

**METABOLIC AND CARDIOVASCULAR BENEFITS OF HIGH-INTENSITY
INTERVAL TRAINING IN TYPE 2 DIABETES**

by

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Abstract

High-intensity interval training (HIIT), comprised of alternating periods of brief high- and low-intensity exercise, has been shown to rapidly improve metabolic and cardiovascular health. As such, HIIT may provide a sufficient exercise stimulus to reduce cardiovascular risk in type 2 diabetes (T2D). Several studies have shown that HIIT is effective for improving glucose control and cardiorespiratory fitness. However, the acute and chronic effects of HIIT on the vasculature of individuals with T2D have yet to be established. The addition of post-exercise nutritional supplementation with high-quality protein from milk has previously been shown to augment favorable body composition changes with exercise interventions, which could promote further improvements in glycemic control and reduce cardiovascular risk factors in older adults with T2D. This thesis examines the impact of acute HIIT on vascular endothelial function, and the chronic metabolic and cardiovascular adaptations to HIIT with or without a post-exercise nutritional beverage, in individuals with T2D. In a randomized clinical trial, fifty-three individuals with T2D completed 12 weeks of low-volume HIIT (4-10 X 1 min intervals) with post-exercise nutritional support of non-fat milk, milk-protein concentrate or flavored water (placebo) beverage. Separately, thirty-five age-matched older adults (12 T2D, 12 untrained normoglycemic, and 11 trained normoglycemic) completed an acute session of cycling HIIT, leg resistance HIIT, or resting control. The principal findings of this thesis were: a) 12 weeks of HIIT improved glucose control, body composition, cardiorespiratory fitness and vascular function in individuals with T2D, b) no additive effects were observed with post-exercise milk or milk-protein supplementation, and c) acute resistance or cardio-based HIIT improves endothelial function in individuals with T2D. Noteworthy, the changes in blood pressure, arterial stiffness, femoral intima-media thickness and endothelial function were likely clinically beneficial based on magnitude based inferences that are associated with ~15-30% reductions in future cardiovascular events. Therefore, HIIT may be an effective means to reduce the burden of cardiovascular complications in T2D.

Lay Summary

Exercise and nutrition are the major lifestyle strategies that can help treat type 2 diabetes (T2D). This thesis examined whether a time-efficient exercise protocol, termed high-intensity interval training (HIIT), would improve overall health in individuals with T2D. In addition, we examined whether consuming a glass of milk after exercise, which provides high-quality protein to support muscle adaptations, could further improve the expected benefits of HIIT. Fifty-one individuals with diagnosed T2D completed twelve weeks of HIIT, with or without 500 mL of milk or milk-protein after exercise. HIIT involved 1 minute of exercise (e.g., walking briskly or lifting weights) requiring a hard effort, followed by 1-minute recovery, progressing to 10 intervals per session. In this thesis, we show that HIIT is effective in reducing many cardiovascular risk factors, with no additive effect of post-exercise milk or protein supplementation. Therefore, HIIT represents a novel form of exercise that could be effective in the treatment of T2D.

Preface

I declare that the composition of this thesis in its entirety is my own, in the case of co-authored work all sources have been acknowledged and my contribution to each study is outlined below.

Chapter 2: Francois ME, Durrer CD, Pistawka K, Halperin F, Chang C and Little JP. Combined Interval Training and Post-Exercise Nutrition in Type 2 Diabetes: A Randomized Control Trial. *Under Review 2017*

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Candidate Contribution

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Data Collection: The candidate contributed solely to the scheduling and organization of all aspects of pre-screening, data collection and exercise training. The candidate supervised exercise training for the majority (37/51) of participants, with help from undergraduate and masters students for the remaining participants. Furthermore, the candidate performed and analyzed all study outcomes presented herein.

Manuscript: The candidate prepared all figures and tables and drafted the first version of the manuscript. The candidate was also responsible for circulating to authors and subsequently revising the manuscript. Finally, the candidate submitted the manuscript to the journal and led any revisions and rebuttal.

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Planning: The Candidate planned and added all outcomes to the main study that are presented in this manuscript. This involved learning and implementing the appropriate measurement techniques available. In addition to contributing to the writing of ethics, information sheets and consent forms the candidate planned the sequence and timing of physiological measures.

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List of Abbreviations

ASR = Antegrade Shear Rate
AUC = Area under the curve
CGM = Continuous glucose monitoring
CVD = Cardiovascular disease
DBP = Diastolic Blood Pressure
ECG = Electrocardiogram
FMD = Flow mediated dilation
HbA_{1c} = Glycated hemoglobin
HIIT = High-Intensity Interval Training
HR_{max} = Peak heart rate
ICAM-1 = Intracellular Adhesion Molecule 1
IMT = Intima-Media Thickness
MAGE = mean amplitude of glycemic excursions
MAP = Mean arterial blood pressure
PWV = Pulse-Wave Velocity
QoL = Quality of Life
RER = Respiratory Exchange Ratio
RPE = Rating of perceived exertion
RSR = Retrograde Shear Rate
SBP = Systolic Blood Pressure
SRAUC = Shear Rate Area Under Curve
T2D = Type 2 diabetes
TR-NG = Normoglycemic highly trained adults
UN-NG = Normoglycemic untrained adults
VAT = Visceral Adipose Tissue
VCAM-1 = Vascular Cell Adhesion Molecule 1 VC Vascular conductance
VO_{2peak} = Peak oxygen consumption

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‘Chase your dreams but always know the road that’ll lead you home again’. (Tim McGraw)

Chapter 1: Introduction

The prevalence of type 2 diabetes (T2D) and related complications are increasing, undoubtedly fueled by the Westernized lifestyle characterized by insufficient exercise and processed foods (108, 116). Indeed, those with T2D are less active (151) and have a lower cardiorespiratory fitness (107) than their non-T2D counterparts. Furthermore, just one day of zero physical activity or several days of reduced activity can cause the metabolic abnormalities reminiscent of T2D in healthy adults (185, 231). In addition to hypertension, dyslipidemia, and obesity, T2D is an established and modifiable risk factor for cardiovascular disease (CVD) (281). T2D greatly accelerates the progression of atherosclerosis (238, 279) and thus individuals with T2D are 2-4 times more likely to suffer from early myocardial infarction (81). Importantly, exercise can prevent or attenuate the cardiovascular and metabolic dysfunction that occurs with aging and T2D (176, 224). In addition to lowering traditional cardiovascular risk factors (e.g., blood lipids, body mass), a large proportion of the cardio-protective benefits of exercise can be attributed to improvements throughout the circulatory system (142). Furthermore, such improvements may be achieved with low-volume high-intensity interval training (HIIT), even though the time spent exercising is often lower than recommended public health guidelines (54). Renewed interest in HIIT has occurred in the last decade and within this the application of HIIT to improve health has emerged as a key theme. Specifically, the widespread changes in metabolic and cardiovascular ('cardiometabolic') risk factors that occur with HIIT have significant potential to reduce the incidence of diabetes complications in those with T2D.

In this literature review, the benefits of exercise (specifically in the context of HIIT) on glucose control, cardiorespiratory fitness and vascular function in individuals with T2D are addressed. In

this regard, the need for further randomized controlled trials of HIIT in individuals with T2D will be emphasized, particularly for examining the effects on vascular function. The acute impact of a bout of HIIT on endothelial function is unclear, and thus the potential mechanisms underlying the adaptive changes in endothelial function with exercise are briefly discussed. The acute response is important because the adaptations to exercise training are often dependent on the accumulated effects of individual exercise sessions. Finally, the potential of post-exercise nutrition to strategically augment the adaptations to HIIT are presented.

1.1 Etiology of type 2 diabetes and cardiovascular disease

Characterized by hyperglycemia, T2D diagnosis ensues following years of multiple physiological defects ('the ominous octet') alongside a background of insulin resistance (i.e., decreased responsiveness of peripheral tissues to the actions of insulin) (66). Indeed, pancreatic beta cell dysfunction and the loss of first phase insulin secretion both occur during the early development of diabetes (66). In view of this, multiple drugs are typically necessary to control hyperglycemia (without lifestyle modification), and eventually patients with T2D require exogenous insulin (158). However, even those treated with oral glucose lowering drugs can experience hyperglycemia (blood glucose >10 mmol/L) for more than 13 hours per day (207). T2D encompasses both fasting and postprandial hyperglycemia, the latter largely influenced by the former, which together contribute to the development of cardiovascular disease (65, 152). However, there is evidence to suggest that postprandial hyperglycemic 'spikes' may be an independent risk factor for CVD, as a result of being mechanistically linked to the pathological processes of oxidative stress and inflammation (44). Furthermore, hyperglycemia in patients with T2D is also compounded by excess cardiovascular risk factors such as inflammation,

dyslipidemia and hypertension (225). In this context, T2D is known to accelerate the arterial stiffening that occurs normally with aging (238). Indeed, 71% of individuals with T2D have hypertension and 40% have three or more coexisting chronic conditions (43). Consequently, CVD is the leading cause of death for those with T2D, occurring 2-4 fold more often than in people without it. Thus, treatments to lessen the risk or impact of CVD in individuals with T2D are warranted (81).

Traditional cardiovascular risk factors (i.e., blood lipids, blood pressure) contribute similarly to macrovascular disease in individuals with T2D and those without diabetes (174). Consequently, other factors such as hyperglycemia have been the focus of intensive research over the last decade. Several clinical trials have sought to determine whether intensive glucose lowering with multiple pharmacological approaches (drug combinations with and without exogenous insulin) can reduce the incidence of micro- and macrovascular complications (1, 3, 71, 255). Although there has been some success in reducing microvascular complications, the effect on macrovascular diseases is unclear (18, 70). Exercise, although underappreciated, is one of the most effective means to improve skeletal muscle insulin sensitivity and improve macrovascular health (123). In fact, lifestyle interventions including diet and exercise are more effective than pharmacotherapy for reducing diabetes incidence over a 10 year follow up (67) and physical activity is strongly associated with reduced cardiovascular mortality (30). Therefore combined diet and exercise interventions may be the most effective strategy to reduce the burden of CVD in individuals with T2D. As such, exercise strategies that improve both metabolic and cardiovascular aspects of health, for example high-intensity interval training, may be the most effective approach for T2D management.

1.2 Exercise and type 2 diabetes

Regular exercise increases insulin sensitivity, mitochondrial function, endothelial function, immune function, lean body mass, cardiorespiratory fitness and lowers blood pressure [reviewed in: (5)]. Undoubtedly, exercise influences the physiological functioning, strength and health of most (if not all) bodily systems. For that reason, exercise remains fundamental in the treatment of many chronic diseases (203), including T2D (54). In individuals with T2D, exercise can immediately increase glucose uptake in the absence of insulin and can enhance insulin-mediated glucose uptake (i.e., increase insulin sensitivity) for up to 48 h after a single session (184, 227). Taken together, exercise can potentiate glucose uptake, even in insulin-resistant tissues and can contribute to long-term glycemic control via increases in insulin sensitivity. In this regard, regular exercise is important for maintaining the glucose lowering effects, since a large proportion of the benefits of exercise are due to the cumulative effects of each individual exercise session (98). However, favorable changes in body composition (increases in lean body mass and reduced fat mass) also contribute to improved glycemic control in individuals with T2D (53).

Low-to-moderate intensity continuous exercise (e.g., walking) has traditionally been recommended for the prevention and treatment of T2D and CVD (53). In the Look AHEAD (Action for Health in Diabetes) trial, moderate exercise (based on public health guidelines (53)) and modest caloric restriction substantially reduced cardiometabolic risk factors, diabetes complications and health costs in 5,000 individuals with T2D (273). However, there was no effect on cardiovascular morbidity or mortality. In fact, no large, longitudinal studies evaluating the effects of exercise training in T2D (prescribed using the public health guidelines) have shown

reduced cardiovascular mortality (273). In view of this, low-to-moderate intensity exercise may not provide a sufficient stimulus to reduce cardiovascular disease in T2D (19). Consequently, over the last decade there has been increasing interest toward the inclusion of higher-intensity exercise for improving cardiometabolic health in T2D (19, 42, 269). Indeed, the prevalence of sedentary lifestyles, poor adherence to traditional exercise guidelines and the failure of low-to-moderate intensity exercise to substantially reduce chronic disease have all contributed to the investigation of other more effective exercise modalities, such as high-intensity interval training (HIIT) (19).

1.2.1 High-Intensity Interval Training (HIIT)

There is accumulating evidence that HIIT can provide similar or greater cardiometabolic health benefits than long-duration continuous exercise (135, 210, 269). While there are numerous HIIT protocols (which vary in interval intensity, length and iteration), they are all characterized by some form of an alternating pattern of high and low intensity exercise. Sprint interval training (SIT) is an extreme form of interval training, involving brief periods of ‘supramaximal’ or ‘all-out’ exercise interspersed with longer periods of recovery (269). Although various studies have shown improved cardiometabolic outcomes with SIT (94), the intensity required is largely impractical and likely not feasible for most clinical populations (120). In contrast, HIIT involves ‘near maximal’ exercise intensity (~90% of maximal heart rate), and typically high-intensity periods of effort are interspersed with equal or shorter periods of recovery (269). Three common and highly effective HIIT protocols that have been used in clinical populations are: i) Low-volume HIIT (10 X 1 min at ~90% maximal heart rate, 1 min recovery; (59, 163, 166)), ii) Interval walking (60 min, alternating 3 min >70% $\dot{V}O_{2\text{ peak}}$ with 3 min recovery ~40% $\dot{V}O_{2\text{ peak}}$;

(144, 145)), and iii) the Norwegian model (4 X 4 min at ~90% maximal heart rate, 3 min recovery; (233, 249, 275)). It has been demonstrated that each of these HIIT protocols can confer significant health benefits, but each has not been compared in a specific controlled trial. As such, there is no consensus as to which protocol is superior for improving cardiometabolic health outcomes. In this regard, the general appeal and novelty of low-volume HIIT is its ability to stimulate similar (and in some cases superior) adaptations to traditional continuous training (165), with less time commitment.

In the last decade, there has been a significant interest in HIIT to improve metabolic and cardiovascular disease risk factors in T2D. While there remains a paucity of randomized controlled trials involving HIIT interventions in T2D, early evidence supports HIIT as a suitable option to improve cardiometabolic risk factors including glycemic control, cardiorespiratory fitness and vascular function (Figure 1.2). A summary of all 14 HIIT studies involving participants with T2D (total of 175 T2D participants completing HIIT across all studies for an average n=12.5 in the HIIT intervention group) is provided in Table 1.1.

Table 1.1. Interventions using high-Intensity Interval Training in Patients with T2D.

Author	HIIT protocol	Intervention length	Sample size in HIIT	Main outcomes HIIT group
Alvarez et al. (4)	8-12 x 30-54 sec at 15-17 RPE separated by ~2 min recovery	16 weeks (3 d/wk)	HIIT = 13 CON = 10	↓HbA _{1c} (by 0.6%), fasting glucose, BP, body fat VO _{2 peak} not reported
Apostolopoulou et al. (10)	4 X 4 min intervals at 85% of maximal heart rate, three min recovery, cycling	12 weeks (3 d/wk)	HIIT = 10	↑Insulin sensitivity, ↓plasma fatty acids & liver fat, ↑mitochondrial function
Cassidy et al. (41)	5 x 2-4 min intervals (based on RPE 16-17 (CR-20) recovery included resistance band exercises	12 weeks (3 d/wk)	HIIT = 12 CON = 11	↓HbA _{1c} (by 0.3%), Liver fat, ↑Cardiac structure and function VO _{2 peak} not reported
Hollekim-Strand et al. (126)	Home-based 4 X 4 min interval, 90- 95% maximal heart rate, 3 min recovery	12 weeks (3 d/wk)	HIIT = 20 MICT = 17	↑FMD (by 9.5%), VO _{2 peak} (by 14%), ↓HbA _{1c} (by 0.4%)
Karstoft et al. (145)	60 min (as 3 min fast and 3 min slow walking)	16 weeks (5 d/wk)	HIIT = 12 MICT = 12 CON = 8	↓CGM mean (by 0.7 mmol/L) & max glucose, fat mass, ↑ VO _{2 peak} (by 14%) No change HbA _{1c}
Karstoft et al. (144)	60 min (as 3 min fast and 3 min slow walking)	16 weeks (5 d/wk)	HIIT = 12 MICT = 12 CON = 8	↑Insulin Sensitivity Index, glucose disposal, disposition index, Insulin signaling.
Karstoft et al. (143)	60 min (as 3 min fast and 3 min slow walking)	2 weeks (5 d/wk)	HIIT = 14 (crossover MICT)	No change CGM mean, ↓max glucose & MAGE
Little et al. (162)	10 X 1 min 90% HR max, 1 min recovery	2 weeks (3 d/wk)	HIIT = 8	↓CGM mean (by 1.0 mmol/L) & sum 3 h postprandial glucose,

Author	HIIT protocol	Intervention length	Sample size in HIIT	Main outcomes HIIT group
Madsen et al. (166)	10 X 1 min 90% HR max, 1 min recovery	8 weeks (3 d/wk)	HIIT = 10	↓HbA _{1c} (by 0.5%), fasting glucose, 2-h OGTT, fat mass. ↑B Cell function (↓HOMA and ↑Disposition) ↑ $\dot{V}O_{2\text{ peak}}$ (by 12%)
Mitranun et al. (187)	Moderate intensity and then 4 x 1 min 80% $\dot{V}O_{2\text{ peak}}$, total 30 min.	12 weeks (3 d/wk)	HIIT = 14 MICT = 14 CON = 15	↑FMD (by 2%), vascular reactivity, ↓□□A _{1c} (by 0.5%), fasting glucose, ↑ $\dot{V}O_{2\text{ peak}}$ (by 21%)
Parpra et al. (201)	6 x 2 min 80-90% maximal heart rate, with 2 min recovery	12 weeks (4 d/wk)	HIIT = 14	↑□□□
Revdal et al. (213)	10 X 1 min 90% vs SIT (2x 20 s 10 min total)	12 weeks (3 d/wk)	HIIT = 10 SIT= 11	↑ $\dot{V}O_{2\text{ peak}}$ (by 10%) and ↓body fat No change HbA _{1c}
Stoa et al. (233)	4 X 4 min of walking or running uphill at 85–95% of maximal heart rate	12 weeks (3 d/wk)	19 HIIT 19 MICT	↑ $\dot{V}O_{2\text{ peak}}$ (by 21%), ↓□□A _{1c} (by -0.6%). ↓body fat,
Terada et al. (239)	7 X 1 min intervals at 100% $\dot{V}O_{2\text{ peak}}$ with 3 min recovery	12 weeks (4 d/wk HIIT + 1 d/wk MICT)	HIIT = 7 MICT = 8	↓body fat No change HbA _{1c} or $\dot{V}O_{2\text{ peak}}$

T2D = Type 2 Diabetes, CON= Control no exercise, MICT = Moderate-Intensity Continuous Training, CGM = continuous glucose monitoring, MAGE = Mean Amplitude of Glycemic Excursions, SIT = Sprint Interval Training, HbA_{1c} = Glycosylated Hemoglobin, FMD = Flow-Mediated Dilation, HRV = Heart Rate Variability

1.2.2 HIIT and glucose control

HIIT is regarded as an efficacious alternative to continuous training for improving glycemic control. In a recent meta-analysis of fifty studies, Jelleyman et al. (135) demonstrated that HIIT was superior to continuous training for improving fasting glucose and HbA_{1c} (only 11 studies with T2D participants were available for inclusion). In those with T2D, HIIT reduced fasting glucose by 0.92 mmol/L and HbA_{1c} by 0.5% (135); which is inline with common pharmacological lowering (89), and similar to HbA_{1c} reductions (-0.6%) following combined resistance and continuous training interventions (243). The mechanisms underlying the effectiveness of HIIT for improving glycemic control may be related to the increased recruitment of muscle fibers, glycogen depletion and AMP-activated protein kinase (AMPK) signaling, increased skeletal muscle glucose transporter 4 (GLUT4) protein content, and higher oxidative capacity [reviewed in: (135, 165)]. While changes in body composition and cardiorespiratory fitness are likely to contribute to long-term benefits, a large proportion of the glucose lowering effects of HIIT do not appear to be explained by changes in body mass and fitness (135).

HIIT appears to be especially effective at lowering postprandial hyperglycemia [reviewed by Francois and Little: (161)]. Indeed, skeletal muscle is responsible for a significant proportion of insulin-stimulated glucose uptake in the postprandial state (65). Advancing technologies, such as continuous glucose monitoring (CGM) allow researchers to examine the influence of interventions on postprandial hyperglycemia and glycemic variability. Through assessing the fluctuations in glucose throughout the course of the day CGM provides more than a ‘snapshot’ of glucose control given by traditional fasting glucose or oral glucose tolerance test methods (148). For example, Little et al. (162) showed that two weeks of HIIT reduced mean glucose and the sum of the 3-h postprandial areas under the glucose curve for breakfast, lunch, and dinner in

individuals with T2D. Therefore, the combination of CGM with HbA_{1c} (which reflects three month average glycemic control) during longer-term trials can provide important information on the effectiveness of exercise interventions on glycemic control.

A landmark study of HIIT-based exercise in T2D by Karstoft et al. (145), randomized individuals with T2D to control (n=8), continuous-walking (n=12) or interval walking (n=12) for sixteen weeks. The exercise was free-living (unsupervised), and involved 60 min sessions on five days per week; with accelerometer data uploaded every second week to monitor compliance. Changes in cardiorespiratory fitness, body composition and glycemic control (CGM 24-h mean and peak glucose, fasting insulin) were superior after interval walking compared to continuous walking. Furthermore, to examine the potential mechanisms governing the improved glycemic control after interval training, study participants completed a three-stage hyperglycemic clamp with glucose isotope tracers and skeletal muscle biopsies before and after training (144). Consistent with the superior changes in glycemic control reported in Karstoft et al. (145), the interval walking only group increased the insulin sensitivity index and peripheral glucose disposal during the hyperglycemic clamp (144). Furthermore, only interval walking improved insulin signaling in skeletal muscle, demonstrated by an increase in insulin-stimulated Akt substrate of 160 kDa (AS160; also known as TBC1D4) phosphorylation (144). The findings from both investigations (144, 145) suggest that the pattern of high and low intensity exercise is superior to continuous exercise, as both groups were matched for training duration and energy use. However, while adherence to the prescribed duration and frequency was ~90% for continuous and interval walking (which in itself is quite remarkable for an unsupervised exercise intervention), the pattern of high and low intensity (% of maximal heart rate) achieved in both groups appeared relatively similar (continuous walking: 66% vs. interval walking: high 69% and

low 63% maximal heart rate) (145). In view of this, it appears that even slight changes in the pattern of high and low intensity exercise can stimulate improvements in metabolic outcomes in T2D.

The improved glucose control after interval walking described above, clearly supports the use of interval exercise in individuals with T2D. However, the volume of exercise used (300 min/wk) is far greater than usually attained by the general population, many of whom cite a 'lack of time' as a considerable exercise barrier (151). Moreover, it appears that interval length strongly influences perceptions of enjoyment, such that 30-s or 60-s intervals result in significantly greater enjoyment than 120-s intervals (175). Considering this, it is of interest to ascertain whether low-volume HIIT protocols (i.e., 10 X 1 min) can elicit similar benefits for glucose control. Indeed, evidence from small short-term trials show that low-volume HIIT can rapidly improve glycemic control in T2D (162, 166). For example, Madsen et al. (166) showed that eight weeks of low-volume HIIT in 13 individuals with T2D improves the homeostatic model assessment of insulin resistance (HOMA-IR), two hour oral glucose tolerance (assessed by the 100g oral glucose test), and beta cell function (assessed by the disposition index). However, further research is needed to confirm these preliminary findings in studies conducted over several months with larger sample sizes. Furthermore, additional cardiometabolic benefits of HIIT may be influenced by changes in fitness. For example, Stoa et al. (233) showed that the change in fitness after HIIT was significantly correlated to the change in HbA_{1c} (R= -0.52, P<0.01).

1.2.3 HIIT and cardiorespiratory fitness

It is perhaps not surprising that higher exercise training intensities generally result in greater increases in cardiorespiratory fitness (34, 115), and more vigorous exercise has been shown to reduce the incidence of cardiovascular diseases (157, 237). Indeed, in a meta-analysis of exercise training in T2D, the studies that used higher exercise intensities resulted in greater increases in cardiorespiratory fitness [maximal oxygen uptake ($\dot{V}O_{2\text{ peak}}$)] (34). Cardiorespiratory fitness is an important vital sign, as it is a strong and independent predictor of cardiovascular and all-cause mortality (149). Interval training is emerging as a robust and effective strategy to increase $\dot{V}O_{2\text{ peak}}$ (269), to the extent that the recent explosion of HIIT studies showing increases of 0.4 to 0.9 L/min are challenging ideas about the trainability (and existence of exercise non-responders) of $\dot{V}O_{2\text{ peak}}$ (17). Taken together, it appears that exercise intensity is an important modifier of improvements in cardiorespiratory fitness and thus may be an important factor underpinning the superior improvements in cardiometabolic health experienced following HIIT (135, 210, 269).

In a recent meta-analysis of HIIT studies Weston et al. (269) found that there was a two-fold superior increase in $\dot{V}O_{2\text{ peak}}$ with HIIT compared to moderate continuous training in patients with lifestyle-induced chronic disease. Of importance, the increase after HIIT was nearly one metabolic equivalent (MET) higher (+3 mL/kg/min, 9%) than after moderate continuous training (269). The potential significance of a superior improvement in $\dot{V}O_{2\text{ peak}}$ is highlighted by data showing that every 1 MET (3.5 mL/kg/min) increase cardiorespiratory fitness is associated with a 15% risk reduction in cardiovascular disease (149). One of the largest reported increases in $\dot{V}O_{2\text{ peak}}$ with HIIT was a ~21% (from 25.6 to 30.9 mL/kg/min) improvement observed after 12 weeks of training in 19 individuals with T2D (233). However, generally HIIT increases $\dot{V}O_{2\text{ peak}}$

in the order of 10% in individuals with T2D (Table 1.1). Importantly, such increases in cardiorespiratory fitness may mediate superior changes in cardiovascular risk factors (149), as interventions that increase cardiorespiratory fitness mitigate the arterial stiffening that accompanies aging (256). In this context, vascular adaptations to exercise training may explain a large proportion of the cardioprotective effects of cardiorespiratory fitness (142).

1.3 Exercise and vascular function

Exercise has well established cardioprotective effects that extend beyond any changes in traditional risk factors (i.e., blood lipids, blood pressure) (142). In addition to autonomic function, two proposed mechanisms that may explain a large proportion of the benefits of exercise are reduced arterial stiffness and improved endothelial function (106, 142). Previous research has shown that adults who exercise regularly have lower arterial stiffness (224) and preserved endothelial function (263), and importantly robust increases in these measures are seen with exercise interventions (105, 235, 280). Mechanisms of exercise-induced improvements in vascular function are multifactorial, but include improvements in lipid profiles, increased antioxidant capacity, reduced sympathetic tone, and lowered inflammation (286). However, the repetitive increases in hemodynamic stimuli (i.e., shear stress and transmural pressure on the vessel wall) that occur during the accumulation of individual training bouts are believed to be a major driver of vascular adaptations to exercise training (104). Indeed, when the exercise-induced increases in shear stress are prevented during exercise the improvements in endothelial function are abolished (247). Furthermore, improvements in vascular function with exercise training can occur independent of changes in body composition, glucose control, blood lipids and blood pressure (28, 142, 149).

Several studies have shown superior improvements in cardiovascular function following HIIT compared to traditional continuous training (41, 110, 274). For example, carotid-femoral pulse wave velocity (PWV) was reduced after HIIT, but not continuous training, in patients with essential hypertension (110). Accordingly, the gradient of shear stress augmentation during the pattern of low and high-intensity exercise (which occurs during each bout of HIIT) appears favorable for improving vascular function (275).

1.3.1 HIIT and vascular function

Flow mediated dilation (FMD) of the brachial artery is a widely used index of endothelial function that can be used as a barometer for future cardiovascular disease risk (131, 234). Encouragingly, a recent meta-analysis showed that HIIT improves endothelial function (210), with a two-fold greater increase in FMD than continuous exercise training (210). To date, there have been two studies that have investigated the vascular adaptations to HIIT in individuals with T2D (126, 187). Hollekim-Strand et al. (127) compared 12 weeks of HIIT (4 x 4 min at 90% of maximal heart rate, 3x/wk; Norwegian model) to home based moderate continuous training (≥ 10 min/bout, 210 min/wk) in T2D patients with diastolic dysfunction. Hollekim-Strand et al. (127) observed a very large increase in FMD after 12 weeks of interval training (from 9.2% to 18.5% in the interval training group), without any change in the continuous training group (127). The second study by Mitranun et al. (187) randomized individuals with T2D to energy matched HIIT or continuous exercise three times per week for twelve weeks. In addition to FMD, cutaneous reactive hyperemia (microvascular function) and several markers of oxidative damage and thrombosis were measured. Mitranun et al. (187) observed significant improvements in FMD and microvascular function in both training groups, but the magnitude of improvement was greater in

the interval training group (187). However the HIIT protocol used by Mitranun et al. (187) (summarized in Table 1.1) was a mixture of continuous and interval training, involving just 4 X 1-min intervals in a 30 min session. Collectively, these findings are encouraging but further research is needed to determine the impact of HIIT on the vascular structure and function in individuals with T2D. With this in mind, the acute impact of individual HIIT exercise sessions on vascular function are likely important in promoting long-term adaptations to exercise training.

1.3.2 Acute effects of HIIT on the Vasculature

It is now well-known that a single session of HIIT acutely improves glucose control (83, 92, 134) and blood pressure (52, 153) in individuals with, and at risk for, T2D. However, despite the clear benefits of HIIT interventions on endothelial function (as discussed above), the impact of an acute session of HIIT on endothelial function is unclear. The majority of studies that have assessed endothelial function after acute exercise have examined FMD at a single timepoint following continuous moderate or continuous high-intensity exercise [Reviewed in: (63)]. Typically a biphasic response in endothelial function is reported after a single session of exercise (Figure 1.1), whereby there appears to be a transient depression in FMD immediately after (the degree of which appears dependent on exercise intensity) followed by a super compensation response 1-24 h post-exercise (63). The transient impairment in FMD may be due to increases in oxidative stress, larger baseline diameter, retrograde shear stress or heightened sympathetic activity (63).

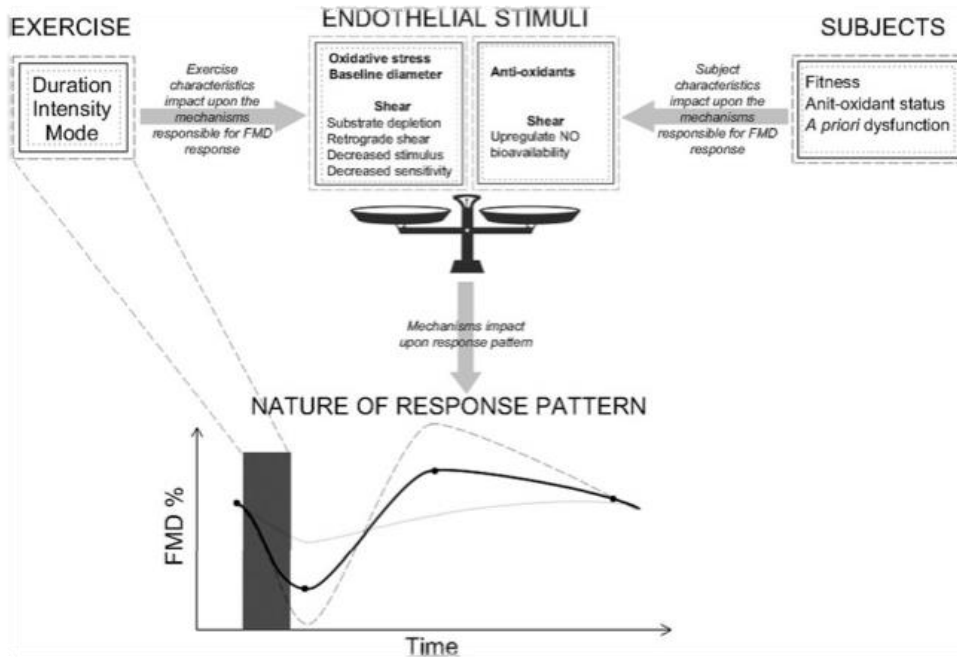


Figure 1.1 Illustration of the factors that influence the purported biphasic pattern of the flow-mediated dilation response to an acute session of exercise, from Dawson et al. (63).

The impact of acute exercise on FMD also appears to be influenced by training status (i.e., activity level or cardiorespiratory fitness) and/or the presence of other pathologies (e.g., obesity, diabetes). Harris et al. (121) examined the FMD response to three different intensities of acute exercise in overweight active compared to inactive men. The active subjects were classified as those completing 30 min of moderate exercise on most days of the week, but interestingly the $\dot{V}O_{2\text{ peak}}$ between active and inactive groupings was not significantly different. Regardless, Harris et al. (121) found that FMD was improved more in the active group compared to the inactive group, independent of exercise intensity. In another study, Hallmark et al. (117) measured FMD before, 1, 2, and 4 h after 30 min of moderate or high-intensity continuous exercise in inactive obese ($\text{BMI} >30 \text{ kg/m}^2$) or lean ($\text{BMI} <25 \text{ kg/m}^2$) males and females. High-intensity, but not moderate-intensity, continuous exercise improved FMD in lean participants,

however neither intensity improved FMD in obese participants (117). The mechanisms underlying the divergent response seen in lean versus obese participants was not investigated but was suggested to be due to the influence of excess adipose tissue and inflammation on nitric oxide production and availability (125).

Given the relative importance of nitric oxide bioavailability on FMD and endothelial function (103), determining the pattern of exercise that elicits an improvement in oxidative status and endothelial-dependent dilation will help elucidate the relationship between acute exercise and endothelial function. Indeed, two studies have shown that interval exercise improves FMD and markers of nitric oxide bioavailability (249, 252). Tyldum et al. (252) showed that a single session of HIIT (but not moderate-intensity continuous exercise) afforded complete protection from the detrimental effects of a high-fat meal, and the improvement in FMD was significantly correlated with an exercise-induced increase in antioxidant status. Tjonna et al. (249) measured FMD before, immediately, 24, 48 and 72 h after a single bout of interval or continuous exercise (4 x 4 min at 90% of maximal heart rate) in individuals with the metabolic syndrome. The single session of HIIT improved FMD for the entire 72 h after exercise, compared to a smaller increase observed for just 24 h following continuous exercise (249). Furthermore, the increase in FMD after HIIT was accompanied by a 35% increase in nitric oxide bioavailability (249). Therefore, it appears that high-intensity exercise performed in an interval pattern may improve endothelial function (249, 252), and certainly does not transiently impair FMD in contrast to high-intensity continuous exercise (13). However, further studies are needed to confirm improve FMD following HIIT including investigations in older, untrained and clinical populations. In addition, the influence of other local factors during HIIT that may impact endothelial adaptations, such as shear stress, warrants examination.

Local factors including changes in hemodynamic forces (e.g., shear stress) and metabolites, such as those that occur with exercise, can modulate changes in endothelial function (104). In recognizing the influence of shear rate patterns on endothelial function, research has focused extensively on determining which factors (magnitude, profile, and frequency) influence atherosclerotic plaque formation. While increases in antegrade shear rate (as discussed above) are known to improve endothelial function, increases in retrograde and/or oscillatory shear stress (distinct from reductions in antegrade flow) have been shown to alter endothelial cell gene expression toward a more inflammatory thrombotic phenotype (47, 192, 229). For example, oscillatory shear stress upregulates endothelin-1 (a potent pro-inflammatory vasoconstrictor) (285) and vascular cell adhesion molecule-1 (VCAM-1; a vascular adhesion molecule that facilitates monocyte adhesion, an initial step in the formation of atheromas) (47, 192). These effects of oscillatory shear stress have been shown extensively in cell culture experiments (47, 192, 285), and more recently humans (100, 242, 246).

In humans the vascular adaptations to exercise are not localized to the exercising limb (101, 198). For example, Green et al. (100) examined the blood flow and shear rate patterns in the forearm (inactive muscle bed) during 15 min of incremental cycling in healthy adults, with or without Monomethyl arginine infusion (L-NMMA; to inhibit nitric oxide production). The blood flow in the vasculature of the inactive upper-limb increased with step-wise increases in exercise intensity during cycling (101), and this increase was predominately nitric oxide mediated (101). The increased shear rate throughout the vasculature during exercise potentially explains the widespread systemic antiatherogenic effects of exercise, but the stimulus may differ depending on the exercise mode. In contrast to rhythmic cycling and walking, leg kicking (40, 209, 240) and handgrip (102) resistance-based exercises have been shown to attenuate brachial artery

endothelial function. It appears that some degree of oscillatory shear stress during exercise may be required to mediate nitric oxide release from the endothelium (102, 240). However, whether there is an optimal range for the level of oscillatory shear stress needed during exercise to improve endothelial function and the relative effects of other exercise factors (muscle mass, pattern and duration) are unknown. Using an elegant bilateral cuff design to manipulate blood flow in order to match brachial artery mean shear rate, Tinken et al. (246) showed that FMD is increased after both cycling and handgrip exercise when antegrade shear rate is increased to the same extent during both exercise modes. This suggests that changes in endothelial-dependent dilation are dependent on the magnitude of antegrade shear rate increase, rather than the exercising muscle mass (and perhaps relative intensity). However, future research is warranted to examine the influence of exercise mode, matched for the intensity pattern and duration, on endothelial-dependent dilation.

1.4 Post-exercise nutrition and cardiometabolic health

The combination of, and interplay between exercise and nutrition may be important for maximizing the adaptations to exercise training. In addition to exercise parameters (intensity, duration, mode), post-exercise nutrition is also important to consider when prescribing lifestyle interventions in individuals with T2D. Diminished lean muscle mass, in the face of excess adiposity, is a hallmark feature of T2D (9). In this context, T2D has been shown to accelerate the age-associated decrements in lean muscle (200). This is disconcerting because skeletal muscle is essential for maintaining functional capacity, provides a large glucose sink following a meal and regulates whole-body metabolism (9, 65, 232).

Exercise (150) and protein (205) are two stimuli that directly increase protein synthesis; the anabolic stimulus for lean muscle accretion. Endurance training (119), resistance training (204) and combined training modalities (167) all promote gains in lean mass and reduce fat mass in older adults. Additionally, HIIT has recently been shown to increase protein synthesis in the skeletal muscle of young and older adults, an effect linked to improved insulin sensitivity and mitochondrial function (215). In a meta-analysis of 22 studies, Cermak et al. (45) showed that protein supplementation during an exercise training intervention significantly augments the gains in lean body mass and muscle cross sectional area. Protein intake is important for the maintenance of protein balance during exercise training (51). Specifically, the ingestion of protein in close proximity to exercise has been shown to maximize skeletal muscle adaptations in healthy adults (50, 78). This strategy may be particularly important for individuals with T2D who have profound skeletal muscle insulin resistance and are at risk for accelerated muscle loss with aging. However there is a lack of research regarding post-exercise nutritional supplementation in patients with T2D.

The type of protein ingested in the post-exercise period may also be important for promoting maximal improvements in body composition. For example whey (rapidly digested) or casein (slowly digested) proteins can differentially modulate the anabolic response as whey stimulates muscle protein synthesis immediately and casein helps to inhibit protein breakdown over several hours (31, 62). Therefore, the combination (i.e., as present in milk; 80:20% casein:whey) may be more effective for improving body composition. Indeed, Wilkinson et al. (271) observed greater increases in protein synthesis and net protein balance when milk was ingested after resistance exercise, compared to isoenergetic soy protein. Consistent with this, others have shown that milk after resistance training promotes greater lean muscle accretion than

a soy or carbohydrate control beverage in healthy adults (122, 141). In addition to providing high-quality protein, milk also contains other nutrients such as vitamin D and calcium that have been shown to improve insulin sensitivity (206, 214). Furthermore, the consumption of milk may have distinct effects on the gut that promote fat loss (283). For example, increased calcium intake from dairy sources has been shown to enhance adipose tissue lipolysis (283) and promote fecal lipid excretion (48). These mechanisms may explain findings from some (140, 284) but not all (245) studies showing reduced central and visceral adiposity with high-dairy diets. Therefore, the inclusion of post-exercise milk to an HIIT intervention has the potential to augment the cardiometabolic adaptations to exercise training in T2D. So far the vast majority of studies examining post-exercise milk and/or protein supplementation have focused on resistance training. However, given the potency of HIIT to improve body composition (23, 215, 220) and elicit cardiometabolic adaptations (discussed above), milk supplementation after HIIT may be beneficial for individuals with T2D. To the best of my knowledge, the combination of HIIT exercise and post-exercise milk (protein) supplementation has not yet been studied.

1.5 Summary

Exercise and dietary interventions present effective frontline treatment options for improving glycemic control and cardiovascular risk factors in individuals with T2D. However, there is poor adherence and potentially limited efficacy of the current lifestyle recommendations to reduce the cardiovascular risk in individuals with T2D. As such, HIIT is being promoted as an innovative and time efficient strategy to improve cardiometabolic health. Early evidence consistently shows that HIIT can reduce hyperglycemia, increase cardiorespiratory fitness and improve endothelial function. However there is a paucity of randomized controlled trials of HIIT

and the small sample sizes of previous studies limit the translation of the findings to a larger population. Moreover, the impact of HIIT on other ‘subclinical’ measures of atherosclerotic vascular disease, such as arterial stiffness and intima-media thickness, have not been investigated in individuals with T2D. Furthermore, T2D incidence is often associated with ‘sarcopenic obesity’, characterized by diminished lean muscle mass in the face of increased adiposity. Thus the strategic addition of post-exercise milk protein ingestion to improve skeletal muscle protein balance may promote favorable body composition changes and augment the cardiometabolic health adaptations to HIIT.

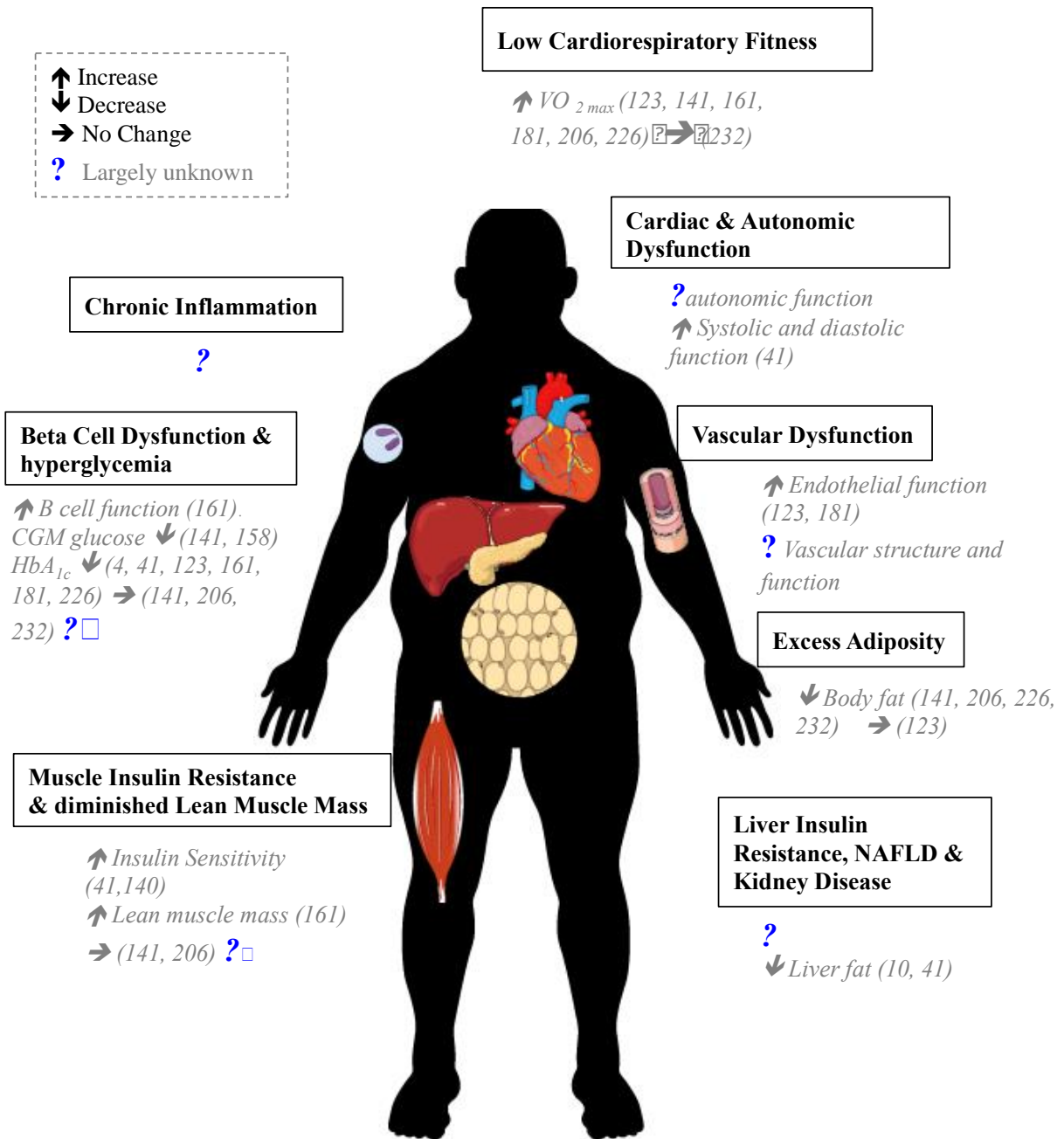


Figure 1.2. The known and unknown effects of HIIT on the physiological defects that contribute to the excess cardiovascular mortality in type 2 diabetes.

1.6 Aims and Hypotheses

The overall purpose of this thesis was to examine the metabolic and cardiovascular adaptations to HIIT in individuals with T2D. Specifically, for Chapter 2 a randomized clinical trial was conducted to examine whether a 12-week HIIT intervention could improve cardiometabolic health markers while determining if a milk or protein beverage consumed within 1 h after each HIIT session could augment cardiometabolic adaptations. Examining whether this same 12-week HIIT intervention could improve measures of vascular structure and function was the primary focus of Chapter 3. Finally, the acute impact of HIIT on vascular endothelial function in individuals with T2D, age-matched untrained and trained control participants was explored in Chapter 4.

Chapter 2

Aim: In a randomized clinical trial, we aimed to investigate whether post-exercise milk or protein supplementation improves cardiometabolic health more than placebo (flavored water) during 12 weeks of HIIT in individuals with T2D. The primary outcome was glycemic control, assessed using continuous glucose monitoring. Secondary outcomes included body composition, HbA_{1c}, fasting blood parameters, fitness, blood pressure and endothelial function.

Hypothesis: Post-exercise milk and protein will result in greater improvements in glucose control, body composition and fitness when compared to placebo supplementation during HIIT.

Chapter 3.

Aim: To examine the impact of 12 weeks of HIIT on cardiovascular health in individuals with T2D. The primary outcome was arterial stiffness, measured using carotid-femoral pulse wave velocity. Secondary outcomes included resting and maximal heart rates, vascular adhesion molecules, intima-media thickness and resting femoral blood flow profiles.

Hypothesis: HIIT will improve arterial stiffness, resting heart rate and femoral blood flow in T2D.

Chapter 4.

Aim: To examine the acute impact of resistance and cardio-based HIIT on endothelial function in age-matched T2D, untrained and highly-trained normoglycemic older adults. The primary outcome was endothelial function, measured by flow-mediated dilation, immediately, 1 and 2 h post-exercise. Secondary outcomes included resting shear stress, blood flow and blood pressure.

Hypothesis: Both acute cardio- and resistance-based HIIT will lead to similar improvements in endothelial function compared to a time-matched control.

**Chapter 2: Combined Interval Training and Post-Exercise Nutrition
in Type 2 Diabetes: A Randomized Control Trial**

Worldwide more than 257 million people have T2D, a figure projected to reach 395 million by 2030 (215). Of those 71% have hypertension and 40% have three or more coexisting chronic conditions, with cardiovascular disease the leading cause of mortality (226). Accordingly, interventions that improve both glycemic control and reduce cardiovascular risk factors are central to reducing the burden of T2D (43). Lifestyle interventions, including exercise and nutrition are at the forefront for the prevention of diabetes complications (133). Intensive glucose lowering with multiple pharmacological treatments leads to reduced microvascular complications (133), but the effect on macrovascular complications is unclear.

Large controlled trials and numerous experimental studies reveal the widespread benefits of exercise for people with T2D (255). The Look AHEAD (Action for Health in Diabetes) trial showed that moderate exercise coupled with dietary intervention leads to sustained reductions in cardiometabolic risk factors, diabetes complications and health care costs (160, 177). However, the number of cardiovascular events observed between the intervention and control groups was not different. The addition of vigorous exercise may be required to elicit substantial changes in cardiovascular function (273), as it appears that vigorous, but not low-moderate exercise, reduces incident cardiovascular disease (19). Studies using higher exercise intensities, such as interval and resistance exercise, show strong effects on cardiometabolic outcomes (157, 237).

Cardiorespiratory fitness is an independent predictor of all-cause mortality and cardiovascular events (269, 275). A recent meta-analysis revealed that the increase in cardiorespiratory fitness after interval training is ~2-fold greater than continuous training (149). In the longest trial to date comparing interval and continuous exercise in diabetes, Karstoft et al. (269) randomized participants to four months interval walking (n=12), energy and time-matched continuous walking (n=12; 60-min, 5 days/week) or non-exercise control (n=8). Greater improvements in fitness, body fat, and glycemic control were observed after interval compared to

continuous walking and control (145). These findings clearly support the benefit of interval exercise, however the volume of exercise (300 min/wk) is far greater than usually attained by the general population, many of whom cite lack of time as a considerable exercise barrier (145). Emerging evidence from small short-term trials show that low-volume high-intensity interval training (HIIT) rapidly improves glycemic control in T2D (151). Low-volume HIIT involves alternating brief periods of vigorous exercise with periods of recovery, typically taking ~20 minutes per session and performed three times per week (162, 166). Further research is needed to confirm changes in cardiometabolic health outcomes after several months of low-volume HIIT in studies with larger sample sizes.

Sarcopenic obesity disproportionately affects people with T2D (162). Diminished lean muscle leads to poor physical functioning, glycemic control and cardiovascular health (200). The anabolic effects of vigorous exercise and consumption of high-quality protein are important for counteracting the age-associated decline in muscle, and when combined provide synergistic effects on muscle protein synthesis (9). In particular, it appears that consuming milk protein after exercise promotes significant lean mass accretion and fat loss (75, 122). HIIT has recently been shown to increase protein synthesis in the skeletal muscle of young and older adults, an effect linked to improved insulin sensitivity and mitochondrial function (215). Thus the combination HIIT and strategically timed milk protein may be a novel strategy to improve cardiometabolic health in T2D. Protein supplementation after HIIT in type 2 diabetes has, to our knowledge, never been tested.

The purpose of this study was to determine whether post-exercise milk consumption augments the cardiometabolic benefits of low-volume HIIT in individuals with T2D. The primary outcome of glycemic control was assessed across three days before and after the intervention using continuous glucose monitoring (CGM). Secondary outcomes of body composition, glycosylated

hemoglobin, fasting blood parameters, cardiorespiratory fitness, blood pressure and endothelial function were also examined to determine how low-volume HIIT impacted key cardiometabolic health parameters.

Research design and Methods

Study design

This double-blind, randomized clinical trial was conducted at The University of British Columbia Okanagan between January 2015 and December 2016 (Trial registration #NCT02251301, clinicaltrials.gov). The Clinical Research Ethics Board (CREB number H14-01636) approved the study and participants provided written informed consent. Randomization was by a third-party using variable permuted block sizes with computer-generated random numbers and sealed envelopes. Beverages were prepared each morning by a research assistant not involved in data analysis, such that participants and study personnel were blinded to the beverage condition.

Participants

Men and women, aged between 40-75 years with physician-diagnosed T2D (>6 months) were recruited from the Kelowna Diabetes Program via mail-out advertisements and sign-up sheets. Exogenous insulin users, diagnosed cardiovascular disease, neuropathy, nephropathy, lactose intolerance or contraindications to exercise (122, 141) were excluded. After initial telephone/email interviews interested participants attended a screening visit, which included a medical history questionnaire, physical activity readiness questionnaire-plus (PARQ+) and informed consent. Eligible participants then completed a 12-lead exercise stress test using a modified Bruce protocol and a cardiologist provided clearance for vigorous exercise.

Intervention

Experimental Protocol Overview

Fifty-three participants were randomized to one of three beverage groups; (i) low-fat milk, (ii) milk protein and lactose matched (macronutrient control), or (iii) placebo (water), consumed after exercise during a 12 week HIIT intervention (details below). Baseline and post-intervention outcomes were assessed over five days before and after the intervention (48-72 h after the last training session). Fasted blood and body composition measures were obtained on day 1 and CGM was performed across days 2-4 while participants followed a standardized diet. Blood pressure, endothelial function and fitness were assessed on Day 5. Body weight, waist circumference, blood pressure and endothelial function were also assessed at six weeks (Mid).

Exercise Training

All groups performed supervised low-volume HIIT 3d/wk for 12 wk. To be consistent with exercise recommendations by the American Diabetes Association and the American College of Sports Medicine (244) both resistance and cardio-based exercises were included in the HIIT program. The first and last session per week were cardio-based performed using a cycle ergometer, treadmill, or elliptical involving one minute bursts of exercise at 85-90% of the participants' maximum heart rate (HR_{max} ; obtained during baseline $\dot{V}O_{2\ peak}$ test) with one minute of easy recovery in between. The middle session each week involved whole-body resistance exercises (using resistance bands or multi-gym). Similar to cardio-based HIIT, each resistance exercise was performed for one minute (as many repetitions as possible) at an intensity eliciting an RPE of 5 'hard' on the CR-10 scale (54) followed by one minute of recovery. The number of one-minute intervals in each session progressed from four in week one to ten in week six of training. Thereafter,

10 X one-minute intervals eliciting ~90% of HR_{max} (cardio-based) or RPE ~5 (resistance) with one-minute recovery were completed in each session through to week 12. Previous short-term training studies in individuals with, and at risk for, diabetes, have shown that this low-volume HIIT protocol is effective for improving cardiometabolic health markers (33). Each session began with a three-minute warm-up and finished with a three-minute cool-down. A heart rate monitor was worn to closely prescribe intensity, and capillary blood glucose and blood pressure measures were obtained before and after each exercise session.

Post-exercise nutrition supplementation

After each session participants consumed 500 mL of either: (i) low-fat milk; (ii) milk protein macronutrient-matched control; or (iii) placebo (water), within one hour. The beverages were designed to look and taste similar and distributed in opaque containers. To accomplish this, one-teaspoon of cocoa powder and ¼ teaspoon of stevia (Stevia In The Raw[®], Cumberland Packing Corp; containing ~28 mg stevia) were added to each beverage. Low-fat milk was prepared from skim-milk powder (MedallionMilk Co., Canada) providing 187 calories, 19 g protein, 26 g carbohydrate and < 1 g of fat. Macronutrient-matched control (milk protein concentrate; Vitalus Nutrition Inc., Canada plus lactose; NOW[®] Foods, IL, US) provided 186 calories, 21 g protein, 24 g carbohydrate and < 1 g of fat; i.e., providing the same macronutrient and protein composition as milk but without the micronutrients and other bioactive factors. The placebo beverage provided < 10 calories from the cocoa powder.

Outcomes

Continuous glucose monitoring (CGM)

A continuous glucose monitor (iPro 2, Medtronic Inc.) was used to continuously measure blood glucose across three days before and after the intervention. CGM provides valuable insight (that a one-off fasting blood or HbA_{1c} sample cannot) into glycemic variability and the magnitude of postprandial excursions across several days under free-living conditions (84, 162). The CGM continuously samples interstitial fluid from the abdomen, measuring glucose concentration every 5-min using the glucose oxidase reaction (148). Participants took capillary glucose samples (4X/d), which were used to retrospectively calculate retrospective blood glucose concentration via an algorithm within the online software program (CareLink Pro, Medtronic) (218). All food and drink were recorded across the three-day monitoring period (including time eaten, amount, brand) for pre-testing, and then replicated exactly for post-intervention. Medication dose and timing were standardized during each CGM wear period.

The primary outcome was 24-h average glucose (from 00:00 to 23:55), calculated as the mean of the three CGM days. Standard deviation of 24-h blood glucose and mean amplitude of glycemic excursions [MAGE; (218)] were calculated from the same 24-h periods to assess glycemic variability.

Body Composition

Waist circumference (190), height and weight (Seca 700, Hamburg, Deutschland) were measured to the nearest 0.1 cm and 0.1 kg, respectively. Percent fat, visceral adipose tissue (VAT) and lean body mass (LBM) were measured by dual-energy X-ray absorptiometry (Hologic

Discovery DXA, MA, USA). All measures were performed and analyzed by the same researcher, with calibrations and quality control testing performed daily.

Cardiorespiratory fitness ($\dot{V}O_{2\text{peak}}$)

$\dot{V}O_{2\text{peak}}$ was assessed using an incremental ramp maximal exercise test on a cycle ergometer (Lode Excalibur, Netherlands) with continuous sampling of expired gases (Parvomedics TrueOne2400, USA). Beginning at 30 W, the test increased by 1 W every 4 s (15 W/min) until volitional exhaustion or contraindication (270). $\dot{V}O_{2\text{peak}}$ and RER were calculated from the highest 30-s average, while HR_{max} was recorded as the highest value obtained during the test.

Biochemical analyses

Fasting blood samples were collected by venipuncture into EDTA containing tubes, centrifuged for 15 min (1550g at 4 °C) and the plasma stored at -80 °C for subsequent batch analyses. Fasting glucose was measured by the hexokinase method, high-sensitivity C-reactive protein (CRP) by latex particle enhanced immunoturbidimetric assay and triglycerides by the enzymatic glycerol kinase and glycerol phosphate oxidase method. All were analyzed in duplicate (average coefficient of variation 6.8%) on a clinical chemistry analyzer (Chemwell 2910, Awareness Technologies) using assays from Pointe Scientific (MI, USA). Pre and post-intervention samples for each participant were analyzed concurrently. Glycosylated hemoglobin (HbA_{1c}) was analyzed from a separate EDTA tube by a medical laboratory that routinely performs this analysis according to the National Glycohemoglobin Standardization Program (NGSP).

Blood pressure and endothelial function

All measures were assessed four hours postprandial, after abstaining from alcohol and caffeine for 12 h and, within participants, at the same time of day with meal and medication standardized. After 20 min of rest in a supine position, blood pressure was measured manually using the auscultatory method, at least twice to the nearest 2 mmHg.

Flow-mediated dilation

Brachial artery flow-mediated dilation (FMD) is an important prognostic indicator of endothelial function and incident cardiovascular disease (79). The ability of the vessel to dilate (%FMD) is measured in response to a physiological (shear stress) stimulus (278). In the current study, brachial artery FMD was assessed according to current guidelines (241). Briefly, simultaneous measures of diameter and blood velocity were obtained with high-resolution ultrasound (Terason 3200), 2 cm from the antecubital fossa. Data were collected over a one-minute baseline, for the last 30 s of a 5 min period of forearm ischemia (pneumatic cuff inflated 60 mmHg above systolic blood pressure) and for 3 min thereafter.

Brachial Artery Dilatory Capacity

The peak blood flow and diameter response to ischemic handgrip exercise provides an index of resistance artery size or remodeling and the maximal dilatory capacity (241). This is important since changes in artery function (%FMD) with exercise training are thought to occur rapidly (i.e., first few weeks) after which are superseded by changes in structure, potentially concealing further changes in function (191). After 15 min of rest, following the FMD procedure, baseline diameter and blood velocity were recorded for 1 min. This was followed by 5 min of forearm ischemia (as

above), including 3 min of isotonic handgrip exercise (1 constriction every 2 s using a dynamometer) between one-minute periods of ischemia alone (247). Again recording resumed 30 s before cuff deflation and continued for 3 min thereafter.

Absolute FMD (peak diameter – baseline diameter), %FMD (peak – baseline diameter/baseline diameter), and time to peak diameter were measured using custom designed edge-detection and wall-tracking software, to minimize user bias (191). This protocol is routinely performed in our lab using the methods outlined in Francois et al. (276); coefficients of variation for diameter and %FMD are 2.1% and 7.3%, respectively.

Quality of Life (QoL)

Participants completed the Medical Outcomes Study Short Form 36 (SF-36) questionnaire before and after the intervention (84). The SF-36 is a self-report QoL questionnaire; the scores are used to provide two norm-based T scores, physical component summary (PCS) and mental component summary (MCS).

Standardization

Participants were instructed to maintain their usual diet, lifestyle and medication habits, verified by physical activity and diet records. Baseline activity was examined using both accelerometry (Actigraph GT3x, FL, USA) over a seven-day period to assess minutes of moderate-vigorous physical activity (MVPA) [Freedson et al. (182) cut-points] and a Godin leisure-time exercise questionnaire (87) (Table 2.1). Baseline dairy consumption was assessed using a food frequency questionnaire, and dietary intake before and during the study was assessed using three-day food records analyzed using FoodWorks16 (The Nutrition Company, NJ, USA).

Statistical Analyses

Sample size

Using means and standard deviations from previously published data on the impact of short-term HIIT for reducing hyperglycemia assessed by CGM in T2D (96), power calculations determined that $n=17$ per group would be sufficient to detect a 30% reduction in glucose (Cohen $d=0.7$) with a power of 80% and alpha of 0.05.

Statistics

Analyses were performed on all participants that completed the intervention. Characteristics of the intervention groups are shown in Table 2.1. Linear mixed models using SPSS 22.0 (SPSS, Chicago, Illinois) examined changes in trial outcomes (pre-post or pre-mid-post) between groups. Significant interactions were probed with pre-planned contrasts comparing the change within each group, whereas isolated significant main effects of time were examined by pairwise comparisons with groups collapsed using Least Significant Difference (LSD) test (162). Results are reported as means and standard deviations with 95% confidence limits. Magnitude based inferences were used to identify clinically meaningful changes in major outcomes using techniques described by Hopkins and Batterham (128). The threshold for clinically beneficial changes in 24-h glucose and HbA_{1c} was a reduction of 0.5 mmol/l and 0.7%, respectively, based on the reduced risk for diabetes complications (22). For cardiorespiratory fitness an increase of 1 metabolic equivalent (MET) was used, a 1 MET increase is associated with a 15% risk reduction in cardiovascular disease (178). For %FMD +1% was used, based on a recent meta-analysis showing a 13% risk reduction in future cardiovascular events (149). In line with previous studies, a 2 mmHg reduction in MAP was

considered to be the smallest clinical threshold change for blood pressure (131). The clinically meaningful difference in QOL was determined as a change greater than 3 points (56).

Table 2.1. Baseline characteristics of participants.

	Milk (n=18)	Macronutrient Control (n=16)	Placebo (n=19)
Sex	11 F	12 F	11 F
Age (y)	62 ± 8	56 ± 9	55 ± 9
BMI (kg/m ²)	36 ± 7	35 ± 6	33 ± 6
Years of diagnosis	6 ± 6	7 ± 7	5 ± 6
Medications			
Lifestyle only	5	5	3
Metformin	10	11	13
Sulfonylureas	6	1	3
SGLT2 inhibitors	1	2	3
DPP4 inhibitors	1	2	3
GLP1 analogs	1	2	0
Lipid lowering	9	7	7
Antihypertensive	7	6	8
Baseline Physical Activity			
LTPA score	17 ± 15	14 ± 10	21 ± 17
MVPA (min/day)	14 ± 15	13 ± 13	30 ± 19
Dairy intake (servings/day)	2.3 ± 2.4	2.7 ± 2.1	2.1 ± 1.6

F = Females, LTPA = Leisure-Time Physical Activity, MVPA = Moderate-Vigorous Physical Activity

Results

Participant Compliance and Adverse Events

A CONSORT flow diagram of the study progression is shown in Figure 2.1. Fifty-three participants were eligible after initial screening; four required additional 24-h blood pressure monitoring (n=2) and stress echo (n=2) cardiologist clearance following the 12-lead ECG stress test. Baseline characteristics of randomized participants are shown in Table 2.1. The majority were of European descent (51/53), while two were Southeast Asian (2/53). Of the 53 participants randomized, 51 successfully completed thirty-six sessions of HIIT in 12 ± 1 wk. One participant

suffered a non-fatal myocardial infarction in week eight (after 23 HIIT sessions) and one dropped out due to personal reasons. There were no reports of hypoglycemia after exercise or at home throughout the intervention. Exercise sessions were rescheduled on ten occasions (n=6 due to sickness and n=4 due to systolic blood pressure >144 mmHg prior to exercise). No musculoskeletal injuries were reported as a result of the training. $\dot{V}O_{2peak}$ testing was truncated in three participants because systolic pressure exceeded 250 mmHg during the test (265). For CGM analyses three participants were excluded due to sensor failure (n=1) and medication changes (n=2; required reduced medication). All other analyses are reported for n=51 unless otherwise stated. Overall the percent of HR_{max} achieved during cardio-based intervals was $88 \pm 7\%$. Overall RPE was 4.7 ± 1.2 and 4.2 ± 1.0 , for cardio- and resistance-based intervals respectively.

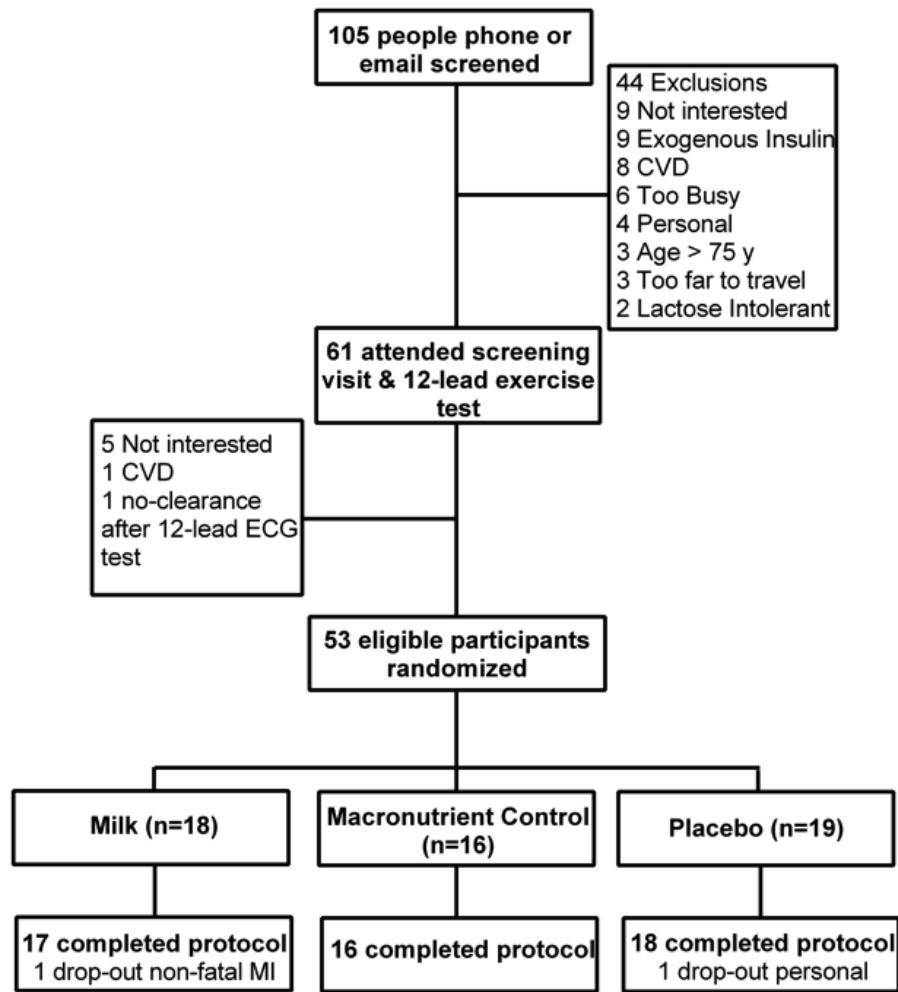


Figure 2.1. Consolidated standards of reporting trials (CONSORT) flow diagram.

Glycemic Control

There was a significant reduction in mean 24-h glucose following 12 weeks of HIIT (by -0.5 ± 1.1 mmol/L, Figure 2.2) with no difference between groups (Table 2.2). The probability that the change in glucose was clinically beneficial was 54% (95% CI: $-0.8, 0.1$ mmol/L). Glycemic variability assessed by both SD (by -0.33 ± 0.78 mmol/L) and MAGE (by -0.98 ± 2.27 mmol/L) was significantly reduced, with no differences between groups (Table 2.2). Glycosylated hemoglobin was significantly reduced after 12 weeks of HIIT (by $-0.22 \pm 0.39\%$, Figure 2.3) with no differences

between groups (Table 2.2). The probability that the change in HbA_{1c} was clinically beneficial was 0% (95% CI: -0.33, 0.16%), with the change being most likely trivial. Fasting blood glucose levels were not significantly different after HIIT in all groups (Table 2.2).

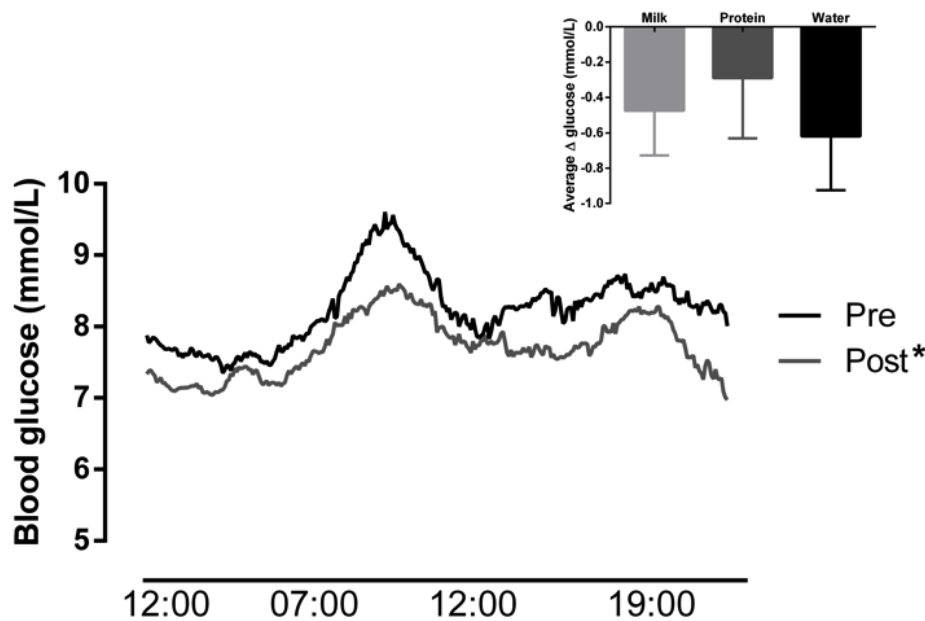


Figure 2.2. Continuous blood glucose across 24-h (n=48) before and after the intervention (groups collapsed, *main effect of time effect: p=0.01).

Inset: Change in blood glucose after the intervention in the milk, protein and water groups.

Body Composition

There was a significant reduction in body mass across 12 weeks of HIIT (Table 2.2) with no difference between groups. Mean values for groups collapsed were Pre: 94.2 ± 18.3 kg, Mid: 93.5 ± 18.2 kg, Post: 93.3 ± 18.4 kg. There was a significant reduction in waist circumference after 12 weeks of HIIT (by -2.9 ± 3.5 cm, main effect of time: p<0.01) with no difference between groups (Interaction: p=0.21, Figure 2.4). Percent body fat was significantly reduced (by -0.76 ± 1.63%,

main effect of time: $p=0.02$) and lean body mass significantly increased (by $+1.07 \pm 2.76$ kg, main effect of time: $p=0.01$) after 12 weeks of HIIT, with no difference between groups (Interactions: all $p>0.83$, Figure 2.3).

Cardiorespiratory Fitness ($\dot{V}O_{2\ peak}$) and Blood Pressure

$\dot{V}O_{2\ peak}$ significantly increased 9.8% after 12 weeks of HIIT (main effect of time: $p<0.01$, Figure 2.3) with no difference between groups (Interaction: $p=0.55$). The probability that the change in fitness was clinically beneficial was 5% (95% CI: 1.8, 3.1 mL/kg/min), with the change being 95% very likely trivial.

Mean arterial blood pressure was significantly reduced after 12 weeks of HIIT (by -5.7 ± 7.0 mmHg, main effect of time: $p<0.01$) with no difference between groups (Interaction: $p=0.11$, Figure 2.4). The probability that the change in MAP pre-post intervention was clinically beneficial was 99% (95% CI: -9, -2 mmHg).

Flow-mediated dilation

%FMD significantly increased after 12 weeks of HIIT (by $+1.4 \pm 1.9\%$, main effect of time: $p<0.01$), with no difference between groups (Interaction: $p=0.72$, Figure 2.4). The probability that the change in %FMD post-intervention was clinically beneficial was 94% likely (95% CI: 0.86, 1.94%). Absolute FMD was also increased after HIIT (main effect of time: $p<0.01$) with no difference between groups (Table 2.2). Time to peak dilation was significantly reduced after 12 weeks of HIIT (by 9.1 ± 31.1 s) with no difference between groups (Table 2.2). Peak dilator capacity did not change across the intervention; Pre: $9.6 \pm 5.2\%$, Mid: $8.1 \pm 4.2\%$, Post: $10.4 \pm 3.6\%$ (main effect of time: $p=0.36$).

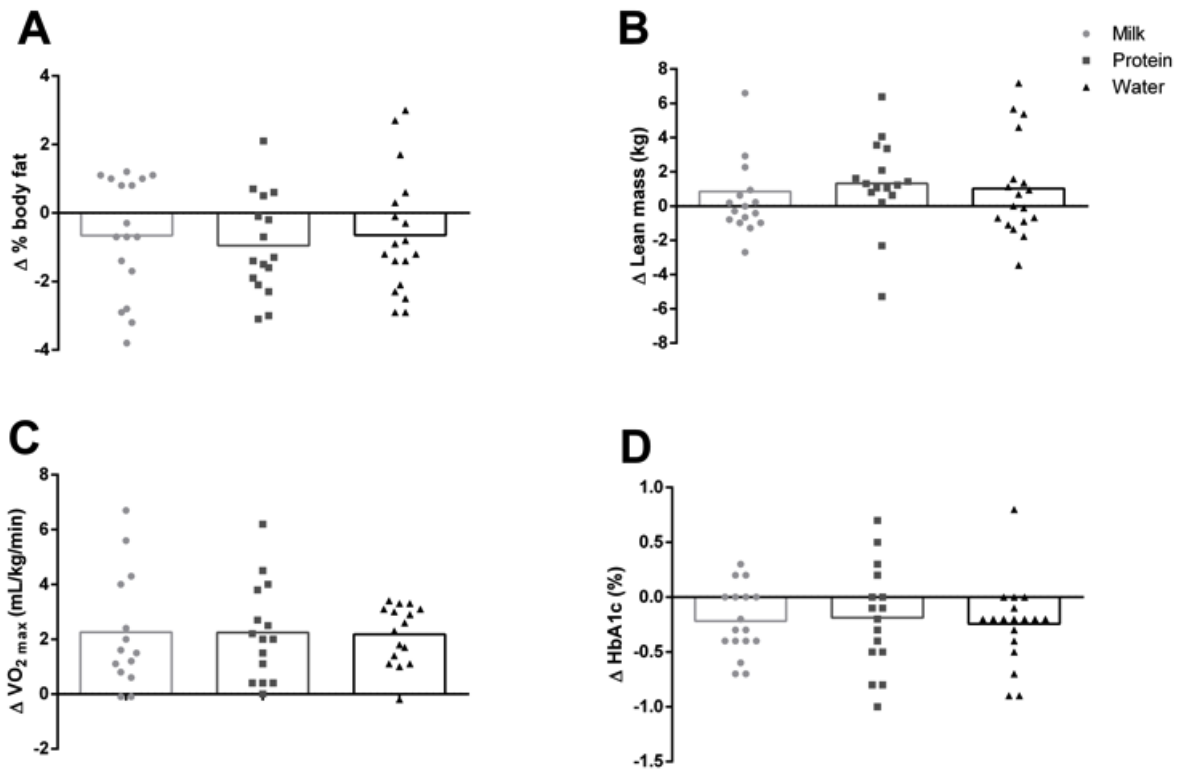


Figure 2.3. Change from pre intervention for (A) % body fat, (B) lean body mass, (C) cardiorespiratory fitness ($VO_{2\text{peak}}$) and (D) glycated hemoglobin (HbA_{1c}) in the milk, protein and water groups (*all main effect of time $p < 0.05$, #no group interaction $p > 0.05$).

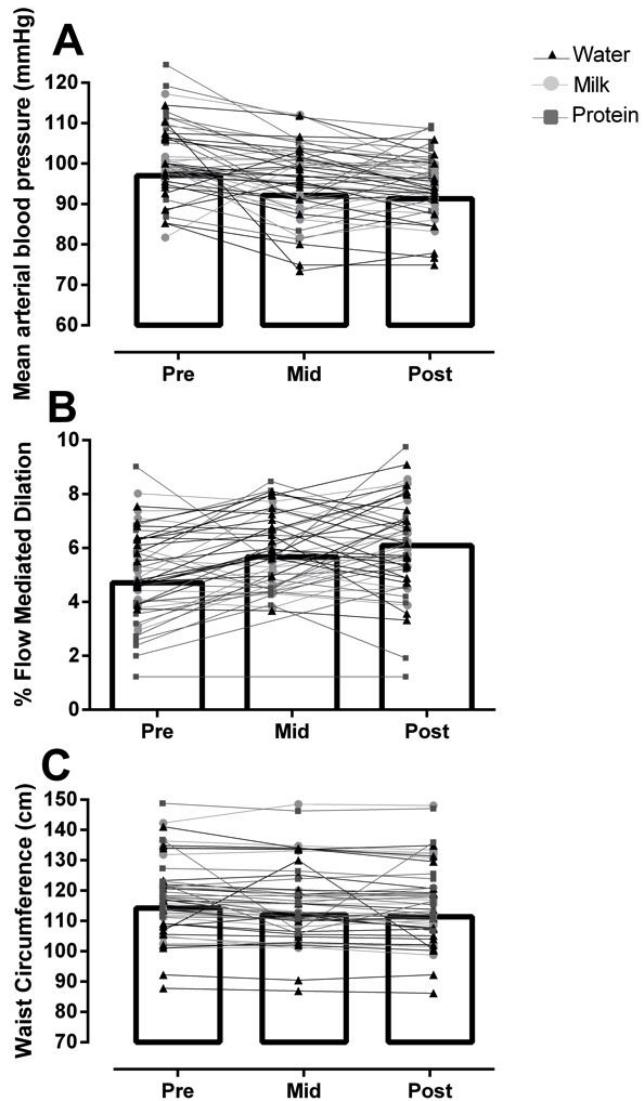


Figure 2.4. Data for (A) mean arterial blood pressure (MAP), (B) % flow-mediated dilation (%FMD), and (C) waist circumference, before (Pre), after six weeks (Mid) and 12 weeks (Post) mean for all participants (bar graph, *all main effect of time $p < 0.05$) and individual data (line and symbols per beverage group, #no group Interaction $p > 0.05$).

Quality of Life

PCS scores significantly increased after 12 weeks of HIIT (n=49, by 8.1 ± 12.1 , main effect of time: $p < 0.01$) with no difference between groups (Interaction: $p = 0.11$). The probability that the change in PCS pre-post intervention was clinically beneficial was 99% likely (95% CI: 4.4, 11.8). The change in MCS post-intervention was different between groups (n=49, Interaction: $p = 0.02$); post hoc testing revealed significant improvements in the milk protein group ($+12.1 \pm 9.69$, $p < 0.01$) but not skim milk (-1.1 ± 13.5 , $p = 0.79$) or placebo ($+5.6 \pm 10.7$, $p = 0.06$).

Dietary Intake Records

Analysis of the three-day diet records collected before and during the last week of the intervention showed no difference in the total daily energy intake between groups and/or across time (Table 2.2). Macronutrient composition of the diet was not different between groups ($p = 0.32$), or across time: for % carbohydrate (Pre: $48.0 \pm 12.5\%$ vs Post: $48.4 \pm 13.0\%$ of total energy, $p = 0.47$), % protein (Pre: $20.4 \pm 4.9\%$ vs Post: $19.9 \pm 4.9\%$ of total energy, $p = 0.15$) and % fat (Pre: $30.3 \pm 12.5\%$ vs Post: $30.7 \pm 13.3\%$ of total energy, $p = 0.49$).

Table 2.2. Body composition, cardiorespiratory fitness, blood pressure, flow-mediated dilation, triglycerides, C-reactive protein and glycemic control measures before and after 12 weeks of HIIT and nutritional beverage.

	Milk (n=18)		Macronutrient Control (n=16)		Placebo (n=19)		P value	
	Pre	Post	Pre	Post	Pre	Post	Interaction	Time
Body Composition								
Mass (kg)	97.7 ± 19.3	96.8 ± 20.5	95.9 ± 17.3	94.5 ± 17.3	89.5 ± 21.1	89.1 ± 20.9	0.46	0.03*
VAT (g)	1057 ± 335	1033 ± 316	1007 ± 260	981 ± 212	815 ± 337	802 ± 285	0.75	0.25
Cardiorespiratory fitness								
$\dot{V}O_2$ peak (L/min)	1.7 ± 0.4	2.0 ± 0.7	1.8 ± 0.5	2.0 ± 0.6	1.9 ± 0.5	2.1 ± 0.5	0.53	< 0.01*
Bloods								
HbA _{1c} (%)	7.1 ± 0.8	6.9 ± 0.7	6.9 ± 0.8	7.0 ± 0.7	6.9 ± 0.8	6.6 ± 0.9	0.92	<0.01*
(mmol/mol)	54 ± 9	52 ± 8	54 ± 8	53 ± 8	51 ± 8	49 ± 9		
Fasting glucose (mmol/L)	8.6 ± 2.3	8.3 ± 1.7	9.2 ± 1.9	9.5 ± 2.3	8.9 ± 2.7	8.5 ± 2.1	0.35	0.53
Triglycerides (mg/dL)	149 ± 82	152 ± 70	161 ± 62	139 ± 65	152 ± 93	142 ± 80	0.36	0.17
C-reactive protein (mg/dL)	7.1 ± 10.3	4.4 ± 5.3	4.7 ± 4.3	4.9 ± 4.7	3.7 ± 4.1	3.1 ± 3.6	0.33	0.21
CGM glucose concentration								
24-h mean (mmol/L)	8.4 ± 1.4	7.7 ± 1.2	8.1 ± 1.4	7.8 ± 1.7	8.4 ± 2.1	7.8 ± 1.5	0.74	0.01*
SD (mmol/L)	1.6 ± 1.0	1.3 ± 0.5	1.6 ± 0.6	1.1 ± 0.4	1.7 ± 0.8	1.5 ± 0.7	0.51	0.01*
MAGE (mmol/L)	4.3 ± 3.5	3.1 ± 1.3	4.1 ± 2.0	2.8 ± 1.3	4.1 ± 2.2	3.7 ± 1.6	0.60	0.02*
Blood Pressure								
Systolic (mmHg)	130 ± 10	119 ± 7	132 ± 13	129 ± 9	128 ± 13	117 ± 11	0.03#	< 0.01*
Diastolic (mmHg)	79 ± 6	75 ± 5	83 ± 11	79 ± 6	81 ± 7	75 ± 7	0.20	< 0.01*
Flow-mediated dilation								
Absolute FMD (mm)	0.020 ± 0.01	0.027 ± 0.01	0.018 ± 0.01	0.024 ± 0.01	0.019 ± 0.01	0.023 ± 0.01	0.61	< 0.01*
Baseline diameter (mm)	0.41 ± 0.10	0.41 ± 0.09	0.41 ± 0.08	0.41 ± 0.07	0.41 ± 0.07	0.42 ± 0.07	0.77	0.71
Time to peak (s)	64 ± 26	57 ± 25	60 ± 30	46 ± 23	56 ± 21	50 ± 21	0.75	0.05*
Total Energy Intake (Kcal/day)	2053 ± 881	2039 ± 898	1810 ± 525	2017 ± 706	1912 ± 629	1888 ± 710	0.35	0.25

HbA_{1c} = Glycosylated Hemoglobin, BMI = Body Mass Index, VAT = Visceral Adipose Tissue, MAP = Mean Arterial Pressure, FMD = Flow Mediated Dilation, TE = Total Energy,

* time effect p < 0.05

interaction group*time p < 0.05

Discussion

This study comprehensively examined the cardiometabolic benefits of HIIT in individuals with T2D. We show for the first time that 12 weeks of low-volume HIIT, with or without post-exercise milk or protein, improves glycemic control, blood pressure, cardiorespiratory fitness, body composition and endothelial function. Low-volume HIIT therefore appears to be a feasible and efficacious lifestyle intervention, involving minimal time and resource, to improve health in individuals with T2D. Reducing the interval length and total exercise time has previously been shown to increase enjoyment and compliance (79). To this end, we experienced very low dropout rates and high compliance to low-volume HIIT. In addition, we show that 12 weeks of HIIT improves quality of life, similar to previous studies in hypertensive (175) and heart failure (189) patients.

Exercise interventions generally result in modest weight loss, however exercise can promote lean mass accretion; which has important implications for whole-body metabolism, glucose disposal and quality of life (275). Indeed, in the current study HIIT significantly increased lean mass and reduced body fat, with a trivial reduction (~1 kg) in body mass. Consuming high-quality protein after exercise is known to further potentiate muscle protein synthesis (9). Despite this, comparable changes in body composition and cardiometabolic health were seen with post-exercise milk, milk-protein or water. In agreement, Parr et al. (75, 122) found changes in body composition after a combined resistance training and diet intervention were independent of the amount and type of protein (high/low dairy). Epidemiological data shows an inverse relationship between low-fat dairy consumption and the risk of T2D (202) and the addition of four servings of low fat dairy per day has been shown to improve insulin resistance (15). Therefore, additional milk/protein supplementation (e.g. on non-exercise days)

may have been needed to elucidate effects of nutritional supplementation. Indeed, some previous studies showing benefits on lean mass have provided milk/protein after exercise five days per week (214). However, ~20 g of post-exercise protein (similar to the current study) has been shown to maximize skeletal muscle protein synthesis (122, 141). To this end, a non-exercising control group may be required to detect effects of post-exercise protein added to a potent training intervention such as HIIT. However, we feel a non-exercise control group in T2D is unethical since numerous studies have shown worsening of glycemic control and cardiovascular risk factors in control group participants (50).

Current research suggests that HIIT is more effective than continuous training for improving insulin resistance (49, 145). A recent meta-analysis revealed that absolute changes in glycosylated hemoglobin (HbA_{1c}) are 0.5% and 0.25% greater with HIIT than control and continuous exercise, respectively (135). The small, yet significant change in HbA_{1c} in the current study is in line with previous HIIT interventions (135) yet robust changes in 24-h glucose were observed (Figure 2.2). Interestingly, the changes in 24-h average glucose are similar to Karstoft et al. (41, 166) after four months of high-volume HIIT (300 min/wk). This is an important finding given the perceived time barrier to exercise participation in T2D (145). The use of CGM is a strength as it allows for additional insight into the changes in postprandial hyperglycemia and overall glycemic variability (151). Mean 24-h glucose and glycemic variability were reduced by 7% and 23% respectively after HIIT, regardless of post-exercise nutritional supplementation. Glycemic variability may be a stronger predictor than HbA_{1c} for diabetes complications (148). Previous research also shows that low-volume HIIT has the potential to improve beta cell function as Madsen et al. (207) demonstrated an increase in the oral disposition index and HOMA-%β after eight weeks of training. The mechanisms underlying the improvements in

glycemic control could not be ascertained from the present study design but likely involve a combination of improvements in peripheral insulin sensitivity, beta cell function and hepatic insulin resistance (166). Collectively, these findings show the potential of low-volume HIIT to improve several underlying aspects of glycemic dysfunction in T2D.

The added benefits of vigorous exercise for cardiovascular health are well known (41, 144, 166) and many studies have demonstrated superior cardiovascular effects of HIIT compared to continuous training (19, 177). Extending on this work, we observed an ~10% increase in cardiorespiratory fitness, a 6 mmHg reduction in MAP and ~1.4% improvement in FMD following 12 weeks of low-volume HIIT in individuals with T2D. In itself cardiorespiratory fitness is a strong predictor for cardiovascular mortality with each MET increase associated with a 10-20% improvement in survival (177, 269, 275). Although only a 0.7 MET increase was observed, this is in line with previous low-volume HIIT studies (149) and participants are likely to have gained significant health benefits given their low baseline fitness (<6 MET). A meta-analysis of 33 studies showed that the greatest mortality benefits occur for even small increases in fitness for those progressing from the least fit category (166). Furthermore, the low-volume nature of the HIIT protocol involved only 45-78 minutes of exercise per week with one session being resistance training. The combination of resistance and cardio exercise may be superior to either type alone for improving health in T2D (149). Indeed in hypertensive patients blood pressure is reduced more with combination training than cardio alone (49); the 5-6 mmHg reduction is in line with the current study. Our findings suggest that HIIT performed as combined aerobic and resistance exercise clearly promotes beneficial cardiovascular adaptations in type 2 diabetes patients.

In conclusion, we show that low-volume HIIT, with or without post-exercise milk or protein supplementation, improves metabolic and cardiovascular risk factors in individuals with type 2 diabetes. The combination of resistance and aerobic-based HIIT increases lean mass, reduces fat mass and improves endothelial function. This study, the largest and longest low-volume HIIT study in type 2 diabetes to date, provides further evidence that HIIT is a feasible and efficacious exercise intervention to improve glycemic control, cardiovascular fitness, and body composition.

Chapter 3: Cardiovascular Benefits Of Combined Interval Training and Post-Exercise Nutrition In Type 2 Diabetes

Lifestyle interventions, including exercise and nutrition, remain the frontline treatment option for patients with type 2 diabetes (T2D) in order to reduce the burden of cardiovascular disease (CVD) (154). The high cardiovascular mortality in individuals with T2D is largely owing to the development of atherosclerosis, accelerated by arterial stiffening, vascular inflammation and reduced perfusion (133, 176). Importantly, improvements in vascular function may explain a large proportion of the cardio-protective effects of exercise training (58, 179, 222). Interventions including both exercise and nutrition components appear to show stronger effects on cardiovascular risk factors, particularly those that encourage favorable changes in body composition (142). On this note, consuming high-quality protein after high-intensity interval exercise (a promising time-effective and powerful exercise stimulus) is one novel and promising combination. Given the poor adoption of current lifestyle recommendations, studies combining simple and effective diet and nutrition strategies are needed.

Exercise exerts direct effects on the vasculature, indeed, elevated blood flow (and associated shear stress and pressure) during and following exercise mediate favorable structural and functional vascular adaptations (16, 196). A growing body of literature has demonstrated the effectiveness of HIIT to improve several cardiovascular risk factors in individuals with T2D (247). Recently, two intervention studies reported promising results demonstrating that HIIT can improve endothelial function, measured by flow-mediated dilation, in participants with T2D (42). However the impact of HIIT on arterial stiffness, intima-media thickness, and basal blood flow profiles in T2D remains to be elucidated. Interestingly, the combination of high-intensity resistance and aerobic training appears to promote superior cardiometabolic health benefits (126, 187). We have previously shown that a single bout of cardio-based and resistance-based HIIT improves endothelial function in individuals with T2D (155, 168, 228). Thus, incorporating both

resistance and cardio-based HIIT may help to optimize health benefits (84). The addition of high-quality protein supplementation in the post-exercise recovery period has been shown to enhance body composition changes (171, 176). Whether the combination of resistance and cardio-based HIIT with post-exercise protein supplementation can enhance cardioprotective effects is currently unknown.

Accordingly, the aim of the present study was to examine the impact of 12 weeks of HIIT, with or without post-exercise protein supplementation, on measures of vascular structure and function in individuals with type 2 diabetes. We also assessed resting and peak exercise heart rates as global indices of cardiovascular function.

Methods

Overview and Ethical Review

A double-blind clinical trial conducted between January 2015 and December 2016 randomized adults with T2D to 12 weeks of HIIT with a post-exercise skim-milk, milk-protein concentrate or water placebo beverage after exercise (Trial registration #NCT02251301 clinicaltrials.gov). Participants first provided written informed consent and all study protocols were approved by the University Clinical Research Ethics Board (CREB number H14-01636).

Participants

Fifty-three individuals with physician-diagnosed T2D (> six months) not on exogenous insulin, and without diagnosed cardiovascular disease, neuropathy or nephropathy were recruited after a baseline screening visit that included a 12-lead cardiologist cleared exercise test.

Characteristics and medications of participants are described in Table 3.1. Of the fifty-three participants who were randomized, two participants did not complete the trial; one due to personal reasons and one due to a non-fatal myocardial infarction occurring in week eight of the intervention.

Table 3.1. Baseline characteristics of participants.

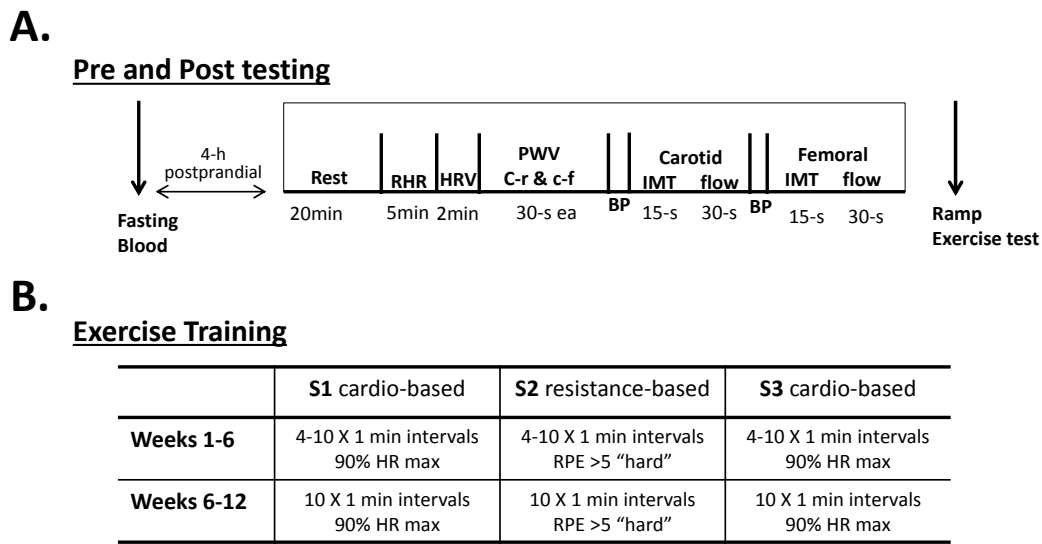
	Milk (n=18)	Macronutrient Control (n=16)	Placebo (n=19)
Sex	11 F	12 F	11 F
Age (y)	62 ± 8	56 ± 9	55 ± 9
BMI (kg/m ²)	36 ± 7	35 ± 6	33 ± 6
HbA _{1c} (%)	7.1 ± 0.8	6.9 ± 0.8	6.9 ± 0.8
$\dot{V}O_{2\text{ peak}}$ (mL/kg/min)	18 ± 3	19 ± 4	22 ± 5
Years of diagnosis	6 ± 6	7 ± 7	5 ± 6
Medications			
Lifestyle only	5	5	3
Metformin	10	11	13
Sulfonylureas	6	1	3
SGLT2 inhibitors	1	2	3
DPP4 inhibitors	1	2	3
GLP1 analogs	1	2	0
Lipid lowering	9	7	7
Antihypertensive	7	6	8

F = Females, BMI = Body Mass Index, HbA_{1c} = Glycated Hemoglobin, $\dot{V}O_{2\text{ peak}}$ = Cardiorespiratory fitness

Intervention

All participants performed 12 weeks of supervised low-volume HIIT, thrice weekly (Figure 3.1). Training sessions involved both cardio-based (2 sessions/week; elliptical, treadmill, or cycle based on participant preference) and resistance-based (1 session/week; whole body resistance exercises performed in the same interval pattern) interval exercise, which progressed from 4 X 1 min intervals per session in week one to 10 X 1 min intervals through week six to twelve. During each exercise session, blood pressure, heart rate and rating of perceived exertion [CR-10 (141)] were monitored. The intensity of the 1 min intervals was prescribed as 85-90% of maximal heart rate achieved at the end of each interval for cardio-HIIT and an RPE of ~5-7 ('hard') for resistance-HIIT, with 1 min of low intensity recovery between intervals (32). Within

one hour after each session participants consumed 500 mL of either: (i) low-fat milk (skim milk powder; MedallionMilk Co., Canada); (ii) milk-protein macronutrient-matched control (milk protein concentrate; Vitalus Nutrition Inc., Canada with lactose; NOW® Foods, IL, US); or (iii) placebo (flavored water). The participants and lead investigator were blinded to the beverage condition, thus all beverages were masked with 1 tsp of cocoa powder and ¼ tsp of stevia (Stevia In The Raw®, Cumberland Packing Corp). Normal medication and dietary intake were maintained throughout the intervention, assessed with 3-day food and activity records.



Cardio-based = 1 min intervals on treadmill, elliptical or cycle; with 1 min recovery between
Resistance-based = 1 min intervals using body weight exercises, resistance bands or multi-gym machine (as many reps as possible in 1 min); with 1 min recovery between
HR = Heart rate, RPE = Rating of perceived exertion (Borg: CR-0-10)

Figure 3.1. Schematic of the (A) pre and post-testing measures, and (B) HIIT exercise intervention.

Experimental measures and analyses

Measurements were obtained before and 48-72 h after the last training session, at the same-time of day within participants (Figure 3.1). Participants were tested four hours

postprandial, with medication and the preceding meal standardized within participants. Measurements were made with participants having abstained from caffeine and alcohol (for 12 h) and exercise (>48 h) before data collection. All measures (described below) were performed after 20 min of supine rest, in a quiet and dimly lit room. To ensure the chronic, rather than acute, effects of HIIT were investigated fasting blood was obtained 48 h after the last training session, and measures of heart rate, vascular function and peak power were obtained 60-72 h after the intervention.

Resting heart rate and heart rate variability

Heart rate was monitored continuously by three-lead electrocardiography and detection of the R wave made using LabChart software (LabChart Pro v. 7.1, ADInstruments, Dunedin, New Zealand). The resting heart rate was calculated as the average of the last three min of a 5-min block where the participant rested silently and uninterrupted. Time and frequency domain indices of heart rate variability (HRV) were calculated as per the task force guidelines (84) from the last minute of a 2-min controlled breathing block (15 breaths/min), using the HRV macro in LabChart. From the time-domain analysis two measures were recorded: a) the SD of all RR intervals (SDNN), and b) the square root of the average of sum of squares of difference between adjacent filtered RR intervals (rMSSD). The two frequency domain variables, low frequency (LF) and high frequency (HF), were analyzed and calculated in normalized (nu) and absolute (ms^2) units, while the ratio of LF to HF was calculated as a measure of autonomic balance (258). Individuals with irregular and erroneous heart rhythms (n=6) were excluded from HRV analysis.

Arterial stiffness: pulse wave velocity (PWV)

Peripheral (carotid-radial) and central (carotid-femoral) PWV, as surrogate measures of arterial stiffness, were measured using applanation tonometry (SPT-301, Millar Instruments, USA) according to consensus guidelines (258). Pulse transit times were calculated from 30-s of data collection at each site using the foot-to-foot method with a macro in LabChart (ADI Instruments). The distance was measured between each site (i.e., carotid-radial and carotid-femoral) minus the distance from the carotid site to the suprasternal notch (257). PWV was then calculated from the distance / time for each of the peripheral and central measures.

Vascular conductance and compliance

Manual brachial blood pressure was measured in duplicate using a sphygmomanometer before ultrasound measurements. Femoral vascular conductance was calculated as mean femoral blood flow/mean arterial blood pressure ($\text{mL}\cdot\text{min}^{-1}/\text{mmHg}$), whereas femoral vascular resistance was calculated as mean arterial pressure/femoral blood flow ($\text{mmHg}/\text{mL}\cdot\text{min}^{-1}$) (266).

Femoral blood flow profiles and hemodynamics

Measures of femoral blood flow and shear rate profiles were calculated from synchronized Doppler velocity and diameter recordings obtained using high-resolution ultrasound (Terason 3200, MA, USA). From a longitudinal section 2-5 cm below the bifurcation, velocity and diameter recordings were made with a 10-MHz multi-frequency linear array probe for 30-s, exact locations were marked and repeated for post-testing. All measures were performed on the right side with an insonation angle of 60° . Offline analyses for blood flow, diameter and shear rates were made using wall-tracking edge detection software, which has been

validated and described previously (8). Blood flow was calculated as velocity*cross sectional area, while antegrade (ASR) and retrograde (RSR) shear rates were calculated as $4*velocity/diameter (s^{-1})$; where all velocities >0 were used for ASR and <0 were used for RSR.

Wall thickness of conduit arteries

The common carotid artery was imaged ~ 2 cm distal from the carotid bulb, while the superficial femoral artery was imaged 2-3 cm distal to the bifurcation. Measurements at each site were recorded for 15 sec (at least 10 cardiac cycles) focusing on the far wall, to obtain clearly demarcated intima-medial boundaries. Video recordings were later analyzed offline using DICOM-based edge detection IMT software (276). Measurement locations, body position and ultrasound settings were replicated for pre and post measures.

Adhesion molecules

Fasting blood was obtained pre and post intervention using venipuncture into EDTA collection tubes. Blood was centrifuged at 1550g and a portion of the plasma stored at -80° Celcius for later analysis. Intracellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1) were assessed using magnetic bead immunoassay (Milliplex HCVD2, Millipore Corporation, Billerica, MA, USA) following the manufacturer instructions and measured on a BioPlex MAGPIX multiplex reader (BioRad, Mississauga, ON, Canada).

Peak power exercise test

Peak power and heart rate were measured as the maximum value achieved during a ramp-style maximal exercise test on a cycle ergometer (Lode Excalibur, Groningen, The Netherlands).

After a four minute warm up at 30 W, the power was increased 1 W every 4 s until volitional exhaustion.

Percent of age predicted maximal heart rate was calculated using the formulas $220 - \text{age}$ and $208 - 0.7 \times \text{age}$ as described by Tanaka et al. (106). Heart rate range (maximal HR – resting HR) was calculated using the Karvonen formula (236).

Statistics

All image acquisition and data analysis was performed by a single investigator blinded to the group assignment. All fifty-one participants who completed the intervention were included in the analysis, unless otherwise stated. Data were first tested for normality using histograms and Shapiro-Wilk test and non-normally distributed data were natural log transformed before statistical analyses. To examine whether training (time) and/or post-exercise nutrition (group) impacted the change in study outcomes, a linear mixed model analysis was performed using SPSS 22.0 (SPSS, Chicago, Illinois). Significance was set at $p < 0.05$. Significant main effects of exercise training (time) were examined by pairwise comparisons of groups collapsed with Fisher LSD and magnitude-based inferences to determine clinically meaningful changes in selected outcomes (146). A 1 m/s reduction in carotid-femoral PWV was deemed to be the clinically meaningful threshold based on a 21% reduced cardiovascular mortality (129). A 0.08 mm reduction in IMT was used as the clinically meaningful threshold for the carotid and femoral conduit arteries as this magnitude of reduction in carotid IMT has been shown to lower cardiovascular events by 46% (109). Finally, a reduction of 3 bpm for resting heart rate was deemed clinically meaningful as this is associated with a 20-40% reduction in mortality (282). Data are presented as means \pm standard deviation (SD) or mean and 95% confidence intervals.

Results

Exercise testing and heart rate data

Twelve weeks of HIIT increased peak power output from 141 ± 33 W to 157 ± 38 W ($p < 0.01$). Participants achieved $94 \pm 8\%$ of age predicted maximal heart rate using the 220-age formula, and $91 \pm 8\%$ using the $208 - 0.7 \times \text{age}$ formula, with no difference between groups or across time ($p > 0.51$). For the ramp maximal exercise test, 35/48 (73%) achieved $\geq 90\%$ of age predicted maximum heart rate (based on 220-age), 45/49 (92%) achieved a respiratory exchange ratio ≥ 1.1 , and 35/49 (29%) achieved a plateau in oxygen consumption. Data from three participants were excluded for % age predicted heart rate due to use of beta-blocker medication.

Resting heart rate was reduced by 2.5 bpm (main effect of time, $p = 0.01$) after 12 weeks of HIIT, with no difference between groups (group \times time interaction: $p = 0.92$, Figure 3.2). The likelihood that this change in resting heart rate is clinically beneficial is 30% (95% confidence interval [CI]: -4.4 to -0.6 bpm). Peak exercise heart rate achieved during the maximal exercise test was unchanged after 12 weeks of HIIT (Pre: 151 ± 16 bpm vs Post: 152 ± 15 bpm; main effect of time, $p = 0.58$). The heart rate reserve was significantly increased after 12 weeks of HIIT (by 3 ± 7 bpm, main effect of time, $p = 0.01$), with no difference between groups (group \times time interaction: $p = 0.58$, Figure 3.2). There were no significant changes in time and frequency domain measures of heart rate variability (Table 3.2, all $p > 0.07$).

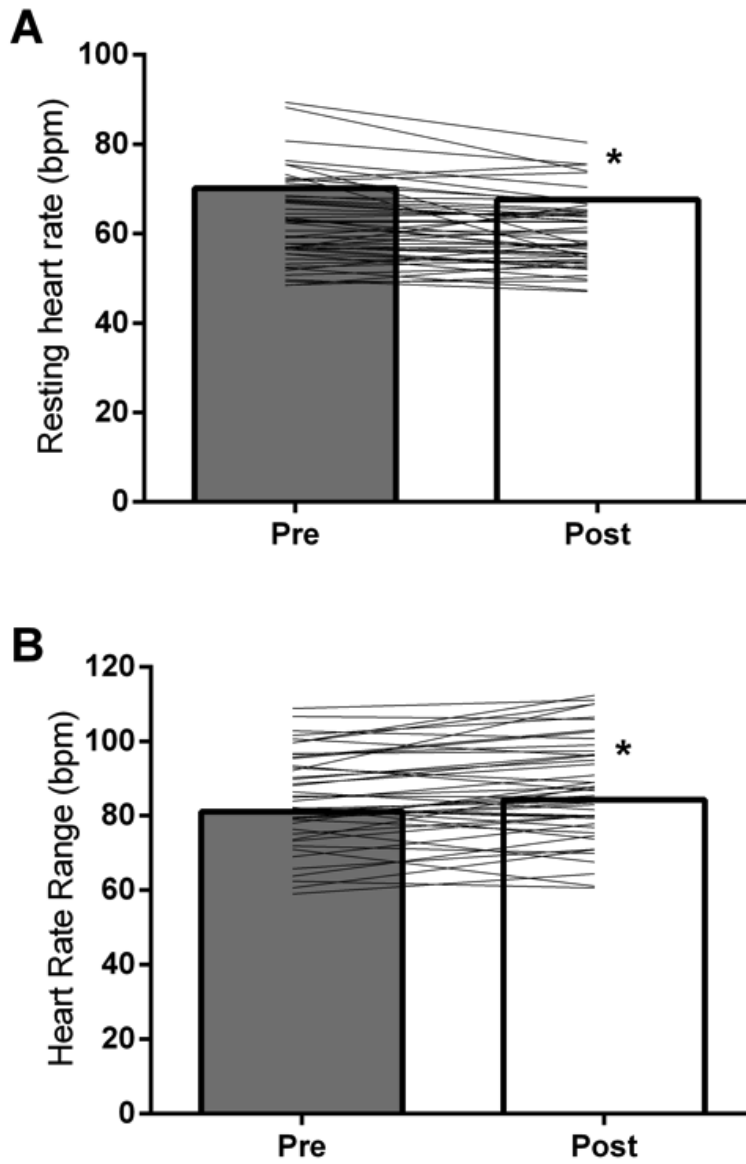


Figure 3.2. Resting heart rate (A) and heart rate range (B) before (Pre) and after (Post) 12 weeks of high-intensity interval training (HIIT). Individual data (lines) and group mean (drink groups collapsed) of n=51.

Arterial stiffness: pulse wave velocity (PWV)

Central pulse wave velocity was significantly reduced by 1.6 m/s ($p < 0.01$, Figure 3.3) following 12 weeks of HIIT, with no group differences (Interaction: $p = 0.11$). The likelihood that this change in arterial stiffness is clinically beneficial is 92% (95% CI: -2.4 to -0.67 m/s). Peripheral PWV was unchanged after 12 weeks of HIIT ($+0.19 \pm 1.0$ m/s; main effect of time, $p = 0.21$) and no difference between groups (Interaction: $p = 0.43$). Data are the average of $n = 39$ for central stiffness and $n = 50$ peripheral stiffness.

Femoral blood flow profiles and hemodynamics

Superficial femoral artery diameter and basal blood flow were unchanged after 12 weeks of HIIT (Table 3.2). Resting mean shear rate and ASR were significantly increased, by 17% and 7% respectively, after 12 weeks of HIIT (main effects of time, both $p < 0.05$, Table 3.2), with no difference between groups. RSR was unchanged after 12 weeks of HIIT (-4%, Table 3.2). However, there was a group*time interaction ($p = 0.049$), post hoc revealed RSR was significantly reduced in the HIIT plus water group only (by $-21 \pm 3\%$, $p = 0.02$). Vascular conductance ($p = 0.26$) and resistance ($p = 0.14$) at rest were not significantly different after 12 weeks of HIIT, and there were no group differences (Table 3.2).

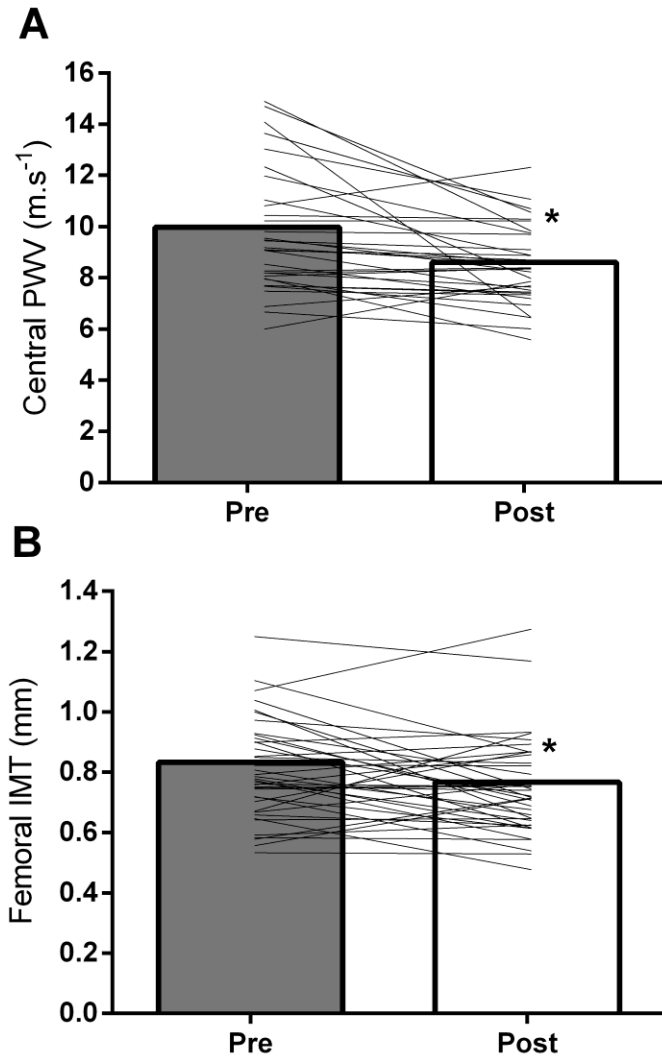


Figure 3.3. Carotid-femoral pulse wave velocity (PWV) (A) and femoral intima-media thickness (IMT) (B) (Pre) and after (Post) 12 weeks of HIIT. Individual data (lines) and group mean (drink groups collapsed) of n=39 A and n=45 B.

Wall thickness of conduit arteries (IMT)

There were no significant changes in carotid IMT following 12 weeks of HIIT (Table 3.2). However, there was a significant reduction in femoral IMT (by -0.07 ± 0.18 mm, main effect of time, $p=0.03$, Figure 3.3) following 12 weeks of HIIT, with no difference between groups (Interaction: $p=0.67$). The likelihood that this change in femoral IMT is clinically beneficial is 38% (95% CI: -0.13 to -0.007 mm).

Vascular Adhesion molecules

No significant changes in soluble circulating ICAM-1 (Pre: 183 ± 66 ng/mL vs. Post: 178 ± 72 ng/mL, main effect of time, $p=0.52$) or VCAM-1 (Pre: 588 ± 199 ng/mL vs. Post: 566 ± 183 ng/mL, main effect of time, $p=0.18$) were seen after 12 weeks of HIIT and there were no differences between groups (data separated by group not shown).

Table 3.2. Femoral artery flow profiles, vascular conductance, carotid intima-media thickness (IMT) and indices of heart rate variability before (Pre) and following (Post) 12 weeks of high-intensity interval training (HIIT) in individuals with type 2 diabetes.

	Pre	Post	Time (p value)	Group X Time (p value)
SFA diameter (cm)	0.67 ± 0.11	0.66 ± 0.09	0.43	0.23
SFA blood flow (mL/min)	168 ± 80	167 ± 75	0.97	0.88
SFA Mean shear rate (s ⁻¹)	81 ± 51	98 ± 52	0.03	0.12
SFA Antegrade shear rate (s ⁻¹)	178 ± 56	190 ± 55	0.03	0.56
SFA Retrograde shear rate (s ⁻¹)	-97 ± 32	-93 ± 28	0.54	0.05
Vascular Conductance (U)	1.7 ± 0.8	1.8 ± 0.8	0.26	0.82
Vascular Resistance (U)	0.71 ± 0.29	0.64 ± 0.25	0.14	0.92
Carotid IMT (mm)	0.84 ± 0.21	0.81 ± 0.16	0.38	0.19
Heart rate Variability				
SDNN (ms)	44 ± 22	49 ± 24	0.32	0.50
RMSSD (ms)	33 ± 22	40 ± 30	0.19	0.45
LF (nu)	75 ± 22	73 ± 24	0.12	0.46
LF (ms ²)	2298 ± 5073	1477 ± 1652	0.53	0.76
HF (nu)	18.3 ± 17.2	22.3 ± 20.0	0.07	0.50
HF (ms ²)	936 ± 2988	408 ± 644	0.27	0.76
LF:HF ratio	7.9 ± 7.2	7.1 ± 6.0	0.54	0.41

Data are shown for all participants with post-exercise nutritional supplement group collapsed. HIIT = High-intensity interval training, SFA = Superficial femoral artery, IMT = intima-media thickness, LF = Low Frequency, HF = High frequency

Discussion

Exercise remains a cornerstone treatment in T2D and CVD (82). This study adds to the growing body of literature showing significant benefits of HIIT for improving health and highlights that HIIT is a promising exercise strategy to improve measures of vascular structure and function (133, 176). The cardiovascular benefits of HIIT were not further augmented by post-exercise milk or protein supplementation. The principal findings of this study were that 12 weeks of HIIT, with or without post-exercise nutritional supplementation: i) Reduced arterial

stiffness, ii) Increased the heart rate range and lowered resting heart rates, and iii) Improved the femoral blood flow profile and reduced intima-media thickness (IMT). The carotid IMT, resting femoral blood flow or vascular adhesion molecules were unchanged. This is the first study, to our knowledge, to show reduced arterial stiffness and femoral IMT after HIIT in participants with T2D. Importantly, because arterial stiffness is implicated in the progression of CVD in T2D, our findings show that HIIT may be an effective means to prevent or delay the progression of CVD.

Impact of HIIT on Arterial Stiffness

The loss of elastic properties of arteries, as with aging and T2D, contributes substantially to increases in blood pressure and the development of CVD (42). Indeed, individuals with T2D have elevated blood pressure and increased arterial stiffness, and have a high incidence of cardiovascular mortality (69, 261). We used carotid-femoral pulse wave velocity (PWV), considered as the gold-standard assessment of arterial stiffness, to demonstrate a clinically meaningful reduction in arterial stiffness (of 1.6 m/s). A 1 m/s reduction in arterial stiffness is associated with a 21% and 29% reduced risk of cardiovascular and all-cause mortality, respectively (130, 156). Guimaraes et al. (109), previously demonstrated a significant 0.54 m/s decrease in carotid-femoral PWV with HIIT in hypertensive individuals who had similar levels of baseline arterial stiffness as the present study. The smaller reduction in carotid-femoral PWV relative to the present study may have been due to the low exercise adherence (just 61%) in Guimaraes et al. (110). Regardless, there was a marked reduction in the 24-h blood pressure with 16 weeks of HIIT (110). In addition others have shown significant improvements in endothelial function (110), thus highlighting the widespread effects of HIIT on several indices of vascular

health. One major factor potentially underlying the favorable effects of HIIT on the vascular function is the episodic increases in blood flow (shear stress and blood pressure) during individual HIIT sessions (126, 187).

Shear rate profiles and intima-media thickness

We have previously shown that an acute session of resistance and cardio-based HIIT improves endothelial-dependent dilation, brachial blood flow and resting shear rate profiles for at least 1 hour after interval-based exercise (104). Herein, we report that 12 weeks of HIIT increases the resting mean shear stress and antegrade shear rate in the femoral artery. Furthermore, there was a significant reduction in the femoral IMT (a marker of subclinical atherosclerosis). This is important because the femoral artery is highly susceptible to the development of atherosclerosis, largely due to reduced flow profiles, especially in those with T2D (84). As such an improved resting shear rate profile (increased antegrade, decreased retrograde shear stress) is known to induce the expression of anti-atherogenic proteins (i.e., eNOS, SOD) and may lower vascular adhesion molecules (86, 173). Limberg et al. (95, 192, 286) demonstrated that a 12 week diet and aerobic exercise intervention improves resting femoral blood flow, antegrade shear rates and vascular adhesion molecules in individuals at risk for the metabolic syndrome. Research by Tinken et al. (159) has shown in humans that increases in antegrade shear stress is an important stimulus for improving endothelial function. Therefore, it is reasonable to suggest that the improved resting shear profiles, lower arterial stiffness and improved endothelial function with HIIT will attenuate the progression of atherosclerosis in T2D. However, we did not see a change in the carotid IMT or plasma adhesion molecules. This may be due to the intervention only being 12 weeks long and largely involving the lower limb

vasculature (although shear rates are known to increase in the upper limbs during exercise (247)). Furthermore, the lack of change in carotid IMT is consistent with other exercise interventions in T2D (198). Of note, the impact of intensive pharmacological treatment in T2D may attenuate exercise-induced cardiovascular adaptations (37, 230). For example, oral antihyperglycemic (i.e., metformin) and lipid lowering drugs (i.e., statins) have been shown to blunt the effects of exercise training on carotid IMT (37, 230). Therefore it is possible that medications used by the T2D participants in the present study (although unchanged during the study) may have inhibited some aspects of the vascular adaptive response to HIIT.

Resting and peak exercise heart rate

Chronotropic incompetence (e.g., an inability to reach peak exercise heart rate) and increased resting heart rate (due to increased sympathetic and/or decreased parasympathetic tone) are two common features associated with T2D (7, 46). Both contribute to exercise intolerance and the elevated CVD risk in T2D (38, 136, 147) but are, unfortunately, often an overlooked complication (36, 82). Following 12 weeks of HIIT, we found a significant decrease in the resting heart rate and a concomitant increase in the heart rate reserve (HRR). Since there was no change in peak exercise heart rate, one can attribute the increased HRR to the reduction in resting heart rate. Interestingly, 38/48 (79%) of the T2D individuals in the present study achieved >85% of age-predicted peak exercise heart rate and 39/48 (81%) achieved >80% of age-predicted HRR, which is higher than previously reported (147). However, it is important to recognize that the participants with T2D in this study completed a 12-lead ECG exercise stress test to screen out the presence of CVD, although chronotropic incompetence was not evaluated. To examine chronotropic competence participants must achieve maximal effort as indicated by a

peak exercise heart rate >90% of predicted, RER >1.1, and/or plateau in oxygen consumption (118, 147); participants in the current study met most of these criteria (see details in results section). The clinical and practical significance of these findings is noteworthy for two reasons as it suggests that a) HIIT may improve autonomic function (broadly indicated by reduced resting heart rate), and b) prescribing HIIT based on % heart rate maximum (from a baseline ramp exercise test) appears reliable across a training intervention whereas HRR is increased over the duration of the HIIT intervention.

Lack of effect of post-exercise nutrition

The maintenance of lean muscle mass is essential for physical functioning and cardiovascular health in older adults with T2D (36). Protein ingestion after exercise is one strategy to enhance the anabolic effect of exercise to attenuate muscle breakdown and increase protein synthesis (200). In this regard, ~20 g of milk-protein after resistance training has been shown to improve body composition changes (50). Additionally, milk-protein supplementation (2 x 28 g/d) alone improves vascular function in hypertensive adults (141). For these reasons we tested the hypothesis that milk-protein supplementation following HIIT would augment cardiovascular adaptations. However, supplementation with ~20 g of protein from fat-free milk or milk-protein concentrate (i.e., same milk proteins without the micronutrients and added vitamins in milk) following each exercise session did not appear to have any additional benefits beyond HIIT alone. It is possible that a greater amount of protein is needed to see effects in participants with T2D due to the presence of insulin resistance (77). Meta-analyses of

prospective cohort studies have reported reduced risk of stroke and cardiovascular disease with an increase in dairy product consumption of two servings per day (211). It is therefore possible that daily supplementation, as opposed to only after exercise three times per week, is required to see any effects. To determine whether greater amounts of protein supplementation are needed to see an additive effect, future research should undertake studies with added protein on non-exercise days and/or provide greater amounts of protein across several hours of recovery following exercise (74, 208).

Clinical implications and future research

Arterial stiffness and the development of atherosclerosis are hallmark features of T2D, and contribute to the increased cardiovascular mortality (50). Accordingly, interventions that attenuate the progression of atherosclerosis and improve cardiovascular risk factors, without significant side effects or financial burden, are of clinical importance. To date, intensive pharmacotherapy (80, 279) and moderate-continuous exercise (254) appear largely unsuccessful in reducing macrovascular outcomes in T2D. Regardless, the findings of this investigation demonstrate that HIIT can induce clinically meaningful reductions in arterial stiffness, femoral IMT and resting heart rate after just 12 weeks. Thus, HIIT may provide a potent stimulus for promoting cardiovascular adaptations in T2D (273) and holds potential for attenuating atherosclerosis and the development of cardiovascular disease in T2D. Larger randomized controlled trials are warranted to confirm the clinical cardiovascular benefits of HIIT in T2D.

**Chapter 4: Resistance-Based Interval Exercise Acutely Improves
Endothelial Function In Type 2 Diabetes**

The benefits of regular exercise are far more pervasive than the effect on traditional cardiovascular risk factors alone; improvements in endothelial function may explain a large proportion of the risk reduction (19, 41). The endothelium plays a pivotal role regulating the many factors that determine vascular tone, tissue perfusion, coagulation and inflammation (142). Endothelial dysfunction is an early manifestation in many chronic diseases, including diabetes (64), and contributes to the ~2-4 fold greater risk of cardiovascular disease in T2D (114). Exercise interventions involving aerobic and resistance exercise can improve endothelial function (114), a response largely mediated by acute elevations in blood flow and laminar shear stress during individual exercise bouts (168, 221). The effect of an acute bout of cardio- or resistance-based exercise, performed in an interval pattern, on the endothelium of adults with T2D has not been investigated. It is known that different exercise modes and intensities modify the shear stress stimulus and may result in distinct responses in endothelial function (247) but the impact of exercise mode, in addition to T2D or training status is unclear.

There is continued widespread interest in high-intensity interval exercise (HIIT) because it has been shown to improve cardiometabolic health with relatively minimal time-commitment (240, 247) (93). HIIT alternates high and low intensity exercise periods, often in a 1:1 work:rest ratio (26, 269). This pattern of exercise may be attractive and makes vigorous exercise attainable for most individuals because it incorporates built in rest/recovery periods (85, 269). A single session of HIIT has been shown to improve endothelial function in coronary artery disease patients (aged ~66 y) (85) and lower 24-h glucose in T2D (60). Resistance exercise may be more effective than cardio for improving vascular function and remodeling (92), although this is not a universal finding (195, 221, 262). Resistance and cardio exercise can be effectively performed as HIIT; for example, in insulin resistant individuals combined resistance- and cardio-based interval

exercise was just as effective as cardio-based HIIT for improving glucose control (188). It is possible that the addition of resistance exercise to the oscillatory pattern of high- and low-intensity HIIT exercise may offer a prophylactic effect on the vasculature (83). Despite this, no study has investigated the effects of leg resistance HIIT alone and most of the literature has investigated the endothelial responses after cardio-based continuous exercise [reviewed in: (275)].

In addition to exercise parameters, inconsistent findings surrounding acute exercise and endothelial function [reviewed in: (63)] may be due to vascular risk factors (e.g., T2D) and/or training status. For example, Hallmark et al. (63) found that while high-intensity exercise improved endothelial function in lean adults, there was no effect in obese adults (117). Similarly, in inactive overweight men endothelial function was decreased after exercise, independent of exercise intensity, compared to an increase in active overweight men (117). These studies suggest that presence of vascular risk factors and/or habitual activity levels may modulate the impact of acute exercise on endothelial function.

Given the clinical and functional importance of changes in endothelial function, we sought to examine the effect of two common exercise modes performed as HIIT in age matched T2D, untrained, and highly-trained normoglycemic adults. The primary purpose was to examine the effects of cardio- and resistance-HIIT on endothelial function measured by flow-mediated dilation. The secondary aim was to examine the influence of HIIT mode on shear stress, blood flow and blood pressure. We tested the hypothesis that both acute cardio- and resistance-HIIT would lead to improvements in endothelial function compared to a time-matched control.

Methods

Study overview and pre-screening

A randomized crossover design was used to compare the vascular response to cardio-HIIT (C-HIIT) and resistance-HIIT (R-HIIT) relative to a time-matched control condition (CTL) in age-matched T2D, normoglycemic adults who met current physical activity guidelines but were not participating in a structured exercise training program (UN-NG), and highly-trained normoglycemic adults (TR-NG). The study protocol was approved by the University of British Columbia Clinical Research Ethics Board and all participants provided written informed consent. Prior to participation T2D participants were screened using a 12-lead ECG exercise stress test and cleared for vigorous exercise by a cardiologist. All participants then completed a maximal exercise test on a cycle ergometer to determine cardiorespiratory fitness ($\dot{V}O_{2 \text{ peak}}$). The T2D patients had been familiarized with six sessions of exercise (two R-HIIT and four C-HIIT sessions involving 4-6 X 1-min intervals at a rating of perceived exertion [RPE] corresponding to ~5 on the CR-10 scale (121)) across two weeks in order to introduce them to HIIT and build up to the exercise protocols for testing days. Baseline investigations were performed after 48 h of rest from a previous exercise session to avoid the acute effects of exercise on baseline values. UN-NG and TR-NG maintained their typical physical activity habits throughout the study but similar to T2D participants refrained from exercise for 48 h prior to testing sessions. UN-NG and TR-NG were screened using a Physical Activity Readiness Questionnaire-Plus (PAR-Q+) and a health-screening questionnaire that included a Godin Leisure Time Physical Activity Questionnaire. TR-NG were defined by completing >7 hours of endurance training per week and were in the >80th percentile for age- and gender-adjusted $\dot{V}O_{2 \text{ peak}}$ based on data from the NHANES and Aerobics Centre Longitudinal Study (33) (range 37-63 mL/kg/min). UN-NG self-

reported performing 213 ± 145 min/wk of light and/or 115 ± 145 min/wk of moderate physical activity (29, 39, 183) and had a $\dot{V}O_{2\text{ peak}}$ in the 20-50th percentile (range 20-35 mL/kg/min).

Participants

Thirty-five participants (40% male, 60% female, average age 56 ± 7 y, range 40-66 y) volunteered to participate and completed two initial and three experimental testing sessions. Baseline characteristics of participants in the three groups are shown in Table 4.1. All participants were non-smoking and were instructed to replicate any vitamin or supplement intake exactly prior to each experimental session (verified by food records and interviews). T2D participants were on stable medications and were physician diagnosed for at least six months (range 2-17 y) prior to the study, were well controlled ($HbA_{1c} < 8.0\%$) and not on exogenous insulin. In addition, exclusion criteria included diagnosed diabetic neuropathy, chronic kidney disease, heart and coronary artery disease and any other contraindication to vigorous exercise. T2D participants on oral hypoglycemic medications followed normal prescriptions, which were replicated exactly for all experimental sessions. Diabetes medications included; Metformin only (n=9), DPP4 inhibitor only (n=1), SGLT2 inhibitor+GLP-1 agonist (n=1), Sulfonylurea+GLP-1 agonist (n=1). Hypertensive medications included; Ace-inhibitor (n=7), Angiotensin receptor blocker (n=2), calcium channel blocker (n=1). All non-T2D participants were free from any diagnosed chronic disease and not taking medications, except one participant in the UN-NG group who was taking 5 mg of felodipine (calcium channel blocker) daily for hereditary elevated blood pressure. All females were postmenopausal (no menstruation for >12 mo), except for two females in the TR-NG group.

Table 4.1. Baseline characteristics of type 2 diabetes (T2D), untrained normoglycemic (UN-NG) and trained normoglycemic (TR-NG) adults.

	T2D	UN-NG	TR-NG
n=	12 (6 males)	12 (6 males)	11 (7 males)
Age (y)	57.5 ± 5.0	55.3 ± 9.1	55.1 ± 7.0
BMI (kg/m²)	35 ± 7	26 ± 5	23 ± 3*
Body fat (%)	32.4 ± 7.5	23.9 ± 4.2*	15.8 ± 5.9*†
$\dot{V}O_{2\text{ peak}}$ (mL/kg/min)	19 ± 4†	29 ± 6*	45 ± 7*†
HR_{peak} (bpm)	161 ± 12	160 ± 20	170 ± 9

Values are mean ± SD. * p < 0.05 vs. T2D. † p < 0.05 vs. UN-NG.

Experimental protocol (Figure 4.1)

Pre testing

Height and weight were measured using a stadiometer and balance beam scale (Seca 700, Hamburg, Deutschland) and body composition assessed by DXA (Hologic Discovery DXA, MA, USA). A maximal incremental exercise test (increasing 1W every 4 s) to volitional exhaustion was performed on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to determine maximal oxygen uptake ($\dot{V}O_{2\text{ peak}}$), heart rate (HR_{peak}), and power output (W_{peak}). The test began at 30 W for T2D and UN-NG participants and 100 W for TR-NG participants.

Experimental trials

Participants completed three, 3-h experimental trials in a randomized order with at least 48 h recovery between (Figure 4.1). Exercise was controlled for 48 h prior to each trial, which began at either 1100 or 1600 (same time within participants) 4 h after consumption of a standardized meal. No food or drink other than water was consumed throughout the trial.

Physiological measures were taken at baseline, immediately (within 5 min), 1 and 2 h after exercise/sitting-control. Between measurements participants remained in the lab in a resting seated position. Baseline measurements for each experimental trial were taken after 15 min of supine rest. All measurements were performed in a temperature controlled, quiet and dimly lit room.

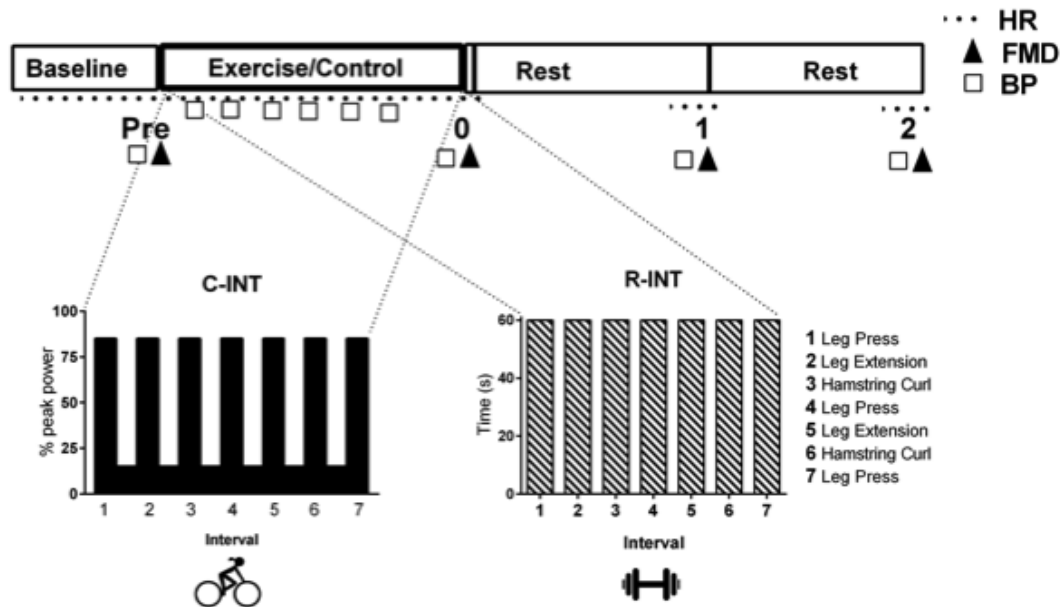


Figure 4.1. Schematic illustrating the timeline of the experimental trials; including a figure illustrating the cardio-based (C-HIIT) and resistance-based (R-HIIT) interval exercise protocols which were performed in a random order with a sitting-control condition (CTL). Flow-mediated dilation and blood pressure were measured before (Pre), immediately (0), 1 and 2 hours after each experimental trial.

Cardio-based interval exercise (C-HIIT)

All participants completed 7 X 1-min intervals on the aforementioned cycle ergometer at 85% W_{peak} , alternated with 1-min recovery at 15% W_{peak} (Figure 4.1). Participants were instructed to increase their cadence to between 80-100 revolutions per minute (rpm) during the

vigorous intervals. Heart rate (continuous 12-lead ECG), manual blood pressure (obtained in last 30-s of alternate work and rest intervals) and RPE (250) were recorded at the end of each interval.

Resistance-based interval exercise (R-HIIT)

All participants completed 7 X 1-min intervals of leg resistance exercise with 1-min recovery; with matched duration, pattern and muscle groups as C-HIIT (Figure 4.1). Familiarization for the three leg resistance exercises involved one set of 6-8 repetitions, the weight was selected out of three levels with the goal to complete each exercise for 1-min with an RPE of ~5 ('hard'). For each 1-min 'hard' interval participants completed as many reps as possible of each exercise, alternated with 1-min recovery where participants walked to the next exercise station. Resistance level, repetitions, heart rate, blood pressure and RPE were recorded for each interval. This R-HIIT protocol was designed to target the same major muscle groups in a similar 1-min on:off pattern as C-HIIT, while eliciting a similar RPE (Table 4.2). Blood pressure (manual BP in last 30-s of each 1-min interval), heart rate (Polar H1, Kempele, Finland), and RPE were recorded in the last 10-s of each interval. Both exercise protocols began with a 3 min warm-up and ended with a 3 min cool-down performed on a cycle ergometer at a self-selected pace (rpm) at 30-50 W.

Control condition (CTL)

In the control condition participants sat upright for 20 minutes in place of the exercise time. Everything else including activity between the measurements and the timing thereof was the same as the exercise trials (Figure 4.1).

Physiological Measures

Flow-mediated dilation (FMD)

Brachial artery FMD was examined as an index of endothelial function using high-resolution ultrasound (Terason 3200) as per published guidelines (33). Briefly, the right arm of each participant was extended 80° from the torso and a longitudinal image of the artery was obtained 2-3 cm from the antecubital fossa. A rapid inflation and deflation cuff was positioned on the forearm 1-2 cm distal from the olecranon process. Once the image was optimized in B-mode, simultaneous B-mode image and Doppler velocity measurements (insonation angle maintained at 60°) were obtained. Ultrasound data was recorded for a 1-min baseline, 30 s before cuff deflation and continued for 3 min thereafter. The cuff was inflated to >60 mmHg above systolic blood pressure for 5-min to induce forearm ischemia and the subsequent hyperemic stimulus. Probe placement and ultrasound settings were maintained for each participant across each experimental trial. Heart rate (single-lead ECG) and brachial blood pressure (manual sphygmomanometer) were measured before each FMD measurement (Figure 4.1). Mean arterial blood pressure (MAP) was calculated as $1/3 \times \text{systolic blood pressure (SBP)} + 2/3 \times \text{diastolic blood pressure (DBP)}$.

Brachial artery diameter and blood flow analysis

Analyses of brachial artery diameter and blood velocity measures were performed using edge detection software, which reduces user bias and increases accuracy (57, 241). Blood flow ($\text{mL} \cdot \text{min}^{-1}$) was calculated from the product of cross-sectional area and Doppler velocity

$((\text{velocity} \cdot \pi \cdot (\text{diameter}^2/4) \cdot 60)$ and shear rate (s^{-1}) was calculated as (four times velocity/diameter) from synchronized diameter and velocity recordings (101, 276). The shear rate area under the curve (SRAUC) for the hyperemic stimulus was calculated from simultaneous diameter and velocity data from cuff release to peak arterial dilation. Baseline antegrade and retrograde shear rates (s^{-1}) were calculated from antegrade and retrograde mean blood velocities (four times mean baseline antegrade or retrograde velocity \div mean baseline diameter). Vascular conductance ($\text{mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) was calculated as the ratio of mean blood flow to mean arterial pressure. The coefficients of variation of brachial artery diameter and %FMD were 2.1% and 7.3%, respectively, based on baseline measurements pre-exercise between experimental trials. FMD is expressed as the absolute change in artery diameter (absolute FMD = $\text{postocclusion}_{\text{peak diameter}} - \text{preocclusion}_{\text{mean diameter}}$), the percent change in artery diameter from baseline ($\% \text{FMD} = 100 \cdot (\text{absolute FMD} / \text{preocclusion}_{\text{mean diameter}})$), and to adjust for the potential confounder of baseline diameter (D_{base}) allometric scaling was used ($D_{\text{base}} - \text{adjusted FMD}$) (101).

Statistics

Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, Illinois). One-way ANOVA was used to examine baseline differences between groups. A 3-factor (Group X Condition X Time) ANOVA with repeated measures on condition and time were used to assess significant differences between groups and conditions across time. Post-hoc analyses with Bonferonni corrections were used to evaluate significant interactions and main effects (using $p < 0.05$). Specifically, significant Group X Condition X Time interactions or Condition X Time interactions were probed for differences within groups between R-HIIT and C-HIIT, relative to CTL, at each time point. All data were first tested for normality and are reported as mean and

standard deviation (SD). For the primary outcome of %FMD, and for MAP, magnitude-based inference analyses were performed according to contemporary views on statistical reporting, allowing for clinically meaningful inference (14, 241). For this, the spreadsheet for confidence limits and inferences was downloaded from www.newstats.org. The smallest clinically beneficial threshold for %FMD was +1%, based on a recent meta-analysis which showed a 13% reduced risk of future cardiovascular events for every 1% improvement in %FMD (95% CI: 9% to 17%) (21). In line with previous studies, a 2 mm Hg reduction in MAP was considered to be the smallest clinical threshold change for blood pressure (132).

Results

Characteristics of C-HIIT and R-HIIT exercise sessions

Participants successfully completed both the C-HIIT and R-HIIT protocols with no reports of discomfort or excessive changes in blood pressure. All participants completed 7 X 1-min intervals; however, for C-HIIT two T2D participants and one UN-NG participant reduced their workload by 10 W for the final two or three 1-min intervals because their RPE was >8 and HR was >95% of maximum. Analyses performed with and without the two non-postmenopausal women were not significantly different and did not change the interpretation of the results. Peak heart rate during the C-HIIT intervals was higher than R-HIIT ($p=0.01$), with no difference between groups (Table 4.2). Diastolic blood pressure was significantly higher during R-HIIT compared to C-HIIT ($p<0.01$) and in T2D participants compared to UN-NG and TR-NG ($p<0.01$, Table 4.2). Systolic blood pressure did not significantly differ between C-HIIT and R-HIIT exercise protocols or between groups (Table 4.2).

Table 4.2. Blood pressure, heart rate and RPE during the high-intensity intervals for cardio and resistance HIIT (R-HIIT) for T2D, UN-NG and TR-NG participants.

Variables	C-HIIT			R-HIIT		
	T2D	UN-NG	UN-NG	T2D	UN-NG	TR-NG
Rating of perceived exertion	5 ± 1	5 ± 2	5 ± 1	5 ± 2	5 ± 2	5 ± 1
% of HR_{peak}	88 ± 6 †	90 ± 6 †	87 ± 6 †	67 ± 7	70 ± 10	64 ± 8
Systolic Blood Pressure (mmHg)	192 ± 15	177 ± 18	174 ± 16	196 ± 18	178 ± 25	191 ± 24
Diastolic Blood Pressure (mmHg)	87 ± 6	79 ± 3*	77 ± 8*	95 ± 6†	87 ± 6†	90 ± 6†

Values are mean ± SD. T2D = Type 2 diabetes, CTL = Control, C-HIIT = cardio-based interval exercise, R-HIIT = Resistance-based interval exercise RPE= rate of perceived exertion, * p < 0.05 vs. T2D. † p < 0.05 vs. C-HIIT.

Brachial artery %FMD

There was a significant Group X Condition X Time interaction for %FMD (Figure 4.2, p<0.01). No change in %FMD was seen across time in CTL nor was it significantly different at baseline between trials within-individuals. TR-NG had a higher baseline %FMD (average of three pre-measures) than UN-NG (7.8 ± 2.2% vs. 6.6 ± 2.3%, p=0.03) and T2D (5.7 ± 1.6%, p=0.01), with no difference between T2D and UN-NG (p=0.32). When adjusted for baseline diameter using allometric scaling (D_{base}-adjusted FMD) there was a significant difference between groups at baseline (TR-NG: 7.7 ± 2.2% vs. UN-NG: 6.6 ± 2.5% vs. T2D: 5.3 ± 1.4%, all p<0.05).

T2D: Post-hoc and inferential analyses indicated that in T2D %FMD was significantly higher immediately (95% Confidence Interval: 3.0 to 5.9%), 1 h (CI: 0.8 to 4.2%), and 2 h (CI: 0.7 to 3.1%) after R-HIIT compared to CTL; the probability that these effects were most likely beneficial/negligible/harmful were 100/0/0%, 96/4/0% and 94/6/0%, respectively. After C-HIIT

compared to CTL, %FMD in T2D was unchanged immediately (CI: -0.5 to 3.1%), higher at 1 h (CI: 0.2 to 3.0%) and unchanged 2 h (CI: -4.5 to 4.3%) following exercise; probability of beneficial/negligible/harmful were 64/35/1%, 81/19/0%, and 30/37/33%, respectively.

UN-NG: %FMD after R-HIIT in UN-NG was unchanged immediately (CI: -5.1 to 4.5%) and 1 h (CI: 0.3 to 2.8%), and higher 2 h following exercise (CI: 0.38 to 5.5%) compared to CTL; probability of beneficial/negligible/harmful were 28/34/38%, 64/35/0.4% and 94/6.0/0.3%, respectively. After C-HIIT compared to CTL %FMD in UN-NG was unchanged immediately (CI: -0.08 to 0.10%), 1 h (CI: -0.6 to 3.2%) and 2 h (CI: -0.06 to 0.02%) following exercise; probability of beneficial/negligible/harmful were 0/100/0%, 63/36/1% and 0/100/0%, respectively.

TR-NG: %FMD after R-HIIT in TR-NG was unchanged immediately (CI: -0.48 to 0.12%), but higher 1 h (CI: 0.36 to 2.0%) and 2 h following (CI: 1.2 to 2.8%) compared to CTL; probability of beneficial/negligible/harmful were 0/100/0%, 68/32/0% and 99/1/0%, respectively. After C-HIIT compared to CTL %FMD in TR-NG was unchanged immediately (CI: -0.3 to 3.6%), and 1 h (CI: -0.4 to 3.6%) and higher 2 h (CI: 1.4 to 3.4%) following; probability of beneficial/negligible/harmful were 74/25/1%, 74/25/1% and 99/1/0%, respectively.

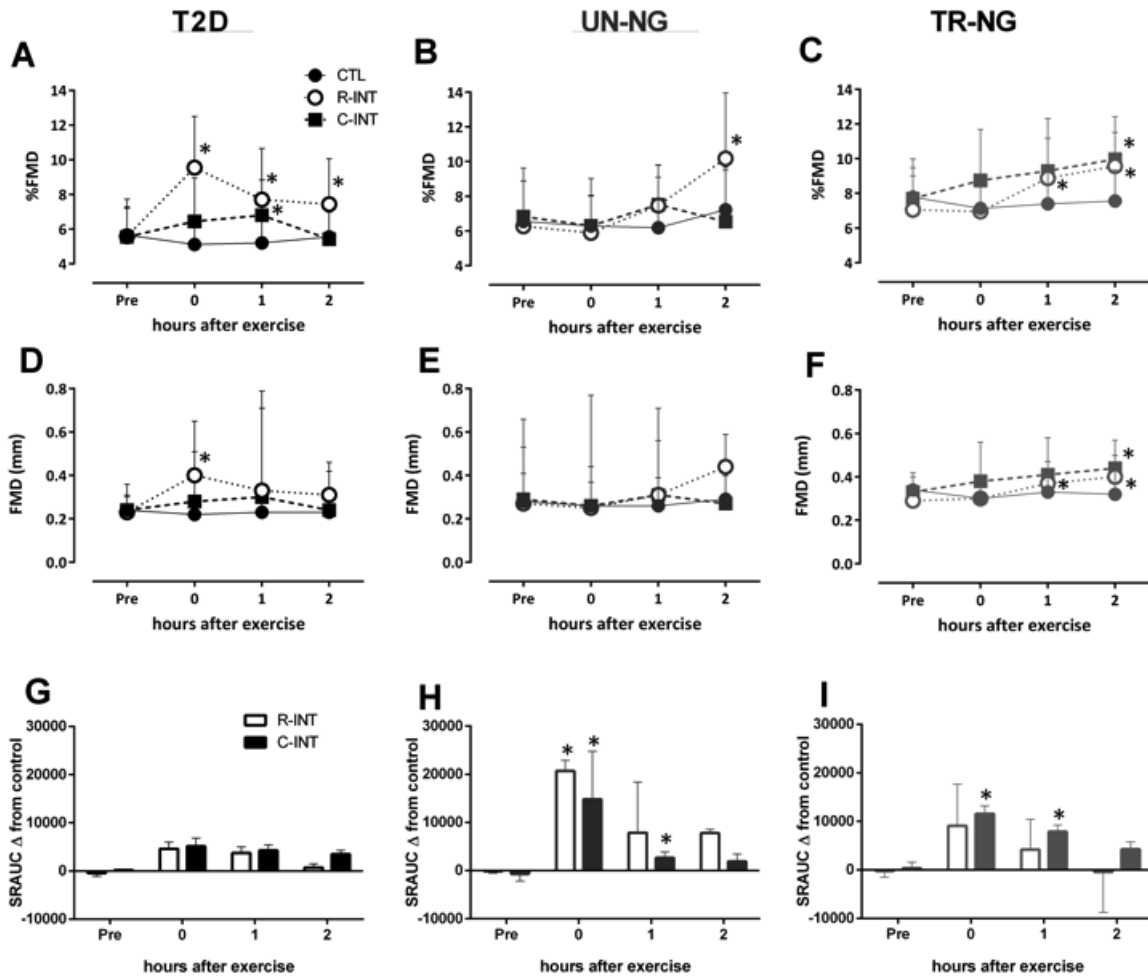


Figure 4.2. %FMD, absolute FMD (mm) and shear rate AUC before, immediately 1 h and 2 h (mean and SD) after control (CTL), resistance-HIIT and cardio-HIIT in type 2 diabetes (T2D: A, D, G), untrained (UT-NG: B, E, H) and trained (TR-NG: C, F, I) normoglycemic participants. * $p < 0.05$ compared to CTL.

Absolute FMD (mm), D_{base} – adjusted FMD and Shear rate AUC

There was a Condition X Group interaction (Figure 4.2, $p=0.05$) for absolute FMD (mm). Post-hoc analyses indicated that in T2D absolute FMD was higher immediately after R-HIIT compared to CTL ($p=0.03$). In TR-NG participants absolute FMD was higher 1 h ($p=0.02$) and 2 h ($p=0.01$) following R-HIIT compared to CTL, and higher 2 h ($p=0.01$) after C-HIIT compared to CTL. There was no change in absolute FMD in UN-NG participants (Figure 4.2). There was a significant Group X Condition interaction for D_{base} – adjusted FMD (**Table 4.3**, $p=0.03$). In T2D D_{base} – adjusted FMD was higher immediately ($p=0.05$) and 1 h ($p=0.01$) after R-HIIT compared to CTL, and higher 1 h ($p=0.01$) after R-HIIT compared to C-HIIT. In UN-NG and TR-NG participants there were no significant differences for R-HIIT compared to CTL, or C-HIIT compared to CTL, for D_{base} – adjusted FMD at any time point (**Table 4.3**). In UN-NG D_{base} – adjusted FMD was higher after R-HIIT than C-HIIT immediately post-exercise ($p=0.05$). Time to peak diameter was not significantly different between conditions or groups (data not shown). There were significant Condition X Time ($p<0.01$) and Condition X Group interactions ($p=0.04$) for the hyperemia induced shear rate area under the curve (SRAUC). SRAUC did not change in the CTL condition and was not different pre-exercise between groups or visits. Post-hoc analyses indicate significantly higher SRAUC immediately and 1 h after C-HIIT and immediately after R-HIIT compared to CTL in UN-NG and TR-NG participants (Figure 4.2, all $p<0.05$) but no significant changes in SRAUC were seen comparing CTL, C-HIIT or R-HIIT at any time point in T2D participants (Figure 4.2).

Blood flow and shear rate

There were Condition X Time interactions (**Table 4.3**, $p<0.05$) for baseline blood flow

and baseline shear rate (Figure 4.3, $p < 0.05$). Post-hoc analyses indicate in T2D and TR-NG participants baseline shear rate was significantly higher immediately after C-HIIT ($p < 0.05$) and R-HIIT ($p < 0.05$), compared to CTL. There was a significant Condition X Time interaction ($p = 0.05$) for antegrade shear rate. Post-hoc analyses indicate antegrade shear rate was higher in UN-NG 1 h after C-HIIT compared to CTL ($p = 0.047$). In TR-NG participants antegrade shear rate was higher immediately after R-HIIT ($p < 0.05$) and C-HIIT ($p = 0.02$), compared to CTL. There was a significant Condition X Time X Group ($p = 0.048$) interaction for retrograde shear rate. Post-hoc analyses indicated a significantly lower retrograde flow after R-HIIT ($p = 0.05$) compared to CTL in UN-NG participants.

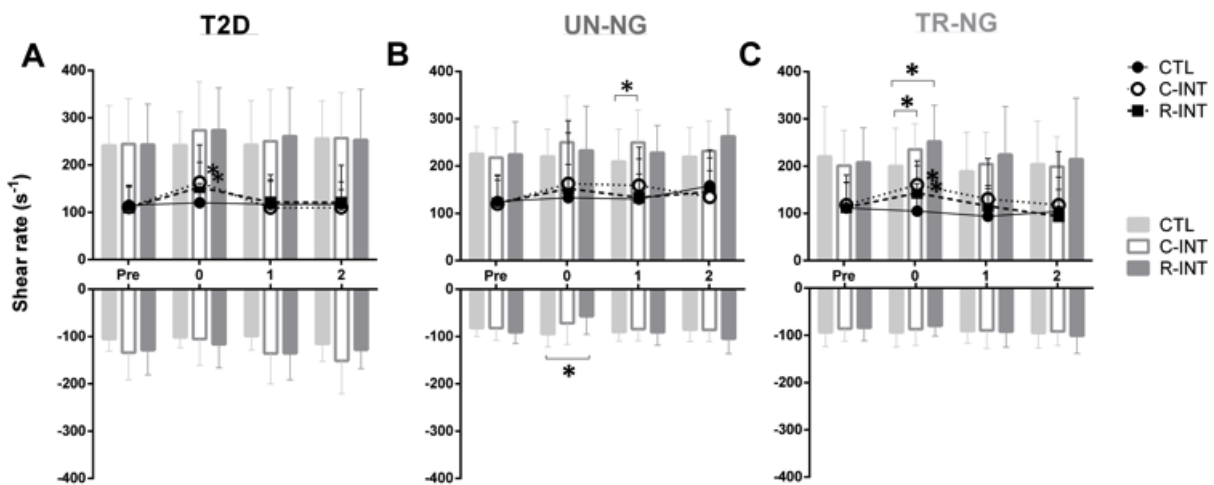


Figure 4.3. Baseline mean (lines), antegrade and retrograde shear rate (s⁻¹; bars) before, immediately, 1 h and 2 h after control (CTL), C-HIIT and R-HIIT for T2D (A), UN-NG (B) and TR-NG (C) participants. * $p < 0.05$ compared to CTL.

Blood Pressure and Vascular Conductance

There was a significant Condition X Time interaction (Figure 4.4, $p < 0.01$) for Mean Arterial Blood Pressure (MAP).

T2D: Post-hoc and inferential analyses indicated that, in T2D participants, MAP after R-HIIT was unchanged immediately (CI: -5.6 to 0.57 mmHg), lower at 1 h (CI: -6.2 to -0.51 mmHg), and 2 h (CI: -5.8 to -0.03 mmHg) following exercise compared to CTL; the probability that these effects were most likely beneficial/negligible/harmful were 64/36/0.4%, 84/16/0% and 75/25/0%, respectively. After C-HIIT, MAP in T2D was unchanged immediately (CI: -5.7 to 0.5 mmHg), 1 h (-5.0 to 0.7 mmHg) and 2 h (CI: -3.9 to 0.5 mmHg) following compared to CTL; probability of beneficial/negligible/harmful were 66/36/0%, 55/45/0% and 39/61/0% respectively.

UN-NG: MAP after R-HIIT in UN-NG was unchanged immediately (CI: -6.7 to 3.7 mmHg), lower at 1 h (CI: -10 to 0.2 mmHg) and unchanged 2 h (CI: -8.5 to 2.3 mmHg) following compared to CTL; probability of beneficial/negligible/harmful were 42/50/8%, 89/11/0% and 70/30/3%, respectively. After C-HIIT exercise compared to CTL MAP in UN-NG was lower immediately (CI: -12 to -1 mmHg), 1 h (CI: -9.9 to -1.9 mmHg) and 2 h (CI: -9.4 to -1.4 mmHg) following; probability of beneficial/negligible/harmful were 95/5/0%, 97/3/0% and 96/4/0%, respectively.

TR-NG: MAP after R-HIIT in TR-NG was unchanged immediately (CI: -11 to 6.5 mmHg), 1 h (CI: -11 to 5.5 mmHg) and 2 h (CI: -13 to 8.3 mmHg) following compared to CTL; probability of beneficial/negligible/harmful were 54/31/15%, 56/32/12% and 51/29/20%, respectively. After C-HIIT compared to CTL, MAP in TR-NG was unchanged immediately (CI: -3.7 to 0.3 mmHg), 1 h (CI: -4.1 to 0.7 mmHg) and 2 h (CI: -1.7 to 0.2 mmHg) following;

probability of beneficial/negligible/harmful were 38/63/0%, 40/60/0% and 1/99/0%, respectively.

There were significant Condition X Time interactions for both SBP ($P < 0.01$) and DBP ($p = 0.01$; **Table 4.3**). There was a significant Condition X Time interaction (Figure 4.4, $p = 0.05$) for Vascular Conductance (VC). Post-hoc analyses indicate in T2D and TR-NG participants VC was higher immediately after R-HIIT and C-HIIT (all $p < 0.03$) compared to CTL. In UN-NG participants VC was higher 1 h ($p = 0.03$) and 2 h ($p = 0.04$) after C-HIIT compared to CTL.

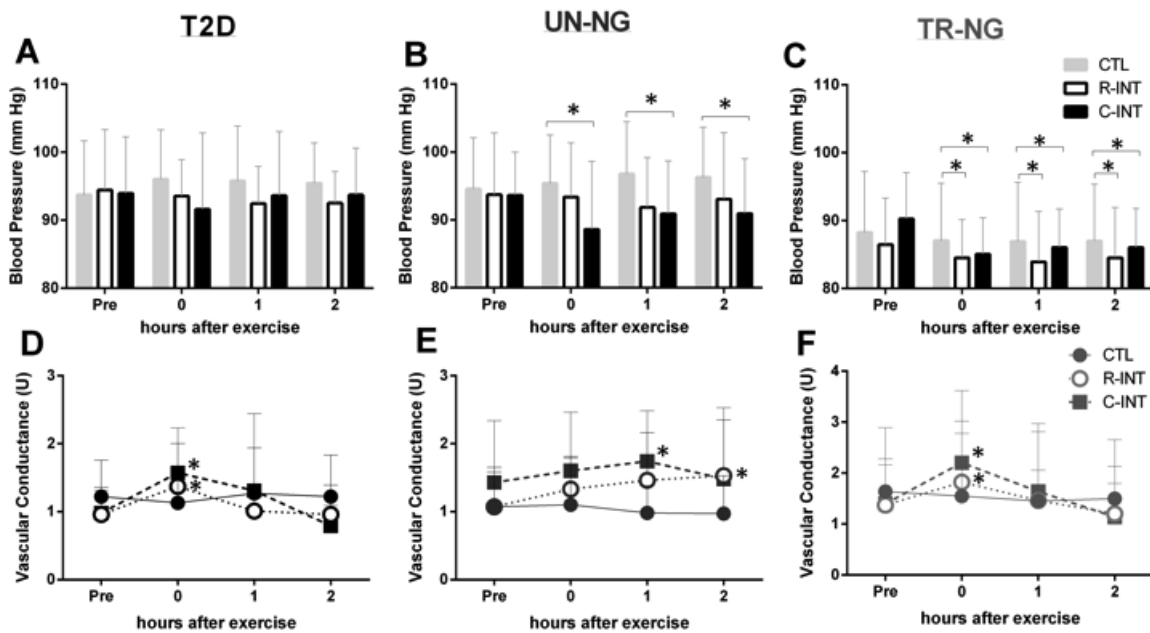


Figure 4.4. Mean arterial blood pressure (MAP) and vascular conductance before, immediately, 1 h and 2 h after control, C-HIIT and R-HIIT in T2D (A, D), age-matched UN-NG (B, E) and TR-NG (C, F) participants. * $p < 0.05$ compared to CTL.

Table 4.3. Flow-mediated dilation and hemodynamic responses across time during the sitting control (CTL), acute cardio-based and resistance-based HIIT conditions in T2D, age-matched untrained (UN-NG) and trained normoglycemic (TR-NG) participants.

T2D	CTL				C-HIIT				R-HIIT			
	Baseline	Immed-ex	1 hour	2 hour	Baseline	Immed-ex	1 hour	2 hour	Baseline	Immed-ex	1 hour	2 hour
Baseline diameter (mm)	4.3 ± 0.9	4.3 ± 0.9	4.3 ± 1.0	4.3 ± 0.9	4.4 ± 0.9	4.4 ± 1.0	4.4 ± 1.0	4.3 ± 1.0	4.3 ± 0.8	4.2 ± 0.9	4.3 ± 0.9	4.2 ± 1.0
Peak Diameter (mm)	4.5 ± 0.9	4.6 ± 1.0	4.6 ± 1.0	4.5 ± 0.9	4.6 ± 0.9	4.7 ± 1.0	4.7 ± 1.0	4.5 ± 1.0	4.5 ± 0.8	4.6 ± 1.0	4.6 ± 1.0	4.5 ± 1.0
D_{base}-adjusted FMD	5.7 ± 1.6	5.1 ± 1.6	5.2 ± 1.3	5.6 ± 1.4	6.0 ± 2.2	7.1 ± 5.6	4.7 ± 6.2	6.8 ± 3.1	5.0 ± 1.6	8.6 ± 5.8*	9.9 ± 9.1*†	5.8 ± 5.1
Blood flow (mL.min⁻¹)	117 ± 55	109 ± 47	124 ± 65	117 ± 58	93 ± 26	148 ± 61*	130 ± 23	74 ± 28	93 ± 38	130 ± 62*	96 ± 36	91 ± 42
Systolic BP (mmHg)	124 ± 11	126 ± 12	128 ± 12	127 ± 11	128 ± 13	124 ± 21*	124 ± 20*	124 ± 11*	125 ± 12	125 ± 12	123 ± 9*	122 ± 7*
Diastolic BP (mmHg)	79 ± 8	81 ± 6	80 ± 7	80 ± 5	77 ± 8	76 ± 8	79 ± 6	79 ± 6	79 ± 9	78 ± 5	77 ± 5	78 ± 4
UN-NG												
Baseline diameter (mm)	4.2 ± 0.8	4.1 ± 0.8	4.1 ± 0.7	4.1 ± 0.9	4.3 ± 1.0	4.1 ± 0.9	4.2 ± 1.0	4.1 ± 0.8	4.4 ± 0.8	4.4 ± 0.8	4.4 ± 0.9	4.4 ± 0.9
Peak Diameter (mm)	4.5 ± 0.9	4.4 ± 0.9	4.4 ± 0.8	4.3 ± 0.9	4.6 ± 0.9	4.4 ± 0.9	4.5 ± 0.9	4.4 ± 0.9	4.5 ± 1.2	4.5 ± 1.0	4.6 ± 1.1	4.7 ± 0.9
D_{base}-adjusted FMD	6.5 ± 2.0	6.8 ± 3.1	6.2 ± 3.0	7.1 ± 2.2	6.0 ± 4.1	6.3 ± 3.6	6.0 ± 4.9	6.5 ± 3.1	7.5 ± 5.1	9.6 ± 6.2†	8.9 ± 5.1	8.8 ± 6.5
Blood flow (mL.min⁻¹)	102 ± 53	104 ± 65.57	94 ± 41	93 ± 56	134 ± 95	141 ± 76	160 ± 73*	138 ± 102*	100 ± 58	122 ± 44	134 ± 72	142 ± 83
Systolic BP (mmHg)	122 ± 12	124 ± 12.00	125 ± 13	125 ± 12	123 ± 10	118 ± 15	120 ± 11*	122 ± 13	123 ± 14	123 ± 15	119 ± 12*	122 ± 16
Diastolic BP (mmHg)	81 ± 7	81 ± 6	83 ± 7	82 ± 6	79 ± 5	74 ± 9*	76 ± 7*	76 ± 7*	79 ± 8	79 ± 6	78 ± 5	79 ± 8
TR-NG												
Baseline diameter (mm)	4.4 ± 0.8	4.3 ± 0.8	4.4 ± 0.9	4.3 ± 0.8	4.2 ± 0.4	4.3 ± 0.9	4.4 ± 0.8	4.3 ± 0.8	4.4 ± 0.8	4.4 ± 0.8	4.4 ± 0.9	4.4 ± 0.9
Peak Diameter (mm)	4.7 ± 0.8	4.6 ± 1.0	4.7 ± 0.9	4.7 ± 0.9	4.7 ± 0.8	4.8 ± 0.9	4.8 ± 0.9	4.8 ± 1.0	4.5 ± 0.8	4.6 ± 0.9	4.7 ± 0.9	4.7 ± 0.9
D_{base}-adjusted FMD	8.4 ± 2.0	7.7 ± 1.9	7.5 ± 2.1	7.3 ± 2.7	8.3 ± 2.0	9.2 ± 4.1	9.5 ± 2.6	10.4 ± 2.7	7.5 ± 1.9	7.1 ± 1.7	8.8 ± 2.1	9.3 ± 1.8
Blood flow (mL.min⁻¹)	144 ± 109	133 ± 104	139 ± 113	128 ± 98	127 ± 86	186 ± 122*	128 ± 84	100 ± 53	116 ± 64	153 ± 95*	129 ± 55	94 ± 74
Systolic BP (mmHg)	116 ± 9	114 ± 11	104 ± 34	105 ± 34	117 ± 10	109 ± 9*	101 ± 33*	101 ± 33*	113 ± 8	111 ± 6	99 ± 32*	100 ± 32*
Diastolic BP (mmHg)	74 ± 9	74 ± 8	68 ± 22	68 ± 22	77 ± 6	74 ± 6	68 ± 22	68 ± 22	74 ± 6	71 ± 7	66 ± 22	67 ± 22

Values are mean ± SD. T2D = Type 2 diabetes, CTL = sitting-control, C-HIIT = cardio-based interval exercise, R-HIIT = Resistance-based interval exercise, Immed-ex = immediately after exercise/control, FMD = Flow-mediated dilation, D_{base}-adjusted = Allometric scaled FMD to diameter, BP = Blood Pressure. * p < 0.05 vs. CTL. † p < 0.05 vs. C-INT.

Discussion

The main novel finding of this study is that resistance interval exercise (R-HIIT) acutely improves brachial artery endothelial function in age-matched T2D, UN-NG and TR-NG participants. In T2D participants, %FMD was 4, 2 and 2% higher respectively immediately, one and two hours after R-HIIT compared to CTL. In UN-NG and TR-NG participants, %FMD was not changed immediately after but was 2-4% higher at one and/or two hours after R-HIIT exercise. %FMD was higher two hours after C-HIIT in TR-NG participants and one hour after C-HIIT in T2D, compared to CTL. The exercise-induced increases in blood flow and shear stress were similar following R-HIIT and C-HIIT, suggesting that these parameters did not fully explain the differential improvements in endothelial function. In contrast to previous research on continuous high-intensity exercise (55), we found no evidence of a transient period of FMD impairment following HIIT. These findings are important given the increasing popularity of interval exercise in clinical and non-clinical populations. Our data indicate a potential therapeutic effect of leg resistance exercise performed as HIIT for improving endothelial function, particularly in people with T2D. These findings warrant the examination of the long-term impact of R-HIIT on vascular function.

Effect of Acute Resistance HIIT on FMD

When compared to a time-matched seated control condition, R-HIIT led to higher %FMD at all time points after exercise in T2D and one and two hours following R-HIIT in UN-NG and TR-NG participants. To the best of our knowledge this is the first study to show improved endothelial function after an acute bout of resistance type exercise. The favorable effect of R-HIIT for T2D and UN-NG participants may be attributed to the pattern of shear stress during

resistance-based leg exercise. Indeed, it is known shear rate patterns *during* exercise modulate changes in endothelial function after exercise (13, 63, 138). Unfortunately due to technical limitations of obtaining quality images using vascular ultrasound we were not able to measure blood flow and shear rate during exercise. However, diastolic and mean arterial blood pressures were higher during R-HIIT compared to C-HIIT, suggesting the potential for greater hemodynamic-mediated shear stress during R-HIIT. Previous work has demonstrated that changes in endothelium-dependent dilation depend on combined increases in blood pressure and heart rate, not heart rate alone (246). However, whether there is an upper threshold for beneficial increases in pulse pressure and rate during exercise is unknown. Previous studies have shown higher exercise blood pressure with greater intensities of handgrip exercise impairs local vascular function (99). In the current study endothelial-dependent dilation was consistently improved after R-HIIT, despite significantly elevated MAP, however the increase in MAP was ~50% lower than Okomoto et al. (97, 180, 193) after handgrip exercise (peak change in MAP +17 mmHg in T2D). Discrepancies in the endothelial response to resistance exercise in our study compared to others (193) may also be attributed to the dynamic interval nature of the resistance exercise used in the current study, which involved a light load lifted for many repetitions (37 ± 12 reps/min) to induce fatigue and a perceived effort of 'hard' (RPE of ~5) in the last 10-s of each 1-min interval, which was followed by 1-min of recovery each time. Additionally endothelial function was measured away from the active muscle bed and it has previously been shown that upper, but not lower, limb resistance exercise increases arterial stiffness (97, 180, 193).

Other potential mechanisms mediating FMD responses to HIIT

The underlying factors modulating the changes in endothelium-dependent vasodilation after HIIT remain unclear. Due to the systemic nature of exercise, including interval exercise, various neurogenic, local and hormonal stimuli may determine endothelial function. In the current study, blood flow, SRAUC (shear stimulus), baseline mean and antegrade shear rates were elevated after both C-HIIT and R-HIIT exercise. The largest increases in blood flow and shear rate were immediately after exercise (excluding during exercise), with a time dependent return to baseline when measured again 1 and 2 h after exercise. Shear stress is a potent stimulator of nitric oxide production and improves endothelial-dependent dilation *in vivo* and *in vitro* (194). The elevated SRAUC after C-HIIT and R-HIIT relative to CTL was lower in T2D than TR-NG and UN-NG participants (Figure 4.3), but the changes in baseline blood flow, mean, antegrade and retrograde shear rates were similar between groups and after C-HIIT and R-HIIT (Figure 4.3). Similar to previous research (246) we saw no relationship between SRAUC and FMD after exercise ($r=0.00$, $p=0.95$). In the current study the largest improvements in FMD were seen when the hyperemic and baseline shear rate had returned near pre-exercise levels (Figure 4.2). Elevated blood flow, shear rate, and SRAUC provide a strong stimulus for increasing endothelial nitric oxide production, mediating vasodilation (164). It is plausible that the subsequent post-occlusion hyperemia immediately after exercise may not be able to cause further vasodilation as it may already be near maximally stimulated. This may explain why in the current study most improvements in endothelial function were seen one and/or two hours into recovery.

Time-course and mediators of the FMD response to HIIT

It is generally reported that vigorous activities ($>80\% \dot{V}O_2$ peak) result in a transient depression in FMD immediately after exercise (246). The current study saw no significant reduction in FMD after HIIT when performed as cardio or resistance exercise. It is thought that the transient reduction in FMD after high-intensity exercise is due to elevated sympathetic activity, changes in arterial diameter and/or oxidative stress [reviewed in: (13, 63, 138)]. The consistent improvements seen one/two hours compared to immediately after HIIT in the current study may be due to reduced sympathetic activity one/two hours post-exercise and hence an improved vasodilator response. Meaningful reductions in blood pressure were seen in UN-NG participants across the two hours after C-HIIT and R-HIIT. In addition vascular conductance was improved immediately after exercise in all groups. The sustained hyperemia after HIIT in the current study is an important finding and may reflect a longer lasting stimulus for favorable artery remodeling and function (63). Importantly, this response was similar in T2D, UN-NG and TR-NG participants.

Potential influence of training status

In TR-NG participants endothelial function was improved two hours after C-HIIT, and one and two hours after R-HIIT. In contrast %FMD was only significantly improved two hours after R-HIIT in UN-NG participants. This finding is in agreement with others (247), who show cardio-based exercise consistently improves FMD in more active participants compared to less active participants. Improvements in %FMD after both R-HIIT and C-HIIT in highly trained participants may be due to a higher antioxidant capacity to scavenge oxidants produced during high-intensity exercise, thereby increasing nitric oxide bioavailability (121, 264). It is also

important to note that the highly-trained TR-NG participants in the current study performed a greater volume of exercise (higher absolute intensity but same relative intensity), for example 85% of W_{peak} for TR-NG participants was +119 W greater than T2D and +94 W greater than UN-NG participants. Although we cannot rule out any influence of higher total work, previous studies have shown the acute endothelial response does not appear to be mediated by total energy use (137). Indeed Currie et al. (60, 121) showed that %FMD was improved similarly after continuous and HIIT exercise, despite ~50% lower total work for HIIT exercise. It is inherently difficult to match the work between groups and between resistance and cardio-based exercise. Matching the muscles used and the time and pattern of exercise was deemed more important and appropriate for this study.

Study Limitations

A consideration in the current study is that we did not measure endothelial-independent dilation (vascular smooth muscle function). However, previous studies, including two after HIIT exercise, show there is no change in endothelial-independent dilation following an acute bout of exercise (60). The current study design precluded endothelial-independent dilation measures to avoid potential confounding factors of repeated maximal stimulations with nitroglycerin and interactions with exercise over time.

The groups in this study are matched by age only, therefore we cannot rule out any influence of body mass, medications or long-term diet on blood flow and endothelial responses to exercise. Age was considered by the authors to be the most important and pragmatic variable to match whilst examining whether the presence of T2D and/or fitness (training status) influenced the changes in endothelial function after two modes of acute interval exercise. It would be quite

difficult to find obese adults with no metabolic or cardiovascular risk factors that engaged in 2.5-5 hours and >7 hours of exercise training per week so groups were matched on age only.

Increases in blood flow and shear rate during exercise can cause vasodilation through local regulatory mechanisms that may influence baseline diameter, which may confound the %FMD calculation (60, 138, 180, 246, 252). To adjust for changes in baseline diameter allometric scaling was used according to current recommendations (197). The same significant relationship as %FMD was seen for FMD corrected for diameter in T2D after R-HIIT. However, for TR-NG participants the changes in FMD after R-HIIT and C-HIIT when corrected for diameter were no longer significant, despite similar trends as %FMD.

It is important to note that the T2D participants had completed a brief familiarization period prior to these acute investigations, as were participating in a longer-term study (NCT02251301). This involved six sessions of HIIT; 4 X 1-min intervals eliciting an RPE of ~5 were performed in the first three sessions, thereafter the number increased by one interval each session until reached 6 intervals. This was deemed necessary to ensure the T2D participants could complete 7 X 1-min interval sessions, were accustomed to this type of vigorous exercise, and did not experience any abnormal HR or blood pressures responses to HIIT. Endothelial function measured before and after the two-week habituation period was unchanged ($+0.5 \pm 2.4\%$, $p=0.50$, data not shown), however the endothelial responses seen in the current study may not generalize to inactive T2D participants or those completely naïve to HIIT.

In conclusion, this study shows that resistance-based interval exercise is a time-efficient and effective exercise method to acutely improve endothelial function in T2D, age-matched UN-NG and TR-NG participants. This is the first study to investigate the acute effect of this novel

form of HIIT and demonstrates its potential utility in older adults with and without T2D. Although the mechanisms underlying the changes in endothelial function with cardio- and resistance-based HIIT are unclear, the pattern of high-and low-intensity exercise stimulates an increase in blood flow and shear rate post-exercise and did not cause a transient decrease in endothelial function as found previously for continuous vigorous exercise. The chronic effects of repeated resistance-based versus cardio-based HIIT warrants investigation to elucidate whether these acute responses transpire to long-term vascular adaptations in these groups.

Chapter 5: General Discussion

5.1 Main findings

The studies outlined in this thesis have sought to enhance our understanding of the metabolic and cardiovascular responses following high-intensity interval training (HIIT) in individuals with T2D (Figure 5.1). A randomized controlled trial was conducted to examine whether strategically timed post-exercise milk (protein) supplementation would augment the cardiometabolic health benefits of HIIT in individuals with T2D. The primary findings of this study were that HIIT, with or without post-exercise protein, significantly improves glycemic control, body composition, cardiorespiratory fitness and endothelial function. In contrast to our hypothesis, there was no additive effect of post-exercise milk or protein on these outcomes. This study reinforced the notion that HIIT is a potent strategy to improve cardiometabolic health in persons with T2D (Figure 5.1). In this regard, Chapters 3 and 4 sought to investigate the effect of HIIT (chronic training and acute, respectively) on the vasculature. Given that the high cardiovascular mortality in T2D is largely due to vascular dysfunction (14), and that the impact of HIIT on the vasculature is unclear, this was deemed an important aspect to examine. Moreover, changes in vascular function may explain a large proportion of the cardiovascular risk reduction afforded by continuous aerobic exercise training (58, 222). In congruence with our hypotheses, both acute and chronic HIIT improved vascular endothelial function. The findings in Chapter 3 show that twelve weeks of HIIT leads to clinically meaningful changes in arterial stiffness, blood pressure and femoral intima-media thickness in individuals with T2D. Interestingly, an acute session of resistance-based HIIT in Chapter 4 robustly enhanced

endothelial function across two hours after the session. Taken together, HIIT may be an effective means to reduce the burden of cardiovascular complications in T2D.

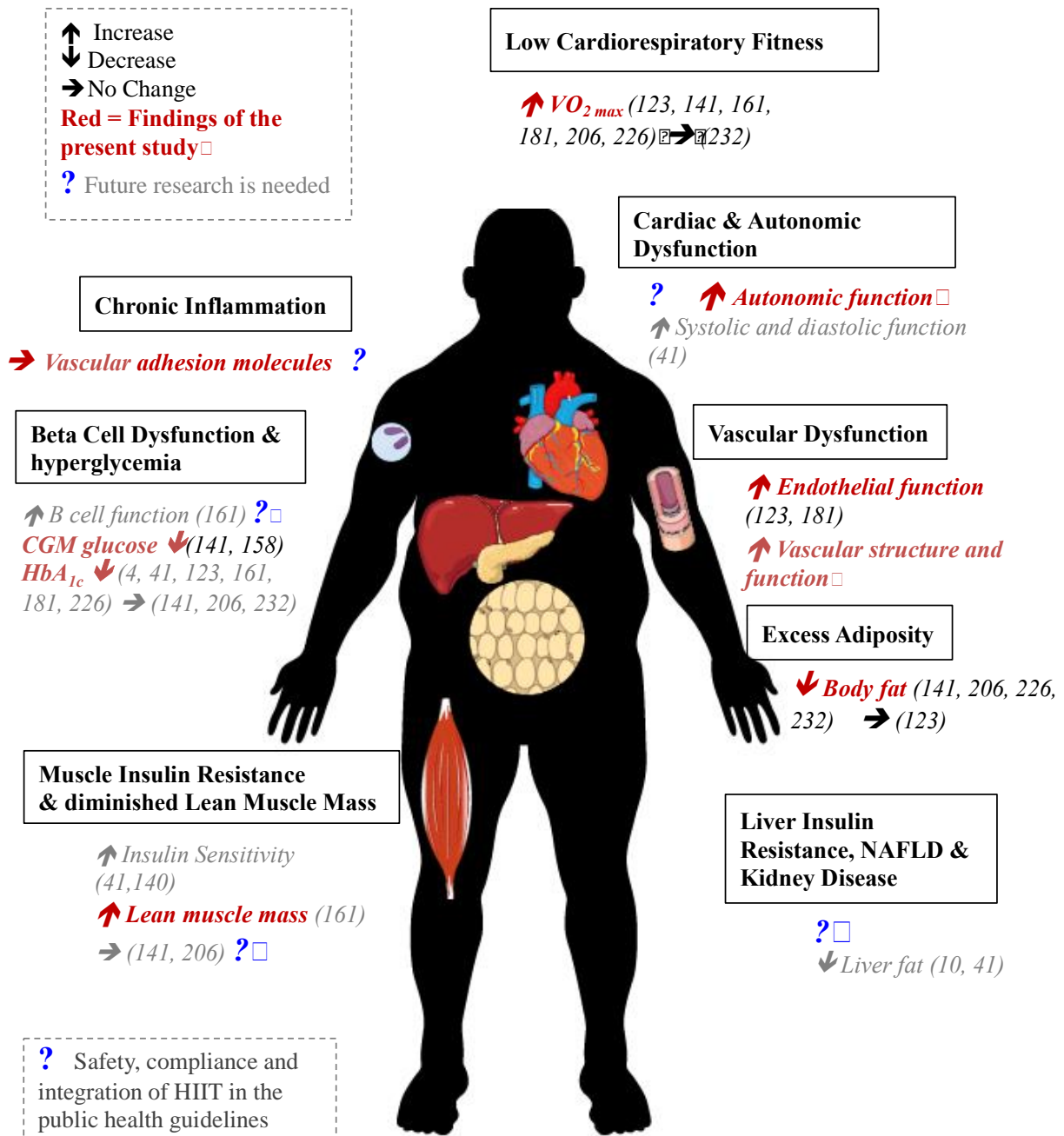


Figure 5.1. Inclusion of the present findings to the known effects of HIIT on the physiological defects that contribute to the excess cardiovascular mortality in type 2 diabetes. The unknown aspects for future research are also highlighted.

5.2 Impact of HIIT on metabolic health in T2D

Glycemic control

It is well established that acute and chronic exercise can improve insulin resistance [reviewed in: (142)], however less apparent is the long-term impact of low-volume HIIT on glucose control in individuals with T2D. In Chapter 2, we showed that twelve weeks of low-volume HIIT significantly improved glycemic control, as indicated by reduced 24-h mean glucose, glucose variability (MAGE and SD (98, 123)) and HbA_{1c}. As such, HIIT improved both the free-living glucose responses to a standardized diet, and the average glycemia across the preceding three months. Karstoft et al. (216) previously showed lower 24-h mean and maximal glucose responses with continuous glucose monitoring, but no change in HbA_{1c}, following four months of 300 min/wk HIIT-based exercise in 12 T2D individuals. We now show that glucose control (both CGM and HbA_{1c}) can be improved with twelve weeks of low-volume HIIT (42 – 78 min) per week. This is important given ‘lack of time’ is commonly perceived as a significant barrier to exercise participation in individuals with T2D (145), and because HbA_{1c} is the gold standard assessment of glycemic in T2D (151). Furthermore, the current study extends upon early findings from short-term low-volume HIIT (<80 min/wk) showing similar improvements in glycemic control with a ~five-fold greater sample size (6). Although speculative, it is plausible to suggest that the improved glucose control observed with HIIT in the present study is due to enhanced skeletal muscle insulin sensitivity and improved beta cell function; based on previous studies (162, 166). Furthermore, because we did not see a reduction in fasting plasma glucose this suggests hepatic insulin resistance was not improved [reviewed in: (144, 166)]. The goal of this study was to investigate the effect of 12 weeks HIIT on cardiometabolic health so potential underlying mechanisms were not directly investigated. The present study observed significant

improvements in metabolic control and body composition, but it is unknown how these changes compare to other interventions. Further to this, determining whether such changes are clinically relevant, in the context of the public health recommendations is important.

HIIT as a potential lifestyle therapy for T2D

To reduce the onset of diabetes complications, individuals with T2D are recommended to engage in lifestyle interventions that result in body mass losses of ~7% and achieve a HbA_{1c} target of <7.0% (42). In clinical practice these markers, in addition to blood pressure and lipids, are typically evaluated by physicians every three months (6, 253, 273). Based on combined results from several intensive pharmacological trials in T2D, a 0.7-0.9% reduction in HbA_{1c} is associated with a 15% reduction in cardiovascular mortality (133). In this context, the 0.22% reduction in HbA_{1c} in the present study is likely trivial, and if conclusions were based on the HbA_{1c} change alone, our intervention would be unlikely to have a large clinical impact (i.e., small effect on reducing mortality risk based on HbA_{1c} alone). On average, HbA_{1c} was lowered from 7.03 to 6.81% after twelve weeks of HIIT which suggests that low-volume HIIT may play a role in assisting T2D participants who are well-controlled (and already receiving standard care) achieve the HbA_{1c} target of <7%. This is of interest, as currently only ~40% of T2D patients meet the recommended HbA_{1c} goal, despite multiple pharmacological options (212, 251). However on closer inspection, of the 27 individuals who had a baseline HbA_{1c} >7% only 33% (9/27) reduced their HbA_{1c} to below 7% with our intervention. There appeared to be no threshold to which benefits were seen with HIIT based on baseline HbA_{1c} (Appendix A). In regard to body composition changes, we observed on average a 0.9 kg (~1%) reduction in body mass, a 1.1 kg increase in lean body mass and a small change in total percent body fat (-1%), with no change in

visceral adiposity. All were not altered by post-exercise milk or protein supplementation. These changes in glycemic control and body composition are inline with some (172, 219), but lower than other HIIT studies (41, 127, 187). A meta-analysis showed that collectively HbA_{1c} is reduced by 0.5% and body mass by 2.3 kg, with HIIT in individuals with T2D and the metabolic syndrome (4, 166, 233). These changes in glycemic control and body mass are similar to that observed with traditional continuous exercise in T2D (meta-analysis of 12 moderate-continuous and 2 resistance exercise studies) (135). In contrast, studies that directly compare HIIT and moderate-continuous training, with matched exercise volume, tend to find greater improvements with HIIT (35). However, before HIIT can be implemented in clinical practice guidelines future research is needed to compare HIIT against standard care (i.e., the current public health guidelines). The reduction in hyperglycemia with exercise alone appears similar to the findings from a large intensive lifestyle intervention trial, The Look Ahead Trial; which is based on the exercise and diet public health guidelines for T2D. The one year results from the Look Ahead Trial (187, 248, 274) showed a reduction in HbA_{1c} of 0.6%, with ~12 kg reported weight loss. To this end, it appears that HIIT can improve glycemic control in T2D without substantial changes in body composition. This is in agreement with Karstoft et al. (76) who found that changes in body composition explained only 25% of the improvement in insulin sensitivity following HIIT. However, based on our findings, the effects of HIIT on metabolic health (i.e., HbA_{1c}, c-reactive protein, visceral adiposity) should not be over-emphasized with respect to other lifestyle interventions in T2D; it is likely that larger benefits would come from a combined intervention involving HIIT and comprehensive dietary strategies to promote more robust weight loss.

5.3 HIIT Plus Milk: No effect of post-exercise milk or protein

The novel aspect of the present investigation was the addition of post-exercise milk or milk-protein to potentially augment the adaptations to HIIT in T2D. This is the first study to test the addition of strategically timed protein to a promising exercise intervention such as HIIT. In contrast to our hypothesis, there was no additive effect of post-exercise milk or protein supplementation on the cardiometabolic responses to HIIT in individuals with T2D. Previous studies in healthy individuals have shown greater muscle protein synthesis and favorable body composition changes with the addition of post-exercise milk to resistance exercise (144). For example, Hartman et al. (122, 141, 271) and Josse et al. (122) showed that 500 mL of skim-milk consumed immediately and 1 h after resistance exercise (5 d/wk for 12 weeks) increased lean body mass and reduced fat mass more than, isoenergetic soy protein or carbohydrate beverages. Furthermore, in an acute study by Wilkinson et al. (141) muscle protein synthesis was substantially increased with the consumption of 500 mL of skim-milk immediately after resistance exercise; which across a training intervention should promote greater increases in lean muscle mass (271). However, discrepancies between the findings of our study and the abovementioned interventions may be due to the age and/or clinical population we studied. Indeed, older adults have reduced sensitivity to the anabolic effects of protein and exercise, and as such may require greater amounts of post-exercise protein (>20 g) to stimulate muscle protein synthesis and inhibit protein breakdown [reviewed in: (75)]. In this context, this may be further exaggerated by the presence of insulin resistance (i.e., T2D) as reduced muscle protein synthesis with aging, may in part, be due to skeletal muscle resistance to the anabolic effects of insulin (50, 51). However, providing individuals with T2D with a greater amount of milk has to be balanced against the risk of hyperglycemia (for every 250 mL there is 13 g of sugar, albeit lactose) and

increased caloric intake. Thus, isolated milk-protein (which does not contain lactose) may be a better alternative to milk for stimulating post-exercise muscle protein synthesis, without causing significant hyperglycemia. For example, Manders et al. (211) showed that milk-protein (30g + 10 g leucine) ingestion after each meal decreased the prevalence of hyperglycemia using continuous glucose monitoring in T2D. Importantly though, the T2D individuals in the present study still responded to the anabolic stimulus of HIIT, with a 1.1 kg increase in lean body mass, which is in line with previous cardio and resistance training interventions in normoglycemic adults (170). In this context, the combination of resistance and aerobic training appears to improve glycemic control, more than either type alone, in individuals with T2D (272). In consideration of this, the benefits of HIIT may be too potent to allow further detection of any additive effects of post-exercise nutrition, which are likely smaller in magnitude.

5.4 Impact of HIIT on cardiovascular health in T2D

Is HIIT Safe?

A recent narrative review (49, 228) highlighted the paucity of available large randomized controlled trials of HIIT in T2D, ostensibly limiting the recommendation of HIIT in the public health guidelines. We attempted to address this by studying the metabolic and cardiovascular responses to HIIT in fifty-three individuals with T2D. However, we acknowledge larger multicenter trials are needed to confirm the safety of HIIT in individuals with T2D. In this context, to our knowledge, we are the first to report a cardiac event following HIIT in T2D. One participant experienced a non-fatal myocardial infarction during week 8 of the intervention. This served as a reminder of the known excess cardiovascular risk in T2D individuals when

participating in vigorous exercise (277). However, it is known that the risk of an exercise-related cardiovascular event decreases with regular participation in high-intensity exercise (72). Speculatively, the prior eight weeks of HIIT may have improved the outcome (non-fatal), despite a 90% blockage of the left anterior descending coronary artery, in this individual. Despite the recent explosion of HIIT studies in clinical populations, there have been no fatal cardiovascular incidents reported. Several preventative measures were undertaken by us to prescribe high-intensity exercise in this population (72). First, all participants were 12-lead ECG screened and cardiologist cleared. Second, all T2D were not diagnosed with any diabetes complications and had no other contraindications to exercise (244). Third, blood pressure and glucose levels were monitored throughout the intervention; importantly exercise was not permitted if blood systolic blood pressure exceeded 144 mmHg at rest and >240 mmHg during exercise (244). Accordingly, our findings may not apply to those T2D who have not been cardiovascular screened or monitored throughout training. There is limited data on the safety of HIIT in clinical populations (244), however currently several ongoing studies are attempting to address this in cardiac rehabilitation centers (85). Acute investigations have shown that markers of acute cardiac damage following moderate-intensity exercise and HIIT are similar (73, 181) and that no undesirable ECG changes are seen in patients with established CVD (24, 111, 112). Furthermore, after 46,364 h of HIIT and 129,456 h of continuous training in cardiac rehab settings, Rognmo et al. (112, 113) observed just two non-fatal cardiac events after HIIT and one fatal event after moderate exercise, highlighting the low frequency of events. The long-term safety of HIIT in patients with T2D is unknown and thus needs to be further examined. Larger and longer research trials are needed, and perhaps sharing of data and collaboration amongst researchers who are working in this area (e.g., standardized protocols and reporting) could help towards the safe

adoption of HIIT in clinical populations. That said, the available evidence shows that several months of HIIT strongly improves cardiovascular risk factors, and thus there is potential for reduced cardiovascular mortality and morbidity. On a final note, the risk associated with not exercising undoubtedly outweighs the risk associated with high-intensity exercise (217), especially considering the strong improvements in cardiorespiratory fitness with HIIT (72). Thus, even if there is a (slightly) higher risk of an acute cardiovascular event when an individual is performing HIIT it needs to be weighed against the likelihood of that individual participating in HIIT over no exercise at all *and* the potential cardio-protection accrued from adapting to HIIT over time.

Cardiorespiratory fitness

The American Diabetes Association (ADA) recommends at least 150 min/wk of moderate-intensity physical activity for adults with T2D (269). To reduce cardiovascular risk, the American Heart Association (AHA) recommends the above and/or 90 min/wk of high-intensity exercise (54). Furthermore, the AHA acknowledges that including high-intensity exercise will likely yield greater health benefits (177). Indeed, in a study of 267,153 adults (over a mean 6 y follow up) an inverse relationship was found between engaging in high-intensity exercise and mortality (~13% reduction in mortality even after adjusting for total exercise) (124, 176). In part, this is likely due the greater impact of high-intensity exercise on cardiorespiratory fitness (90). Several meta-analyses have highlighted the robust improvement in cardiorespiratory fitness with HIIT, which on average equates to a 1.5 MET increase (34). In line with this we show that 44/49 participants in our study improved their $\dot{V}O_{2\text{ peak}}$ with 12 weeks of HIIT, and importantly the remaining five participants showed no change (no decrease) in $\dot{V}O_{2\text{ peak}}$ (Figure 2.3). In

individuals with T2D, each 1 MET increase in fitness is associated with 25% lower risk for all-cause mortality (20, 186, 269). On average, we observed a 0.7 MET (95% CI: 1.8 to 3.1 mL/kg/min) increase in cardiorespiratory fitness with HIIT. It is important to note that the 0.7 MET increase observed with the present intervention (although smaller than 1 MET) is likely still meaningful, as the data collated by Kodama and others (267) is population-based relying on both treadmill and predictive tests. Moreover, for those with low levels of fitness (<7.8 METs) even small increases in fitness result in significant risk reductions for CVD and mortality (149, 157). For example, based on analyses by Kodama et al. (149), the minimum level of cardiorespiratory fitness that is associated with significantly lower cardiovascular mortality is 5.5 METs for women and 7.5 METs for men (aged 55 years). After 12 weeks of HIIT, the females increased from 5.2 to 5.6 METs and the males from 6.2 to 7.1 METs. However, just 2/15 women with baseline maximal METs <5.5 improved to >5.5 METs, and 5/16 men whose baseline maximal METs were <7.5 increased to >7.7 METs. Similar to HbA_{1c} there appeared to be no threshold for improvements with HIIT based on baseline fitness (Appendix A). Interestingly, the risk reduction associated with higher cardiorespiratory fitness in T2D exists even after adjusting for traditional CVD risk factors (149). Therefore, this suggests that the protective effects of exercise (of a sufficient intensity) extend beyond traditional risk factors, further highlighting exercise training that effectively improves cardiorespiratory fitness as a frontline therapy for individuals with T2D (267).

The need for therapies that reduce multiple risk factors in T2D

T2D is considered to be one of the greatest, yet largely preventable, threats to public health (203). In this context, CVD is the most common cause of mortality and morbidity in

individuals with T2D (287). Given the relatively small impact of intensive pharmacological glucose lowering on cardiovascular events (80), it appears that targeting multiple cardiovascular risk factors is needed to reduce CVD in individuals with T2D (1, 3, 254). Indeed, the Steno-2 study (152) showed that targeting multiple risk factors in T2D (lifestyle and polypharmacotherapy; blood glucose, pressure and lipid lowering drugs) resulted in a 20% reduction in cardiovascular events. However, the financial burden of this treatment intervention is significant, and the current pharmacological cost of T2D is already crippling the US health system (88). Moreover, it has been said that the benefits of pharmacological treatment are at least partly offset by the side effects (i.e., hypoglycemia, weight gain and gastrointestinal distress). Therefore, the improvement in multiple cardiovascular risk factors after HIIT (demonstrated in Chapters 2 and 3) have important practical implications for the treatment and prevention of T2D and related complications. Noteworthy, improvements in vascular function are of significant interest given the prevalence and importance of vascular disease in the development of CVD in individuals with T2D.

5.5 Impact of HIIT on vascular function

Twelve weeks of HIIT improves vascular structure and function in individuals with T2D. This was shown by reduced arterial stiffness, femoral intima-media thickness (IMT), blood pressure, and increased endothelial function. Confirming the findings by Mitranun et al. (68) and Hollekim-Strand et al. (187), HIIT is an effective means to improve endothelial function in T2D, as demonstrated by a significant improvement in brachial artery flow-mediated dilation (FMD). The study described in Chapter 4 is the first to demonstrate a reduction in central arterial stiffness and femoral IMT following HIIT in individuals with T2D.

HIIT and Arterial Stiffness

Stiffening of the arterial wall, notably of the central elastic arteries (i.e., the aorta and carotid), is strongly implicated in the increased cardiovascular mortality of patients with T2D (126). Numerous studies have revealed that exercise interventions can improve micro- and macrovascular function (223, 286). However, while many studies have shown improved endothelial function [reviewed in: (27, 168, 169, 224, 247)], the effect of exercise training on indices of arterial stiffness are more variable [reviewed in: (12)]. This variability may be due to the differences in measurement techniques and/or the measurement site; for example ‘peripheral’ (brachial-ankle or radial-carotid PWV) and ‘central’ (carotid-femoral PWV) should be distinguished. Importantly, central arterial stiffness can be reliably measured by carotid-femoral PWV and has a strong association with the incidence of CVD (11). A carotid-femoral PWV >10 m/s is considered as the threshold above which there is an increased risk for cardiovascular events, although there is additive risk for every 1 m/s increase in PWV (260). In contrast, the relationship of peripheral stiffness to CVD is not well established. A recent meta-analysis (257) revealed that improvements in arterial stiffness with exercise training are greater in those with higher baseline stiffness, and overall these changes seemed to be larger for peripheral than central arterial stiffness. Moreover, it was clear that higher exercise intensities, rather than exercise volume, were associated with larger improvements in arterial stiffness (11). In the present study, HIIT reduced carotid-femoral PWV from an average of 10.2 to 8.6 m/s after 12 weeks of HIIT, interestingly with no change in peripheral stiffness. Based on a meta-analysis of 14 studies (baseline PWV > 8 m/s) by Ashor et al. (11) the observed change in central stiffness is higher than typically demonstrated after continuous moderate training (-1.0 m/s, range: -1.43 to -

0.6 m/s). However, to date only one other study has measured carotid-femoral PWV after HIIT. Guimaraes et al. (11) observed a 0.54 m/s reduction in carotid-femoral PWV with HIIT in hypertensive individuals. The poor adherence (60%) to the HIIT intervention may explain the smaller reduction in PWV seen by Guimaraes et al. (110), compared to our fully supervised and 100% attended intervention. Regardless, the strong improvement in arterial stiffness in the present study and in Guimaraes et al. (110), indicate the potential of HIIT to improve arterial stiffness in clinical populations. Potential mechanisms underlying the reduced arterial stiffness with HIIT may be attributed to an improved collagen:elastin protein ratio; mediated by any combination of reduced inflammation, glycation, endothelial dysfunction and sympathetic activity (110). Indeed, we observed lower blood pressure, wall thickness and improved endothelial function after twelve weeks of HIIT. Therefore this suggests that HIIT has important effects on the vasculature, including improvements in vascular endothelial function.

Long-term HIIT and endothelial function

The importance of the endothelium in maintaining vascular health is well-established [reviewed in: (286)]. Exercise results in marked increases in blood flow and pressure, such increases are known to promote vasodilation and an anti-atherogenic endothelial phenotype (91, 259). In agreement with our findings in Chapter 2, several studies have shown improved endothelial function with HIIT (25, 104). Indeed, a meta-analysis of seven HIIT studies demonstrated on average a 4.31% increase in FMD (126, 187, 249, 252, 275). This is substantially higher than the 1.4% increase in the present study, however the majority of the studies included in the analysis by Ramos et al. (210) were from the same lab group and used the higher-volume 4 X 4 min Norwegian HIIT protocol. It is therefore tempting to speculate that

perhaps higher volume and/or longer intervals may confer larger improvements in endothelial function, however no study has directly compared interval protocols. Regardless, research suggests that the elevation and pattern of shear stress provided during HIIT is therapeutic for promoting favorable endothelial adaptations. Such episodic increases in shear stress, provided by individual exercise sessions, drive the vascular adaptations with regular exercise training (210). In this context, a significant increase in resting femoral antegrade flow and reduction in IMT was observed after 12 weeks of HIIT, suggesting attenuated ‘subclinical’ atherosclerosis in individuals with T2D. Taken together, the improvements in arterial stiffness, endothelial function and IMT suggest that long-term lifestyle interventions involving HIIT may reduce the development of atherosclerosis in individuals with T2D. However, longer and larger clinical trials are needed to confirm these findings and determine if atherosclerosis is indeed reversed or prevented by HIIT.

Acute HIIT and endothelial function

Despite the consistent improvement in endothelial function with HIIT interventions, the acute impact of HIIT on endothelial function remained to be elucidated in individuals with T2D. Thus, the purpose of Chapter 4 was to examine the acute impact of cardio and resistance-based HIIT on endothelial function, measured by flow-mediated dilation (FMD). Furthermore, we investigated whether the acute response was impacted by the presence of disease (i.e., T2D) and/or training status (fitness and habitual activity levels). In agreement with our hypothesis, cardio and resistance-based HIIT improved endothelial function in T2D and highly trained age-matched controls. However, in individuals with T2D, cardio-based HIIT only improved FMD at

1-h post-exercise, whereas resistance-based HIIT improved FMD immediately, 1 and 2 hours after. Interestingly, the resting shear stress profile and increase in brachial blood flow within the hours after both cardio and resistance-based HIIT were similar. Furthermore, these findings persisted when corrected for any changes in baseline diameter. This suggests that other factors may underlie the differing response for endothelial-dependent dilation after cardio and resistance-based HIIT. To this end, the divergent responses may be attributed to elevated sympathetic activity (SNS) immediately after cardio-based HIIT and/or differences in oxidative stress (and nitric oxide bioavailability) (247). Indeed, Atkinson et al. (63) demonstrated that the transient impairment in FMD following 30 min of intense continuous exercise was due to elevated SNS. Using Prazosin (a specific α_1 -adrenoceptor blocker), Atkinson et al. (13) prevented the transient impairment in FMD after high-intensity exercise, instead showing a ~2% increase in FMD immediately after exercise when SNS activity was blocked. In agreement with others, this transient impairment (which appears to be likely SNS mediated) following vigorous exercise typically recovers within one hour of exercise cessation [reviewed in: (13)].

Another significant factor that impacts FMD is shear stress, the magnitude and pattern of shear stress during exercise is an important signal for conduit artery vasodilation (63). For example, during lower limb cycling blood flow and shear stress increases (combination of antegrade and retrograde shear stress) in the upper limb lead to significant brachial artery vasodilation, which has been shown to be largely driven by the relative contribution of shear stress to nitric oxide production (199). Conversely, significant increases in retrograde shear stress alone impair endothelial function in a dose-dependent manner (101, 102). Unfortunately, due to technical limitations of obtaining quality images using vascular ultrasound we were not able to measure blood flow and shear rate *during* cardio-based or resistance-based HIIT exercise.

Therefore the shear stress pattern during HIIT is unknown and may have contributed to the differences in FMD following cardio and resistance-based HIIT in the present study. Future research should examine the shear rates during cycling, walking and circuit-based HIIT as these are becoming commonly performed modes of interval exercise. However, based on our findings and those of several others that demonstrate a robust enhancement in endothelial function (242), we can speculate that the pattern of shear stress during HIIT exercise enhances endothelial function.

5.6 Strengths and limitations

Strengths

The training study described in Chapter's 2 and 3 is presently the largest randomized controlled trial (RCT) involving a HIIT intervention in T2D. Although the findings of previous HIIT studies (summarized in Table 1.1) are promising, the average sample size in the HIIT intervention group is 12.5 participants. Thus, studies involving larger sample sizes are needed to confirm the effectiveness of HIIT in individuals with T2D and allow for more reliable effect size estimates. To this end, the paucity of available RCTs of HIIT in T2D mean only six studies have been included across all recent reviews (84, 126, 139, 187, 249, 252). In addition to the sample size and length of our intervention, our study adds to the current literature of HIIT in T2D with robust improvements in several measures of cardiometabolic health, and includes a novel post-exercise nutrition intervention. Exercise and nutrition are both important, as well complementary, in the treatment of T2D (2, 41, 61, 135, 210, 269). Indeed, adjunct interventions typically accrue greater health benefits than exercise or nutrition interventions alone (133). A further strength of this trial is that, collectively in Chapters 2 and 3, we examined several

metabolic and cardiovascular outcomes together enabling a comprehensive examination of the cardiometabolic health benefits of HIIT in T2D.

In addition to traditional statistics (null hypothesis testing) we have included magnitude-based inferences (268) in an attempt to establish the probability that the changes seen for selected measures of cardiometabolic health are clinically beneficial. This analysis estimates the likelihood that our effect is clinically beneficial using spreadsheets available from www.sportssci.org and the magnitude of change associated with clinically beneficial changes obtained from large randomized clinical trials or appropriate epidemiological data. From a clinical and practical perspective, this allows quantitative descriptions of our research findings for the translation into clinical practice. For example, although the 0.2% reduction in HbA_{1c} was statistically significant (according to $p < 0.05$) the magnitude of this effect was very small and based on a clinically meaningful change was very likely trivial.

Similar to Cassidy et al. (22) we also incorporated resistance exercise. Public health guidelines recommend that both cardio and resistance-based exercise should be performed each week (41). This is based on evidence from several large RCTs showing that the combination of cardio and resistance exercise seems to be more effective than either type alone (54, 177). However, the current health recommendations are very time-intensive (e.g., 150 minutes of aerobic exercise plus 2-3 resistance exercise sessions are recommended per week). Using a combination of two sessions of cardio-based HIIT and one session of resistance-based HIIT we observed several meaningful changes in cardiometabolic health. In particular, we demonstrated an increase in lean body mass, which is commonly attributed to resistance-based training. Therefore it is tempting to speculate that HIIT, particularly applied as it was in the current thesis with a resistance-based interval approach, may be able to substitute for both aerobic and

resistance training. This would make HIIT an even more time-efficient health-promoting exercise strategy and warrants future research.

Limitations

Some may consider the lack of a no-exercise or standard care (e.g., guidelines ‘advice’) control condition a limitation of our study. However, the primary aim of the RCT was to examine whether the addition of post-exercise milk would augment the cardiometabolic adaptations of HIIT in individuals with T2D. In that regard, it was deemed more important to have a placebo and macronutrient control beverage, than a control no-exercise intervention. Several studies have already confirmed that HIIT is more effective at improving health than control no-exercise, standard care advice and moderate continuous exercise (Table 1.1). On this note, the drink groups may have influenced some outcomes in our results, and it is possible that we were underpowered to detect slight differences that might have occurred on top of the potent effects of HIIT. Systolic blood pressure and quality of life showed significant interactions; however post hoc testing revealed no differences for milk or milk-protein compared to placebo control. Thus the influence of post-exercise nutrition seems unlikely, as there were no significant interactions for the primary outcomes, when compared to the placebo-water (no protein) control. It is possible that the post-exercise nutritional interventions did not provide enough protein to augment adaptations as previous studies have incorporated training and nutrition five days per week (49, 154, 228). Furthermore, epidemiological data indicates that an increase in consumption of low-fat dairy (e.g., skim milk), of four servings per day, is associated with lower risk of T2D and improved indices of cardiometabolic health (122, 141). Given that we supplemented with two extra servings of low-fat dairy on training days only (3 days per week) the potential effects of increased dairy/milk may have been limited by the relatively small dose.

5.7 Implications and future directions

Complementing previous studies, we show that low-volume HIIT is feasible for T2D participants over 12 weeks. We observed high compliance to our supervised HIIT intervention, and improved overall quality of life. Anecdotally T2D participants reported strong enjoyment for HIIT and a sense of accomplishment for being able to do this type of exercise. However, because training was supervised and participants were monitored closely with individualized motivation provided throughout, our findings do not necessarily suggest that HIIT is ready to be taken outside of a laboratory setting. Despite two home-based intervention studies supporting good adoption of HIIT in T2D (214), further clinical trials with larger samples and longer interventions are needed to inform the prescription of HIIT for clinical practice guidelines and in general public health. A comprehensive comparison of cardiovascular and metabolic adaptations to different HIIT protocols, along with information on long-term adherence, would be beneficial in this field. However given the potency of HIIT and the myriad of health benefits gained from it, it is unlikely that one protocol will be superior for all indices of health in everyone i.e., not ‘one size fits all’. Moreover, more studies need to incorporate HIIT exercise with combined nutrition interventions; given both are cornerstone therapies for the treatment of T2D. To this end, it is known that including vigorous exercise in an exercise program provides additional benefits for health (41, 145). Thus the combination of moderate-intensity exercise with HIIT and resistance training, with dietary strategies that promote fat loss, maintain skeletal muscle mass, and lower glucose levels might be the most effective exercise prescription for individuals with T2D. Clearly such an approach would require integrating expertise from multiple disciplines but

given the burden of T2D and limited effectiveness of pharmacological approaches future research on such comprehensive lifestyle strategies seems a worthy endeavor.

5.8 Conclusions

The overall aim of this thesis was to determine whether a time-efficient HIIT protocol could improve both metabolic and cardiovascular health in individuals with T2D. Our findings extend upon the growing body of research showing that HIIT is an effective means to improve multiple aspects of cardiometabolic health in T2D. Specifically, we demonstrated in the largest sample size study to date that HIIT improves arterial stiffness and reduces femoral IMT in addition to several other established risk factors of CVD, including glucose control, endothelial function, body composition, and cardiorespiratory fitness in T2D. These beneficial adaptations of HIIT are not impacted by post-exercise protein supplementation. Collectively, our findings suggest that HIIT may be an effective therapeutic strategy to reduce the cardiovascular burden of T2D.

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Appendices

Appendix A Individual data from Chapters 2 & 3 RCT

