SD.
BP: blood pressure; CI: cardiac index; CO: cardiac output; DT: deceleration time of early diastolic mitral flow velocity; E: early diastolic mitral flow velocity; e': early diastolic annular velocity; E/A: ratio of early-to-late mitral flow velocity; EF: ejection fraction; GIT: graft ischaemic time; HR: heart rate from echocardiography; IVRT: isovolumic relaxation time; LV: left ventricle; RV: right ventricle; s': systolic annular velocity; SV: stroke volume.
[a]n = 22 in the GIT <200 min group and n = 22 in the GIT ≥200 min group.
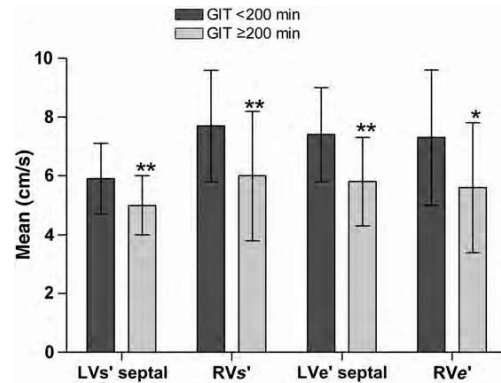[b]n = 25 in the GIT <200 min group and n = 26 in the GIT ≥200 min group.

Figure 2: Graft ischaemic time (GIT) and echocardiographic parameters. Error bars represent ±1 SD. *P < 0.05; **P < 0.01 between GIT ≥200 min and <200 min. LV: left ventricle; RV: right ventricle; e': early diastolic velocity; s': systolic velocity; sep: septal.

Table 3: Donor age and cardiac function

	Donor age <37 years (n = 25)	Donor age ≥37 years (n = 27)	P-value
Systolic parameters			
LVs' septal (cm/s)	5.6 ± 1.2	5.3 ± 1.1	0.28
LVs' lateral (cm/s)	6.2 ± 2.1	6.0 ± 1.6	0.63
RVs' (cm/s)	6.8 ± 2.2	6.8 ± 2.2	0.98
LV septal displacement mm	8.2 ± 2.3	8.3 ± 2.1	0.87
RV displacement lateral mm	9.7 ± 2.7	10.4 ± 2.5	0.36
LV EF biplan (%)	54.4 ± 5	52.4 ± 7	0.31
LV peak-systolic strain (%)[a]	16.0 ± 2.6	15.5 ± 1.8	0.50
LV SV (ml)	62 ± 16	68 ± 14	0.19
LV CO (l/min)	5.1 ± 1.2	5.5 ± 1.0	0.26
LV CI (l/min/m^2)	2.7 ± 0.6	2.7 ± 0.4	0.73
Diastolic parameters			
LVE (cm/s)	68 ± 13	69 ± 10	0.79
LV E/A	2.3 ± 0.7	1.9 ± 0.7	0.065
LV DT (ms)	147 ± 31	165 ± 26	0.025
LVe' septal (cm/s)	7.4 ± 1.5	6.3 ± 2.0	0.043
LVe' lateral (cm/s)	10.1 ± 2.1	8.5 ± 2.3	0.023
LV E/e' septal	9.6 ± 3.4	12.5 ± 6.0	0.040
LV E/e' lateral	6.7 ± 2.1	8.8 ± 2.6	0.003
RVe' (cm/s)	6.7 ± 2.0	6.3 ± 2.7	0.57
Exercise capacity[b]			
VO$_{2peak}$ (ml/kg/min)	28.4 ± 5.8	27.1 ± 6.6	0.46
% of predicted VO$_{2peak}$	85.3 ± 23.7	83.4 ± 19.9	0.75
VO$_{2peak}$ (l/min)	2.25 ± 0.46	2.32 ± 0.60	0.63

Values are expressed as mean ± SD.
CI: cardiac index; CO: cardiac output; DT: deceleration time of early diastolic mitral flow velocity; E: early diastolic mitral flow velocity; e': early diastolic annular velocity; E/A: ratio early-to-late mitral flow velocity; EF: ejection fraction; LV: left ventricle; RV: right ventricle; s': systolic annular velocity; SV: stroke volume.
[a]n = 22 in the donor age <37 years group and n = 22 in the donor age ≥37 years group.
[b]n = 25 in the donor age <37 years group and n = 26 in the donor age ≥37 years group.

Donor age and cardiac function

Due to the known relation between age and diastolic function, we also grouped the HTx recipients according to median donor age (37 years). Recipients with donor age ≥37 years had significantly lower LVe' velocities, both septal and lateral, than those with younger donors (Table 3). Furthermore, E/e' was significantly higher and DT was longer in recipients with older donor hearts. However, no significant differences were found between groups in systolic annular velocities and displacement, EF, LV global longitudinal peak-systolic strain, estimated arterial compliance or exercise capacity. There were no significant differences in gender,

BMI, aetiology of heart failure, medications, time since HTx, warm ischaemic time, incidence of coronary vasculopathy or other clinical characteristics (data not shown). In the group with donor

age <37 years, recipients were younger (median 52 (20–69) vs 61 (19–72) years; $P = 0.15$), and GIT was shorter (189 (46–291) vs 210 (44–301) min; $P = 0.28$). Donor brain death due to intracerebral haemorrhage was significantly more frequent in donors >37 years old (7 vs 15, $P = 0.032$).

Correlation and linear regression analysis

GIT (presented as continuous variable) correlated with septal LVs' ($r = -0.41$, $P = 0.003$), RVs' ($r = -0.42$, $P = 0.004$), septal LVe' ($r = -0.42$, $P = 0.002$) and RVe' ($r = -0.36$, $P = 0.012$), while donor age (presented as continuous variable) correlated with septal LVe' ($r = -0.31$, $P = 0.029$) and lateral LVe' ($r = -0.33$, $P = 0.017$) (Fig. 3). There were no correlations between GIT and VO$_{2peak}$, GIT and donor age, recipient age and donor age or between warm ischaemic time and LVs' and LVe' septal and lateral.

In multiple regression analysis with septal LVe' as a dependent variable, a model was identified (R^2 0.24, $P = 0.002$) with GIT in minutes ($B = -0.008$, $P = 0.004$) and donor age ($B = -0.034$, $P = 0.059$) as predictors, controlling for each other. For the dependent variable septal LVs', we got the following model: R^2 0.17, $P = 0.011$, with GIT ($B = -0.005$, $P = 0.004$) and donor age ($B = -0.006$, $P = 0.64$) as predictors. Similar models were identified for the dependent variables lateral LVe' (R^2 0.127, $P = 0.038$), RVs' (R^2 0.19, $P = 0.011$) and RVe' (R^2 0.13, $P = 0.042$) with GIT and donor age as predictors, adjusting for each other. Finally, we identified a model with several significant predictors and septal LVe' as the depended variable (R^2 0.422, $P < 0.001$, adjusted R^2 0.371) and GIT in minutes ($B = -0.007$, $P = 0.003$), BMI ($B = -0.154$, $P = 0.002$) and donor gender mismatch ($B = -1.309$, $P = 0.008$) as significant predictors, controlling for donor age ($B = -0.021$, $P = 0.193$).

Comparability

The HTx recipients included in the present study were compared with the 135 recipients who were not asked or did not want to participate in the present study. There were no significant differences in age (median 58.0 vs 59.0 years, $P = 0.20$, for attending vs not-attending recipients, respectively), BMI (27.3 ± 5.4 vs 26.9 ± 4.3, $P = 0.68$, for attending vs not-attending recipients, respectively), gender or pretransplant diagnosis between those who attended and those who did not want to participate in the present study.

DISCUSSION

The main finding of the present study is that both systolic and diastolic heart transplant function are reduced in stable HTx recipients with prolonged GIT as assessed by reduced mitral annular velocities of the interventricular septum (IVS) and the RV, as well as LVEF. The present study also demonstrates that increasing donor age, but not recipient age, was associated with an impaired LV diastolic function in stable HTx recipients. These abnormalities were associated with increased NT-proBNP, but neither with exercise capacity nor coronary artery vasculopathy.

Cardiac function and ischaemic time

There are few studies regarding GIT and cardiac function short-term post-HTx. Whereas Fernandez *et al.* [18] reported a lower LVEF 48 h after HTx in recipients with prolonged GIT, Pflugfelder *et al.* [19] did not find any effect of GIT on LVEF, neither after 3 nor 12 months. In paediatric HTx recipients with GIT >4 h, a decreased diastolic posterior wall movement was found in the first week after HTx, whereas no impairment in systolic fractional shortening was found [20]. However, none of these studies used a comprehensive echocardiographic approach with TDI and diastolic cardiac blood-flow velocities, as in the present study.

The occurrence of abnormal IVS motion and RV dysfunction, both in systole and diastole, after cardiac surgery involving cardio-pulmonary bypass have been well documented [21–24]. Eroglu *et al.* [12] found that HTx recipients had lower systolic velocities, strain rate and strain values at septal LV and RV free wall when compared with healthy age-matched controls, while Fyfe *et al.* [25] found both systolic and diastolic dysfunction in basal segments at septal LV and RV free wall in paediatric HTx recipients
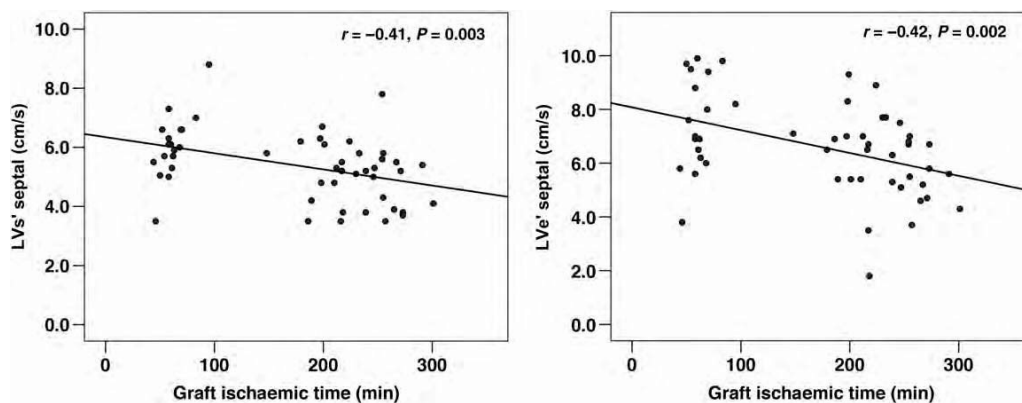

Figure 3: Left ventricular systolic and diastolic function against graft ischaemic time (GIT). e': early diastolic mitral annular velocity; LV: left ventricular; s': systolic mitral annular velocity. r: Pearson correlation coefficient; P: the significance of the Pearson correlation coefficient. The distribution of the GIT reflects the geographical diversity in Norway, where donor hearts with short GIT are from the transplant centre or nearby hospitals, and donor hearts with long GIT are predominantly from distant hospitals.

compared with healthy age-matched controls. Interestingly, these are exactly the same myocardial segments where we found the highest impact of GIT in our study. Thus, it seems likely that a transplanted heart demonstrates similar cardiac abnormalities as observed in native hearts following conventional cardiac surgery with cardiopulmonary bypass, but that this postoperative impairment in myocardial contraction and relaxation patterns is accentuated by a prolonged GIT in HTx recipients.

The underlying mechanism for these changes after conventional cardiac surgery has not been well understood, but hypotheses relating pericardial changes and degree of myocardial preservation (ischaemia) during cardiopulmonary bypass have been proposed [23, 24]. In the present study, all recipients underwent the same surgical procedure. Pericardiotomy therefore, cannot explain the differences between the groups observed in this study. Thus, we assume that the systolic and diastolic impairments were strongly influenced by factors related to the prolonged GIT and preservation of the allograft.

Donor age and cardiac function

Increasing donor age was associated with an impaired LV diastolic function in stable HTx recipients, as assessed by the decreased LVe' (both septal and lateral), the increased E/e' ratio and the prolonged DT. Prior studies have shown that diastolic function is impaired with age, which is in agreement with our finding [6, 7]. Aging is associated with myocardial fibrosis, leading to increased stiffness of the myocardium. Both Dalen *et al.* [6] and Nikitin *et al.* [7] found decreasing LVs' and RVe' with increasing age in a population free from cardiovascular disease, and Dalen found decreased RVs' as well. We did not find any associations between donor age and systolic parameters or RV function. This might be due to the fact that Nikitin used subjects as old as 89 years, while in the present study no donors were older than 58 years.

Clinical implications

By use of sensitive echocardiographic techniques, we found a significant impairment of systolic and diastolic myocardial function in stable HTx recipients of donor hearts with either long GIT or older age. Thus, the duration of graft ischaemia and donor age should be taken into account when evaluating for cardiac dysfunction in HTx recipients. Considering the shortage of available donor hearts, these results do not support refusing donors with prolonged GIT or older age. However, the findings support the practice of the eager matching of younger donor grafts with younger recipients.

Study limitations

The selection criteria may potentially have introduced a selection bias since only clinically stable recipients were included. On the other hand, since the effect of longer GIT and higher donor age were still present in stable HTx recipients, we think that the results underscore the influence of GIT on transplant function. The present study was cross-sectional, thus the time of study after HTx was not equal. However, no significant difference in demographic parameters between the studied groups was present. Although the study population was heterogeneous in age, BMI and physical condition, there were no significant differences in pretransplant

diagnosis, age, gender or BMI between those who attended and those who were not asked or did not want to participate in the present study. Thus, we think that the result of the present study represents stable HTx recipients in general.

It should be noted that possible long-term consequences of the GIT-related graft dysfunction, such as heart failure and mortality, have not been addressed. The recipients in the present study were included at different stages in post-HTx status. It would therefore be of interest to investigate how GIT and donor age influence cardiac function in a prospective study of newly transplanted HTx recipients.

CONCLUSION

Prolonged GIT impairs both systolic and diastolic function measured at the IVS and RV free wall in stable HTx recipients, while increasing donor age impairs LV diastolic function only. Although the impairment is moderate and does not influence exercise capacity, the duration of graft ischaemia and donor age should be taken into account when evaluating for cardiac dysfunction in HTx recipients.

Funding

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