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THESIS FOR THE MASTERS DEGREE OF EXERCISE PHYSIOLOGY
MICHAEL SHELVER

Contents

Acknowledgements

Abbreviations

1-min HRR = 1 minute heart rate recovery

2-min HRR = 2 minute heart rate recovery

ASDNN5 = average standard deviation of the normal sinus beats in 5 min periods in 24 hours

CAN = cardiovascular autonomic neuropathy

CGM = continuous glucose monitoring

CPET = cardiopulmonary exercise test

CV = coefficient of variation

CVD = cardiovascular disease

FPG = fasting plasma glucose

HbA_{1c} = glycosylated hemoglobin

HDL-Cholesterol = high density lipoprotein cholesterol

HF = high frequency

HR = heart rate

HR_{max} = maximum heart rate

HRV = heart rate variability

hs-CRP = high sensitive c-reactive protein

IR = insulin resistance

LDL Cholesterol = low density lipoprotein cholesterol

LF = low frequency

OGTT = oral glucose tolerance test

RER = respiratory exchange ratio

rMSSD = root mean square of differences between successive NN intervals

RR = interval between successive heart beats

SDANN5 = standard deviation of the average sinus beats in five minute periods

SDNN = standard deviation of normal sinus beats

T2D = type 2 diabetes

VO_{2max} = maximal oxygen consumption

VO_{2peak} = peak oxygen consumption

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A randomized controlled trial

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ABSTRACT

BACKGROUND: It is estimated that by 2045 close to 700 million people worldwide will be suffering from Type 2 Diabetes (T2D). Exercise is important in the treatment and prevention of T2D. The frequency of exercise required for optimal effect is still to be explored.

OBJECTIVES: To compare two volume and intensity matched exercise protocols with different frequencies in individuals with T2D and to investigate the effects of these protocols on cardiorespiratory fitness, cardiovascular health and glycemic control.

METHODS: Subjects with T2D were recruited and randomly assigned either a high frequency high intensity interval training protocol (HF) or a low frequency high intensity interval training protocol (LF). Both groups were matched for volume and intensity and the study participants exercised for 120 minutes a week for 12 weeks. Changes in aerobic capacity (VO_{2peak}), glycosylated hemoglobin (HbA_{1c}), heart rate variability, glucose variability, blood lipids and body composition were measured.

RESULTS: Both the HF and LF group significantly improved aerobic capacity by 9% and 10% respectively, with no significant difference between groups. HbA_{1c} improved significantly only in the LF group by 5%. Heart rate variability improved only in the LF group. No significant improvements in glucose variability were seen in either groups.

CONCLUSION: Both HF and LF exercise protocols can be used as effective programs for improving aerobic capacity and reducing cardiovascular risk factors. Longer, less frequent exercise training seems to be more effective in improving heart rate variability and glycemic control provided this is at a frequency of at least four times a week.

1 Introduction

1.1 Overview of Type 2 Diabetes

The number of people with diabetes worldwide has doubled in the last 20 years and is estimated to increase to almost 700 million by 2045 [1, 2]. The dramatic increase in T2D prevalence has been hastened by rapid global urbanization and an increase in sedentary lifestyle [3]. A significant proportion of people living with T2D are undiagnosed including up to 40% of the diabetic population in Europe [4-6]. More than 5% of Norwegians between the ages of 20-79 have diabetes, but with undiagnosed cases this number could be as high as 400,000. The increase in global health expenditure attributed to diabetes is predicted to be unsustainable [2]. Diabetes mellitus is characterized by long term elevated blood glucose levels with type 2 diabetes (T2D) accounting for 90-95% of all cases [7]. T2D is a chronic metabolic disease characterized by hyperglycemia with disturbances in carbohydrate-, fat-, and protein metabolism. T2D is caused by inadequate insulin production by the pancreas, known as relative insulin deficiency, as well as decreased insulin effect on peripheral tissue, known as insulin resistance (IR) [8]. Insulin, the key hormone mediating glucose uptake in skeletal muscle and glucose production in the liver and kidneys, has impaired action and/or is unable to be produced at adequate quantity by the pancreatic β-cells [9]. The lack of insulin action and secretion leads to a decreased uptake of glucose by the skeletal muscle as well as increased hepatic glucose release, leading to chronic hyperglycemia [10]. Reduced insulin action and increased insulin resistance also leads to increased lipolysis in adipose tissue possibly leading to dyslipidemia due to

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inadequate fat lipid storage [11]. The disease onset is often gradual with early stages of insulin

resistance being indicated by excessive post-prandial hyperglycemia. Usually a deterioration of

insulin response to increased blood glucose concentration follows [12]. Lifestyle related factors

such as physical inactivity and obesity are the major contributing factors to T2D [12-14].

Genetics may also play a significant role in dysregulation of insulin action and peripheral insulin

sensitivity [8]. There is also an increased risk in developing T2D as age increases, if previously

diagnosed with gestational diabetes, if a family member has diabetes and with hypertension [7].

1.2 Diagnosis and Treatment of Type 2 Diabetes

T2D is diagnosed by measuring fasting plasma glucose (FPG), glycosylated hemoglobin (HbA$_{1c}$)

or the oral glucose tolerance test (OGTT) [15]. HbA$_{1c}$ is the most common method used to

diagnose T2D as it reflects average blood glucose levels over three to four months. Other

methods used are listed in Table 1.1 [7, 15].

Table 1.1 *Criteria for the Diagnosis of Diabetes*

Measurement	Threshold for Diagnosis
HbA$_{1c}$	≥48 mmol/mol (6.5 %)
Fasting Plasma Glucose (8 hours)	≥7.0mmol/L
Plasma-glucose ≥11,1 mmol/L two hours after an oral glucose tolerance test	≥11.1mmol/L
Random Plasma Glucose	≥11.1mmol/L

Treatment of T2D is multifaceted, involving pharmacological treatment strategies for

hyperglycemia, nutrition therapy, and physical activity [16]. Metformin is the preferred

pharmacological agent with long standing evidence of efficacy in reducing hyperglycemia [17] and additional antidiabetic agents can be used if glycemic goals are not being achieved. With T2D being a progressive disease, insulin therapy may also eventually be implemented to combat hyperglycemia. While usually only considered for more severe cases, self-titrated insulin therapy has been shown to be effective in improving glycemic control [18].

The majority of T2D individuals are obese. Medical nutrition therapy is a strategy designed to decrease weight by reducing caloric intake and optimizing food choices to improve overall health, measured by improvements in blood lipid levels and blood pressure [19]. Nutritional therapy clinical trials have demonstrated absolute reductions in HbA$_{1c}$ of 0.25-2.9% and these trials also indicated that this approach is more effective in the treatment of recently diagnosed T2D compared to chronic cases where diabetes has been treated for years [20, 21]. Physical activity, of moderate intensity aerobic activity (50-70% of HR$_{max}$), for periods of at least 150 minutes per week spread over at least 3 days per week with no more than two consecutive days off, is recommended to all T2D patients without contraindications [16]. Adults with T2D are also encouraged to perform resistance training twice a week. Exercise is clearly an important treatment strategy for T2D and will be discussed in detail in a latter section. Pharmacological, nutritional and exercise therapies are regularly used in combination as the standard method of care for mitigating complications stemming from T2D.

1.3 Type 2 Diabetes and Cardiovascular Disease

Cardiovascular disease (CVD) accounts for 70% of total mortality in the T2D population [9]. All types of CVD including stroke, coronary heart disease, and peripheral vascular disease are considerably more common in patients with T2D compared to nondiabetic patients [22]. While

CVD risk is markedly increased with T2D, so is the fatality of vascular incidents with myocardial infarctions being one third more fatal in patients with T2D than those without [23]. The key risk factor for CVD is glycemic control, with substantial evidence correlating long term hyperglycemia with both micro- and macrovascular complications [7, 9, 16]. HbA_{1c} is a significant predictor of CVD particularly with regard to coronary heart disease and ischemic stroke [24, 25]. A 2004 meta-analysis found that every 1% increase in HbA_{1c} was associated with an 18% increase in the risk of CVD events [26]. Alarmingly, the results of this study also showed that increased risk of CVD occurs at levels of glycemia far lower than the current threshold for diagnosis of T2D and even mild elevation of glucose has a substantial effect on cardiovascular health [9].

Chronic hyperglycemia leads to increased oxidative stress as well as glycosylation of almost every protein systemically, including lipoproteins, apolipoproteins and a variety of clotting factors. This activation of oxidative stress and glycosylation of important vascular proteins trigger dysfunctional metabolic pathways leading to microvascular damage, plaque formation and atherosclerosis [9, 27, 28]. However, it is important to treat the patient more comprehensively than just reducing HbA_{1c} by also reducing cardiovascular risk.

Pharmacological treatment of T2D has shown to reduce or prevent microvascular complications stemming from T2D but less so macrovascular complications. The importance of treating T2D is more than just lowering long term blood glucose. Treating blood glucose as well as reducing CV risk is of best treatment. This can be done by implementing statins, use of anti-hypertensive drugs as well as the use of newer antidiabetic drugs such as SGLT2-i and GLP-1R agonists, that have a mitigating effect on CVD. SGLT2-i work to reduce preload, afterload, vasodilation and epicardial fat while also increasing glucosuria, natriuresis, and uricosuria. This leads to a major

reduction in cardiovascular events, weight, blood pressure and nephropathy [29]. GLP-1R

Agonists increase satiety and insulin release while reducing gastric motility, chylomicron

production and glucagon production. These changes cause reduced postprandial glucose levels

and promote an anti-atherogenic effect with similar reductions to SLGT2-i in cardiovascular

events, blood pressure, weight and nephropathy [30].

The ADVANCE trial was designed to determine whether improving glycemic control of HbA$_{1c}$

concentrations to <6.5% would reduce the risk of macro- and microvascular disease using

standard T2D care in conjunction with more intensive pharmacological treatment [31]. While a

reduction in microvascular damage was demonstrated, the effect of intensive glucose control

showed no significant reduction in macrovascular effects [31, 32]. This trial produced similar

results to the U.K. Prospective Diabetes Study (UKPDS) where the risk of microvascular

complications such as retinopathy were reduced significantly with increased glycemic control yet

macrovascular risk factors had a non-significant change [32, 33]. Reduction in CVD within the

T2D population, especially in macrovascular events such as stroke or myocardial infarction,

appears therefore to require more intervention than just intensive glycemic control.

1.4 Type 2 Diabetes and Exercise

Regular exercise is critical for optimal health in individuals diagnosed with T2D. There is strong

evidence suggesting improved cardiac health, reduced hyperglycemia, and improved insulin

sensitivity are all positively correlated with regular exercise [34]. Physical activity causes

increased glucose uptake into active muscles and depletes glycogen signaling hepatic glucose

production. In T2D, glucose uptake generally outpaces gluconeogenesis and blood glucose levels

decline, reducing hyperglycemia [35]. Acute insulin sensitivity is experienced after mild to

moderate intensity exercise for 2-72h after with longer duration or higher intensity prolonging sensitivity [36, 37].

1.4.1 Aerobic Capacity

Aerobic capacity, measured as VO_{2max}, was first proposed in 1923 by Hill and Lupton [38]. VO_{2max} is defined as the oxygen uptake during maximal intensity that will not increase even with further workload and is considered a valid measurement in determining the cardiorespiratory system's ability to transport oxygen from air into tissue [38]. VO_{2max} can be described using the Fick equation (VO_2 = Cardiac Output * arterial-venous O_2 difference) [39]. Cardiac output (=stroke volume x heart rate) described as the amount of blood that can be pumped by the heart, usually expressed as liters per minute while arterial-venous O_2 difference is the differential in the oxygen content of arterial blood compared to venous blood. A greater cardiac output and arterial/venous difference indicates higher VO_2. While generally used as a predictor of performance within the world of endurance sports, VO_{2max} has been shown to be a more powerful predictor of mortality among men than other risk factor for cardiovascular disease [40]. Further research confirmed the finding of Myers *et al.* with a low peak VO_2 being shown as a strong predictor of long-term cardiac mortality in T2D patients [41]. T2D individuals were found to have an especially low aerobic capacity when evaluated by peak oxygen consumption (peak VO_2) and capacities have been shown to be reduced by 12-15% compared with the reference levels of age-matched healthy subjects [42]. With CVD being the major cause of death in the T2D population, improving aerobic capacity is paramount.

Aerobic exercise has been shown to improve VO_{2peak} as well as glycemic control in T2D patients [43-45]. A study, conducted to evaluate aerobic capacity and glycemic control, found that initial VO_{2peak} value as well as the increase in VO_{2peak} due to exercise was important in improving

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glycemic control [46]. Subjects with a higher baseline VO_{2peak} had better glycemic control, with equivalent exercise instruction of 30 minutes of aerobic exercise 3 times a week, indicating that T2D individuals with reduced VO_{2peak} may require more bouts of exercise to achieve similar improvements in glycemic control [46]. This study demonstrated aerobic exercise as a method for improving hyperglycemia, a known CVD risk factor. Moderate intensity exercise has been the generally accepted recommendation to individuals with T2D [47] although, recent literature has indicated that high intensity training may yield better results. A study, conducted in 2017, found an average 21% increase in VO_{2max} was achieved with high intensity aerobic training which was significantly different from levels measured with moderate continuous training [48]. A correlation between increased VO_{2max} and decreased HbA_{1c} values was demonstrated, thereby further supporting the finding that improved aerobic capacity may be connected to a concomitant improvement in glycemic control. A similar study, investigating metabolic syndrome, found that high intensity exercise increased VO_{2max} to a higher degree than moderate intensity exercise, suggesting that intensity was an important factor in improving aerobic capacity and reducing CVD risk factors [49]. Low volume high intensity exercise was also found to produce similar or even better results than high volume endurance training in T2D individuals with greater VO_{2peak} increases and larger reductions in HbA_{1c} among the high intensity group despite a 45% lower training volume [50].

1.4.2 Glucose Variability

Glucose variability, the shift in daily glucose trends, has recently become of clinical interest in the treatment of T2D. Individuals with T2D who achieve goal mean glucose values may still be at risk for complications from hyperglycemia if they have high levels of glycemic variability

[51]. Variation measures of fasting glucose and HbA$_{1c}$ have been shown to be predictors of all-cause mortality as well as micro- and macrovascular complications thus daily glucose trends have become important in optimal care of T2D [52]. High glucose variability has also be shown to affect cognitive performance in older T2D individuals independent of HbA$_{1c}$, fasting plasma glucose, and postprandial plasma glucose possibly indicating neural degeneration [53].

Recent advancements in continuous glucose monitoring (CGM) have made measuring glucose variability achievable. By the use of systems designed for monitoring of type 1 diabetic patients (Dexcom G4/5, Medtronic iPro2), glucose measurements can be recorded every 5 minutes, providing insight into daily patterns and hyperglycemic excursions.

Mean glucose and standard deviation of the mean can be analyzed to assess change in glucose variability with reduced standard deviation indicative of reduced glucose variability and in turn reduced risks of complications associated with poor control of T2D. T2D individuals may experience short term hypo- and hyperglycemic spikes that could negatively impact organs. Continuous glucose monitoring enables daily glycemic patterns, including spikes, to be observed which is not possible with quarterly HbA$_{1c}$ measurement. Coefficient of variation (CV), the ratio of standard deviation to the mean, is another metric commonly used to analyze relative variance. Reductions in CV indicate lower variation of blood glucose. Modest exercise has a potent effect in reducing blood glucose [54] and a multiple exercise sessions a week may lead to an improvement in glucose variability. However, it is still unknown how different frequencies of exercise can affect glucose variability.

1.4.3 Heart Rate Variability

Heart rate variability (HRV) is the variation in beat-to-beat (RR) interval and is a non-invasive measurement of the autonomous nervous system [55]. In HRV the RR interval is often called the NN interval based on normal sinus to normal sinus interval. The RR interval is measured from the electrocardiogram (ECG) complex from the R-wave to the following R-wave. HRV has been assumed to give information on the balance between sympathetic and parasympathetic nervous systems [56]. Low HRV has been associated with increased mortality [57] and has been identified in T2D individuals, which could reflect a defect in autonomic function [58, 59]. Reduced HRV may be linked to diabetic neuropathy, a progressive degeneration that affects mostly small-diameter nociceptive fibers within cutaneous tissue [60]. Measurement of HRV has been proposed as an important clinical measurement for early detection of autonomic nervous system dysfunction [61] and to assess the degree of cardiovascular autonomic neuropathy (CAN) [62, 63]. It has been reported that between 12% and 22% of T2D patients suffer from some degree of CAN with onset occurring within the first year of diagnosis [64].

HRV can be measured using time domain, frequency domain, and heart rate turbulence methods. It is most common to measure using time or frequency domain as heart rate turbulence measurement are uncommon in most clinical practices. Time and frequency domain are considered the methods of choice for analysis of the autonomic nervous system [65]. Time domain focuses on variability in interbeat intervals, the time between successive heartbeats, while frequency domain is an estimate of relative power in 4 frequency bands [66]. Assessment of time domain HRV variables is a simple method of assessing autonomic function in a variety of patient groups [67]. Time domain variables are used are: the average standard deviation of the normal sinus beats in five minute periods in the 24 hour period (ASDNN5), the standard

deviation of normal sinus beats (SDNN), the standard deviation of the average sinus beats in five minute periods (SDANN5), and the root mean square of differences between successive NN intervals (rMSSD). SDNN is the "gold standard" in regards to HRV and is used in cardiac risk management over a 24 hour period [68]. SDNN values below 50ms are considered unhealthy, 50-100ms as compromised health, and above 100ms as healthy [69]. The rMSSD is the second most useful measurement and is a reflection of beat to beat variance in HR. It is used to assess vagally mediated changes [70]. The rMSSD is more affected by the parasympathetic nervous system and low values are considered to be a higher risk for adverse health outcomes [71]. Age, heart rate, sex, and physical fitness all affect HRV causing a wide range in normal values [66]. Habitual aerobic exercise has been associated with higher HRV in a male population, indicating exercise as an augmentative treatment to improve HRV [72, 73].

Studies regarding exercise and the effect on HRV in T2D populations are limited, especially with regard to frequency. A recent study conducted on 41 T2D subjects found a thrice weekly moderate exercise intervention to be enough to induce significant improvements in SDNN and rMSSD [74]. A systematic review found that more than three days a week of aerobic exercise complemented with strength training was effective in improving HRV [55]. Frequency was noted as possibly the most important factor to improve HRV. A 12 week study showed improvements in weight, aerobic capacity, blood pressure and glucose control and trends for improved HRV [75]. The study concluded that exercise may be an important factor in preventing or stalling CAN, a valid reason to implement exercise in T2D.

1.5 Impact of Exercise Frequency

A review by Umpierre *et al.* examined 26 aerobic and/or resistance exercise studies to determine how frequency, intensity and duration of exercise influenced HbA1c among T2D individuals. The authors defined frequency as number of sessions per week, duration as minutes spent exercising throughout the study, and intensity as a percentage of maximum heart rate, when examining the effects of aerobic exercise [76]. Analysis of pooled data showed that increases in the frequency and weekly exercise duration, was correlated with significant improvement in HbA1c concentrations. Interestingly, when aerobic exercise trials were analyzed separately from resistance or combined aerobic and resistance programs, higher exercise frequency clearly improved HbA1c while exercise intensity showed no correlation with HbA1c improvements [76]. Studies with the highest number of sessions per week showed the greatest improvements in HbA1c. When a meta-regression analysis was performed on the 20 aerobic only trials, frequency accounted for 62% of the variation in glycemia. The observed improved glycemic control is believed to be due to evidence that a single bout of moderate intensity exercise was found to increase GLUT-4 gene expression for at least 3 hours [77] and 7 days of moderate intensity exercise increasing glucose disposal and skeletal muscle GLUT-4 transporter content [78]. GLUT-4 transporters are the principle glucose transporter proteins that mediate uptake of glucose in skeletal muscle [79] and increased GLUT-4 gene expression as well as increased GLUT-4 transporter content in skeletal muscle would likely lead to improved insulin sensitivity and glycogen storage [78, 80]. Increasing the frequency of exercise may lower the exposure of hemoglobin to glycosylation thereby lowering HbA_{1c} [76]. This is thought to be due to each frequent bout of exercise causing constant spikes in GLUT-4 gene expression and long-term improved GLUT-4 transporter content. It should be noted that Umpierre *et al.* assessed only for

HbA1c and did not include aerobic capacity, which is an important outcome particularly when assessing frequency of exercise and CVD. In order to maximize glycemic control, aerobic exercise of more than 3 sessions a week was recommended and may be more beneficial than increasing duration or intensity [76]. It should also be noted that the American Diabetes Association stresses the importance of frequency of exercise, recommending no more than 2 days without exercise, as insulin sensitivity, caused by exercise, is usually diminished after 72 hours [34].

A study, aimed at determining whether short bouts of intense exercise before meals would produce better postprandial blood glucose control compared to a single bout of moderate intensity exercise, found that the small bout regime, multiple times a day, was more successful [81]. Deemed "exercise snacks", the short intense exercise consisted of six 1-minute work bouts at 90% maximum heart rate that were performed 30 minutes before breakfast, lunch and dinner. The moderate intensity group had energy cost matched single daily sessions of 30 minutes at 60% maximum heart rate. 3-hr post prandial glucose was lower in the "exercise snacks" group compared to the moderate intensity single bout group, although the difference was not found to be significant [81]. Interestingly however, significant benefits were observed in 24-hour glucose concentrations with the "exercise snacks" group when compared to the moderate intensity single bout group, which could have been due to the short intensive bouts combating hyperglycemic spikes often seen after meals and frequently the cause of short term insulin resistance [82]. These hyperglycemic spikes may be more predictive than elevated fasting plasma glucose of CVD onset [83] and are strongly associated with increased HbA_{1c} levels in T2D individuals [84]. These spikes cause glycemic instability and may be reduced more effectively with higher frequency of exercise sessions than what is currently being recommended. The increased

frequency of sessions would also induce breaks in sedentary time which has shown to be beneficial for T2D parameters such as waist circumference, plasma triacylglycerol and plasma glucose levels [85]. Francois *et al.* have therefore suggested that the recommended 150 minutes per week of exercise be accumulated in short bouts, generally around meal times, to achieve glycemic control [81]. This study was in alignment with DiPietro *et al*, who found that three, 15-minute, post-meal walks were more effective than one 45-minute walk, for lowering postprandial plasma glucose [86]. It should be noted that both of these studied were aimed at observing postprandial plasma glucose in insulin resistant, but not T2D, individuals.

Looking to investigate whether fractionated exercise would yield differing glucose tolerance results compared to a single session of exercise, Baynard *et al.* had 15 subjects (9 obese and T2D, 6 non-obese and healthy), each perform 2 different exercise sessions and 1 session of no exercise as a control. This was conducted over 3 study days with the first session being 30 minutes at 60-65% of VO_{2peak} and the second being three 10-minute sessions at the same intensity. Interestingly, the investigators found that single or multiple bouts of moderate intensity exercise had no impact on glucose tolerance or insulin sensitivity [87]. Furthermore, glucose tolerance and insulin sensitivity on the no exercise day were no different from the exercise days [87] which may indicate that the exercise intensity of 60-65% VO_{2peak} was not high enough to elicit significant changes. While one day of 3 short sessions of moderate intensity exercise was shown not to improve glucose tolerance and insulin sensitivity, multiple repeated days may provide different results.

Investigating the frequency of training in the cardiovascular field highlights the fact that there are a limited number of published studies. One study investigating the effects of high-frequency training versus low-frequency training in patients with coronary artery disease found peak VO_2

and ventilatory anaerobic threshold increased significantly in the high frequency group [88].

There was also a greater improvement in maximal power output measured in watts, most likely

related to the increased ventilatory anaerobic threshold. A major limitation with this study was

that the exercise groups were not matched for volume, with the high frequency group performing

10 two hour training sessions a week compared to only 2 with the low frequency group [88]. A

strong case could be made that the significant improvements seen within the high frequency

group were due to having an exercise regime volume five times greater over the six-week period.

A study conducted within the faculty at NTNU assessed the effect of 24 sessions of high-

intensity interval training carried out at either high frequency versus moderate frequency and had

interesting findings regarding cardiovascular adaptations [89]. Twenty-one healthy subjects

completed 24 interval sessions in either 3 weeks (high frequency) or 8 weeks (low frequency)

followed by 9 weeks of no training. VO_{2max} gradually improved over the training period in the

moderate frequency group with the highest improvement (10.7%) being achieved at the ending

[89]. The high frequency group had no improvements in VO_{2max} during the training period yet

showed an improvement of 6.1% 12 days into the de-training period. The moderate frequency

group had significantly reduced VO_{2max} after 4 weeks of no training compared to max value,

while the high frequency group did not show reduced VO_{2max} [89]. The lack of immediate

improvement in VO_{2max} in the high frequency group was thought to be due to fatigue

accompanied by such a high exercise dose. With lack of sufficient rest, there was lack of time to

adjust unlike the moderate frequency group which had a continual improvement of VO_{2max} over

the training period, demonstrating adequate recovery and adaption [89]. While the highest

VO_{2max} in the moderate frequency group was measured 4 days post training, the high frequency

group peak VO_{2max} was found two weeks post training, indicating a high intensity load of this

frequency was too severe to cause progressive cardiovascular improvements [89]. Nevertheless, in both cases, improvements were found in aerobic capacity, indicating moderate to high frequency sessions of exercise can be useful in decreasing mortality, especially in regards to T2D individuals with high risk of CVD. The study described above was performed with young, healthy females and therefore the results should be interpreted with caution.

While evidence overwhelming support in aerobic exercise being an important treatment in T2D ideal exercise prescription is still yet to be determined, particularly with regard to the frequency of sessions. While there is strong evidence in support of exercise to improve CVD risk factors [46], particularly with regard to intensity [49], there is a general lack of agreement within the field as to optimal frequency with some studies showing benefits in terms of T2D glycemic control [81] while others suggesting high stress on the cardiovascular system, requiring long recovery periods to elicit improvements [89]. With the major barrier to exercise often being a lack of spare time and motivation, much shorter yet frequent sessions may be more attainable within this population. The importance of frequency cannot be denied, with Umpierre *et al.* showing number of exercise sessions was of greater importance when compared with duration and intensity in T2D HbA_{1c} control. There is a significant knowledge gap with regard to exercise frequency and its effect on T2D, and in particular, the parameters HRV and glucose variability.

1.6 Aim and Hypothesis

The aim of this study was to compare the effects of different frequencies of volume and intensity matched exercise on aerobic capacity, HRV and glucose variability in individuals with T2D. It was hypothesized that low frequency exercise training will improve VO_{2max} and HRV to a greater degree than high frequency training while high frequency training will improve glucose

variability more than low frequency training. We also hypothesized that HbA_{1c} will improve in both groups, but to a greater extent in the high frequency group. A secondary outcome was to determine which treatment would be more sustainable as a long-term exercise program for T2D patients.

2 Methods

2.1 Study Design

This study was a 12-week, single center, parallel-group, randomized study performed at the Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology in Trondheim, Norway. The first inclusion was done in year 2015 by another master's project (REK reference number 2014/1405). From here called Cohort 1. Chart 2.1 (below) illustrates the study design. The same study design was used in both cohorts. The first inclusion (cohort 1) did not reach sample size and therefore a second inclusion was done (cohort 2).

Chart 2.1 *Study Design*

Cohort 1 (REK 2014/1405)

Assessed for eligibilty (n=42)

Excluded due to not meeting inclusion criteria (n=2)

Cohort 2 (REK 2018/995)

Assessed for eligibilty (n=15)

Excluded due to not meeting inclusion criteria (n=1)

Randomized (n=54)

Allocated to High Frequency group (n=29)

Allocated to Low Frequency group (n=25)

- Lost to follow-up (n=2)
- Discontinued intervention (four due to injury/illness, one due to lack of time, one due to personal reason, one due to acute kidney failure unrelated to exercise) (n=9)

- Lost to follow-up (n=0)
- Discontinued intervention (two due to personal reasons) (n=2)

- Analyzed (n=13)
- Excluded from analysis (four due to completing less than 80% sessions within training period, one had participated in both cohorts and only cohort 2 results were used)

- Analyzed (n=19)
- Excluded from analysis (three due to completing less than 80% sessions within training period, one had participayed in both cohorts and only cohort 2 results were used)

2.2 Study Participants

Subjects were recruited via newspaper advertisements, social media platforms, and through local medical professionals in Trondheim, Norway. Subjects were also recruited from a control group in a previous study within the research group. To be included, subjects were required to be

between 30 to 70 years of age and be diagnosed with type 2 diabetes. Subjects were excluded if they were on insulin therapy, had severe chronic diabetic complications, liver dysfunction, renal dysfunction, heart failure, myocardial ischemia, or major cardiovascular event history. If patients reported exercising more than the minimum guidelines of 150 minutes of exercise per week or were unable to perform exercise, they were also excluded. Cohort 1 had the same inclusion criteria. Two subjects from Cohort 2 had previously participated in Cohort 1 and their previous data was excluded from the study.

After screening interviews, 14 of the 15 subjects were found to be eligible for this study. The study protocol was explained and a detailed description of the study was given to each subject. Before beginning the study, all subjects signed a written informed consent approved by the regional ethical committee (REK reference number 2018/995). To be able to use the results from cohort 1 we obtained informed consent again according to the decision of the regional ethical committee.

2.3 Baseline Assessment

Baseline testing took place at St. Olav's Hospital over a two-week period. Medical history was documented. Body weight was measured using a Seca 877 Digital Scale (Seca, Hamburg, Germany). Shoes and outerwear were removed. Body height was measured using a wall mounted height scale with shoes off. Waist circumference was measured from the top of the iliac crest with a measuring tape and was taken in expiration. The average of 3 sequential measurements was recorded.

Systolic and diastolic blood pressure was measured with a CASMED 740 Vital Signs Monitor (CAS Medical Systems Inc., Branford, Connecticut, United States). The subject remained in a

seated position with feet contacting the ground and rested for 2min before the first measurement. Three measurements were taking with a 2min rest in between. The first measurement was deleted and the average of the subsequent two measurements was calculated and recorded. A 12-lead electrocardiogram assessment was taken prior to exercise testing to determine if there were any cardiovascular anomalies. The subject lay in a supine position for five minutes and an auto analysis was performed using a Phillips Page Writer Trim III ECG (Phillips Medical Systems, Andover, MA, USA). The analysis was then viewed by a cardiologist (Dr. Charlotte Björk Ingul) to determine if the subject was appropriate for the study.

2.4 Cardiopulmonary Exercise Test

A cardiopulmonary exercise test was performed to determine maximal oxygen uptake (VO_{2max} or VO_{2peak}) using a treadmill (Woodway PPS5, Woodway, Weil am Rheim, Germany). Before beginning a warm up and subsequent test, subjects were informed of the protocol as well as the procedures for voluntarily stopping the treadmill if needed. After setting the inclination of the treadmill to 2%, subjects began a self-paced 10 minute warm up to familiarize themselves with the treadmill. Once the warm up was completed, the treadmill was stopped and a mask connected to a Cortex Metalyzer II spiroergometric system (Cortex, Leipzig, Germany) was placed on the patient and used to measure oxygen uptake. The mask was tested to ensure an airtight seal. The incline was then increased to 4% and speed increased to the same value that was observed at the end of the warmup period. Speed was then kept constant while inclination increased 2% every 2 minutes until subject exhaustion. If the subject was able to continue at 10% incline, speed was then increased 1km/h every 2 minutes until exhaustion.

An average of the three highest 10 second values of oxygen uptake recorded were averaged to determine VO_{2max}. Criteria for VO_{2max} was as follows: an RER value of >1.05, a plateau in oxygen uptake despite increasing inclination or speed, and a breathing frequency >40. If these were not met, the value was considered a $VO_{2peak.}$

During the treadmill test, heart rate (HR) was recorded using HR monitors (Polar Electro, Kempele, Finland) to determine maximal attainable heart rate. To calculate HR_{max} 5 beats per minute was added to the maximal HR achieved during the CPET [90]. Heart rate recovery was recorded as the difference between peak heart rate obtained during the test and heart rate 1 min and 2 min following termination of the test. Subjects remained standing on the treadmill during these measurements.

2.5 Heart Rate Variability

A Philips DigiTrak XT Holter recorder (Philips, Amsterdam, Netherlands) was worn for 24 hours to record HR and HRV. Electrodes were placed on the subject and the subject was instructed on device use and how to replace the electrodes during the measurement period if required. Data was then analyzed by Philips Holter Software. Time Domain HRV measurements (ASDNN 5, SDANN 5, SDNN, rMSSD) were selected for analysis. In cohort 1, the Firstbeat Bodyguard 2 (Firstbeat Bodyguard, Jybaskyla, Finland) was used to assess 5-day HRV data. The device used 2 electrode pads to measure HRV over the time period. Night time rMSSD data from cohort 1 was selected for analysis.

2.6 Continuous Glucose Measurement

Dexcom G4 and G5 CGM systems were used for continuous glucose measurement (Dexcom, San Diego, California, USA). This system uses a small subcutaneous sensor to determine interstitial blood glucose levels and reports a blood glucose value every 5 minutes. The sensors were inserted in the lower abdomen and worn for a minimum of 72 hours. The devices were calibrated using Contour Next EZ Glucometers (Ascensia Diabetes Care, Parsippany, NJ, USA) twice 2 hours after initial device insertion and every 12 hours after. The data recorded was then downloaded and analyzed using Dexcom Studio and CLARITY software. Cohort 1 used the Medtronic iPro2 system (Medtronic, Minneapolis, Minnesota, USA) and Contour Next EZ Glucometers (Ascensia Diabetes Care, Parsippany, NJ, USA) to obtain CGM data.

2.7 Blood Samples

Blood samples were collected at the laboratory medical clinic, St. Olavs Hospital after fasting for at least 8 hours. Blood glucose, HbA_{1c}, insulin C-peptide, total cholesterol, HDL, LDL, triglycerides and high sensitive c-reactive protein (hs-CRP) were analyzed at baseline and post-testing. Hemoglobin and creatinine were assessed at baseline

2.8 Randomization

After completion of baseline testing, cohort 1 and 2 subjects were randomized using WebCRF, a data collection program provided by Unit for Applied Clinical Research, Faculty of Medicine, NTNU, into one of the following groups for a 12-week training intervention:

1. 10-minute high frequency (12 sessions per week) interval group (HF, n=29)

2. 30-minute low frequency (4 sessions per week) interval group (LF, n=25)

2.9 Exercise Intervention

Each group performed 120 minutes of exercise, matched for workload intensity, with both groups completing 36 minutes of high intensity and 84 minutes of moderate intensity per week. The HF group performed a total of 144 sessions lasting 10 minutes each while the LF group performed a total of 48 sessions lasting 30 minutes each over the intervention period. Completion of 80% of total sessions (HF = 115, LF = 38) was considered acceptable. Due to the HF group having short and frequent sessions, the majority of sessions were completed at home at convenient times. Subjects in the HF were given the option to complete sessions at the hospital if wanted. The LF group completed the majority of interval sessions under supervision in the hospital training center while performing moderate intensity workouts at home. Subjects with long commutes or scheduling issues were allowed to complete sessions at home if necessary. All training sessions were monitored using Polar HR monitors (Polar Electro, Kempele, Finland). Peak HR during interval bouts were recorded on paper by either the subject or study supervisor.

Training sessions in both groups were based on intensity determined from a percentage of HR_{max} found during CPET. Moderate intensity was defined as 70% of HR_{max} and high intensity as 90-95% of $HR_{max.}$

The HF group had 9 high intensity sessions and 3 moderate intensity sessions while the LF group had 3 high intensity sessions and 1 moderate intensity session.

The HF high intensity session protocol involved a 5 minute warm up at 70% HR_{max} and then a 4 minute high intensity interval with the goal of ending at 90-95% of HR_{max}. A 1 minute cooldown

to return to 70% HR_{max} followed the interval. The moderate intensity session protocol was a 10 minute session at 70% HR_{max}. These sessions were performed at least 4 hours apart from each other with a maximum of twice a day. These sessions are illustrated in Figure 2.1 and Figure 2.2.
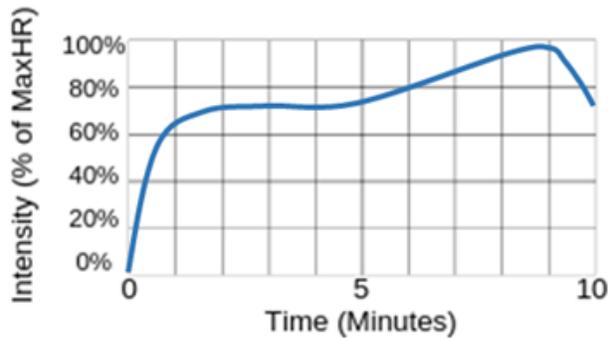

Figure 2.1 *HF High Intensity Interval Session. The figure describes percentage of maximum heart rate (% of MaxHR) at a given time (minutes).*
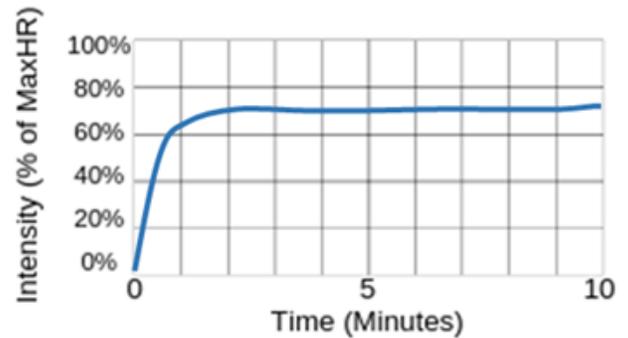
Figure 2.2 *HF Moderate Intensity Session. The figure describes percentage of maximum heart rate (% of MaxHR) at a given time (minutes).*

The LF high intensity session protocol involved an 8 minute warm up at 70% HR_{max} and then three 4 minute high intensity intervals with the goal of ending each at 90-95% of HR_{max}. Between each interval a 3 minute recovery period occurred in order to return HR to 70% HR_{max}. Following the third high intensity interval, a 4 minute warm down at 70% HR_{max} concluded the session. The moderate intensity session protocol was a 30 minute session at 70% HR_{max}. These sessions were performed throughout the week with no more than two days off between sessions. These sessions are illustrated in Figure 2.3 and 2.4.
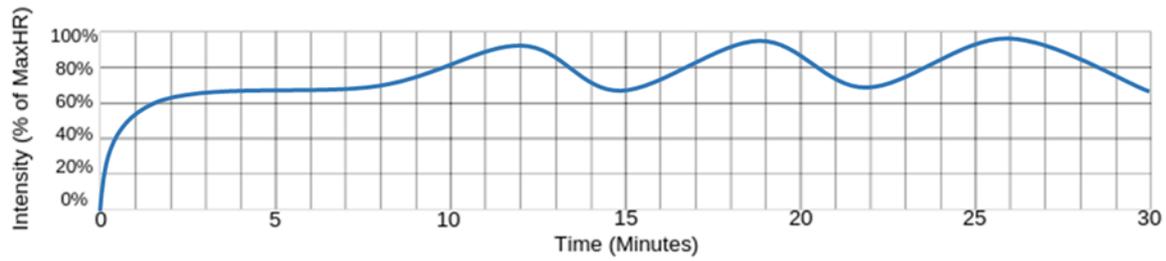
Figure 2.3 *LF High Intensity Interval Session. The figure describes percentage of maximum heart rate (% of MaxHR) at a given time (minutes).*
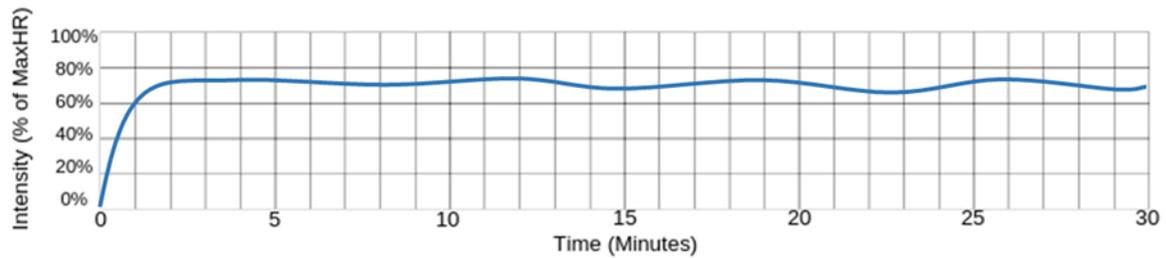
Figure 2.4 *LF Moderate Intensity Session. The figure describes percentage of maximum heart rate (% of MaxHR) at a given time (minutes).*

Exercise sessions were performed mostly on treadmills with some subjects opting for cycling due to familiarity or comfort. As fitness level improved, speed, inclination, or resistance were increased to ensure that HR zones were met.

2.10   Post-testing

Post-testing was completed within 1 week of subjects last training session and followed the same protocol as baseline testing.

2.11   Outcomes

The primary outcome of this study was comparing the effect of a HF exercise intervention on VO_{2max} compared to a LF exercise intervention in individuals with T2D. Secondary outcomes

were the effect of HF exercise training compared to LF exercise training in improving HRV, glucose variability, and HbA$_{1c}$.

2.12 Data Analysis

Data analysis was conducted using IBM SPSS Statistics 25 (IBM, Armonk, New York, USA). Data was checked for normal distribution using quantile-quantile (Q-Q) plots and a One-way Analysis of Variance (ANOVA) was used to check for any significant difference between the two groups at baseline. Within group changes were analyzed for significance using a paired sample t-test while between group changes were analyzed using an independent samples t-test.

3. Results

3.1 Participants

No significant differences in test variables were found at baseline (see Table 3.1). 32 of the 54 participants completed the study with an average compliance of 96.6%. The HF group had 11 drop out or fail to complete post-testing. The LF group had 2 drop out of the study. The HF group had 5 participants excluded from analysis (4 due to less than 80% compliance, 1 participated in both cohort 1 and 2 and only the cohort 2 data was used.) while the LF group had 4 participants excluded (3 due to less than 80% compliance, 1 participated in both cohort 1 and 2 and only the cohort 2 data was used.). There was no significant difference in compliance between groups with the HF group completing 98.3% of sessions and the LF group completing 95.6% of sessions.

Table 3.1 *Baseline Characteristics*

	HF (n=13)	LF (n=19)	P-value of Difference
Age (years)	57.5±8.5	56.9±9.9	0.61
Height (cm)	180.4±8.7	176.7±11.1	0.34
Weight (kg)	100.2±12.4	91.4±16.8	0.67
Waist Circumference (cm)	109.8±10.3 (n=12)	104.9±13.2	0.31
Systolic Blood Pressure (mmHg)	144.1±12.0 (n=10)	141.8±24.3	0.09
Diastolic Blood Pressure (mmHg)	89.2±10.4 (n=10)	87.2±12.3	0.37
HbA1c (%)	6.56±0.60	7.05±0.79	0.14

Baseline values are reported as mean ± standard deviation. P-values show between group differences. HbA1c = glycosylated hemoglobin.

3.2 Aerobic Capacity

A significant improvement in VO_{2peak} (mL/min/kg) was found in both groups with the HF group improving by 8.7% (p=0.002) and the LF group improving by 10.3% (p<0.0005). Absolute VO_{2peak} (L/min) saw similar significant improvements with the HF group increasing by 6% (p=0.001) and the LF group increasing by 7.9% (p<0.0005) respectively. A significant change in 2-min HRR was observed in the LF group (p=0.04). No significant differences were found between group changes after treatment. Changes in aerobic capacity variables can be seen in Table 3.2 and Table 3.3. Changes in VO_{2peak} and mean difference can be seen in Figures 3.1 and 3.2.

Table 3.2 *High Frequency Group Aerobic Capacity and Heart Rate Variability*

	Baseline HF (n=13)	Post HF (n=13)	Mean Change	95% CI	p-value
VO$_{2peak}$ (mL/min/kg)	33.2±5.7	36.1±6.4	3.0±2.7	1.3, 4.6	0.002**
VO$_{2peak}$ (L/min)	3.32±0.54	3.52±0.55	0.189±0.16	.09, 0.29	0.001**
Maximum HR (bpm)	175.4±14.9 (n=12)	170.9±14.3 (n=12)	-4.5±9.5	-10.3, 1.6	0.13
1-min HRR (bpm)	141.0±18.0 (n=4)	130.8±13.1 (n=4)	-10.3±19.3	-41.0, 9.8	0.37
2-min HRR (bpm)	113.3±8.5 (n=3)	111.7±11.0 (n=3)	1.7±4.6	-13.1, 9.8	0.60
Average HR (bpm)	76.0±10.5 (n=3)	66.0±8.7 (n=3)	-10.0±13.9	-44.5, 24.5	0.34
ASDNN5 (ms)	51.5±5.1 (n=3)	68.0±15.0 (n=3)	16.5±9.9	-8.2, 41.2	0.10
SDANN5 (ms)	103.4±6.6 (n=3)	105.5±19.7 (n=3)	2.1±13.1	-30.3, 34.6	0.80
SDNN (ms)	115.1±4.6 (n=3)	131.0±25.9 (n=3)	15.8±21.9	-38.5, 70.2	0.34
rMSSD (ms)	51.4±21.8 (n=3)	59.1±17.8 (n=3)	7.7±15.5	-30.7, 46.2	0.48

Baseline and post values are reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the last column. * indicates a p-value of <0.05 and ** a p-value of <0.01.

VO$_{2peak}$ indicates peak oxygen uptake. 1-min HRR indicates 1-min Heart Rate Recovery. 2-min HRR indicates 2-min Heart Rate Recovery. ASDNN5 indicates average standard deviation of the normal sinus beats in five minute periods in the 24 hour period. SDANN5 indicates the standard deviation of the average sinus beats in five minute periods. SDNN indicates the standard deviation of normal sinus beats. rMSSD indicates the root mean square of differences between successive NN intervals.

Table 3.4 *Low Frequency Group Aerobic Capacity and Heart Rate Variability*

	Baseline LF (n=19)	Post LF (n=19)	Mean Change	95% CI	p-value	p-value of diff.
VO$_{2peak}$ (mL/min/kg)	32.1±7.1	35.4±7.2	3.3±2.3	2.2, 4.3	<0.0005**	0.74
VO$_{2peak}$ (L/min)	2.93±0.62	3.16±0.58	0.227±0.21	0.13, 0.33	<0.0005**	0.46
Maximum HR (bpm)	175.4±9.2 (n=18)	171.9±6.5 (n=18)	-3.6±8.8	-7.9, -3.6	0.10	0.78
1-min HRR (bpm)	133.4±16.8 (n=7)	132.9±14.3 (n=7)	-0.57±10.8	-10.5, 9.4	0.89	0.30
2-min HRR (bpm)	117.0±12.6 (n=7)	110.9±12.5 (n=7)	-6.1±6.3	-12.0, -0.3	0.04	0.31
Average HR (bpm)	70.3±3.1 (n=6)	74.7±11.6 (n=6)	4.3±9.1	-5.2, 13.8	0.29	0.31
ASDNN5 (ms)	58.9±13.1 (n=6)	61.9±14.5 (n=6)	3.0±8.7	-6.1, 12.1	0.43	0.95
SDANN5 (ms)	105.4±24.0 (n=6)	119.7±52.3 (n=6)	14.3±39.6	-27.3, 55.8	0.42	0.13
SDNN (ms)	118.7±29.6 (n=6)	142.4±67.0 (n=6)	23.7±46.3	-24.9, 72.3	0.26	0.23
rMSSD (ms)	37.2±12.7 (n=6)	43.4±7.4 (n=6)	6.2±10.3	-4.6, 17.0	0.20	0.33

Baseline and post values are reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the second to last column. P-values showing between group differences are displayed in the last column. * indicates a p-value of <0.05 and ** a p-value of <0.01.

VO$_{2peak}$ indicated peak oxygen uptake. 1-min HRR indicates 1-min Heart Rate Recovery. 2-min HRR indicates 2-min Heart Rate Recovery. ASDNN5 indicates average standard deviation of the normal sinus beats in five minute periods in the 24 hour period. SDANN5 indicates the standard deviation of the average sinus beats in five minute periods. SDNN indicates the standard deviation of normal sinus beats. rMSSD indicates the root mean square of differences between successive NN intervals.
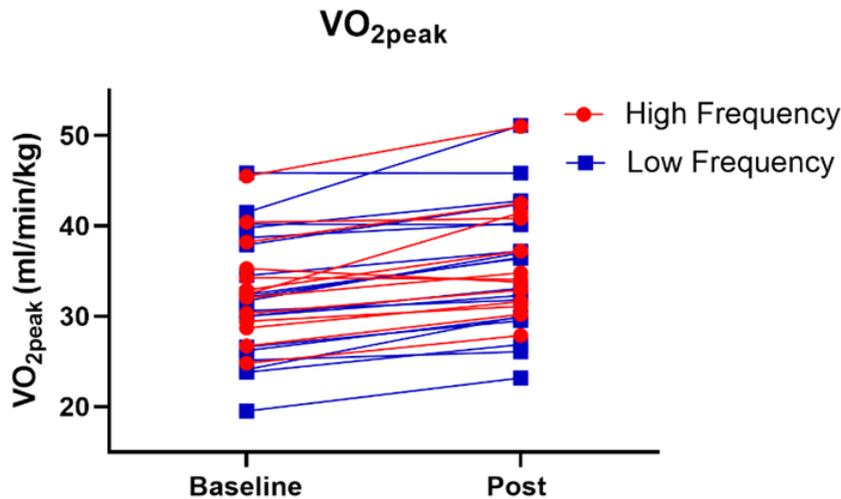
Figure 3.1 *Changes in VO$_{2peak}$*
Displays change in VO$_{2peak}$ (ml/min/kg) in each individual subject from baseline to post testing. The high frequency group individuals are displayed in red with dot. The low Frequency group individuals are displayed in blue with squares.
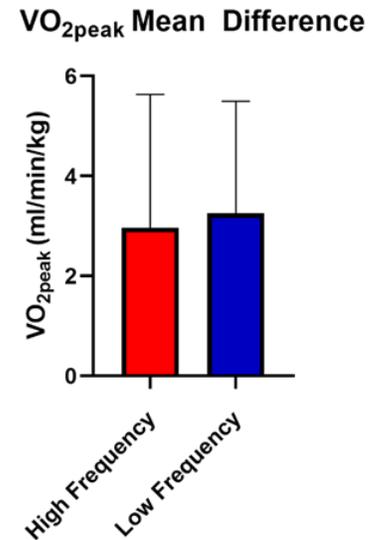
Figure 3.2 *Mean Differences in VO$_{2peak}$*
Displays mean differences in VO$_{2peak}$ (ml/min/kg). The high frequency group is displayed in the first column in red and the low frequency group in the second column in the blue.

3.3 Heart Rate Variability

In cohort 1 a significant decrease in nighttime HR and increase in nighttime rMSSD occurred in the LF group after 12 weeks of training, seen in Table 3.5. No significant change occurred in the HF group. No significant change in 24-hr HRV variables were observed in cohort 2. ASDNN5, SDANN5, SDNN, and RMSSD had mean values higher than pretreatment but failed to reach any significance. Cohort 2 pre- and post-treatment HRV data can be found in TABLE 3 and TABLE 4.

Table 3.5 *Cohort 1 Nighttime Heart Rate Variability*

	Baseline HF (n=9)	Post HF (n=9)	P-value	Baseline LF (n=12)	Post LF (n=12)	P-value
Night HR (BPM)	61±10	61±7	0.405	69±11	64±10	0.005**
Night rMSSD (ms)	29±13	35±17	0.362	27±13	32±17	0.023*

Baseline and post values reported as mean ± standard deviation. P-values of the change from baseline to post are displayed in the last column. * indicates a p-value of <0.05 and ** p-value of <0.01.
Night HR = nighttime average heart rate. Night rMSSD = nighttime root mean square of differences between successive NN intervals.

3.4 Glucose Variability

Mean 3-day average glucose trended lower post-treatment but no significant change was found. SD and CV changes were insignificant. Table 3.6 and 3.7 show pre- and post-treatment glucose variability data.

Table 3.6 *High Frequency Group Glucose Variability and Body Composition*

	Baseline HF (n=13)	Post HF (n=13)	Mean Change	95% CI	p-value
Average 3-day Glucose (mmol/L)	7.71±1.68 (n=12)	7.33±1.37 (n=12)	-0.38±1.23	-1.17, 0.40	0.31
SD of 3-day Glucose (mmol/L)	1.60±0.66 (n=12)	1.40±0.55 (n=12)	-0.20±0.47	-0.50, 0.09	0.16
Coefficient of Variation (%)	20.21±6.06 (n=12)	19.13±7.34 (n=12)	-1.08±7.26	-5.70, 3.52	0.61
Weight (kg)	100.2±12.4	97.6±12.2	-2.5±4.8	-5.4, 0.4	0.08
Waist Circumference (cm)	108.2±12.2	105.4±9.9	-2.8±3.0	-4.8, -0.8	0.01*

Baseline and post values reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the last column. * indicates a p-value of <0.05 and ** p-value of <0.01.

Table 3.7 *Low Frequency Group Glucose Variability and Body Composition:*

	Baseline LF (n=19)	Post LF (n=19)	Mean Change	95% CI	p-value	p-value of difference
Average 3-day Glucose (mmol/L)	8.10±1.67 (n=16)	7.97±1.61 (n=16)	-0.13±1.26	-0.80, 0.54	0.68	0.60
SD of 3-day Glucose (mmol/L)	1.63±0.55 (n=16)	1.76±0.47 (n=16)	0.13±0.57	-0.18, 0.43	0.39	0.11
Coefficient of Variation (%)	19.76±3.19 (n=16)	22.19±4.87 (n=16)	2.43±5.22	-0.36, 5.21	0.08	0.15
Weight (kg)	91.4±16.8	90.0±17.4	-1.5±2.6	-2.7, -0.3	0.02*	0.38
Waist Circumference (cm)	105.4±13.4 (n=18)	104.4±12.3 (n=18)	-1.1±2.6	-2.4, 0.2	0.10	0.80

Baseline and post values reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the second to last column. P-values showing between group differences are displayed in the last column. * indicates a p-value of <0.05 and ** p-value of <0.01.

3.5 Blood Variables and Body Composition

A significant decrease of 3.6% in HbA1c was observed in the LF group (p=0.001). No significant change was found in HbA1c in the HF group. A significant decrease in C-peptide (nmol/L) was seen in the LF group. Both HF and LF groups had a significant increase in HDL-cholesterol (mmol/L) with the HF group increasing by 7.6% (p=0.03) and the LF group by 6.5% (p=0.03). Changes in blood variables can be viewed on Table 3.8 and Table 3.9.

The LF group had a significant change in weight, decreasing by 1.5% (p=0.02) while the HF group did not. The HF group had a 2.6% reduction in waist circumference (p=0.01). Changes in body composition can be seen in Table 3.6 and 3.7. Changes in HbA_{1c} and mean difference can be seen in Figure 3.3 and Figure 3.4.

Table 3.8 *High Frequency Group Blood Variables*

	Baseline HF (n=12)	Post HF (n=12)	Mean Change	95% CI	p-value
HbA$_{1c}$ (%)	6.58±0.62	6.38±0.78	-0.20±0.35	-0.43, 0.03	0.08
HbA$_{1c}$ (mmol/mol)	48.5±6.8	46.4±8.5	-2.08±3.91	-4.6, 0.4	0.09
Fasting Glucose (mmol/L)	7.77±1.46	7.34±1.52	-0.43±0.76	-0.91, 0.06	0.08
C-peptide (nmol/L)	0.99±0.28	0.89±0.28	-0.10±0.23	-0.24, 0.04	0.15
hs-CRP (mg/L)	2.02±1.94	1.98±1.73	0.04±1.03	-0.69, 0.61	0.90
Total Cholesterol (mmol/L)	4.63±1.24	4.52±1.19	0.11±0.51	-0.43, 0.21	0.48
HDL Cholesterol (mmol/L)	1.18±0.27	1.27±0.28	0.09±0.13	-0.01, 0.17	0.03*
LDL Cholesterol (mmol/L)	3.05±1.19	2.87±1.07	0.18±0.45	-0.47, 0.10	0.18
Triglycerides (mmol/L)	1.92±0.96	1.55±0.67	0.37±0.74	-0.84, 0.09	0.11

Baseline and post values are reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the last column. * indicates a p-value of <0.05 and ** a p-value of <0.01.

HbA1c = glycosylated hemoglobin. hs-CRP = high sensitive c-reactive protein. HDL Cholesterol = high density lipoprotein cholesterol. LDL Cholesterol = low density lipoprotein cholesterol.

Table 3.9 *Low Frequency Group Blood Variables*

	Baseline LF (n=19)	Post LF (n=19)	Mean Change	95% CI	p-value	p-value of diff.
HbA$_{1c}$ (%)	7.01±0.79	6.76±9.80	-0.25±0.26	-0.38, -0.12	0.001**	0.21
HbA$_{1c}$ (mmol/mol)	53.2±8.7	50.4±8.8	-2.8±3.0	-4.3, -1.3	0.001**	0.36
Fasting Glucose (mmol/L)	8.00±1.54	7.78±1.78	-0.22±2.00	-1.17, 0.75	0.64	0.07
C-peptide (nmol/L)	1.09±0.46	0.97±0.37	-0.12±0.22	-0.22, -0.01	0.03*	0.90
hs-CRP (mg/L)	2.38±2.67	3.71±7.77	1.33±5.92	-1.53, 4.19	0.34	0.23
Total Cholesterol (mmol/L)	4.47±1.24	4.55±1.17	0.08±0.62	-0.21, 0.38	0.56	0.75
HDL Cholesterol (mmol/L)	1.08±0.25	1.15±0.28	0.07±0.13	-0.01, 0.13	0.03*	0.74
LDL Cholesterol (mmol/L)	2.90±1.16	2.98±1.10	0.08±0.50	-0.16, 0.33	0.47	0.92
Triglycerides (mmol/L)	1.72±0.85	1.65±0.81	-0.08±0.46	-0.30, 0.14	0.46	0.053

Baseline and post values are reported as mean ± standard deviation. Mean change is mean within group change ± standard deviation. 95% CI = 95% confidence intervals. P-values of the change from baseline to post are displayed in the second to last column. P-values showing between group differences are displayed in the last column. * indicates a p-value of <0.05 and ** a p-value of <0.01.

HbA1c = glycosylated hemoglobin. hs-CRP = high sensitive c-reactive protein. HDL Cholesterol = high density lipoprotein cholesterol. LDL Cholesterol = low density lipoprotein cholesterol.
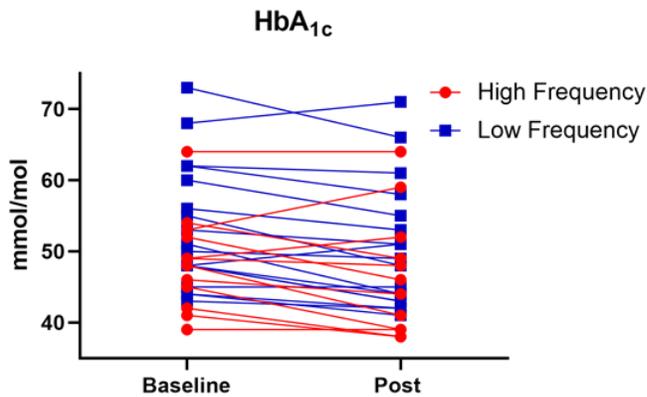
Figure 3.3 *Changes in HbA$_{1c}$* Displays change in HbA$_{1c}$ (mmol/mol) in each individual subject from baseline to post testing. The high frequency group individuals are displayed in red with dot. The low frequency group individuals are displayed in blue with squares.
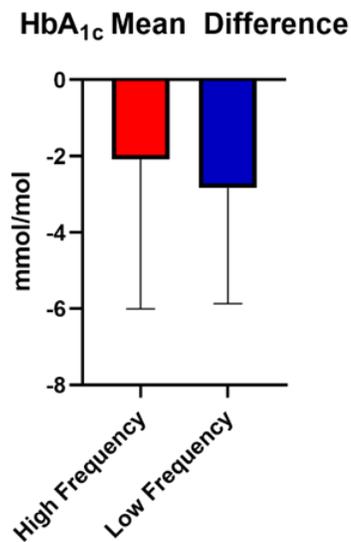
Figure 3.4 *Mean Differences in HbA$_{1c}$* Displays mean differences in HbA$_{1c}$ (mmol/mol). The high frequency group is displayed in the first column in red and the low frequency group in the second column in the blue.

4. Discussion

4.1 Aerobic Capacity

The major finding in this study was that aerobic capacity improved similarly in both the HF and LF groups, disagreeing with the proposed hypothesis that VO$_{2peak}$ would improve to a greater extent in the LF group. Improvements of 8.7% in the HF group and 10.7% in the LF group were seen in VO$_{2peak}$, which is a smaller improvement compared to a recent high intensity interval study where T2D patients improved VO$_{2max}$ by 21% [48]. This is likely due to the Støa *et al.* study having a population with lower baseline VO$_{2max}$ at 25.6 mL/min/kg compared to the HF and LF groups, in the present study, at 33.2 mL/min/kg and 32.1 mL/min/kg respectively. When compared to a study conducted to determine the effects of high intensity interval exercise versus moderate intensity exercise with similar baseline values, the improvements were similar to the

high intensity group. Hollekim-Strand *et al.* used a similar high intensity interval program for 12 weeks with T2D patients and noted a 13% improvement in VO_{2peak} [91]. This aligns with marked improvements observed in both groups in this study. In both the current study and the studies referred to above, VO_{2max} was markedly lower when compared to age-matched reference values [92].

The significant improvements in both the HF and LF group indicate that weekly accumulated exercise duration, rather than session duration, may be the most important factor in improving aerobic capacity. Significant increases in VO_{2peak} were observed in both volume matched groups without significant difference between groups. This could indicate that as long as high intensity intervals cause an increased cardiac load frequently enough, the duration of sessions does not need to be as long as previously thought. Improvements in VO_{2max} have been shown with time efficient exercise programs using high intensity exercise with weekly durations of 81 and 30 minutes per week [93]. Exercise intervals of 10 x 1min at 90% HR_{max} and 2 x 20sec at maximum achievable intensity, have yielded improvements in T2D cardiorespiratory fitness while remaining below the ADA recommended 150 minutes per week of exercise [7, 93]. Individually designed exercise prescriptions concerning duration, frequency, and exercise mode could enhance compliance and long term outcome.

With CVD being identified as the primary cause of death in T2D individuals [94] and VO_{2max} being a strong predictor of mortality for populations [40, 41] the exercise treatments prescribed to both the HF and LF group may be considered as valid options for improving cardiovascular health and reducing CVD risk.

4.2 Heart Rate Variability

The 12-week low frequency exercise training caused significant improvement in nighttime HRV values which aligns with previous studies [64, 75, 95]. Reduced activity during nighttime causing lower activation of the sympathetic nervous system and a more predominant parasympathetic outflow, nighttime provides a clearer indication of changes in the autonomic nervous systems regulation [96]. HRV during 24 hour monitoring periods trended higher post-treatment in both the HF and LF group suggesting that both protocols had a beneficial effect on cardiovascular health and prevention of CAD. A larger sample size might have achieved significant changes in HRV. Variation in daytime activity with each subject may have made detecting changes more challenging. Both training methods influenced 24 hour HRV metrics in a similar way suggesting that volume rather than frequency may have been the major factor in exercise and its effect on HRV. The LF group had significant nighttime HRV changes indicating exercise duration of greater than 10 minutes per session may be required to induce changes in nervous system regulation. Age causes a significant decrease in HRV and therefore could have diminished any improvements that occurred when compared to a healthy and younger population [66]. Our finding of improvement in HRV after LF exercise prescription is clinically important as it may be associated with enhanced cardiovascular health in a population with an elevated risk of cardiovascular morbidity and mortality.

4.3 Glucose Variability

Continuous glucose monitor data showed no significant changes in glucose variability over the 12 week study pre- and post-treatment. There was a trend towards a lower average glucose in the HF group. Changes in SD and CV were not seen which most likely means that a greater training volume or longer duration of intervention is required to reduce glucose variability. The number

of high and low glucose spikes could have been an important end-organ effect. However, in this study sample, few spikes were seen indicating that these T2D individuals were well regulated. This could also explain the non-significant results. Post testing of glucose variability occurred more than 3 days after the last prescribed exercise session. It is known that exercise induced insulin sensitivity diminishes significantly after 72 hours. Due to the delay in testing, the possible effect of exercise on glucose variability were most likely reduced. CGM measurement for the duration of the intervention might have provided better understanding of the effect of exercise and how frequency and duration could impact glucose management. The frequency of glucose concentration recording on CGM devices is high enough to detect immediate post-exercise glucose sensitivity. A study investigating the acute effects of exercise on glucose variability in T2D over a 24 hour period post exercise showed significant improvements indicating analysis during continual exercise may be a valuable future study [97]. Glucose variability in T2D was found to be significantly reduced for 24 hours immediately after a single bout of aerobic exercise, again showing the importance of intra-intervention measurement [98].

4.4 Blood Variables and Body Composition

A significant relative decrease in HbA$_{1c}$ of 5.3% was seen in the LF group post-treatment indicating an improvement in glycemic control due to exercise. This finding is in agreement with previous studies indicating that high intensity interval training reduces HbA$_{1c}$ significantly [48, 93]. More intensive exercise seems to show greater glycemic control with regards to reduction in HbA$_{1c}$ [99]. While the HF group did have a decrease in HbA$_{1c}$, this trend did not reach significance. This may have been due to the 10-minute sessions not providing adequate muscle glycogen depletion and blood glucose reabsorption compared to the longer 30-minute LF sessions. The LF group may also have experienced greater enhancements in insulin sensitivity

due to the repeated intervals during each session compared to a single interval per session in the HF group. These longer bouts could have caused greater depletion of muscle glycogen causing increased GLUT4 translocation, which in turn would decrease blood glucose [100]. Post-exercise insulin sensitivity is noted to last up to 72 hours with highly intensive exercise [34] and the LF group may have achieved greater long term insulin sensitivity compared to the HF group over the 12 week training period, reducing HbA_{1c} percentage to a greater degree.

HDL cholesterol levels significantly increased in both the HF and LF group indicating both modes of exercise may be an effective way of improving blood lipid values. The improvement seen in the present study aligns with results from a meta-analysis observing the effects of aerobic exercise on HDL cholesterol with significant yet modest improvements [101]. It has been shown that every 0.026 mmol/L increase in HDL cholesterol level is associated with a concomitant 2% and 3% decrease in CVD risk for men and woman respectively [102]. Based on the previously cited study, both groups in the present study had a hypothetical decrease of approximately 6-9% in CVD risk. Other studies have indicated that longer exercise sessions have a greater improvement in HDL cholesterol [101]. Exercise intensity, duration and frequency may cause different effects on HDL cholesterol in the T2D population when compared to the normal population.

Body weight decreased in both intervention groups. The LF group had a significant reduction of 1.5% in body weight. The HF group had a significant reduction in waist circumference indicating a change in body composition. The reduction in waist circumference but not weight could be due to decreased visceral adipose tissue and increased skeletal muscle mass [103]. While there was no observed change in Body Mass Index, the possibility that there was a decrease in adipose tissue and an associated increase in muscular tissue mass is seen as favorable in improving

insulin sensitivity as well as general health. Use of a body composition analyzer would have allowed us to view any changes in body composition with these exercise prescriptions.

4.5 Study Limitations

The primary limitation of the present study was the small sample size. Several study variables displayed trends of improvement and a larger sample size may have allowed for significant values to be achieved. The study population was also heterogenous with different diabetes duration, degrees of inflammation and genetics, which all may have influenced the results. While both groups monitored their intensity levels during training, the LF group had supervised training sessions and therefore may have followed the protocol more exactly. Cohort 1 and cohort 2 also had different students testing and supervising the exercise trainings which may also have influenced the results.

While instructed not to change lifestyle or diet habits during the study, a majority of the participants in cohort 2 completed baseline and post-testing during the Christmas and Easter holidays. This may also have affected results, especially with regard to glucose variability.

4.6 Participant Feedback

Almost every participant who participated in the HF group in cohort 2 expressed that the exercise program was impractical and unsustainable. Preparation for exercise and recovering after each session (changing into exercise clothing, showering, going to gym) took a significant amount of time and most stated they would prefer to do longer yet less frequent sessions. While one of the HF group participants stated they would continue the program on their own, the rest either stopped after the 12 weeks or asked for recommendations on programs with less frequent sessions. The LF group were noticeably more motivated to continue after the program with their

four times weekly program. With motivation and lack of time reported as major barriers to exercise and two out of three T2D individuals failing to exercise regularly [104], this study feedback suggests that shorter but higher frequency exercise sessions may be less successful as a long term lifestyle change for those with T2D.

4.7 Conclusions

Both high frequency and low frequency high intensity interval training for 12 weeks were effective in improving aerobic capacity in T2D individuals as well as increasing HDL cholesterol levels. When matched for volume, longer yet less frequent interval training sessions appeared to be superior in improving long term blood sugar control outcomes as measured by HbA_{1c} concentration. HRV improved in the low frequency group which implies that longer aerobic interval sessions are required for significant changes to be accurately measured. The present study provides additional evidence that high intensity interval training is a safe and time effective exercise method for improving health and reducing the risk of cardiovascular disease in individuals with T2D.

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