An Examination of Sex Specific Differences in Glucose Responses Using the Exercise – Physical Activity and Diabetes Glucose Monitoring (E-PAraDiGM) Protocol

by

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Abstract

Background/Objective. Continuous glucose monitors (CGM) allow researchers to examine various aspects of circulating glucose profiles in response to exercise. Exercise studies using CGM in individuals with type 2 diabetes (T2D) vary in regards to the type and timing of exercise, making it difficult to compare inter-individual differences to the same bout of exercise. Furthermore, the majority of acute exercise studies have been conducted in males making it difficult to examine sex specific differences. As a consequence of these challenges, the *Exercise-Physical Activity and Diabetes Glucose Monitoring* (E-PAraDiGM) *Protocol* has been proposed and implemented across eight sites in Canada to provide a standardized comparison in prospective exercise studies using CGM. Results from this thesis form the preliminary analysis of the E-PAraDiGM protocol with data collection from the University of Alberta sites.

Methods. Twenty participants diagnosed with T2D wore a CGM during the 6-day protocol and standardized meals were provided for 2 conditions (exercise vs. seated control) lasting 2 days each. Conditions were separated by a 72-hour washout period and their order was assigned according to a randomized crossover design. Exercise involved a 50-minute walk at 5.0 km/hr and 0.5% incline (~3.5 metabolic equivalents [METs]) performed 3 – 5 hours after lunch and prior to the evening meal. The 24-hour period following exercise was analyzed and compared to the control condition in which exercise was replaced by a time-matched 50-minute seated control condition.

Results. Twenty participants (11 males, 9 females) were recruited and completed the protocol. The mean±standard deviation (SD) for age, time since diagnosis of T2D, and glycated

hemoglobin (A1c) were 61.9 ±9.1 years, 9.3 ±6.9 years, and 6.8% ±0.7%, respectively. On average, exercise did not affect 24-hour mean glucose (exercise 7.0 ±1.6, control 7.2 ±1.5, p=0.343) with the difference between the exercise and control conditions ranging from -1.7 mmol/L to +2.0 mmol/L. There was no difference between sexes (p=0.265), and no sex by exercise interaction in 24-hour mean glucose (p=0.300). There was a difference in 50-minute mean glucose during the exercise and seated control conditions (exercise 6.4 ±1.5 control 7.3 ±1.6 p<0.0001) and between sexes (males 7.0 ±1.5 , females 5.6 ±1.0, p<0.0001). No differences were found between the exercise and seated control conditions or between sexes in time spent above 10 mmol/L or below 4 mmol/L, postprandial glucose, fasting glucose, or glycemic variability.

Conclusion. This was the first study to examine sex differences following an acute bout of exercise. Interestingly and contrary to previous findings**,** there was no effect of exercise on 24-hour mean glucose. Females had lower glucose levels during 50-minutes of exercise compared to males, but no differences were found in other outcome variables. Future analysis using the full E-PAraDiGM sample size will allow for further investigation of sex specific differences. Moreover, the examination of additional predictors of the glycemic responses (e.g. age, medication use, and body composition) to exercise will be examined.

Preface

This thesis is an original work by Jordan Laurel Rees. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, "Exercise Physical Activity and Diabetes Glucose Monitoring Protocol", March 1, 2016 (Pro00059779).

Some of the research conducted for this thesis forms part of a national research collaboration, led by Dr. Normand Boulé at the University of Alberta, and Dr. Jonathan Little at the University of British Columbia. The introduction in chapter 1 and the literature review in chapter 2 are my original work. The research methodology referred to in chapter 3 was designed by a team of researchers from across Canada. The data collection for this thesis took part at the University of Alberta; on North campus under my supervision within the Physical Activity and Diabetes Laboratory. Data collection also took place at the University of Alberta Augustana Campus under the supervision of Drs. Gary Snydmiller and Jane Yardley. The data analysis in chapter 4 and the discussion in chapter 5 are my original work. Tables and figures included in Chapter 2, *Literature Review*, are included with permission.

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Abbreviations

%	Percent
>	Greater than
<	Less than
A1C	Glycated hemoglobin
AMP	Adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CEP	Certified exercise physiologist
CGM	Continuous glucose monitors
cm	Centimeters
Crtl	Control
CSEP	Canadian society for exercise physiology
CONGA	Continuous overall net glycemic action
DBP	Diastolic blood pressure
DC	Diabetes Canada
DPP-4	Dipeptidyl peptidase-4
E-PAraDiGM	Exercise-Physical Activity and Diabetes Glucose Monitoring
Ex	Exercise
F	Females
FEV	Forced expiratory volume
FFA	Free fatty acid
FFM	Fat free mass
FM	Fat mass
FPG	Fasting plasma glucose
GLTEQ	Godin leisure-time exercise questionnaire
GLUT4/2	Glucose transporter type 4/2
GOx	Glucose oxidase
HDL	High density lipoprotein
HIT	High intensity training
HOMA	Homeostatic model assessment
HR	Heart rate
HRR	Heart rate reserve
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
ISF	Interstitial fluid
JNK	c-Jun N-terminal kinase

kg	Kilogram
LDL	Low density lipoprotein
M	Males
METs	Metabolic equivalents
min	Minutes
ml	Millilitres
mmHg	Millilitres of mercury
mmol/L	Millimols per litre
mTor	Mechanistic target of rapamycin
NA	Not applicable
NEFA	Non-esterfied fatty acids
O2	Oxygen
OGTT	Oral glucose tolerance test
PHQ-8	Personal Health Questionnaire Depression Scale
PKC	Protein kinase c
PSQI	Pittsburgh Sleep Quality Index
QOL	Quality of life
R_a	Rate of appearance
R_d	Rate of disappearance
RER	Respiratory exchange ratio
RHR	Resting heart rate
RMR	Resting metabolic rate
RPE	Rate of perceived exertion
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SD	Standard deviation
SKM	Skeletal muscle
TEE	Total energy expenditure
TG	Triglycerides
TNF-a	Tumor necrosis factor-alpha
T2D	Type 2 diabetes
umol/L,	Micromole per litre
VAT	Visceral adipose tissue
$VO2_{max}$	Maximal oxygen consumption
W	Watts
WC	Waist circumference
wt	Weight
yrs	Years
2hrPG	2-hour postprandial glucose

CHAPTER 1 - INTRODUCTION

1.1 Background

The worldwide prevalence of diabetes mellitus continues to rise with numbers increasing from 366 million in 2011 to estimates of 552 million by the year 2030 [1]. In Canada, over 2 million individuals have type 2 diabetes (T2D) and recent reports indicate that Canada has reached a tipping point with numbers expected to rise to 3.7 million by the year 2019 [2]. The consequences of this sharp increase in diagnosis have led to expenditure projections reaching 5 billion dollars by the year 2026 in Canada alone [3]. This increase in T2D prevalence poses many challenges to the health care system due to the vast number of health complications associated with prolonged exposure to T2D.

As a heterogeneous disease, the pathogenesis of T2D is complex with a number of changes contributing to the progressive nature of the disease. Foremost, individuals with T2D experience chronic high blood sugar (hyperglycemia) due to defective insulin secretion, defective insulin action, or both [4]. A number of complications may develop upon prolonged exposure to diabetes and can be divided into microvascular (nephropathy, neuropathy, and retinopathy) and macrovascular (stroke, peripheral arterial disease, and coronary artery disease) complications [5]. Due to the increasing prevalence and progressive nature of the disease, the need for different treatment and management methods for diabetes care is critical. The development of more comprehensive management methods will ultimately lead to improvements in quality of

life (QOL) for many living with diabetes. Currently, physical activity, nutrition counselling and the concomitant use of an oral hypoglycemic agent (often metformin) are considered the first line therapies for T2D management [6, 7].

In exercise studies, researchers and clinicians often rely on measures such as glycated hemoglobin (A1c) to characterize the effects that exercise has on glycemic control [8]. Glycated hemoglobin can be a useful measure under a number of conditions as it is simple to assess, with a single blood sample indicating average blood glucose concentrations over the previous 2-3 months [9]. Furthermore, A1c is independently associated with risk of developing diabetic complications [10] and is therefore useful in assessing the efficacy of different treatments. Despite its many advantages, the ability of A1c to assess variability in blood glucose levels in free-living conditions and acute response to exercise is limited. For example, individual glucose profiles may differ in terms of time spent in hyperglycemia versus hypoglycemia; yet A1c levels for these individuals may be very similar due to the averaging of glucose levels over an extended period of time.

Continuous glucose monitors (CGM) are small, minimally invasive devices which allow researchers to assess individual responses to an acute bout of exercise. These devices are typically worn on the abdomen area where a small flexible filament is inserted into the subcutaneous fat. This filament takes interstitial glucose readings every 10 seconds and outputs an average glucose reading averaged over a 5 minute interval. These readings are collected and

stored for several consecutive days in the CGM (e.g. iPro®2) device attached to the sensor. Once removed, the data stored in the CGM can be uploaded to a computer and analyzed for a variety of parameters including time spent above 10 mmol/L, time spent below 4 mmol/L, and fasting glucose. The advent of CGM allows for numerous glucose measurements to occur in a variety of laboratory-based or free-living conditions. This dramatically reduces the burden and costs for participants and researchers (i.e. numerous blood samples are not required), and allows for various aspects of a glucose profile to be analyzed. In recent years, CGM have become more readily available and their role in diabetes care is expanding. Ultimately, they present a practical and promising way to examine the effects of exercise on an individual's glucose profile.

A recent meta-analysis by MacLeod et al. (2013) identified 11 studies within the last 10 years examining the effects of exercise in T2D as assessed by CGM [11]. The protocols in these studies differed in regards to type and timing of exercise, meal composition, outcome measurements, and laboratory-based versus free-living conditions [11]. Small sample sizes were often utilized in these studies (mean sample size within studies =12), which adds an additional challenge (low power) when comparing inter–individual differences in blood glucose responses [11]. Consequently, the lack of viable comparisons and small sample sizes significantly decreases the potential impact of CGM use as a measurement tool in exercise studies conducted to date.

In addition to the methodological variability between exercise studies, sex specific differences were not examined in any of the 11 studies included in the meta-analysis and very

few females were included in the samples [11]. This is an important aspect to consider as the world-wide prevalence of diabetes affects both sexes similarly [12], yet many physiological differences exist between males and females which may ultimately affect glycemic response to exercise. For example, it is well known that males and females exhibit differences in terms of adipose tissue distribution, sex hormones, and appetite regulatory hormones [13] which may all have important implications when it comes to regulating glucose levels in the body. The current treatment and management recommendations for individuals with T2D, including clinical practice guidelines [6, 14], consider the population as a homogeneous group, neglecting the physiological differences that exist between males and females. As a result, many individuals, particularly females who have been excluded from many studies, may not be receiving the appropriate quality of care to manage their T2D. To date, exercise studies examining sex specific differences in response to an acute bout of exercise have been limited.

As a consequence of these challenges, the *Exercise-Physical Activity and Diabetes Glucose Monitoring* (E-PAraDiGM) *Protocol* has been proposed to help provide a standardized comparison in prospective exercise studies using CGM. The protocol was developed with investigators from seven sites in May of 2015. See Appendix A for details on this meeting and collaborators involved with the project. Currently, eight sites are involved with the E-PAraDiGM protocol. As the lead site, the University of Alberta (North and Augustana Campuses) was the first site to test the protocol in participants with T2D. The University of Alberta site also added

additional steps to the E-PAraDiGM protocol as a part of this thesis. Chapter 3, *Methodology,* explains the E-PAraDiGM protocol in full, including the additional steps.

1.2 Objectives

While following the guidelines set forth by the E-PAraDiGM protocol, the specific objectives of the present thesis were:

1) To examine the acute effects of a single bout of walking on 24-hour mean glucose concentrations as assessed by CGM in individuals with T2D.
2) To examine associations between sex (male vs. female) and the glycemic response to a single bout of walking in participants in the E-PAraDiGM protocol.

1.3 Hypothesis

1) A single bout of walking will lower 24-hour mean glucose as assessed by CGM in individuals with T2D.
2) Males will have a greater reduction in 24-hour mean glucose than females as assessed by CGM in individuals with T2D.

1.4 Limitations and Delimitations

A primary limitation in this sub-study is the small sample size. With a sample of 20 participants, this study is underpowered. Although, the present analysis has more participants than all but one of the 8 acute studies included in the meta-analyses by Macleod et al [11] and has

the largest number of females. The cross-over (repeated measures) design increases the statistical power to detect overall differences between the exercise and control conditions. However, the differences between males and females will be more difficult to detect due to the small sample size. Nevertheless, this preliminary analysis can help identify if the protocol is feasible for both sexes, and to identify other confounders that could improve the planning of more definitive analyses once the larger sample of the E-PAraDiGM is available.

Another potentially confounding factor in this study is the self-report method of standardized meal consumption outside of the laboratory environment. Participants were required to record the time and quantity of their standardized meals in their log books and this makes it difficult to monitor adherence and accuracy of their records. In order to account for this, standardized training sessions with each participant were included in the baseline visit to the laboratory, when the importance of adhering to the standardized meals was emphasized. Participants were also given specific instructions on how to record their dietary consumption.

The chosen exercise (50-minutes of walking on the treadmill) may also be another limitation to this study as this exercise was not intended to be representative of all exercise protocols. The selected exercise protocol was prescribed according to a fixed walking speed and grade. This was not attainable by all participants and represented a different relative exercise intensity for each participant. Different intensities may result in diverse hormonal responses with the potential for different glycemic responses. However, walking is the most common activity in

people with diabetes [15] and is a realistic mode and intensity of exercise for this population. Furthermore, the inclusion of indirect calorimetry during the exercise session provided an indicator of exercise intensity for each participant.

1.5 Study Significance

The examination of sex specific differences in response to an acute bout of exercise using CGM opens up an avenue of research which has not been investigated previously. By examining data from both the University of Alberta North and Augustana Campuses, this study will include a larger sample size (n=20) than most exercise studies using CGM. This work will act as the preliminary analysis (i.e. interim analysis) for the E-PAraDiGM Protocol across Canada. As a multi-site study, the E-PAraDiGM is the first study of its kind that serves to standardize the recruitment, collection, analyses, and reporting of CGM data across eight sites. It will allow researchers to combine results from different sites and serve as the basis for comparisons with other exercise protocols. The implications of this are advantageous from an evidence-based perspective with the results from the E-PAraDiGM protocol determining whether the recommended exercise guidelines of walking lead to acute benefits in glucose control in people with T2D. The long-term implications of this study will enable follow-up studies to examine more in-depth how age, sex, and medications may alter the response to exercise. Additionally, the multi-site aspect of the E-PAraDiGM protocol may allow for the inclusion of participants from diverse backgrounds (e.g. diversity in socio-economic status, ethnicity, age, and sex) which may ultimately enhance the generalizability of results.

Ultimately, the E-PAraDiGM protocol will lead to an improved understanding of why and when some individuals have greater improvements in glucose control compared to others following exercise. With a better understanding of how different characteristics such as age, medication use and sex, may affect glycemic responses to exercise, the E-PAraDiGM has the potential to contribute to improved management methods for T2D through the development of individualized lifestyle interventions. As a lead site for the E-PAraDiGM protocol, this preliminary work will act as a model for use of the protocol across Canada and internationally.

CHAPTER 2 – LITERATURE REVIEW

2.1 Type 2 Diabetes Mellitus: Classification, Diagnosis, and Management

2.1.1 Classification and Diagnosis of Type 2 Diabetes

Type 2 diabetes is the most commonly diagnosed form of diabetes, accounting for 90-95% of all diabetes diagnoses [4]. In 2016, it was estimated that 3.4 million Canadians, or 9.3% of Canada's population were diagnosed with diabetes [16]. By the year 2025, estimates project an additional 1.6 million Canadians, or 12.1% of Canada's population to be diagnosed with T2D [16]. Not only is the rate of diagnosis increasing in the adult population, but T2D prevalence is also increasing in individuals 18 years and younger [17]. These statistics are alarming and require action in the form of varied management methods, support networks, and diabetes education programs to reduce the burden on Canada's health care system, and ultimately enable individuals with T2D to successfully manage their disease and sustain a high QOL.

To be diagnosed with diabetes, an individual must fit within a specific diagnostic criteria. Type 2 diabetes can be diagnosed if an individual meets one or more of the following criteria: fasting plasma glucose (FPG) $\geq$ 7.0 mmol/L, A1c $\geq$ 6.5%, 2-hour plasma glucose (2hrPG) in a 75g oral glucose tolerance test (OGTT) $\geq$ 11.1 mmol/L, or a random plasma glucose $\geq$ 11.1 mmol/L [17]. Individuals may also be diagnosed as having prediabetes, which places them at an increased risk of developing T2D and the associated complications. An individual can be diagnosed with prediabetes if they meet one or more of the following criteria: impaired fasting

glucose (IFG) between 6.1 – 6.9 mmol/L, impaired glucose tolerance (IGT) 2hrPG in a 75g OGTT between 7.8 – 11.0 mmol/L, or an A1c between 6.0 – 6.4% [17].

2.1.2 Management Targets in Type 2 Diabetes

Once diagnosed with diabetes, treatments often focus on improving glycemic control. Depending on an individual's age and duration of time since diabetes diagnosis, different glycemic targets may be recommended to reduce the risk of cardiovascular disease, while also reducing the risk of becoming hypoglycemic. For most individuals, the recommended target for A1c is ≤7% [18]. Glycated hemoglobin is an important aspect to consider as reductions in A1c have been associated with reductions in diabetes complications [10]. In addition to attaining a specific A1c target, it is also important to consider other aspects of glycemic control, such as fasting glucose and postprandial glucose, as both of these are also correlated with diabetes complications [19, 20] . Higher FPG and postprandial glucose levels will ultimately contribute to increased A1c values, therefore it is recommended that individuals achieve a FPG level between 4.0 – 7.0 mmol/L and 2hrPG level between 5.0 – 10.0 mmol/L [18] .

In order to achieve these targets, individuals diagnosed with T2D are often initially prescribed an oral hypoglycemic agent such as metformin [7]. Metformin, classified as a biguanide, has been in use for over 40 years [21]. As the most commonly prescribed drug to treat T2D, metformin is often administered due to is effectiveness, safety, and low cost. A variety of other oral hypoglycemic agents may also be prescribed such as sulfonylureas or dipeptidyl

peptidase-4 (DPP-4) inhibitors, depending on the patient's needs. Physical activity is also recommended, with specific guidelines stating that individuals should accumulate at least 150 minutes of moderate to vigorous physical activity spread over at least 3 days per week [22]. It is recommended that individuals complete a combination of aerobic and resistance exercise sessions each week [22]. In addition to the physical activity recommendations, it is also recommended that individuals receive nutrition counselling by a registered dietitian [23, 24]. Specific nutrition targets recommend that individuals achieve a macronutrient breakdown of 45 – 60% carbohydrates, 15 – 20% protein, and 20 – 35% fat (percentage of total energy) while also considering the timing and spacing of meals [23, 24].

There is no doubt that the management of tight glycemic control requires strict adherence to lifestyle interventions and that this may pose challenges for many with T2D. Different tools and strategies have therefore been recommended by DC to help individuals with self-care practices [25]. Strategies such as problem solving, goal setting and self-monitoring are encouraged, and often required, for many individuals to achieve the appropriate glycemic control targets.

2.2 Blood Glucose Homeostasis

To understand how glucose homeostasis is impaired in individuals with T2D, it is first important to understand how the body regulates blood glucose levels under normal, resting conditions. It is also important to understand the physiological changes that occur to maintain normal blood glucose levels under different conditions such as in the fasted state, postprandial

state, and during exercise. This section briefly examines blood glucose homeostasis during each of these conditions.

Under normal conditions the human body maintains tight control of blood glucose concentrations with levels ranging between 4.0 mmol/L and 8.0 mmol/L [26]. Since glucose is the main energy source for the brain [27] and major organs, it is important to maintain this tight range of circulating blood glucose levels. When glucose levels extend beyond this range on a regular basis (i.e. chronic hyperglycemia or hypoglycemia) there is the potential to induce irreversible damage to a number of tissues [28] [29]. In order to maintain blood glucose levels within this range, the body is constantly working through a number of physiological processes [26]. Many organs contribute to maintaining euglycemia including the pancreas, liver, kidney, adipose tissue, skeletal muscle (SKM), gastrointestinal tract, and brain [30]. Due to the complexity of blood glucose homeostasis, this section focuses mainly on the role of the pancreas and SKM.

The pancreas is a key organ for maintaining euglycemia, with the Islets of Langerhans containing both alpha cells (α-cells) and beta cells (β-cells), among others [31]. Pancreatic β-cells play a major role in glucose homeostasis through the secretion of the glucose-lowering hormone insulin [32]. As an anabolic hormone, insulin acts to increase glucose uptake into peripheral tissues (e.g. SKM) during periods of hyperglycemia or elevated blood glucose levels, such as after a meal. In healthy individuals, insulin is always present in the blood, but an increase in blood glucose caused by food ingestion requires an increased release of insulin from pancreatic β-cells

to maintain normal levels of blood glucose. In brief, insulin secretion is dependent on the ability of β-cells to sense increases in blood glucose levels. The subsequent uptake and metabolism of glucose in the β-cells leads to the induction of electrical activity and trafficking of the insulin secretory granules to the plasma membrane for insulin exocytosis [32, 33 34 35].

It is important to note that insulin is secreted in a biphasic pattern, with the first phase initiated rapidly, followed by a slower and sustained second phase [32]. The first phase lasts between 10-15 minutes and is essential for normal glucose tolerance [36-38] with this phase often reduced or absent in T2D [38]. Once in the blood stream, insulin stimulates the transport and uptake of glucose into peripheral tissues, which is important for cell metabolism [39]. In SKM and adipose tissue, insulin stimulates glucose transporter type 4 (GLUT4) translocation to the cell membrane for glucose uptake [39]. Like the pancreatic β-cell, glucose uptake into the liver occurs via GLUT2 [40].

With higher circulating levels of insulin after a meal, SKM, adipose tissue and the liver are the main sites of insulin action and glucose metabolism [31]. Despite all of these tissues contributing to glucose metabolism, SKM acts as the predominant site for peripheral glucose uptake [41] accounting for 70-80% of glucose disposal under euglycemic hyperinsulinemic conditions [42]. Specifically, insulin action in SKM stimulates glucose uptake and glycogen synthesis [39] through a number of insulin-dependent enzymatic steps [41]. These steps can be found in Figure 1. When insulin binds to an insulin receptor, phosphorylation of three tyrosine

molecules on the insulin receptor occurs [41]. This phosphorylation of the insulin receptor allows for insulin receptor substrate (IRS)-1 to move to the cell membrane and also become phosphorylated by the tyrosine molecules. Subsequently, activation of p85, a subunit of phosphatidylinositol (PI)-3 kinase, occurs and leads to the further activation of p110. The result of these primary steps leads to the activation of protein kinase B (PKB), also called Akt, and the phosphorylation of the Akt substrate, AS160. This leads to the facilitation of GLUT4 translocation to the cell membrane for glucose uptake [43]. Maintenance of this pathway is critical to maintain normal glucose uptake into SKM [44] with dysregulation ultimately contributing to insulin resistance. Interference with this pathway is thought to be associated with dysregulated intramyocellular triglyceride (IMT) metabolism, and thus increased free fatty acids (FFA)[45] often seen in T2D.

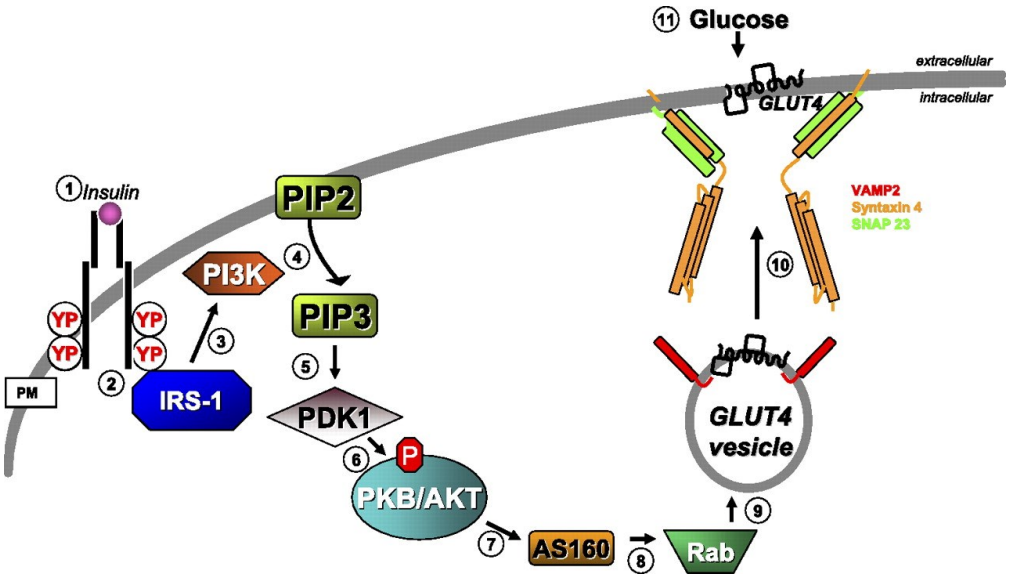


Figure 1. Insulin independent pathway for glucose uptake in skeletal muscle [46]

During periods of fasting or hypoglycemia, pancreatic α-cells secrete glucagon, a key counterregulatory hormone [31]. Glucagon opposes the glucose lowering action of insulin by exerting its main effects in the liver. Here, glucagon stimulates glucose production from substrates such as amino acids (hepatic gluconeogenesis), and the breakdown of glycogen stores (glycogenloysis) to release glucose into the blood stream [31]. It can therefore be thought that β-cells and α-cells function in a reciprocal fashion to achieve normoglycaemia [47]. For example, when blood glucose levels start to rise, such as in the postprandial state, insulin secretion from the β-cells is stimulated in a tightly coupled manner with blood glucose levels, a mechanism known as stimulus-secretion coupling [34]. Simultaneously, glucagon is inhibited to reduce glucose production in the liver.

During prolonged periods of rest, where a number of hours have elapsed since a meal was consumed, or glucose levels decrease below ~4.4 mmol/L, insulin is inhibited and glucagon secretion is stimulated to maintain a normal level of circulating glucose in the blood [48]. If the fasting state is prolonged, the balance between insulin inhibition and glucagon stimulation may not be sufficient to maintain normal glucose levels. In this state, other endocrine hormones come into action such as growth hormone, cortisol [49], epinephrine and norepinephrine [48]. These hormones assist in increasing blood glucose levels through the stimulation of glycogenolysis and gluconeogenesis and inhibition of insulin-stimulated glycogenesis [50, 51].

2.2.1 Blood Glucose Homeostasis During Exercise

During exercise, the body must ensure adequate fuel sources to maintain proper function, while also maintaining normal blood glucose levels. Substrate utilization to provide energy depends on the intensity and duration of exercise [52], as well as the fuel (i.e. food) consumed prior to exercise [53]. During the first 5-10 minutes of moderate intensity aerobic exercise, muscle glycogen provides the majority of energy required to complete work. After this, circulating glucose and non-esterfied fatty acids (NEFA) become the main fuel source for working muscles [54]. If this moderate intensity aerobic exercise is sustained over a prolonged period of time (i.e. several hours), NEFA become the primary fuel source. Alternatively, during a shorter, intense bout of exercise, carbohydrate metabolism becomes the main fuel source [55].

In healthy individuals, glucose levels will remain relatively unchanged during an acute bout of moderate intensity aerobic exercise, despite the increased demands of glucose by working muscles [56]. This can be attributed to reductions in insulin secretion and increases in hepatic glucose production to match the increased glucose uptake [54]. This increase in glucagon secretion and subsequent gluconeogenesis allows for adequate substrate availability for working muscles, and aids in maintaining normal glucose levels. Increased blood flow is also an important regulatory response allowing for sufficient substrate (e.g. glucose) availability during exercise [57]. If exercise persists for a prolonged period of time without adequate carbohydrate intake, glucose levels may decrease due to increased glucose utilization compared to hepatic glucose production [58]. Similar to the mechanisms required to maintain glucose levels during

prolonged fasting (e.g. increased growth hormone, cortisol, [49] epinephrine and norepinephrine [48]) these hormones also come into action during exercise to assist in the maintenance of normal blood glucose concentrations.

2.2.2 Insulin Independent Pathway for Glucose Uptake

Exercise is a major mediator of GLUT4 activity, the common carrier protein for glucose uptake in SKM [57]. Evidence suggests that the increase in glucose uptake during exercise occurs due to an insulin independent translocation of GLUT4 to the cell surface [59-61]. This exercise-induced, or SKM contraction-induced translocation of GLUT4 results in an increase in the plasma membrane content of GLUT4 isoform [61, 62]. Due to increased blood flow during exercise, it is reasonable to conclude that increased insulin transport to SKM is the cause for this increased GLUT4 translocation, but in-vitro studies examining SKM contraction in the absence of insulin have demonstrated increased plasma membrane GLUT4 [63-65]. The findings from these studies support an insulin-independent pathway for glucose uptake in SKM during exercise. Furthermore, evidence from rat models suggests that exercise- and insulin-induced GLUT4 translocation occur from distinct intracellular GLUT4 pools [62], perhaps contributing to the additive effects of glucose transport and uptake observed during exercise in individuals with T2D.

The intracellular signaling mechanisms which occur to facilitate this SKM contraction-induced GLUT4 translocation remain elusive. AMP-activated protein kinase (AMPK) has gained attention in recent years and may help to explain how exercise aids in increasing insulin

sensitivity with both acute and chronic exercise contributing to adaptations in this enzyme [66]. AMP-activated protein kinase acts as an energy sensor in the cell, indicated by the ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) [67]. An increase in AMPK activity and glucose transport has been observed in both animal models [68, 69] and individuals with T2D post-exercise [70].

Supporting an insulin-independent pathway for glucose uptake, most studies examining the SKM contraction pathway have not been successful in demonstrating associations with early components of the insulin-dependent pathway such as IRS and P13K [71]. Rather, studies have observed AS160 to be an important regulator of AMPK during SKM contraction, which may be a linking point between the two pathways [72]. Additionally, protein kinase-c (PKC), which is a calcium-dependent signaling intermediary, has been observed to increase with muscle contractions [73, 74] but the second messenger required to activate this protein remains unknown and there is little research analyzing the association between PKC and AMPK [71]. It is proposed that PKC, like AS160, may represent a point of convergence between the insulin and muscle contraction pathways [71], although additional research is required.

2.3 Pathophysiology of Type 2 Diabetes

2.3.1 Overview of the Pathogenesis of Type 2 Diabetes

The pathophysiology of T2D is complex with a combination of genetic and environmental factors contributing to adverse effects on β-cell function and insulin sensitivity [75].

As a heterogeneous disease, its pathogenesis is not restricted to impaired β-cell function, but extends further with alterations occurring in many of the body's tissues, such as in the pathways discussed previously. Perhaps one of the most well-documented and researched characteristics of the etiology of T2D begins with insulin resistance. With insulin resistance, or decreased insulin sensitivity, the major sites for insulin action (i.e. SKM, adipose tissue, and the liver) do not respond effectively to insulin. As a result, higher circulating levels of insulin are required to maintain normoglycaemia [76]. The compensatory hyperinsulinemia that is observed upon insulin resistance may allow for an individual's blood glucose levels to remain within the prediabetes range for a number of years [17], but places an increased demand on the pancreatic β-cells. Upon appropriate lifestyle interventions some individuals may not progress to T2D [17], yet this requires strict management and many will likely progress to T2D. Specifically it is estimated that the conversion rate of prediabetes to T2D is between 5-10% each year [77]. A depiction of insulin secretion and action during the early and late stages of T2D can be found in Table 1. The following section discusses more specifically the mechanisms contributing to insulin resistance.

Table 1. Insulin secretion and insulin action in the early and late stages of development of type 2 diabetes (T2D) [78].

STAGE OF DIABETES	INSULIN SECRETION	INSULIN ACTION
Early stage T2D	High	Low
Late stage T2D	Low	Low

T2D=type 2 diabetes

2.3.2 Insulin Resistance

The development of insulin resistance is multifactorial and can be linked to many factors such as excess caloric intake, obesity, inflammation, and physical inactivity [79]. It is possible that a dominant pathway plays a role in the pathophysiology of insulin resistance, but it is more viable that a complex interplay between many cellular pathways exists. Individual factors such as hereditary risk factors and lifestyle habits may also influence disequilibrium in these cellular pathways and subsequent insulin resistance [79]. The cellular mechanisms contributing to insulin resistance in SKM, adipose tissue, and the liver are unique yet their contributing roles to whole body insulin sensitivity are interconnected with alterations in one tissue effecting alterations in another tissue [79]. Recognition of cross-talk between insulin secretion and the major sites of action is therefore essential to the development of insulin resistance.

In muscle, liver, and adipose tissue, abnormal interaction of insulin with insulin receptors on the surface of cell membranes and the intracellular signaling pathway that follows this interaction are thought to be major contributors to insulin resistance [76, 80]. More recent data suggest a disruption in the pathway molecules as a major contributor to insulin resistance with a number of molecules potentially causing this disruption [76]. Firstly, the insulin receptor substrate (IRS) is a key protein involved in the initial insulin signaling cascade and excessive serine phosphorylation of IRS proteins may alter or attenuate signaling of downstream molecules [76, 81, 82]. Excessive serine phosphorylation may be triggered by a number of molecules including molecular target of rapamycin (mTOR), tumor necrosis factor-alpha (TNFa), c-Jun N-terminal

kinases (JNK), and protein kinase C (PKC) which are often increased in states such as obesity [76]. Ultimately, the result of excessive serine phosphorylation of IRS has been shown to reduce the strength of insulin signaling, thus resulting in decreased glucose uptake in peripheral tissues [83-85].

The role of adipose tissue in the pathogenesis of insulin resistance has gained great interest and it has been well-documented that obesity is strongly correlated with insulin resistance and the development of T2D [86]. Now recognized as an active endocrine organ, the cells in adipose tissue, adipocytes, play a major role in regulating nutrient homeostasis [87] through the secretion of various hormones and cytokines known as adipokines [88]. In a state of energy surplus, triglyceride storage may shift from storage in the subcutaneous area to ectopic fat deposition (fat deposits around the organs) and accumulation of visceral adipose tissue (VAT) [89]. Storage of triglycerides around different organs has a number of implications in regards to insulin sensitivity with intramyocellular and intrahepatic lipids coupled with insulin resistance [90-92]. For example, VAT is known to be more metabolically active and contain an increased number of immune and inflammatory cells (i.e. macrophages) compared to subcutaneous adipose tissue (SAT) [89]. Additionally, VAT has a greater capacity than SAT to generate NEFA [89]. Adipokine release from adipocytes may become modulated upon ectopic fat storage and affect many of the body's pathways, including the insulin signaling pathway mentioned previously [86, 87]. An increased infiltration of macrophages observed with increased levels of adipose tissue may contribute to increased levels of circulating cytokines, such as TNF-a and interleukin-6 (IL-6) which negatively affect insulin action [86]. Furthermore, insulin may no longer inhibit lipolysis to

the same extent, which leads to increased circulating NEFA with accumulation of further lipid intermediates occurring in non-adipose tissue such as SKM and the liver.

As a major site for glucose disposal, SKM plays an integral role contributing to the body's overall resistance to insulin. Accounting for up to 40 % of an individual's body mass, SKM is responsible for a large portion of postprandial glucose disposal [41]. As mentioned previously, during euglycemic hyperinsulinemic clamp, it has been found that approximately 80% of glucose uptake occurs in SKM [93]. Therefore, ectopic fat storage and impaired insulin signaling in SKM may contribute significantly to increased levels of circulating glucose, particularly in the postprandial state.

Not only does insulin resistance affect glucose uptake in SKM and adipose tissue, but it also affects glucose uptake in the liver. But, perhaps more importantly, insulin resistance at the level of the liver may lead to uninhibited glucose production [94], particularly during times of fasting. Since insulin inhibits gluconeogenesis in the liver, glucose production in the liver may become uninhibited upon insulin resistance, further contributing to chronically high levels of blood glucose.

Ultimately, insulin resistance in the body's three main sites of insulin action increases the demand on pancreatic β-cells. This chronic demand on β-cells to increase insulin secretion may eventually lead to β-cell dysfunction, and ultimately β-cell death [86], further enhancing the

pathogenesis of T2D. An overarching diagram of the pathogenesis of insulin resistance and β-cell dysfunction can be seen in Figure 2.

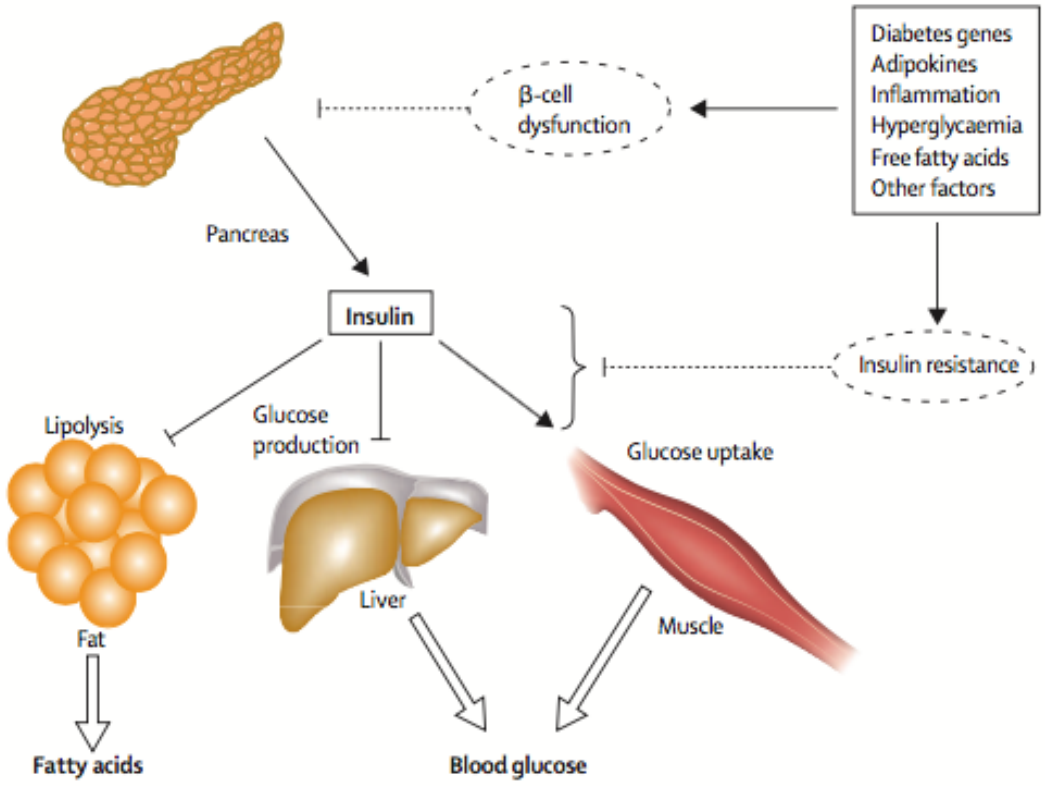


Figure 2. Factors contributing to insulin resistance and β-cell dysfunction [86]

2.3.3 Assessment of Insulin Resistance

Insulin secretion and sensitivity can be assessed in a number of ways depending on the specific tissue being examined. The hyperglycemic clamp is a useful method that allows for examination of β-cell sensitivity to circulating plasma glucose concentrations [95]. With the hyperglycemic clamp, glucose concentrations are acutely raised above basal concentrations and are then tightly monitored to maintain a constant plasma glucose concentration [95]. The rate at

which subsequent glucose must be infused signifies an index of glucose metabolism (i.e. glucose is being metabolized in the β-cells resulting in insulin secretion and the subsequent glucose uptake into peripheral tissue) [95]. The euglycemic insulin clamp, or euglycemic hyperinsulinemic clamp, is a method to determine insulin sensitivity in peripheral tissue, such as SKM. With this method, insulin is infused into the blood to reach a desired concentration while plasma glucose concentrations are held at a basal level by a variable glucose infusion [95]. Under steady state, the rate at which glucose must be infused to maintain a specific concentration equals the rate of glucose uptake into peripheral tissues, providing an indication of the body's peripheral sensitivity to insulin [95].

Although the hyperglycemic clamp and hyperinsulinemic clamps are useful and direct measures, they can be expensive and often difficult to perform. Additional methods for quantifying insulin sensitivity also exist and are commonly used in clinical settings, particularly as diabetes diagnostic tools. These methods often involve calculating the insulin-glucose ratio after measuring each of their plasma concentrations during different conditions such as fasting or in the postprandial state [96]. The homeostasis model assessment (HOMA) is another useful and common tool used to evaluate β-cell function and insulin sensitivity. This model is of great utility as it considers both glucose and insulin together. Specifically this model measures insulin and glucose concentrations in the fasted state [97, 98]. Only requiring a single plasma blood sample, it is a relatively simple test to administer with its validity tested against a number of other physiological methods, such as the euglycemic clamp and hyperglycemic clamp [98]. By

measuring insulin and glucose in the fasted state, HOMA allows the examination of the relationship between hepatic glucose output and insulin secretion, which is maintained through a feedback loop between β-cells and the liver [98].

Other commonly used tests, but perhaps less indicative of insulin sensitivity or resistance include the assessment of FPG and OGTT. For the FPG test, an individual is tested in the fasted state (minimum of 8 hours without consuming food) [17]. As mentioned previously, FPG concentrations ≥ 7.0 mmol/L indicates the presence of diabetes and more specifically, may indicate hepatic insulin resistance with uninhibited hepatic gluconeogenesis contributing to higher levels of FPG. OGTT's are also commonly used with a 2hrPG value ≥11.0mmol/L after a 75g load indicating impaired glucose tolerance.

2.4 Exercise and Type 2 Diabetes

It is well known that regular physical activity or exercise enhances SKM glucose uptake and can improve insulin sensitivity at rest [99-101]. Many cellular pathways may be modulated during regular exercise to promote this glucose uptake and improve insulin sensitivity [102]. This section seeks to better understand both the acute and chronic effects of exercise in individuals with T2D.

2.4.1 Exercise Training and Glycemic Control

Both aerobic and resistance exercise aid in the management of T2D [103, 104] and the

benefits of exercise are multi-faceted, extending beyond glycemic control. It has been demonstrated that exercise training can improve an individual's glucose control, decrease insulin resistance, decrease blood pressure (BP), improve blood lipid profile, improve cardiorespiratory fitness, and aid in meaningful changes in body composition [104-106]. With regards to glucose control, a number of studies have identified reductions in A1c following exercise training in individuals with T2D, yet variability exists among exercise modality and study protocols [8, 105, 107]. Different modes of exercise have been examined with aerobic training, resistance training, and a combination of both contributing to meaningful decreases in A1c [107]. A randomized trial consisting of 22 weeks of exercise training in individuals with T2D (combined aerobic and resistance training, aerobic training alone, or resistance training alone) reduced A1c values by 0.9, 0.43 and 0.30 percentage points, respectively [107]. It is important to note that the combined group in this study performed the full aerobic and resistance training programs and therefore completed a higher quantity of exercise, which could in part account for the larger reduction in A1c observed. A meta-analysis by Snowling & Hopkins also concluded that combined training reduced A1c values more than either intervention alone, yet the benefit was small to moderate [105]. Additionally, a randomized control trial by Church et al. found that only combined exercise resulted in significant reductions in A1c compared to either modality alone [108].

Synthesizing many of the studies examining the effect of exercise on A1c levels, a large systematic review by Umpierre et al. found that all modalities decreased A1c levels and greater reductions were found in structured exercise training interventions which accumulated greater

than 150 minutes of exercise a week [8]. Despite variability between studies it has been well documented that exercise training can decrease A1c values between 0.5% - 0.9%, with larger decreases observed when both aerobic and resistance training are combined, and the duration of this training exceeds 150 minutes a week [8, 105, 107-109]. Greater reductions may also be observed in individuals with higher baseline A1c values [107]. These reductions are comparable to some oral hypoglycemic agents (e.g. alpha-glucosidase inhibitor ~0.6% reduction, DPP4-inhibitor ~0.7% reduction, metformin ~1-1.5% reduction) [7] and can often be in addition to the glucose lowering effects of medication.

2.4.2 Glycated Hemoglobin as an Outcome Measure

Glycated hemoglobin is a useful clinical tool and is a simple and easy measure, which can be taken at any time point (i.e. fasting or postprandial state) to gage improvements in glycemic control over a period of time. In addition, reductions in A1c (1%) are associated with large reductions in microvascular complications (37%) [10]. Despite these clinical benefits, its practicality in exercise studies is limited. More specifically, changes in A1c take a long time to become fully apparent as it is an average measure of blood glucose over a 2-3 month period [9]. Due to the heterogeneity of T2D, two individuals with the same A1c value may have vastly different glucose profiles in terms of time spent in hyperglycemia and time spent in hypoglycemia [110]. In addition, the practicality of A1c as the main measure in exercise is limited and researchers are often required to take numerous blood samples to gage the physiological changes occurring during and after an exercise session. The advent of CGM allows for the

examination of a number of additional outcome variables including 24-hour mean glucose, postprandial glucose, fasting glucose, and glucose variability (e.g. mean amplitude of glycemic excursions [MAGE]).

2.4.3 Acute Exercise and Glycemic Control

Discussed previously, acute exercise plays a role in improving insulin sensitivity, particularly in SKM. These insulin-sensitizing effects are thought to last up to 48 hours following the acute bout of exercise [111]. A recent meta-analysis by MacLeod et al. included 11 exercise studies utilizing CGM. Eight of these studies were short term (<2 weeks), while three were long term studies (>2 months) [11]. Despite variability between protocols (i.e. sample size, population [medications], exercise duration, dietary intake) the acute benefits of exercise were observed in 24-hour mean glucose and time spent in hyperglycemia [11]. Specifically, average glucose levels (Figure 3) decreased by 0.8 mmol/L ($p<0.01$) and time spent in hyperglycemia (Figure 4) decreased by a total of 129 minutes in a 24-hour period ($p<0.01$)[11]. This decrease in time spent above 10.0 mmol/L may have important implications as postprandial hyperglycemia has been recognized as a strong independent risk factor for the development of diabetic complications [20, 112].

A study by Winnick et al. observed improvements in peripheral insulin sensitivity after one week of exercise training [113] which may reduce postprandial glucose due to the association between SKM insulin sensitivity and postprandial glucose uptake. Interestingly, there was no

effect on hepatic insulin sensitivity, suggesting that exercise more readily affects the postprandial aspect of glycemic control [113]. This supports the findings by MacLeod et al., with no changes in fasting glucose levels observed. Studies observing changes in fasting glucose following exercise could be attributed to the weight loss that is often accompanied with the exercise training [114, 115], rather than the exercise training itself. Therefore a single bout of exercise would likely not affect fasting glucose concentrations.

Only one study [116] assessed glucose variability (continuous overall net glycemic action [CONGA]) in the meta-analysis by MacLeod et al. [11]; which was not found to decrease following an acute bout of exercise. This represents an aspect of glycemic control that has been given little attention in previous studies, requiring further investigation. It should also be noted that many of the short-term exercise studies have been male dominated, with very few females included in the study samples. This greatly reduces the ability to generalize results to the female T2D population. Ultimately, results from previous studies, particularly the meta-analysis by MacLeod et al., suggest that further examination is required to better understand inter-individual differences in response to the same bout of exercise [11]. Further examination of fasting glucose and glycemic variability are of particular interest due to the heterogeneity of outcomes in the literature. As well, the addition of sex specific differences must be examined; this is discussed in detail in the following section.

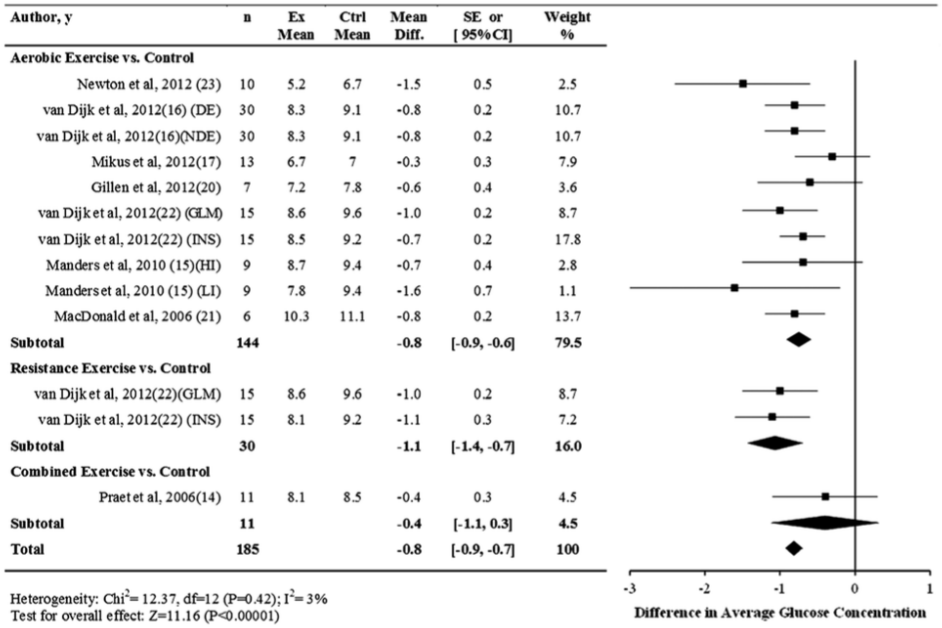


Figure 3. Effects of exercise on average glucose concentrations as measured by a continuous glucose monitor [11] .

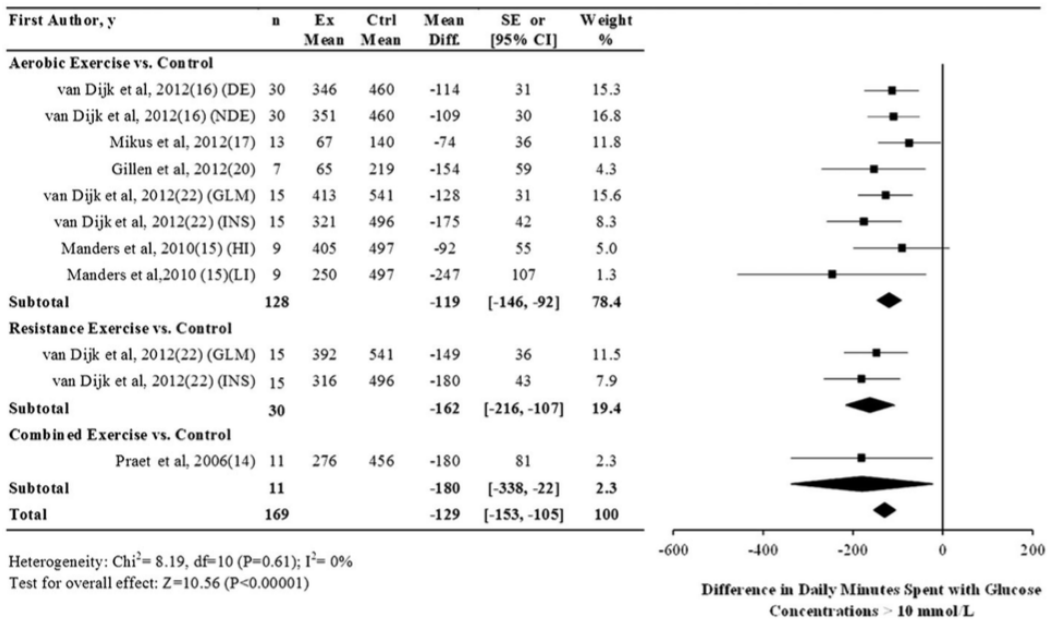


Figure 4. Effects of exercise on daily time spent in hyperglycemia (>10.0mmol/L) as measured by a continuous glucose monitor [11].

2.5 Sex Specific Differences in Type 2 Diabetes & Exercise

Literature examining sex specific differences in glycemic response to the same bout of exercise is limited and deserves further examination, particularly due to the increasing incidence of T2D in older, ethnic-minority female populations [117]. Despite well-known physiological differences between males and females (e.g. hormone levels and adipose tissue distribution [13]) the exclusion of females from many T2D study populations has led to recommendations that may not benefit both sexes to the same extent, as reviewed by Legato et al [117].

Before discussing sex differences, it is important to differentiate between the terms "sex" and "gender". Sex refers to the biological differences between males and females caused by sex chromosomes, gene expression, sex hormones and their effects on the human body [118]. It differs from the term gender, which refers more closely to the sociocultural practices which may influence an individual's behaviours [119]. This work only discusses sex differences.

2.5.1 Sex Differences in Body Composition

As mentioned, recognition of the many biological and physiological differences that exist between males and females is critical. A review article by Geer & Shen investigated differences in insulin resistance, body composition, and energy balance between sexes. It was reported that, as a whole, males have higher lean soft tissue (e.g. SKM), while females tend to have lower levels of SKM and increased adiposity levels [13]. Interestingly, the distribution of adipose tissue also differs between males and females, with males having a higher ratio of VAT and females

having a higher ratio of SAT [13, 120]. Adipose tissue distribution in males and females is depicted in Figure 5.

These differences in body composition could have important implications since adipose tissue is an endocrine organ which plays an integral role in an individual's metabolic profile [121]. A strong body of evidence supports the role of VAT and hepatic adiposity in the development of insulin resistance [120, 122, 123]. Adipocytes in VAT have increased sensitivity to catecholamine-induced lipolysis and have decreased sensitivity to insulin when compared to adipocytes in SAT [124]. Considering the fact that males tend to have higher levels of VAT, this may be a factor contributing to increased risk for insulin resistance in males. Interestingly, it has been found that males are more commonly diagnosed with diabetes at a lower age and body mass index (BMI) compared to females [119] and VAT may play a contributing role in this earlier diagnosis. Additionally, males often exhibit worse FPG levels compared to females supporting the role of increased VAT and hepatic adiposity in the pathogenesis of insulin resistance in males.

Alternatively, the increased SKM mass exhibited by males may be associated with improved peripheral insulin sensitivity and response to an OGTT when compared to females. Methodological practices may influence this varied response to an OGTT due to a 75g glucose load administered to both males and females, despite females often having a shorter stature and less total body weight. A study by Faerch et al. examined the role of body composition in response to an OGTT and found that indeed, height is associated with 2hrPG response [125] with

no differences found between males and females when height was adjusted for. This suggests that sex differences in glucose tolerance are likely not due to the physiology of glucose regulation, but rather differences in body size and SKM mass.

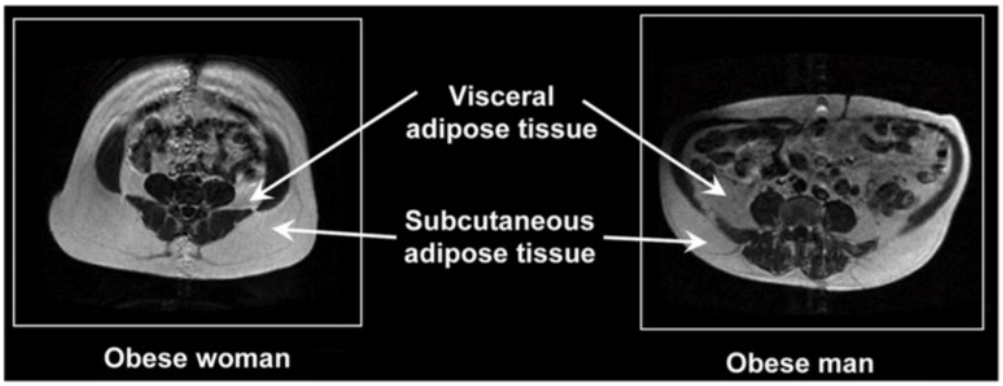


Figure 5. Cross-sectional abdominal magnetic resonance images of an obese female and an obese male [13].

2.5.2 Sex Differences in Hormones

Not only does body composition play a role in the differing metabolic profiles exhibited by males and females, but hormone levels may also play a contributing role. Estrogen and testosterone are two fundamental hormones to consider when examining sex specific differences in insulin resistance.

Despite females having increased total adiposity, the female sex hormone, estrogen, plays a protective role favoring glucose homeostasis and adipose tissue distribution [126]. There may be a number of pathways in which estrogen enhances insulin sensitivity and in animal models it has been observed to decrease hepatic glucose production as well as enhance glucose transport in

SKM [127, 128]. This finding may have important implications for differences in FPG concentrations between sexes due to alterations in hepatic glucose production. Interestingly, upon menopause, many of the protective effects of estrogen decline with notable decreases in insulin sensitivity and increases in visceral adiposity [126, 127, 129]. This is of interest as females have been found to exhibit characteristics more similar to males following menopause [130]. For example, increases in VAT and decreases in lipid oxidation have been observed in females post menopause [130] . Being pre or post-menopausal may therefore have important implications when examining sex specific differences.

The role of testosterone may also play a role in an individual's metabolic profile with higher levels of testosterone in females correlating with increased waist circumference and abdominal adipose tissue, and with decreased insulin sensitivity [131]. Alternatively, increased testosterone levels in males is associated with decreases in visceral adiposity and improved glucose disposal rates [132].

Taken together, both body composition and hormone levels may contribute to variation in metabolic homeostasis between sexes. Differences observed in metabolic profiles between females who are pre-menopausal and females who are post-menopausal appear to be related to changes in hormones and body composition occurring during menopause [130].

2.5.3 Sex Differences in Response to Exercise

Studies examining sex specific differences following exercise are limited. One study examining the effects of an exercise training intervention (20 weeks) on glycemic control in healthy individuals found that improvements in insulin sensitivity post exercise were greater in males compared to females [133]. This study included a large sample size (males = 280, females = 316) and measured insulin sensitivity utilizing an intravenous glucose tolerance test [133]. In contrast, a 6-month resistance training study in healthy elderly adults (n=53) found no differences in A1c or oral glucose tolerance between males and females after exercise training [134]. The reasons for these findings are somewhat elusive and deserve further examination as both of these studies were conducted in a non-T2D population.

Interestingly, studies have been conducted examining differences in body weight following exercise training interventions with males often displaying greater reductions in body weight [135, 136]. Reasons for this may be attributed to females having a greater compensatory response in order to protect adipose stores and reproductive function [137, 138]. Weight loss in males following exercise, but not in females also suggests the role that appetite regulatory hormones (e.g. ghrelin which plays a role in satiety, and insulin and leptin which regulate long term energy balance and adipose tissue)[138] may play in this varied response. It has been suggested that variation in energy intake following exercise may contribute to these differences in body weight [137]. Since body composition may ultimately affect an individual's metabolic profile, this is an important aspect to consider when examining sex differences in glucose control.

Studies examining sex differences in T2D following an acute bout of exercise are limited, particularly with regards to glucose regulation. Interestingly, sexual dimorphism in counterregulatory response during hypoglycemia has been more thoroughly documented. These studies have found females to have a reduced counterregulatory responses during hypoglycemia compared to males [139] [140, 141]. Since exercise and hypoglycemia may share similar counterregulatory mechanisms, this reduced counterregulatory response in females may also be present during and following exercise [142]. A study by Henderson et al. determined the rate of glucose appearance (R_a) and disappearance (R_d) before, during, and after 3-hours of 2 different exercise intensities (45% VO2peak and 65% VO2peak)[143]. It was found that there were no differences between males and females in glucose R_a or R_d during each of the exercise intensities, but the glucose R_a and R_d remained elevated in males following exercise [143]. Males were also found to have significantly higher glucagon levels following exercise, which was thought to have contributed to enhanced hepatic gluconeogenesis, thus the increased glucose R_a [143]. The elevated R_d was thought to be driven by increased glycogen synthesis [143] perhaps due to greater glycogen depletion during exercise and greater SKM mass [144] . Taken together, this increased glucose metabolism (also referred to as glucose flux) following exercise suggests that females regain glucose control, or return to resting values, quicker than males.

This hypothesis is supported by Hedrington & Davis' findings in their recent review paper examining dimorphism in hypoglycemic and fasting states [145]. It was observed that females experience significantly lower levels of epinephrine, norepinephrine, glucagon, growth hormone,

pancreatic polypeptide, and hepatic glucose production during hypoglycemia [146]. Additionally, substrate utilization during exercise was examined and females were found to exhibit higher levels of plasma FFA and glycerol versus males who exhibited higher reliance on carbohydrate oxidation [146]. Figure 6 includes details of substrate utilization during hypoglycemia and exercise.

Differences in counterregulatory responses and substrate utilization during exercise may ultimately affect glycemic control, thus the findings from these studies are of great interest and could have important implications for the type and timing of exercise that is most beneficial for males and females. Further investigation of the counterregulatory response to an acute bout of exercise will ultimately lead to a greater understanding of how sex may affect glycemic control.

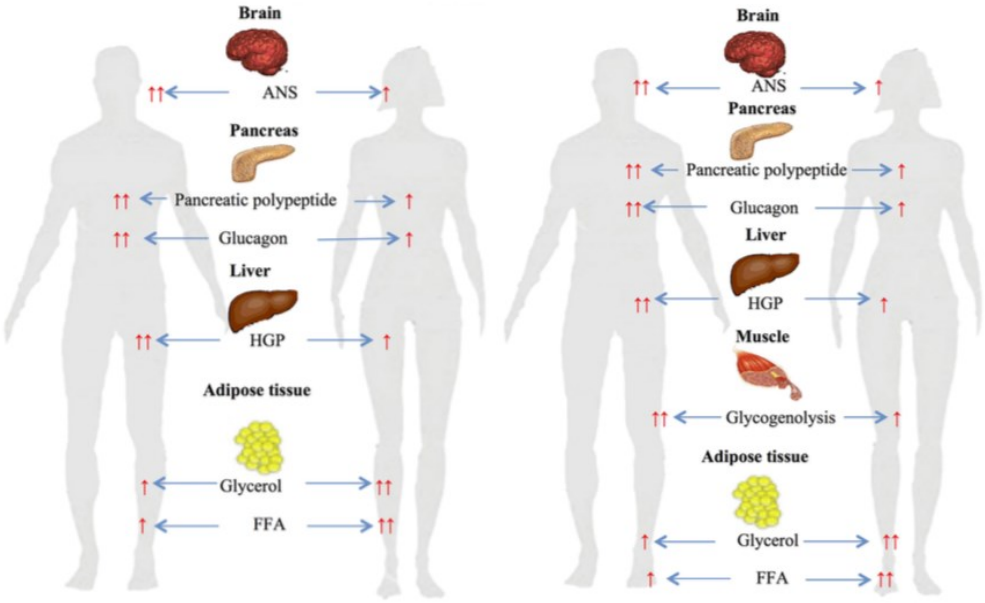


Figure 6. Sex differences in glucose and lipid metabolism during hypoglycemia (on the left) and during exercise (on the right) (↑=increased, ↑ ↑=significantly increased, ANS=autonomic nervous system, HGP=hepatic glucose production, FFA=free fatty acids) [146]

2.5.4 Sex Differences in Cardiovascular Profiles

Despite limited literature on how glucose control following exercise may be affected by sex, the examination of differences in cardiovascular risk factors has been more thoroughly documented. A large observational study using a cross-sectional survey was conducted in older individuals with T2D and found females to have a higher prevalence of abdominal obesity, higher A1c values, increased low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, and increased systolic blood pressure (SBP) [147]. Similarly, a retrospective cross-sectional study examined sex specific differences in older individuals and identified females as having higher levels of LDL and HDL cholesterol as well as SBP and diastolic blood pressure (DBP), but not A1c values [148]. Females were also identified as less likely to meet cardiovascular treatment goals [148]. Similar findings were identified by Kautzky-Willer et al. in a study examining sex specific differences in individuals with T2D with only slight differences found in A1c values, favoring males [149]. Despite similar findings between studies, these results may not apply to the general population of individuals diagnosed with T2D as many of these studies were conducted in individuals who were older (e.g. in one study, the mean age of females was 70.9 and the mean age of males was 69.56 [148]) and thus could differ depending on the age of an individual.

In conclusion, these findings suggest that differences' between sexes are present with regards to body composition, hormone levels, substrate utilization, and cardiometabolic profiles. Furthermore, differences in A1c favouring males [147, 149] suggest that males may have improved

glycemic control compared to females. Due to limited literature examining sex specific differences and the exclusion of females from many exercise studies, it is difficult to conclude whether males or females may have greater improvements following an acute bout of exercise. Since exercise is a cornerstone to diabetes management and is associated with decreases in cardiovascular risk factors [133] and A1c values [8] examination of sex differences is essential for proper exercise prescription. Closer examination of the effects of an acute bout of exercise on glycemic control is essential.

2.6 Continuous Glucose Monitoring

Biosensor technology has gained momentum in recent years with a wide range of point-of-care devices available and the emergence and use of CGM. Over the past 15 years, CGM have been increasingly used in diabetes care, clinical practice and research settings. Due to their user-friendly design and ability to provide additional information to the traditional retrospective A1c measure; they can provide individuals with a more comprehensive picture of their glycemic control. This section briefly discusses CGM technology, their accuracy and reliability, future directions, and their place in diabetes management.

2.6.1 Continuous Glucose Monitor Technology

In Canada, there are currently 2 approved models of CGM (from the companies Medtronic and Dexcom). Table 2 includes details of different CGM models available from these companies. These monitors are equipped with 2 - 3 parts, depending on the model. All CGM

models include a small flexible sensor (~1 cm in length), which is inserted under the skin, and a data storage device (about the size of a quarter) that sits on the surface of the skin and receives and stores the sensor's information. Figure 7 shows an image of a CGM (from Medtronic Canada) including the sensor and data storage device. Some models may also include a small data display unit (about the size of a small cell phone), which receives wireless signals from the data storage device to display real-time interstitial glucose measures.

Real-time models are particularly useful for individuals with type 1 diabetes (T1D) as they can facilitate informed decision making about insulin timing and dosage. For many individuals the fear of hypoglycemia may interfere or reduce the capacity to tightly control blood glucose levels [150, 151]. In this regard, real-time CGM may assist with improved management of diabetes, as these monitors are equipped with alarms, alerting individuals when their glucose levels are low or high (e.g. < 4mmol/L or >10 mmol/L). The retrospective models (no display unit) may be more useful in situations where immediate behaviour change is not required. The retrospective models without monitors can also be useful in research settings where individuals may need to be blinded to their glucose levels.

The sensors used in CGM are smaller than an intravenous needle, are flexible and are inserted into the SAT, generally on the abdomen or arm [152, 153]. Unlike glucose samples obtained from venous blood, CGM technology obtains glucose readings from the interstitial fluid (ISF) [152], which are correlated to blood glucose levels [154, 155]. That is, the glucose readings are obtained

from fluid that surrounds cells in the SAT. The sensors contain glucose oxidase (GOx) (like capillary glucose test strips)[156] which reacts with glucose, converting it to hydrogen peroxide [156]. This newly formed peroxide then reacts with the platinum inside the sensor creating an electrical signal which travels to the storage device for frequent readings (~every 10 seconds) [157]. The interstitial glucose readings are then calculated via a computer program in the storage device.

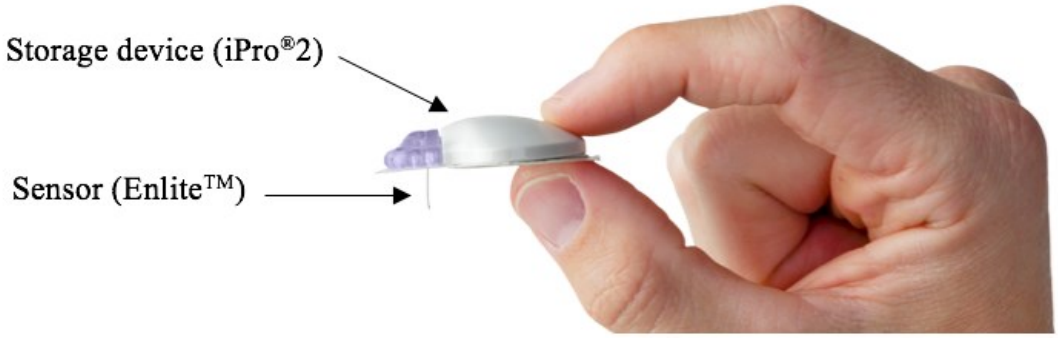


Figure 7. Image of continuous glucose monitor, including the sensor and storage device

The current approved CGM models give an average interstitial glucose reading every 1 – 5 minutes and can be worn for 3-7 days [158]. After this time period, the deterioration of the sensor may not allow for accurate readings and the battery of the storage device may need to be charged. Once the sensor is removed, the data stored on the device can be uploaded to a computer software program for analyses or patient counselling with their physician.

2.6.2 Continuous Glucose Monitor Accuracy and Reliability

Since CGM do not take glucose measurements directly from the venous blood, there is a lag time between venous blood glucose and interstitial glucose measures [159]. This lag time is thought to be prolonged during periods of rapid glucose fluctuations [153]. Furthermore, the accuracy of CGM measurements from the ISF has been examined in comparison to venous blood glucose. One study found the correlation between venous and interstitial glucose readings throughout a CGM sensor life-time to be r=0.73 [160]. Interestingly, the correlation was reduced over the sensor life time with reductions from 0.77 on day one of sensor wear to 0.65 on day three of sensor wear [160]. This is an important aspect to consider, especially if sensors are worn for the entirety of their recommended lifespan. The accuracy has also been shown to decrease during hypoglycemia [161] or rapid fluctuations in glucose [152] .

Accuracy of CGM is measured by the mean absolute relative difference (MARD) between CGM and blood glucose readings [162] or relative absolute difference (RAD) and may vary slightly between the specific CGM device [163] (e.g. Medtronic iPro®2 Enlite™ or Dexcom®G5). Most studies examining the accuracy of CGM have been conducted during sedentary time, with accuracy found to decrease during exercise compared to resting [164, 165]. Although decreased accuracy during exercise has been found, a large portion of the readings (90%) are still considered to be in the normal range [164] , and the use of CGM as a measurement tool is widely accepted. While some variation in monitor accuracy exists (between approximately 11 and 22% as reviewed by Klonoff et al. [152]), the use of CGM has been

associated with reductions in A1c and glycemic excursions [166-170] , and the additional measurements that it permits greatly increases the potential impact of CGM as a research and management tool.

Table 2. Examples of Continuous Glucose Monitors available from Medtronic and Dexcom [171]

Company	CGM	Accuracy (%MARD)	Calibration Requirements	Sensor Lifetime	Retrospective or Real-time
Medtronic	iPro®2 with Enlite™ sensor	~11% [171]	1 every 12 hrs required. 3-4/day recommended	6 days	Retrospective
Medtronic	MiniMed® Paradigm®Veo™ with Enlite™ sensor	13.6 - 14.2%[171]	2/day	6 days	Real-time
Dexcom	Dexcom G4®	13-15%[171]	2/day	7 days	Retrospective or real-time
Dexcom	Dexcom G5™	9-10%[171]	2/day	7 days	Retrospective or real-time

CGM=continuous glucose monitor, MARD=Mean absolute relative difference, hrs=hours

The reliability of CGM has also been tested in individuals with T2D. Under standardized conditions, reliability for mean glucose, postprandial glucose, exercise glucose and nocturnal glycaemia was found to range between 0.77-0.95 [172]. Glycemic variability (discussed below) was also assessed using mean amplitude of glycemic excursions (MAGE), percentage coefficient of variation and standard deviation (SD). It was found that all of these measures of glycemic variability were reliable using CGM [172].

2.6.3 Outcome Variables from Continuous Glucose Monitors

Continuous glucose monitors present a method of glucose assessment which is different from the longstanding methods of glucose assessment such as capillary glucose checks and A1c. For example, the outcome variables which can be obtained for each 24-hour period include: 1) 2-hour postprandial glucose area under the curve for each meal, 2) fasting glucose, 3) time spent above or below a specified mmol/L, and 4) glycemic variability (e.g. MAGE).

Glycemic variability represents a unique and important aspect of glycemic control and is a measure of the glycemic excursions that may occur during a specified time period [173]. Since oxidative stress has been shown to be a mediator of complications associated with diabetes [174] and greater oxidative stress is associated with intermittent hyperglycemia (rather than sustained hyperglycemia), glycemic variability should be considered as an important outcome to assess in the management of diabetes.

Despite there being no 'gold-standard' measure [175], MAGE is a commonly used measure of glycemic variability [173, 176] which was originally designed to assess meal-time glycemic excursions [173]. Mean amplitude of glycemic excursions is calculated by determining the glycemic excursions which exceed 1 SD (i.e. the peak to nadir and nadir to peak of an excursion exceeds 1 SD) [176]. More specifically, the minimum and maximum glucose points are determined within a specified time period. If the difference between the peak and nadir is greater than 1 SD then the measure is included in the final calculation [173]. The included measures are then summed

and divided by the number of data points included in the summation to calculate MAGE [173]. See Figure 8 for an example of how MAGE is determined.

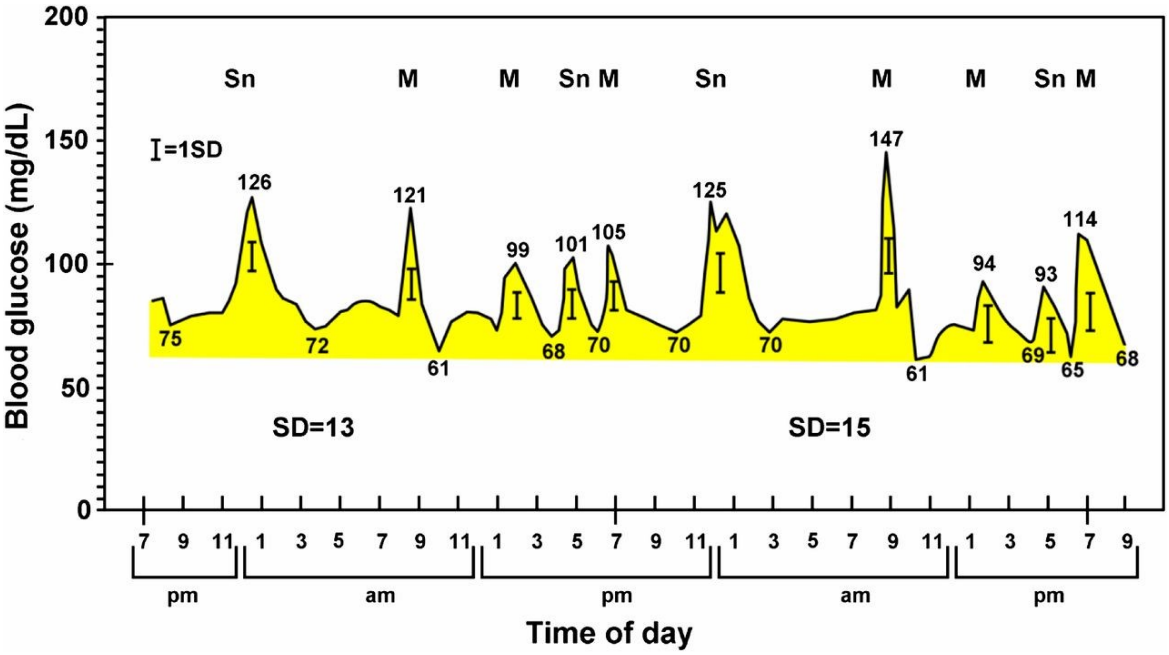


Figure 8. Mean amplitude of glycemic excursion calculation (M=meal, Sn=snack, SD=standard deviation) [176]

Normative ranges for MAGE have not been well established which greatly reduces the current usefulness of MAGE as a clinical tool [177]. One study using 3-day CGM data from 434 healthy adults found an average MAGE±SD of 1.73±0.75 mmol/L [177]. Interestingly, it was found that there were no differences between sexes and MAGE tended to increase with age [177]. From these findings it was suggested that a MAGE±SD of <3.9±1.4 mmol/L was within a normal range, although further examination of MAGE in different population groups is required.

Notwithstanding limited normative data, the measure of glycemic variability obtained from CGM is of great interest and deserves examination in future studies utilizing CGM.

Other measures of glycemic variability exist including the mean of daily differences (MAD), J-index, coefficient of variation, and average daily risk range [176]. Standard deviation can also be used as a measure of variability, but because SD takes into account all data points within a given time period, this makes it difficult to specifically assess glycemic excursions or swings [176]. In addition, SD implies that data are normally distributed, which may not always be the case, particularly in a T2D population where glucose concentrations can commonly be skewed towards hyperglycemia [173]. Due to limited literature comparing the different glycemic variability calculation methods, caution should be taken when choosing which method to use.

Due to their simple and practical design, CGM present a user friendly method for diabetes care and management. Despite their many benefits, CGM technology is still considered relatively new and there remain a number of drawbacks to both patients and researchers. These drawbacks include factors such as the high cost of the devices, making them relatively inaccessible to the majority of individuals with diabetes. Sensor degradation or rejection over time, and the frequent sensor calibrations which are required (3-4 per day) also remain major limitations to the current CGM technology.

Looking to the future, improved CGM technology is emerging with the development of smaller, non-invasive devices (e.g. wearable watch with no needle insertion), and wireless Bluetooth® smartphone applications for real-time glucose monitoring [178]. These newer technologies will ultimately contribute to enhanced diabetes care and management for individuals with T1D and T2D.

To conclude, the practicality of CGM allows for the assessment of glucose in a number of everyday settings such as during sleep, during and after meal times and during and after physical activity. This is advantageous from a management perspective, allowing for the assessment of glycemic control under different conditions. This may ultimately open an avenue to more individualized diabetes management plans. From a research standpoint, CGM allows for the analysis of various outcome variables in 'real-life' scenarios. This may permit the design of more diverse protocols, expanding our knowledge of how glycemic control may be affected under different conditions.

CHAPTER 3 – RESEARCH METHODOLOGY

3.1 Research Design

The E-PAraDiGM protocol took place over a 6-day period, which included exercise and seated control conditions. Each participant completed both of these conditions according to a randomized crossover design. Table 3 illustrates the basic timeline that each participant completed.

Table 3. Timeline of the E-PAraDiGM Protocol

BASELINE	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Questionnaires, Anthropometric measures, & treadmill practice session	**Insert CGM**	**All meals are standardized** **Session #1** (Exercise or Control)	**All meals are standardized**	**WASHOUT**	**All meals are standardized** **Session #2** (Exercise or Control)	**All meals are standardized** Remove CGM (evening)

CGM=continuous glucose monitor

3.2 Outline of the Six-Day Protocol

Following a baseline assessment, participants completed the 6-day protocol:

Day 1 - Participants arrived at the Physical Activity and Diabetes Laboratory (PADL) at the University of Alberta, Main Campus, or at the Exercise Physiology Laboratory at the University of Alberta, Augustana Campus at their designated appointment time. An individual, trained by a Medtronic specialist, inserted the CGM sensor on the abdomen area of the participant (CGM, iPro®2 Medtronic, Northridge, CA, USA and Enlite™ sensor). Participants were then given breakfast, lunch and snacks for the following day, and were reminded on how to

fill out their log books (i.e. record the time of each meal and snack and the quantity of each item consumed).

Day 2 - Participants arrived at PADL or the Augustana laboratory 3 - 5 hours after consuming their standardized lunch for the first of the 2 conditions, i.e. either walking or seated control for 50-minutes. Twenty minutes after the completion of the condition, participants consumed their standardized meal (dinner) in the laboratory. Participants were then given meals for the following day (breakfast, lunch, dinner and snacks) and day 5 (breakfast, lunch and snacks) prior to leaving the laboratory.

Day 3 - Participants were asked to consume the standardized meals (breakfast, lunch, dinner and snacks) at similar times as the previous day and were instructed to also record the time of their meals in the participant log books. There was no visit to the laboratory on day 3.

Day 4 - As a wash out day, participants resumed their typical daily activities and eating habits. There was no visit to the laboratory on day 4. The washout period ensured there was a 72-hour period between the beginning of the exercise and control conditions in order to minimize a potential carry-over effect of the bout of exercise.

Day 5 - Participants arrived at PADL or the Augustana laboratory 3 - 5 hours after consuming their standardized lunch to complete the opposite condition of day 2 (walking or seated control). Twenty minutes after the completion of the condition, participants consumed their standardized meal (dinner) in the laboratory. They were then given meals for the following day (breakfast, lunch, dinner and snacks).

Day 6 - Standardized meals (breakfast, lunch, dinner and snacks) were consumed on day 6. The CGM was removed by the participant on day 6 in the evening, before bedtime. Participants were instructed to return their log books and CGM devices to the lab following day 6 of the protocol (and within 24 hours of removing the CGM).

Throughout the 6-day protocol, participants were required to take 4 capillary glucose samples (OneTouch® Ultra®2 glucose meter, LifeScan Milpitas, CA, USA) within each 24-hour period. Participants were instructed to take these samples immediately after waking up in the morning, before lunch, before dinner, and before going to bed each evening. These readings were recorded in the participant's log book. Participants also wore a pedometer (Yamax DigiWalker 200) to track daily step count. This was recorded in the participant log book. Further details of the protocol, including the standardized meals and exercise conditions can be found in the following sections.

3.3 Inclusion Criteria

Inclusion criteria for the E-PAraDiGM study was as follows

- Between the age of 30-90 years
- Diagnosed with T2D for at least 6 months
- A1c less than 9%
- No changes in weight >5 pounds in the last 3 months
- No changes in diabetes medications in the last 3 months
- Not taking corticosteroids
- No diagnosis of heart disease, stroke, kidney disease (or any other chronic condition that

may have impacted an individual's ability to exercise)

- Able to comply with study requirements (e.g. attend visits during the day and eat the standardized meals)

3.4 Baseline Assessment

Participants were recruited to the E-PAraDiGM study through newspaper and radio advertisements, as well as through a phone database provided by the Alberta Diabetes Institute, Clinical Research Unit. If contacted by phone and the individual was interested, participants were asked basic eligibility questions prior to arranging a date and time to come in for their baseline visit. The phone screening from can be found in Appendix B.

Once an individual expressed interest in participating in the E-PAraDiGM study, they were invited to come into the laboratory for their initial visit. This visit was included to perform pre-screening of participants and ensure participant eligibility. Written informed consent was also completed at that time (Appendix B). Participants were required to fill out a Physical Activity and Readiness Questionnaire (PAR-Q) in order to identify any individuals who may need further medical screening by their physician before completing exercise in the laboratory.

During the initial visit participants completed several questionnaires including a medical screening questionnaire. The medical screening form included questions pertaining to duration of T2D diagnosis, age, medications, previous medical events (e.g. myocardial infarction, stroke), smoking, and alcohol habits. This form is available in Appendix B. Data on the participant's

most recent blood test (A1c, total triglycerides and cholesterol in the last 6 months and creatinine within the last year) was also collected. In addition, participants who completed the protocol at the University of Alberta Main campus had an A1c measurement completed in the lab (Siemens DCA Vantage Analyzer, Frimley Camberley, UK). Following the medical screening questionnaire, the Rose Angina questionnaire was administered to identify individuals who may experience ischemic heart pain upon exercise initiation, and thus be at increased risk for an adverse cardiac event. This questionnaire is moderately associated with disease and risk factors and has been identified as appropriate for use as a pre-screening tool [179]. This form can also be found in Appendix B.

Following the Rose Angina questionnaire, participants completed the Godin Leisure-Time Exercise Questionnaire (GLTEQ) which assesses the frequency of strenuous, moderate and light physical activity that an individual completes during a one week period [180]. The GLTEQ is a commonly used tool and includes a leisure scale (part 1 of the questionnaire) and a sweat scale (part 2 of the questionnaire), with a total score calculated from the two parts [180]. This form can be found in Appendix B.

Additionally, participants completed the Patient Health Questionnaire-8 (PHQ-8) and the Pittsburgh Sleep Quality Index (PSQI). The PHQ-8 is a valid measure of current depression in the general population [181] with a cut point of greater than or equal to 10 identifying current depression. Inclusion of this questionnaire was important as a strong body of evidence suggests

an association between T2D and depression [182]. The PSQI was included as a valid and reliable assessment tool to assess sleep quality and disturbances over a 1 month period [183]. These forms can be found in Appendix B.

Following the questionnaires, anthropometric measurements were administered by a Certified Exercise Physiologist® (CEP). These measures included height, weight, waist and hip circumference using the Canadian Society for Exercise Physiology (CSEP) protocols. Resting heart rate (radial pulse method) and blood pressure (manual) were also taken. This data collection form can be found in Appendix B and the protocols used can be found in Appendix C. In addition, a bioelectrical impedance analysis (BIA) (Tanita Body Composition Analyzer, TBF-300A, Tokyo, Japan) measurement was completed as an assessment of percent body fat and fat free mass (FFM). Bioelectrical impedance analysis was chosen as it is relatively inexpensive and is considered a non-invasive measure [184] which provides an accurate and reliable measure of FFM [185].

During the baseline assessment, participants also completed a 15-minute walking session on the treadmill at the speed and grade of the walking intervention session (5.0 km/hr at a grade of 0.5%). This was included to allow participants to familiarize themselves with the laboratory environment and to become comfortable using the treadmill.

3.5 Standardized Diet

Participants were provided with all of their food (breakfast, lunch, dinner and snacks) for days 2, 3, 5 & 6 of the study. In order to prepare meals with the appropriate total energy needs of an individual, the Harris Benedict equation was used to calculate an individual's resting metabolic rate (RMR)[186]. This equation calculates RMR relative to an individual's body weight, height, age, and sex. The equation is as follows:

Males: RMR (kcal/day) = 66.4730 + 13.7516W + 5.0033H – 6.7750A
Females: RMR (kcal/day) = 665.0955 + 9.5634W + 1.8496H – 4.6756A
(W = weight in kilograms; H = height in centimeters; A = age in years)

Although the Harris Benedict equation has been found to often overestimate RMR, it is considered an efficient and valid prediction as an alternative to indirect calorimetry [187]. To account for an individual's activity levels, or total energy expenditure (TEE) during a 24-hour period, the Harris Benedict equation was multiplied by 1.4 RMR. The chosen macronutrient profile as a percentage of TEE was based from recommendations from the Institute of Medicine dietary reference intakes (45-60% carbohydrate, 20-35% fat and 15-20% protein) [24]. The E-PAraDiGM protocol specifically aimed to achieve within 5% of the following macronutrient breakdown: 55% carbohydrates, 30% fat, and 15% protein [23]. Ideally, each meal would fall within this macronutrient distribution, but the specific goal was to achieve this breakdown for the individual's total daily energy intake. There were no specific targets for the percentage of total daily calories allotted to each meal and no other targets (e.g. total daily sugar, saturated fats, glycemic index, or micronutrient content) were used. All meals and snacks were the same on the

exercise and seated control days (i.e. day 2 meals matched day 5 meals) and the same on the days following the testing days (i.e. day 3 meals matched day 6 meals).

After a participant menu had been created (see Appendix D for examples), it was shown to the participant so they could assess whether the foods included and the meal sizes were appropriate for them. This was done as participant adherence to the standardized meals was essential in order to control for the confounding effects of an unstandardized diet. The standardized meals varied slightly between participants to accommodate personal preferences and total caloric needs, but often similar foods were administered due to their practicality (e.g. easily accessed or prepared) and personal preference. For breakfast, it was common for participants to have milk, a banana, President's Choice (PC)® Blue Menu® steal cut oatmeal, and /or a Dannon Oikos® yogurt cup. Lunch often consisted of a 6 inch turkey, ham, or veggie Subway® sandwich, and depending on the participant's caloric needs, may have also included a piece of fruit, granola bar or almonds. Dinner was commonly a PC® Blue Menu® meal, salad, and/or a slice of bread with butter. Common snack foods included Dannon Oikos® yogurt cups, almonds, an apple, carrot sticks and hummus. When participants had a food sensitivity or allergy (such as a gluten and dairy allergy) this was accommodated.

Participants were permitted to consume caffeine (e.g. coffee) but were instructed to refrain from caffeine consumption 2-hours prior to their laboratory conditions (as specified by

the Canadian Society of Exercise Physiology guidelines [188]) and to record all caffeine intake in their log books. Other than coffee or tea and water, participants were not permitted to consume any calorie containing or non-calorie containing beverages during the standardized meal days, unless these were included as a part of their standardized meal (e.g. milk). Participants were provided with a food log which they filled out to confirm adherence to the standardized meals. They were asked to record the time of each meal and snack, as well as any alterations they made to the standardized diets. For example, if a participant did not eat a snack, they were required to record this in their log book. Other methods for recording the standardized meals could include smartphone applications and the use of photographs to document meals sizes. The more traditional food log approach was used in order to keep things simple for all participants, particularly those who may not be familiar with newer technology.

3.6 Walking & Seated Control Conditions

Three to 5 hours after lunch on days 2 and 5 participants came into the laboratory to complete a standardized bout of 50-minutes of walking (5.0 km/hr and 0.5% incline or about ~3.5 metabolic equivalents [METs] according to the Compendium of Physical Activities [189]), or 50-minutes of sitting. Walking was chosen to represent the typical physical activity prescription for prevention and treatment of T2D and its complications: 150 minutes per week of moderate [3-5.9 METs] to vigorous physical activity performed over at least 3 days of the week [22]. A standardized 5-minute warm-up and cool-down was included at a pace of 3.5 km/hr and 0% grade. If a participant could not comfortably complete the walking session at this intensity, they

were permitted to reduce the speed accordingly and this was recorded. Heart rate (Polar HR monitor) and ratings of perceived exertion (RPE) using the Borg scale were monitored during exercise and recorded every 5 minutes.

Indirect calorimetry was completed (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah, USA) from minutes 5-10 and 40-45 during both the walking and seated control conditions. This allowed for data collection on respiratory exchange ratio (RER), METs, volume of oxygen consumed (VO_2) and volume of carbon dioxide expired (CO_2). Data from the metabolic cart was averaged for each of the 5 minute sessions to quantify participant work rate (i.e. METs) and RER to estimate non-protein substrate utilization (i.e. reliance on fats or carbohydrates). Blood pressure and capillary glucose were assessed before and after each of the sessions. A standardized dinner (as described above) was provided in the laboratory 20-minutes after the completion of each condition before the participant was allowed to leave.

3.7 Statistical Analysis

Hypothesis 1:

Null Hypothesis (H_0): A single bout of walking will have no effect on 24-hour mean glucose concentrations as assessed by CGM in individuals with T2D.

Alternative Hypothesis (H_1): A single bout of walking will lower 24-hour mean glucose concentrations as assessed by CGM in individuals with T2D.

Hypothesis 2:

Null Hypothesis (H_0): The effect of a single bout of exercise on glycemic control will be the same between males and females.

Alternative Hypothesis (H_1): Males will have greater improvements in glycemic control when compared to females.

Data from the CGM were compared between the 24-hour periods, which started immediately prior to the exercise or seated control conditions. Specifically, the 24-hour period commenced at the initiation (minute 0) of the exercise and seated control. In addition to the primary outcomes of 24-hour mean glucose, secondary outcomes of interest included:

- 2-hour post meal area under the glucose curve
- Fasting glucose (defined by DC as at least 8 hours without consuming food)
- Time spent above 10 mmol/L
- Time spent below 4 mmol/L
- Glycemic variability (as measured by MAGE)
- 50-minute mean glucose during exercise

To simultaneously examine hypothesis 1 and 2, a repeated-measures two-way factorial analysis of variance (ANOVA) was performed through the Statistical Package for Social Sciences (SPSS) software version 24.0. The first within-subject factor compared exercise versus seated control (repeated measures) and the second between-subject factor compared males versus

females. The interaction between factors examined if males and females responded in a similar way to exercise.

Prior to conducting a statistical test, it is important to examine the criteria for using the test. Criteria for utilizing an ANOVA include: 1) data are drawn from a normally distributed population, 2) variance within each of the groups is similar, 3) the sample is randomly selected, and 4) the data are absolute, interval, or ratio [190]. Normality assesses whether the dataset is modelled to a normal distribution and can be done by testing it against the null hypothesis (normal distribution). Data was assessed for heterogeneity of variance (Levene's Statistic) and normality of residuals (Shapiro-Wilk). The analysis was also repeated adjusting for covariates or confounders such as the condition order (exercise 1st vs. control 1st), medication (metformin vs. no metformin), and BMI. The unadjusted values are presented unless otherwise stated.

The alpha level was set at 0.05 which is considered liberal (i.e. more liberal than an α-level of 0.01), and was chosen as this study is using a two-tailed test and is somewhat exploratory in nature. Due to the heterogeneity in the literature, we are not as confident in the outcome when compared to conducting a phase III clinical trial for example, where preliminary studies have already been conducted on the topic of research. In choosing this alpha level the chance of committing a type 1 error is 5%. A type 1 error would occur if we rejected the H_o when we should have accepted it. In other words, if we stated that a difference existed when in reality, no difference existed. All results are presented as mean values ±SD.

Sample size was determined a priori. In order to detect a clinically significant difference (1 mmol/L) in 24-hour mean glucose between males and females with a conservatively large SD of 1.5, a sample size of 36 males and 36 females would be required providing 80% power to detect a significant difference with an alpha of 0.05 . Since this was not feasible, a sample size of 20 was determined sufficient due to the exploratory nature of the study design and since this sample size provided 80% power to detect a main effect of exercise versus control. Since this study was underpowered to examine sex differences, the terms “trend” and “tend” were used in instances when a p-value was between 0.05 and 0.09.

CHAPTER 4 – RESULTS

Twenty-nine individuals diagnosed with T2D were screened between May 2016 and February 2017 for inclusion in the E-PAraDiGM protocol at the University of Alberta, with 20 of these individuals completing the full protocol. Reasons for ineligibility were weight loss >5 pounds in the last three months (n=2), inability to adhere to study requirements, particularly the dietary component (n=2), drop-out before study completion (n=3), and medical reasons (n=2).

Participants included in this analysis were diagnosed with T2D for 9.3 ±6.9 years and included 11 males and 9 females. Age and baseline A1c of participants was 61.9 ±9.1 years and 6.8% ±0.7 %. Males were taller than females (176.0 ±7.0 vs 161.4 ±10.6 cm, p=0.002) and had higher serum creatinine (91.2 ±14.4 vs 63.8 ±13.7 umol/L, p=0.001). Female participants who were menstruating (n=1) completed the protocol during the follicular phase of their menstrual cycle. The remaining female participants (n=8) were post-menopausal (9.8 ±5.7 years). No participants were treated with insulin but many were treated with an oral hypoglycemic agent (n=17). Three participants were not taking any oral hypoglycemic agents and controlled their diabetes through diet and exercise. Participants had no changes in diabetes medications within the last 3 months. Descriptive characteristics of participants are listed in Tables 4 and 5.

Table 4. Characteristics of E-PAraDiGM Participants

Baseline Characteristics	Males (n=11) mean±SD	Females (n=9) mean±SD	All (n=20) mean ±SD	p-value (M vs F)
Time since diagnosis, yrs	7.8 ±5.4)	11.1 ±8.4	9.3 ±6.9	0.295
Age, yrs	62.8 ±10.7	60.7 ±7.1	61.9 ±9.1	0.611
Weight, kg	89.6 ±21.6	71.6 ±18.1	81.5 ±21.6	0.062
Height, cm	176.0 ±7.0	161.4 ±10.6	169.4 ±11.3	*0.002
◊Body fat (%)	26.0 ±8.3	33.9 ±9.3	29.8 ±9.5	0.069
◊FFM	63.0 ±9.3	45.3 ±8.6	54.1 ±12.6	0.730
◊FM	27.2 ±15.3	23.8 ±11.1	25.5 ±13.0	0.331
BMI (kg/m^2)	29.0 ±7.1	27.3 ±6.0	28.2 ±6.5	0.589
WC, cm	102.6 ±16.7	96.8 ±14.6	100 ±15.7	0.430
HC, cm	105.6 ±14.4	105.8 ±13.3	105.7 ±13.5	0.976
Waist-hip-ratio (%)	0.99 ±0.1	0.91 ±0.0	0.94 ±0.1	*0.024
SBP, mmHg	131 ±10	128 ±14	129 ±11	0.571
DBP, mmHg	79 ±7	73 ±10	76 ±9	0.132
RHR, bpm	74 ±13	69 ±8	71 ±11	0.310
A1c, %	6.7 ±0.6	6.9 ±0.8	6.8 ±0.7	0.527
HDL mmol/L	1.27 ±0.4	1.51 ±0.4	1.37 ±0.4	0.208
LDL, mmol/L	2.47 ±0.8	2.39 ±0.9	2.44 ±0.8	0.836
TC, mmol/L	4.37 ±1.1	4.49 ±1.3	4.42 ±1.1	0.824
TG, mmol/L	1.60 ±0.8	1.36 ±0.5	1.50 ±0.7	0.462
Creatinine, umol/L	91.2 ±14.4	63.8 ±13.7	79.6 ±19.5	*0.001
GLTEQ Score	44 ±20	37 ±9	41 ±17	0.364
PHQ-8 Score	1.6 ±1.3	1.7 ±2.8	1.8 ±2.0	0.619
PSQI Score	4.4±1.8	5.6±2.5	4.9±2.1	0.470

SD=standard deviation, M=males, F=females, yrs=years, kg=kilograms, cm=centimeters, FFM=fat free mass, FM=fat mass, BMI=body mass index, WC=waist circumference, HC=hip circumference, SBP=systolic blood pressure, DBP=diastolic blood pressure, mmHg=millimeters of mercury, RHR=resting heart rate, bpm=beats per minute, A1c=glycated hemoglobin, HDL=high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TC=total cholesterol, TG=triglycerides, mmol/L=millimole per litre, umol/L = micromole per litre, GLTEQ = Godin Leisure Time and Exercise Questionnaire (physical activity), PHQ-8 = Patient Health Questionnaire , PSQI = Pittsburgh Sleep Quality Index. ◊Body Fat (%): n=10 for males.

Table 5. Medications of E-PAraDiGM Participants

Medication	Males (n=11)	Females (n=9)
Metformin Alone	7	3
Metformin + Other	2	5
No Hypoglycemic Agent	2	1
Blood Pressure Medication	8	6
Lipid/Cholesterol Lowering Agent	4	6

4.1 Dietary Intake

The overall average macronutrient distribution of all standardized meals combined fit within 5% of the target of 55% carbohydrates, 30% fats and 15% proteins for both sexes. There was a difference in the carbohydrate macronutrient distribution administered to males and females (males = 53.0 ±1.5%, females = 55.3 ±2.5%, p=0.025), but there were no differences in fat and protein macronutrient distribution. Since participants consumed slightly different meals on days 2 and 5 compared to days 3 and 6, macronutrient breakdown between these days were assessed. Females consumed less fat than males on days 3 and 6, Table 6. Meals were also examined separately, and males consumed more protein at breakfast and dinner compared to females, Table 6.

The estimated daily mean caloric needs based off the Harris Benedict equation and physical activity factor were 2210 ±451 kcal (males = 2455 ±416 kcal, females =1911 ±288 kcal), Table 6. The actual daily mean caloric content administered to participants was 2178±437, 32 kcals lower than the predicted value, $p<0.0001$. On average, males received 2451±393 (4 kcal

less than the predicted value, $p<0.0001$) and females 1874±244) (37 kcal less than the predicted value, $p<0.0001$). There was a statistically significant difference of kcal administered between the sexes (males and females) ($p=0.001$). Details of the standardized meals can be found in Table 6 and sample menus can be found in Appendix D

4.2 Exercise & Seated Control Conditions

Two participants (one male and one female) were unable to sustain the predetermined speed of 5.0 km/hr for the duration of the 50-minute session. One participant completed the warm-up at a speed of 3.2 km/hr. Speed was then increased to the set 5.0 km/hr at minute 5, but was reduced to 4 km/hr at minute 6. Speed was reduced further to 3.5 km/hr at minute 15 and the cool-down was completed at 3.0 km/hr. The second participant had the speed reduced to 4.5 km/hr at minute 22. Indirect calorimetry was completed for two separate 5-minute periods during each of the sessions, for a total of 10 minutes each session (i.e. during minutes 5-10 and 40-45). Details of the exercise and seated control sessions can be found in Table 7.

4.3 Twenty-four-hour Mean Glucose and 50-minute Mean Exercise Glucose

No differences were found in 24-hour mean glucose between the exercise and seated control conditions ($n=20$, exercise 7.0 ±1.6 mmol/L, seated control 7.2 ±1.5 mmol/L, $p=0.343$) with the intra-individual difference between the exercise and control conditions ranging between -1.7 and +2.0 mmol/L. In addition, there was no difference between sexes (males vs. females)

(p=0.265) and there was no exercise by sex interaction (p=0.300). Figure 9 includes details of 24-hour mean glucose concentrations following each condition.

Fifty-minute mean glucose concentrations during the exercise and seated control conditions were different (exercise 6.4 ±1.5 mmol/L, seated control 7.3±1.6 mmol/L, p<0.0001). There was a difference between sexes (males 7.0 ±1.5 mmol/L, females 5.6 ±1.0 mmol/L, p<0.0001) and there was no exercise by sex interaction (p=0.250). Figure 10 includes details of 50-minute mean glucose concentrations between the seated control and exercise conditions.

4.4 Fasting Glucose

No differences were found in fasting glucose between the exercise and control conditions (exercise 6.7 ±1.5 mmol/L, control 6.8 ±1.7 mmol/L, p=0.565) or between sexes (p=0.417). In addition, there was no exercise by sex interaction (p=0.252). Details of fasting glucose can be found in Figure 11.

4.5 2-hour Area Under the Curve Postprandial Glucose

When all meals were analyzed together (i.e. the 2-hour postprandial glucose area under the curve was averaged from the dinner, breakfast, and lunch following the exercise and control conditions) no difference was found between the exercise and control conditions (exercise 8.2 ±2.1 mmol/L, control 8.5 ±2.1 mmol/L, p=0.140). Furthermore, there was no difference between

sexes ($p=0.097$) and there was no exercise by sex interaction ($p=0.119$). Details of postprandial glucose can be found in Figures 12 and 13.

Table 6 Details of Standardized Meals

Variable	Males (n=10) mean±SD	Females (n=9) mean±SD	All (n=20) mean±SD	p-value (M vs F)
Daily Caloric Content	2451±393	1874±244	2178±437	*0.001
Average Grams of Carbohydrates Per Day	314 ±57	253 ±33	285 ±56	0.158
Average Grams of Fat Per Day	94 ±18	66 ±13	81 ±21	0.796
Average Grams of Protein Per Day	98 ±23	78 ±11	89 ±21	0.382
Overall Average Macronutrient Distribution				
Carbohydrates, %	53.0 ±1.5	55.3 ±2.5	54.1 ±2.3	*0.025
Fat, %	29.2 ±3.7	27.5 ±1.6	28.4 ±2.9	0.231
Protein, %	16.0 ±2.2	14.7 ±2.1	15.4 ±2.2	0.209
Average Macronutrient Distribution: Day 2 & 5				
Carbohydrates, %	52.9 ±2.1	54.9 ±3.0	53.8 ±2.7	0.253
Fat, %	28.8 ±3.5	27.8 ±2.5	28.3 ±3.0	0.197
Protein, %	16.3 ±2.3	15.1 ±2.7	15.7 ±2.5	0.711
Average Macronutrient Distribution: Day 3 & 6				
Carbohydrates, %	53.1 ±1.9	55.8 ±2.4	54.4 ±2.5	0.233
Fat, %	29.5 ±4.2	27.2 ±1.2	28.4 ±3.3	*0.026
Protein, %	15.7 ±2.2	14.3 ±1.9	15.1 ±2.1	0.691
Individual Meal Macronutrient Distribution				
CARBOHYDRATES				
Breakfast, %	65.0±3.7	64.0±7.2	64.5±5.5	0.719
Lunch, %	62.6±9.2	57.1±14.3	60.0±11.9	0.328
Dinner, %	33.1±9.5	40.8±13.7	36.7±12.0	0.164
FATS				
Breakfast, %	15.2±4.2	19.4±8.1	17.2±6.5	0.162
Lunch, %	24.4±10.4	28.2±10.6	26.2±10.4	0.440
Dinner, %	43.3±10.5	38.1±11.7	40.8±11.1	0.323
PROTEIN				
Breakfast, %	17.8±2.6	13.7±3.5	15.8±3.7	*0.010
Lunch, %	11.1±2.9	12.9±4.2	11.9±3.6	0.291
Dinner, %	22.1±2.8	17.6±2.7	19.9±3.6	*0.002

SD=Standard Deviation, M=Males, F=Females

Table 7. Details of Exercise and Seated Control Conditions

Variable	Males (n=11) mean±SD	Females (n=9) mean±SD	All (n=20) mean±SD	Ex vs Crtl	M vs F	Ex by Sex
METs,						
EX	3.8 ±0.39	4.1± 0.38	3.9 ±0.4	*<0.0001	0.425	0.112
CRTL	1.1 ±0.50	1.0 ±1.9	1.1 ±0.4			
VO_2, L/min						
EX	1.2 ±0.29	1.0 ±0.24	1.08 ±0.27	*<0.0001	0.268	0.611
CRTL	0.33 ±0.19	0.24 ±0.05	0.29 ±0.14			
RER						
EX	0.85 ±0.04	0.83 ±0.03	0.84 ±0.04	0.489	0.577	0.839
CRTL	0.87 ±0.08	0.84 ±0.09	0.85 ±0.08			
CAPILLARY GLUCOSE						
PRE EX	7.7±1.7	6.3±1.3	7.1±1.7	NA	0.513	NA
POST EX	6.6±1.7	5.5±1.2	6.1±1.6		0.297	
HR, bpm						
EX	104 ±17	113 ±13	108 ±16	NA	0.208	NA
RPE, borg						
EX	9 ±2.7	11 ±1.7	10 ±2.5	NA	0.556	NA
Step count						
EX	10308 ±1767	11529 ±4041	10886 ±3036	*0.008	0.700	0.076
CRTL	9405 ±3262	7771 ±1650	8631 ±2689			

SD=standard deviation, M=males, F=females, Ex=exercise condition, Crtl=control condition, METs=metabolic equivalents, VO_2=volume of oxygen consumed, L/min=liters per minute, RER=respiratory exchange ratio, HR=heart rate, bpm=beats per minute, RPE=rate of perceived exertion, NA=not applicable

4.6 Time spent above 10 mmol/L and below 4 mmol/L

No difference was found between the exercise and control condition on time spent above 10 mmol/L (exercise 133 ±198 mins, control 172±224 mins, p=0.365). Likewise, no differences were found between sexes (p=0.389) and there was no exercise by sex interaction (p=0.328). No difference was found between the exercise and control condition on time spent below 4 mmol/L

(exercise 55 ±210 mins, control 60±152 mins, p=0.890). No difference was found between sexes (p=0.248) and there was no exercise by sex interaction (p=0.185). Details of time spent above 10 mmol/L and below 4 mmol/L can be found in Figures 14 and 15, respectively.

4.7 Mean Amplitude of Glycemic Excursions

No difference was found in MAGE between the exercise and control condition (exercise 3.9 ±1.4, control 4.4 ±2.1, p=0.330). No difference was found between sexes (p=0.469) and there was no exercise by sex interaction (0.305). Figure 16 includes details of MAGE during the exercise and control conditions.

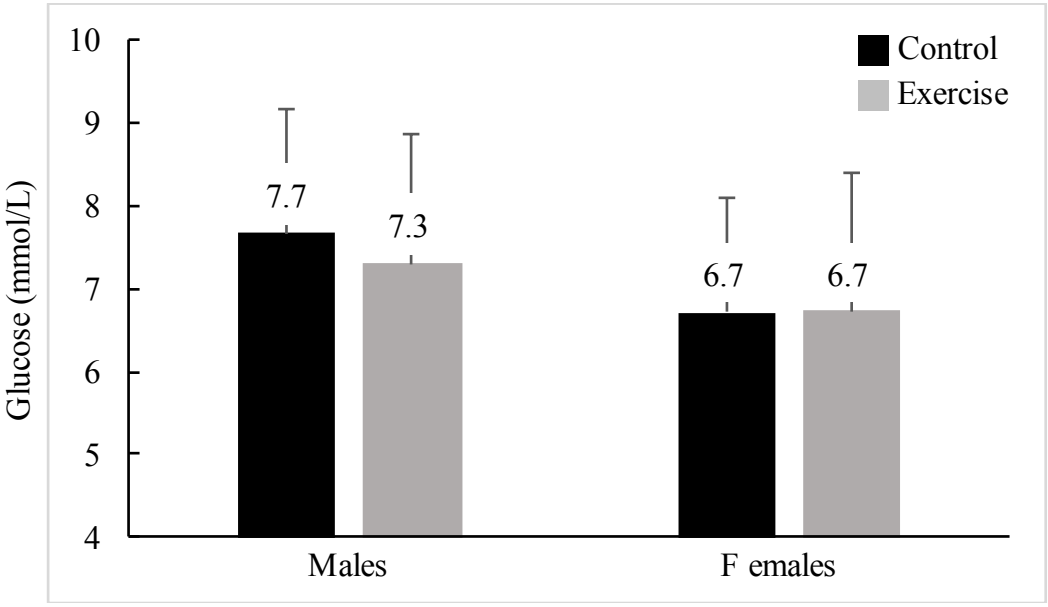


Figure 9. Twenty-four hour mean glucose following the seated control and exercise conditions

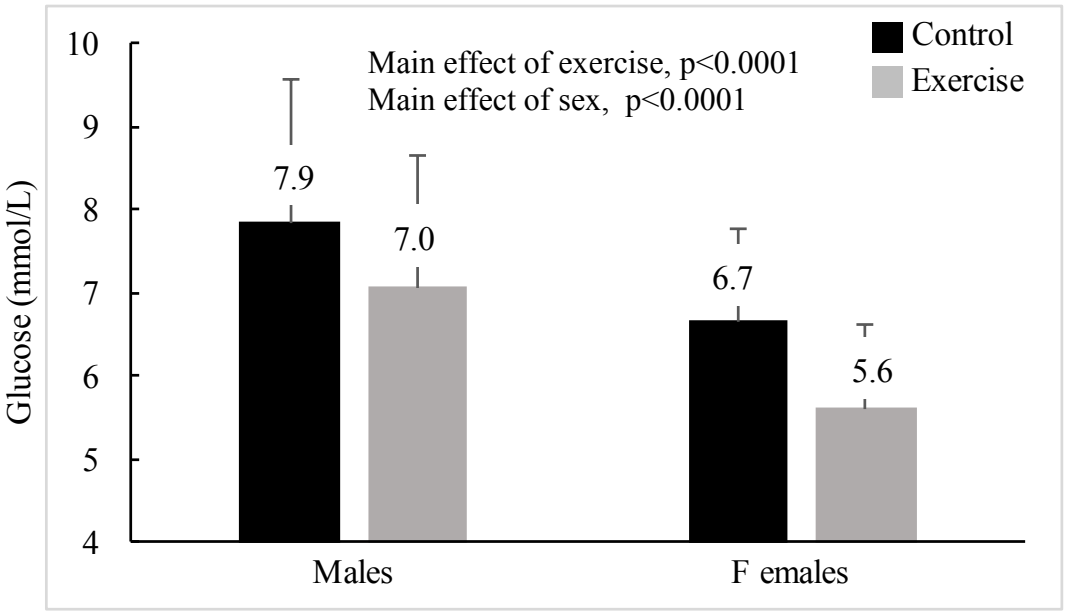


Figure 10. Mean glucose during the fifty-minute seated control and exercise conditions

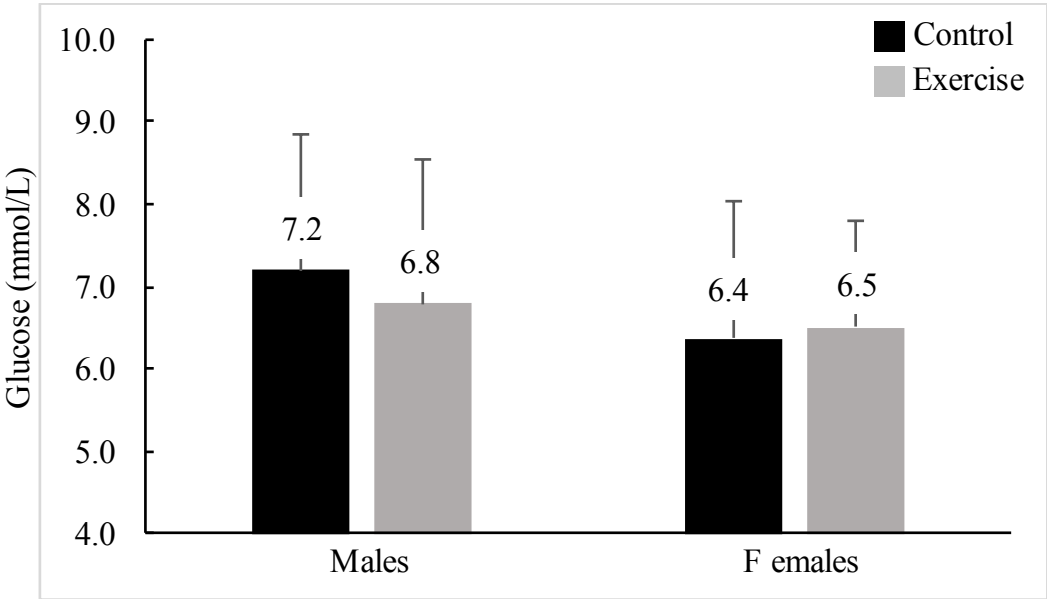


Figure 11. Fasting glucose following the seated control and exercise conditions

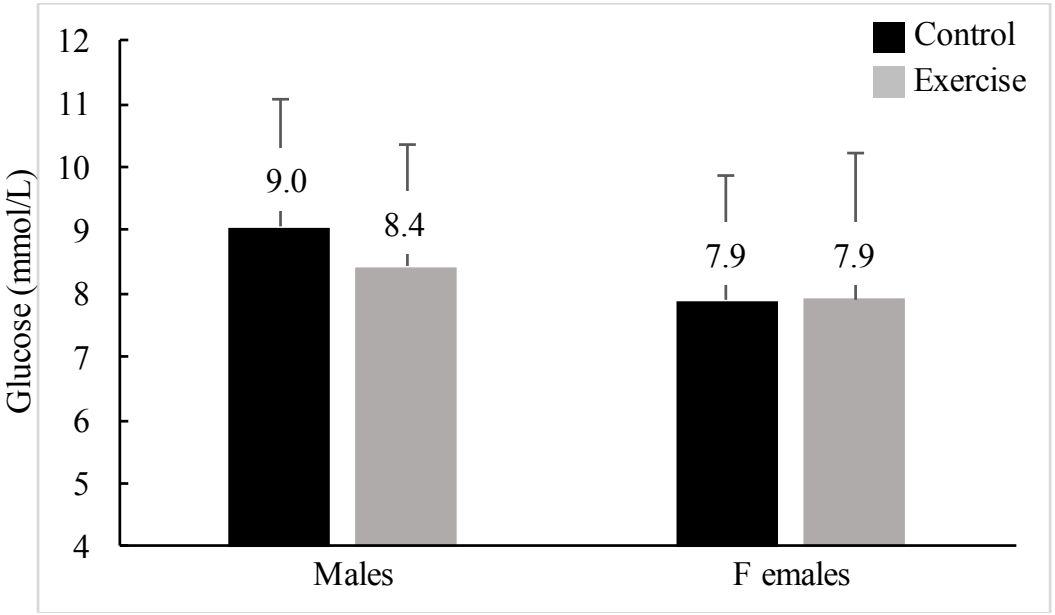
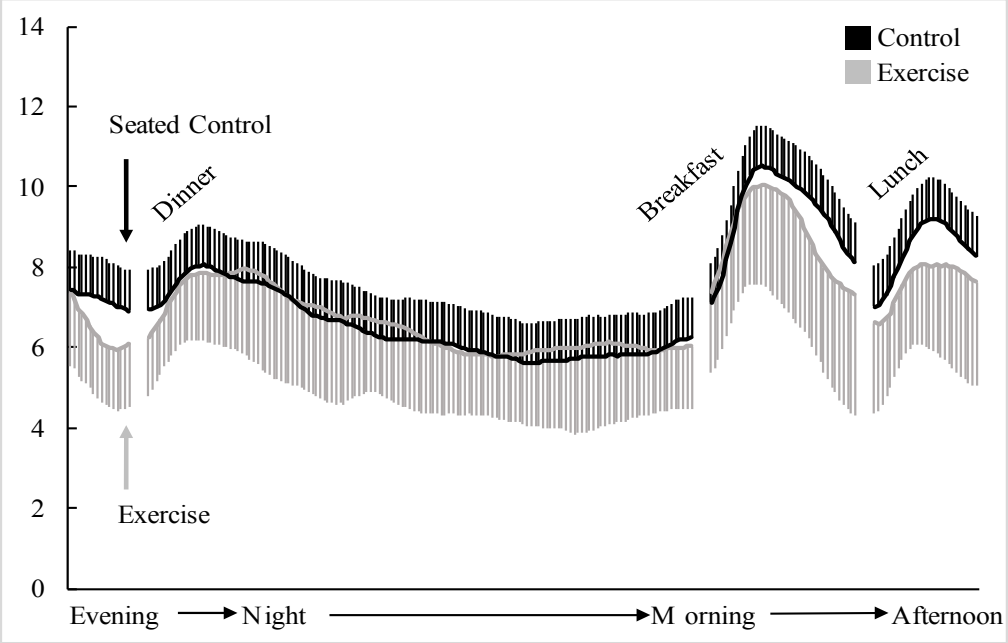
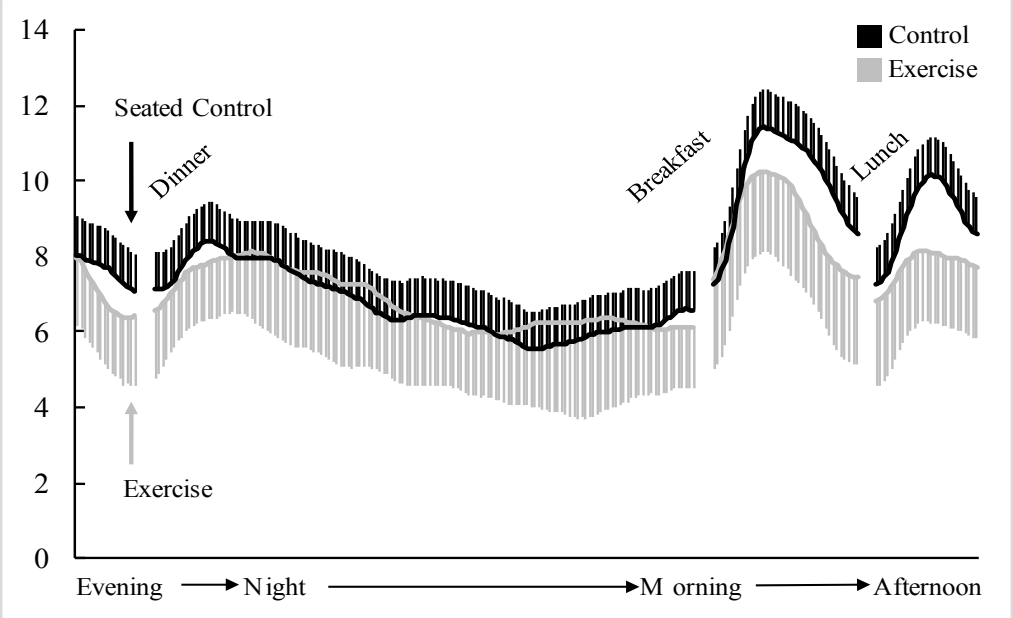


Figure 12. Average postprandial glucose (dinner, breakfast, lunch) following the seated control and exercise conditions

A. Twenty-four hour continuous glucose monitor tracing in all participants



B. Twenty-four hour continuous glucose monitor tracing in males



C. Twenty-four hour continuous glucose monitor tracing in females

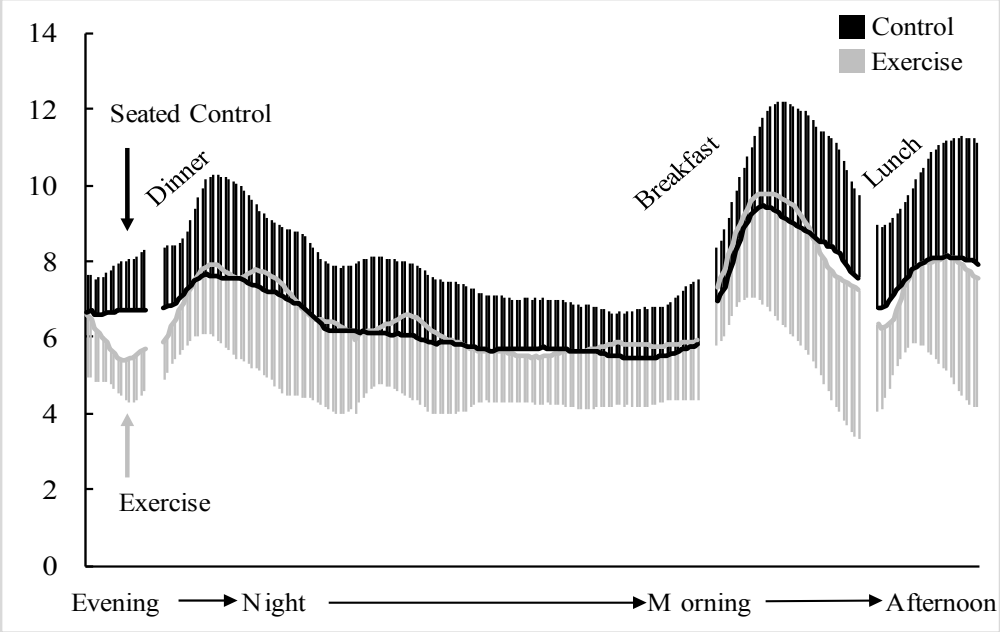


Figure 13. Twenty-four hour continuous glucose monitor tracing in E-PAraDiGM participants, starting at the initiation of the exercise and seated control conditions

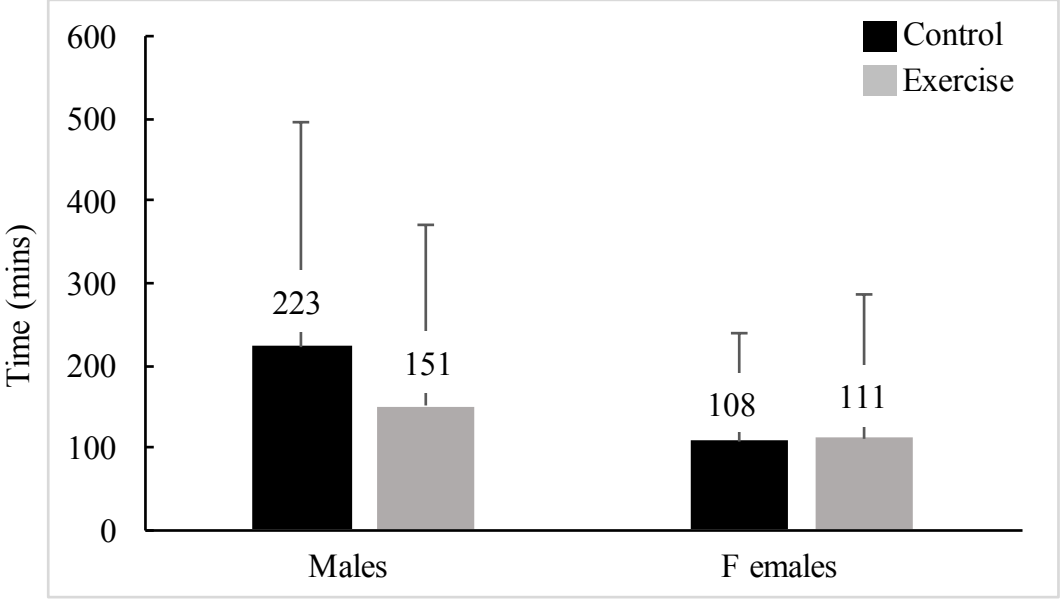


Figure 14. Time spent above 10 mmol/L following the seated control and exercise conditions

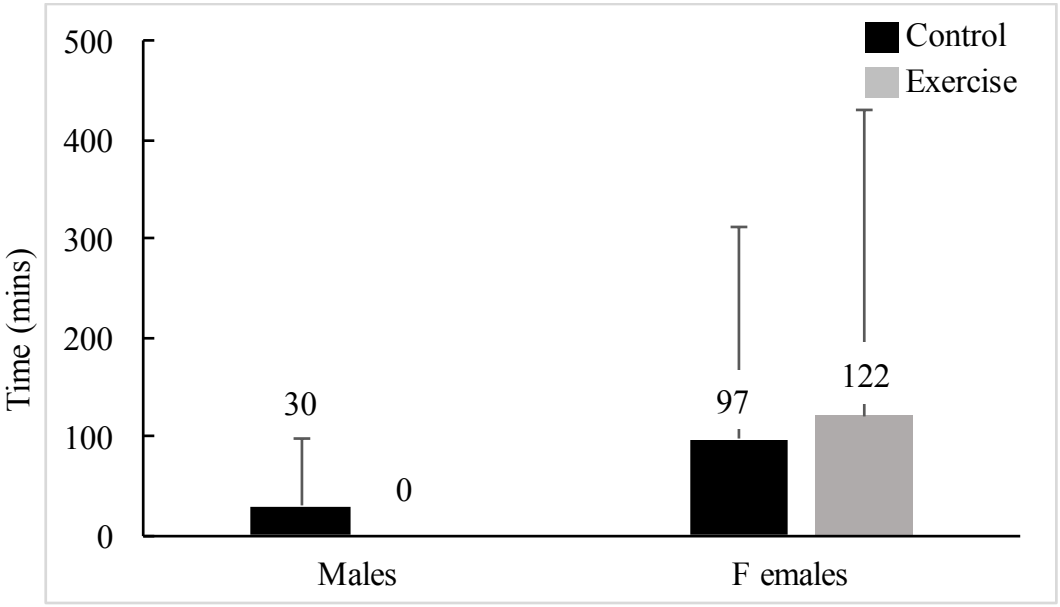


Figure 15. Time spent below 4 mmol/L following the seated control and exercise conditions

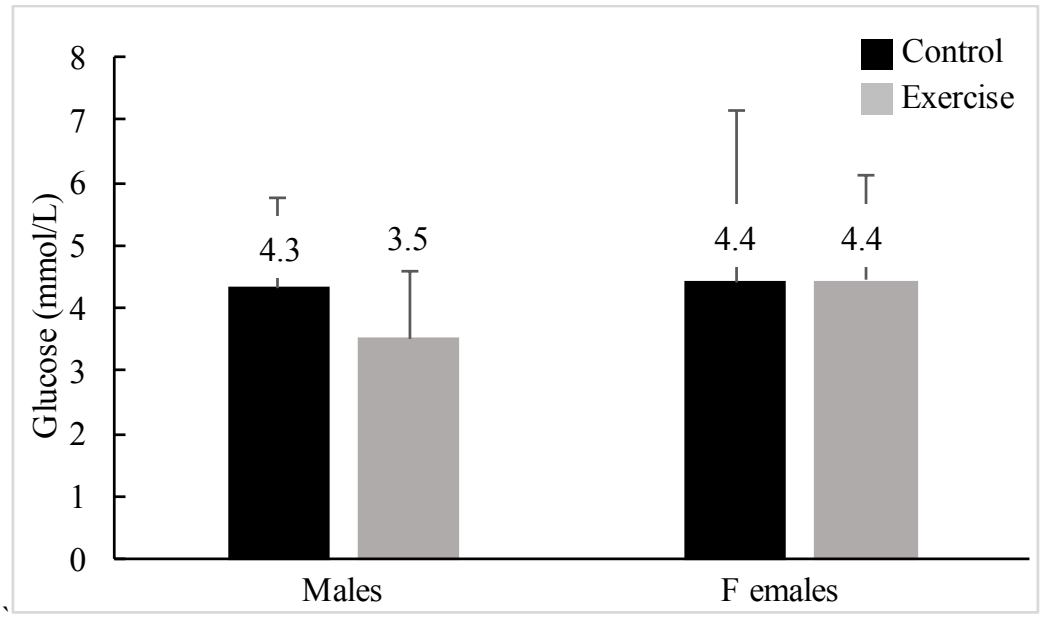


Figure 16. Mean amplitude of glycemic excursions following the seated control and exercise conditions

CHAPTER 5 – DISCUSSION

This is the first exercise study using CGM to examine sex specific differences in responses to an acute bout of aerobic exercise. The primary finding from this study was that no differences in 24-hour mean glucose were observed between males and females in response to the same bout of exercise. However, females exhibited lower average glucose during the 50-minute exercise session. Unlike previous studies [11] and contrary to our hypothesis, exercise did not decrease 24-hour mean glucose concentrations. Two-hour postprandial glucose, time spent above 10 mmol/L and below 4 mmol/L, and fasting glucose were also not improved following an acute bout of exercise. Moreover, glycemic variability was not affected by an acute bout of exercise.

Contrary to previous findings [11], 24-hour mean glucose and time spent above 10.0 mmol/L were not affected by a single bout of aerobic exercise. In the meta-analysis by MacLeod et al, 10 groups from 8 studies were included in the analysis of 24-hour mean glucose concentrations following a single bout of aerobic exercise, with a main effect of -0.8 mmol/L (-0.6 mmol/L greater reduction then our findings) and values ranging from -0.3 to -1.7 mmol/L. Eight groups from 5 studies were included in the analysis of time spent above 10 mmol/L with an average reduction of 119 minutes (81 minutes greater reduction than our findings) and values ranging from -74 minutes to -247. Despite similarities between the E-PAraDiGM protocol and studies included in the meta-analysis (e.g. most studies had an exercise duration between 45 – 60

minutes), differences existed between the E-PAraDiGM protocol and the studies included with regards to type of exercise (continuous walking vs. continuous cycling or cycling intervals), exercise time of day (afternoon vs. morning) and exercise intensity [11]. See Table 8 for details on differences in exercise protocols.

In terms of exercise timing, exercise completed in the postprandial state, such as after breakfast, has been shown to improve glycaemia compared to exercise completed in the post-absorptive state [191] . This could be a potential factor influencing the E-PAraDiGM findings with our exercise protocol completed 3-5 hours post lunch, while the majority of previous exercise studies have often completed exercise sessions in the more proximal postprandial period [11] . For example, 8 out of the 10 groups included in the MacLeod et al. meta-analysis completed exercise soon after breakfast (between 1 and 2.5 hours after), and 2 studies did not specify. It is possible that exercise completed in the early postprandial state may have contributed to greater reductions in total time spent above 10 mmol/L and postprandial glucose. Although it should be noted that the glucose lowering effect of exercise in the postprandial state is thought not to persist to the following meal [192] and would therefore likely have minimal effects on 24-hour mean glucose concentrations. More recently, the timing of exercise around meals has been more thoroughly examined in healthy individuals [193], yet literature in a T2D population remains largely undocumented and is a pertinent area of interest. Despite some studies showing improvements in glycemic control in the postprandial state [191, 194], it is important to take other aspects into consideration. For example, it has been shown that exercise training completed in the fasted state

may lead to increased mitochondrial biogenesis and ultimately improvements in insulin sensitivity over time [195]. This is an important factor to consider as the E-PAraDiGM moves forward, and could be a potential area to expand the protocol.

Table 8. Protocol Differences in Acute Aerobic Exercise Studies Utilizing Continuous Glucose Monitors

Author, yr	Sample Size (m/f)	Exercise Mode	Exercise Duration, mins	Exercise Intensity	Exercise Time
E-PAraDiGM	**20 (11, 9)**	**Walking**	**50**	**~3.5 METs**	**3-5 hrs post lunch**
Gillen et al, 2012	7 (NR)	Cycling, HIT	20 x 1 (1 min rest)	90% Wmax 80%Wmax	1.5 hrs post breakfast
Van Dijk et al, 2012	30 (30/0)	Cycling	60	50% Wmax	1.5 hrs post breakfast
Van Dijk et al, 2012	30 (30/0)	Cycling or Resistance	45	50% Wmax or 55, 65, 75% 1RM	2.5 hrs post breakfast
Manders et al, 2010	9 (9/0)	Cycling	60 or 30	35%Wmax or 70%Wmax	1 hr post breakfast
Mikus et al, 2012	13 (8/5)	Cycling, Walking	60	60-75% HRR	NR
MacDonald et al, 2006	6 (5/1)	Cycling	60	90% LT	9:00 am (meal time NR)

yr=year, m=males, f=females, METs=metabolic equivalent, hrs=hours, NR=not reported, HIT=high intensity training, min=minutes, HR=heart rate, W=watts, RM=repetition maximum, HRR=heart rate reserve

During fasted exercise, substrate utilization may shift to an increased reliance on fat oxidation, and increased breakdown and oxidation of intramyocellular triglycerides [196] . This may be central to diabetes management given the fact that decreased intramyocellular

triglyceride oxidation may lead to insulin resistance [197]. Since the cumulative effects of successive bouts of exercise may ultimately lead to long term improvements in glucose control [198] further examination of exercise timing and its effects on glycemic control in the 24-hours following is essential. This may ultimately lead to greater reductions in important indicators of metabolic health (i.e. A1c).

Exercise intensity and mode may also be factors contributing to the conflicting results in many of our outcome variables. The E-PAraDiGM protocol aimed to capture exercise that was likely to occur in free-living conditions. Since walking is the preferred form of exercise and is often recommended for individuals with T2D [15], it was chosen with the objective of attaining a moderate intensity (~3.5 METs) to fit with DC guidelines [6]. The METs attained by participants during the exercise condition reached an average of 3.9 ±0.4 (0.4 METs higher than our predicted value), as measured by indirect calorimetry. This higher than predicted MET value could be attributed to basing our prediction off the 'factorial method' which is based on the assumption that a male of 70 kg and ~40 years of age consumes ~3.5 ml of O_2/kg/min at rest [199]. Despite its convenience and widespread utility in exercise prescription, this method has been found to underestimate the energy cost of walking in individuals with increased fat mass [199]. Despite this slightly higher intensity, the walking condition still fit into the moderate intensity category of 3.0- <6.0 METs [200] and within the recommended range [6].

Previous acute exercise studies have typically varied in terms of exercise intensity with ranges between 35% of maximum power output [201] to 90% maximum power output [202] and have often used cycling protocols [11]. High intensity exercise studies are a newer avenue of research demonstrating a promising method for improving insulin sensitivity in a T2D population [203]. Gillen et al. utilized an acute high intensity exercise protocol and found significant decreases in postprandial glucose and time spent in hyperglycemia, but not 24-hour mean glucose [204], while Little et al. , also using an acute high intensity protocol, found decreases in both postprandial glucose levels and 24-hour mean glucose [205]. Overall, these studies support the efficacy of high intensity exercise on different aspects of glycemic control and may suggest that the intensity utilized in the E-PAraDiGM protocol was not sufficient to elicit the insulin sensitizing effect of exercise. Although, even at a low intensity (35% maximum power out-put on a cycle ergometer), 60 minutes of continuous cycling was found to reduce 24-hour mean glucose levels and time spent above 10mmol/L, while the high-intensity group (70% maximum power out-put on a cycle ergometer for 30 minutes) did not [201].

It is therefore difficult to conclude whether the chosen exercise intensity was a contributing factor to the minimal differences observed in 24-hour mean glucose and other outcome variables such as postprandial glucose and time spent above 10 mmol/L. Both exercise intensity and mode of exercise should be considered in future exercise studies in order to determine if the recommended DC guidelines and walking recommendations are indeed sufficient to elicit improvements in an individual's glucose profile in the 24-hour period

following. The feasibility of high intensity exercise (i.e. level of motivation, risk of injury, risk of cardiovascular event, access to equipment such as cycle ergometers) must be taken into consideration [206].

In addition to variables such as exercise time of day, mode of exercise and exercise intensity, it is important to examine the populations used in each of these acute exercise studies. Previous studies have typically been male dominated. In the meta-analysis by MacLeod et al, only 6 women were included in the sample size of 144 individuals (121 males, 17 not reported) [11]. Interestingly, in the study that included females (males = 8, females = 5), 24-hour mean glucose was not improved following exercise [207] , while many of the studies including only males found improvements in 24-hour mean glucose [201, 208]. Since males tended to have improved glycemic control following exercise in our study (-0.4 mmol/L, p=0.062) but females did not (0.0 mmol/L, p=0.962), it may be possible that findings from previous studies overestimated the effect of exercise on glycemic control when we consider the population as a homogenous group. Caution must be taken when interpreting our results due to the small sample size. It should also be recognized that improvements were observed in postprandial glucose and glucose variability in the study by Mikus et al. including female participants, and this was not observed in the E-PAraDiGM findings.

Another interesting finding from this study was 50-minute mean glucose concentrations during the exercise and seated control conditions. The main effect of exercise suggests that

glucose was lower during the exercise versus control condition and that overall, glucose was lower for women. The lower levels of glucose seen in females may be due to a reduced counterregulatory response [139, 209], and therefore lower hepatic glucose output. It could be argued that males should experience a greater reduction in glucose during exercise as they tend to metabolize a higher percentage of carbohydrates when compared to females [145]. This is not supported by results from the indirect calorimetry which indicate no differences in substrate utilization between sexes ($p=0.577$). The lower level of glucose seen in the females may therefore be attributed to a reduced counterregulatory response.

Overall, males tended to respond better to exercise than females in all outcome variables except 50-minute mean glucose during the exercise and seated control conditions. Despite minimal statistical differences between sexes, it remains important to investigate the possible mechanisms underlying this trend. There may be a number of factors, such as baseline glucose control, medication use, and outliers contributing to our results.

Firstly, it should be noted that females may have had better glycemic control upon entry into the study. Despite males responding more favourably to exercise, females often exhibited lower glucose levels than males throughout the study. For example, males spent an average of 223 minutes above 10mmol/L following the seated control condition whereas females spent, on average, only 108 minutes in this range. In addition, it was also more common (but not statistically significant) for females to be on a combination of oral hypoglycemic agents versus

just one oral hypoglycemic agent as seen more commonly in the males. Taken together, females may have had better glycemic control upon entry into the study, thus minimal room for improvements, although this is not supported by baseline A1c levels (males = 6.7%, females =6.9%). These A1c values appear counterintuitive when we consider the lower average glucose levels seen in the females throughout the protocol. This raises the question as to whether males were overfed or whether females were underfed during the study, thus contributing to higher levels of interstitial blood glucose. The predicted mean daily caloric content between males and females was significantly different (p=0.004), as was the actual administered daily caloric content (p=0.001) despite no significant difference in total body weight (p=0.062) and age (p=0.611) (two variables used in the Harris Benedict equation). Height is also used in the Harris Benedict equation and was significantly different, which could have contributed to differences in the predicted and administered caloric content given to males and females. Literature examining the utility of energy expenditure prediction equations have indicated overestimation in obese individuals [186, 210, 211], but literature examining whether this is more pronounced in males or females is lacking. Furthermore, recommendations for which equation to use in a T2D population is lacking. When compared with the Mifflin equation, the Harris Benedict equation estimated an average RMR of 1579 ±322 kcal, whereas the Mifflin [212] equation estimated an average RMR of 1495 ±314 kcal, a 5.3% difference which is relatively small and likely not clinically significant.

Moreover, it should be noted that females tended to experience greater variability in response to the same bout of exercise when compared to males. Changes in 24-hour mean glucose in the exercise versus seated control ranged from -1.7 to 2.0 mmol/L in the females but only ranged from -1.2 to 0.3 mmol/L in the males. Greater variability in females may have contributed to females tending to respond worse than the males. This finding is of interest and deserves further investigation with the inclusion of a larger sample size.

Also to be noted are the similar characteristics (e.g. body composition) displayed by the males and females included in the study. Since 8 out of the 9 females included in analysis were post menopause, this could be a contributing factor to similarities in body composition. For example, results from the BIA analysis indicated that males tended to have decreased body fat compared to females, but there was actually no statistical difference ($p= 0.069$). Furthermore, waist circumference measures were very similar between the sexes ($p=0.430$). It is possible that since most females were post menopause, their distribution of adipose tissue and hormone levels were more similar to the males. This is speculative and would require more in-depth analysis of body composition and the analysis of hormone levels. Future studies are encouraged to include a wider age range of individuals with T2D (i.e. also include females who are pre-menopause when the protective effects of estrogen on insulin sensitivity and adipose tissue distribution may be more prominent in females [129] [126]).

Previous acute exercise studies have demonstrated very little impact on FPG [11]. Fasting hyperglycemia is thought to be closely related to hepatic insulin resistance in individuals with T2D [213,214] . Since exercise more readily affects peripheral insulin sensitivity [113], it is not surprising that a single bout of exercise had no effect on fasting glucose concentrations. Exercise training studies which observe decreases in FPG may be the result of decreases in adipose tissue and hepatic fat content as opposed to the actual effects of exercise [114, 115 215]. It may be of interest that there was no difference between sexes in this outcome, as there is extensive literature supporting a decreased counterregulatory response and decreased hepatic glucose production in females [145], which would therefore suggest lower fasting glucose. Alternatively, males in general exhibit higher levels of VAT and hepatic ectopic fat stores [13, 120]. From this it could be hypothesized, that with exercise training and decreased fat mass, males would experience greater improvements in fasting glucose than females due to greater reductions in ectopic fat stores in the liver, and consequently greater hepatic insulin sensitivity. This is speculative and deserves attention in future exercise training studies.

Literature examining glycemic variability following an acute bout of exercise is limited. One study using a resistance exercise protocol examined glycemic variability (CONGA) in 11 males with T2D and found no difference following the acute bout of exercise [116]. Another study found reductions in glycemic variability, but only when both aerobic and resistance exercise were combined (rather than aerobic alone) [216]. In addition, a more recent 2-week exercise training study found reductions in glycemic variability following the interval walking condition,

but not in the continuous walking condition [217]. Due to the heterogeneity in the literature in terms of exercise type and population group examined (e.g. insulin dependent vs. independent) further examination of glycemic variability is needed for a better understanding of how exercise may affect this outcome.

Limitations to this study include a small sample size, which may have restricted sex difference analysis and the analysis of inter-individual differences such as medication use and body composition. For example, the power of our study with a sample size of 11 males and 9 females was low (0.08). It is therefore difficult to conclude that exercise has no effect on glycemic control and that there are no sex specific differences due to the low power of the study. Future analysis utilizing the full E-PAraDiGM protocol will account for this limitation with an N=70. In addition to the small sample size, the participants included in analysis may not accurately represent the T2D population as a whole. It is possible, although speculative, that the individuals who volunteered for this exercise study were highly motivated individuals who were already physically active with good glycemic control (e.g. Godin Leisure scores categorized participants as "active" and average A1c was <7.0%). This phenomenon is known as the healthy volunteer, and may have contributed to our limited findings. There were a number of participants (n=9) who expressed interest in the E-PAraDiGM study, but upon follow-up did not complete the protocol due to various reasons such as lack of time, inability to adhere to the standardized diet, and inability to walk for 50-minutes continuously. It is possible that the inclusion of these participants would have made for a more representative sample of the T2D population.

Another limitation to this study is the consumption of standardized outside of the laboratory setting and the use of a prediction equation to estimate RMR. The feasibility of having participants consume all of their meals in the laboratory would require a greater time commitment on both the part of the participants and researchers and this may have ultimately decreased the willingness of participants to complete the study. Therefore, standardization of the two meals preceding both the exercise and seated control conditions as well as the four meals following each of the conditions was reasoned to be sufficient in order to balance the participants level of interest to the study while also controlling for the effect of diet. In addition, the standardization of meals is likely better than the commonly used self-report methods which have many limitations such as individual subjectivity in terms of the type and quantity of food consumed and level of participant motivation [218]. Furthermore, one of the unique aspects of using CGM is that it allows for the analysis of glucose control in a free-living environment. Giving participants their standardized meals to consume at home and work allowed for the assessment of glucose control to take place during 'real-life' scenarios, which may ultimately contribute to increased generalizability of results.

The selected exercise protocol intensity may have been another limitation to this study as it was prescribed according to a fixed walking speed and grade. This represented a different relative exercise intensity for each participant which could have contributed to variability in glycemic response. For example, it is possible that older individuals may have been exercising at a higher relative intensity compared to the younger participants. Females may have also been

exercising at a higher relative intensity, as indicated by their slightly higher HR values during the 50-minutes of walking (not significant). However, walking is the most common activity in people with diabetes [15] and it was a realistic mode and intensity for the majority of participants. If exercise intensity was higher, it is possible that this would not have been attainable for many of the participants, particularly for those that found the 50-minutes of walking to be challenging. The inclusion of indirect calorimetry during the exercise session helped to control for this limitation and allowed for the examination of exercise intensity. Moreover, it should be noted that the exercise protocol is not representative of how all individuals with T2D may partake in physical activity.

The positive effects of long term exercise training on glucose control (i.e. A1c) have been well established [8, 109] and can be largely attributed to improvements in peripheral insulin sensitivity [113] from the cumulative effects of successive acute bouts of exercise. These cumulative effects contribute to increases in GLUT4 expression and SKM mitochondrial capacity [205] and thus improvements in insulin sensitivity. Perhaps less well understood is the variability in glycemic responses following an acute bout of exercise. Results from this study emphasize the need for a better understanding of how glycemic control may vary following an acute bout of exercise, particularly between sexes. Indeed, the results from MacLeod et al [11] were promising, yet consideration must be given to the variation in protocols, exercise intensity and included population groups. Additionally, results from this study, and others including the well-known Look AHEAD trial [219], indicate that exercise may not always lead to improvements in

glycemic control whether it is an acute bout or long term intervention. Additional studies are required to better understand why exercise is successful in improving glycemic control in some instances, while not in others. Moreover, future studies are encouraged to further examine the role of body composition, age, sex, and medication use on variability in glycemic responses.

As a lead site for the E-PAraDiGM study, the University of Alberta acted as the first site to initiate data collection in participants with T2D. The protocol itself proved to be very reasonable for both the researcher and the participants themselves. For example, there were minimal issues with recruitment of participants (males and females) and very few individuals who were unable to complete the protocol (i.e. broad inclusion criteria, so many individuals were not excluded). Moreover, the timing of the laboratory conditions allowed for many individuals who work during the week to easily participate in the study as they were not required to be in the laboratory during the work day. Additionally, the walking protocol was attainable (both mode and intensity) for most individuals, which is a positive finding. This may allow for the E-PAraDiGM protocol to include a wider range of individuals which may result in a more representative sample of the T2D population when compared to previous exercise studies.

Participant compliance to the protocol, including filling out their log books, taking capillary glucose readings, and complying with the standardized meals, was also a success. Only one participant was excluded from analysis due to lack of adherence to the standardized meals. The feasibility of this pilot study has been very promising with data collection from the other

participating E-PAraDiGM sites already in progress. The preliminary results from this thesis and pilot study demonstrate that response to an acute bout of exercise can be highly variable. Compilation of results from all eight sights will allow for a larger sample size and additional examination of inter-individual differences.

CONCLUSION

The prevalence of T2D in Canada increased by 70% from 1999 – 2009 and has continued to rise in the years following [2]. This poses many challenges for Canadians and places a great burden on the health care system. For years, researchers and exercise physiologists have sought to better understand how exercise may play a role in diabetes management. From this continued pursuit of knowledge, a plethora of studies were conducted, laying down the stepping stones leading researchers towards new ideas, hypotheses and theories. From this framework of knowledge, a number of influential lifestyle intervention studies emerged gaining great recognition and helping to formulate our current understanding of how exercise plays a role in glycemic control and other aspects of health.

Results from this thesis work are contrary to many previous findings and have shown that exercise may not always be beneficial for glycemic control. This opens up the question as to why exercise reduces glucose in some contexts but not in others. Despite the results from this study suggesting no differences between sexes following an acute bout of exercise, it should be noted that the detection of a difference between sexes may have been more likely if the overall effect of exercise was greater. Due to the small sample size and low power of this study it is difficult to say whether a difference could be detected with a larger sample size. Further investigation upon data collection from the remaining E-PAraDiGM sites will help to better understand how exercise may effect glycemic control following a single bout of walking.

As a multi-site study, the E-PAraDiGM protocol is the first study of its kind to standardize, collect and analyze CGM data from eight sites across Canada. This pilot study proved to be viable in terms of recruitment and protocol adherence. The use of the CGM as a measurement tool reduced the burden for researchers and participants, with minimal time required in the laboratory for participants. These findings are promising with regards to buy-in from the other E-PAraDiGM sites. The implications of the E-PAraDiGM Protocol are advantageous from an evidence-based perspective with results from this study determining whether the recommended exercise guidelines of walking lead to acute benefits in glucose control in people with T2D. Ultimately, the E-PAraDiGM protocol will lead to an improved understanding of why and when some individuals have greater improvements in glucose compared to others following exercise. This may contribute to improved management methods for individuals with T2D through the development of individualized lifestyle interventions.

APPENDIX A

Details of the First E-PAraDiGM Protocol Meeting

1. General objectives of the E-PAraDiGM Protocol

The objective of this proposed planning meeting is to develop a standardized protocol for the use of CGM in exercise studies. This protocol will be named the E-PAraDiGM (Exercise-Physical Activity and Diabetes Glucose Monitoring) Protocol.

2. Summary of the E-PAraDiGM Consensus Meeting

2.1 Meeting date and setting

May 22-23, 2015, Kananaskis, Alberta

2.2 Meeting agenda

Day 1 (Friday, May 22)

8:30-9:30 Introduction (Drs. Boulé/Little)

- Vision/mission
- CGM overview from Medtronic (Jonkers and Lambert)

9:45-12:00 Current CGM standardization protocols

- International (Dr. Manders)
- Canadian
  - McMaster U (Dr. Gibala)
  - UBC (Dr. Little)
  - U of C/U of O (Dr. Sigal & Yardley)
  - U of A (Dr. Boulé)
  - U of M (Mrs. MacIntosh for Dr. McGavock)
  - U Laval (Dr. Weisnagel)

2:00-3:30 Discussion: Key issues for standardization (moderators: Drs. Boulé/Little)

-"Basic" protocol

- Inclusion criteria and safety considerations (e.g., screening/fitness)
- Standardizing exercise and other physical activities
  - (Frequency, intensity, type, time, timing)
- Baseline assessment
- Standardizing nutrition (guest: Dr. Prado)
- Behavioral/psychological assessment (guest: Dr. Jung)

3:45-5:00 Discussion: Key issues for standardization (moderators: Drs. Boulé/Little)

-"Deluxe" protocol

- Baseline assessment (Fitness, body comp, blood sample).

- Standardizing nutrition (guest: Dr. Prado)
- Behavioral/psychological assessment (guest: Dr. Jung)
- Data management and analyses
- Guidelines for sharing databank, sub-studies and publications

Day 2 (Saturday, May 23)

8:30-9:30	Summary of progress (Drs. Boulé/Little) • Presentation by Canadian Biosample Repository (Mrs. Lee) • Feedback from Dr. Manders

9:45-12:00	Planning: Grant application
	• Pilot project
	  o Preliminary idea
	  o Feedback: Dr. Jonkers/Medtronic & Manders
	• Establishing a nation-wide cohort study
	• Combining with ongoing work

2:00-3:30	Planning: Publication, dissemination and grants • Protocol manual for investigators • Methods article on Canadian "**E-PAraDiGM**" protocol • Subsequent grant applications • Broadening "**E-PAraDiGM**" to type 1 diabetes (Dr. Yardley, Dr. Weisnagel) • Guidelines for publication and authorship on subsequent results • "Opening it up"
3:45-5:00	Planning: Next steps • Questions/discussion • Summary + key action items

3. Meeting attendees

Physical activity and diabetes experts:

- **Normand Boulé PhD (E-PAraDiGM Co-PI)**, Associate Professor at the University of Alberta, recently started using CGM after conducting the first systematic review in the area[11] and first test-retest reliability study [172]. He has since completed his first two exercise and CGM study in T2D (one presented at ADA conference and under second review in *Diabetes Care*, and the second which will be presented at the CDA conference in the fall of 2014. **Jordan Rees BSc,** will be starting her MSc with Dr. Boulé and will be working on E-PAraDiGM as part of her research was also in attendance.
- **Jonathan Little PhD (E-PAraDiGM Co-PI),** Assistant Professor at the University of British Columbia, is an expert in the area of the effects of exercise and diet on skeletal muscle and whole body metabolism in obesity and T2D. He started using CGM in the context of studies on high intensity interval training [202, 205, 220]. He is Co-PI on a CIHR-funded trial using CGM in pre-diabetes and PI on a Dairy Farmer's of Canada-funded exercise trial using CGM in T2D.
- **Martin Gibala PhD**, Professor at McMaster University, is an expert in the regulation of skeletal muscle energy provision. He is particularly interested in the potential for exercise and/or nutrition to induce metabolic adaptations at the molecular and cellular levels in humans. Recent work in his laboratory has focused on metabolic adaptations to low-volume, high-intensity interval training, with an emphasis on the regulation of oxidative energy provision. He has worked with Dr. Little on several CGM publications [202, 205].
- **Ronald Sigal MD, PhD**, Professor and Endocrinologist at the University of Calgary**,** is a leading expert in exercise and diabetes, having authored Canadian and American guidelines/consensus statements on physical activity/exercise and diabetes [221, 222]. Dr. Sigal is well connected with most investigators at the proposed meeting and has experience in conducting large, multi-centered trials. He has collaborated with many Canadian researchers including **Dr. Yardley** on CGM and exercise studies.
- **Jane Yardley PhD**, Assistant Professor, University of Alberta-Augustana Campus, is an expert in exercise and type 1 diabetes (T1D). She has four publications[223-226] on exercise and CGM which have highlighted the important potential of this technology to identify different response from small changes in exercise interventions. While our meeting will focus on T2D, many of the methodological challenges and considerations are similar to studies in T1D.
- **Jonathan McGavock PhD**, Assistant Professor University of Manitoba & Robert Wallace Cameron Chair in Evidence Based Child Health. Dr. McGavock's research program is focused the prevention and management of T2D in youth, with a focus on physical activity. He is currently the principal investigator for a study using CGM in

youth with T1D. **Andrea Mackintosh BSc,** has been working with Dr. McGavock as a research coordinator and a MSc student at this time, represented Dr. McGavock's lab at this meeting.

- **S. John Weisnagel MD**, Centre Hospitalier Universitaire de Québec et Centre de recherche du Centre Hospitalier Universitaire de Québec. Dr. Weisnagel is an endocrinologist with an active research program in the area of diabetes (type 1, type 2 and gestational diabetes). Many of his publications on exercise have involved patients with T1D. His group is currently using CGM in this population and is considering studies in T2D.

Complimentary expertise

- **Mary Jung PhD,** Assistant Professor, University of British Columbia, is an expert in self-regulation of health behaviours, including the promotion and adherence to physical activity and healthy diets. She uses CGM as a self-monitoring tool to increase exercise adherence in individuals with T2D. She led the discussion on the standardization of questionnaires regarding physical activity levels and determinants.
- **Carla Prado PhD**, Assistant Professor & CAIP Chair in Nutrition, Food and Health, University of Alberta, is an expert in the study of nutritional assessment and status. She led the discussion on the development of the standardized nutritional guidelines for both the laboratory and free living components of CGM studies.
- **Bruce Ritchie MD**, Associate Professor, University of Alberta and Director of the Canadian Biosample Repository (CBSR). Dr. Ritchie is active in both clinical and basic hematology research. Dr. Ritchie was not able to attend the meeting. His assistant, **Ami Lee**, presented on the high quality processes for collecting and storing biologic samples for translational research.

International perspective

- **Ralph Manders PhD,** Lecturer in Exercise Physiology, University of Surrey. Dr. Manders is recognized as an international leader in CGM studies. He has published over 10 peer-reviewed manuscripts with exercise and CGM, many of which were as part of highly recognized team from Maastricht University in the Netherlands. He has collaborated with Co-PI (**Dr. Little**) on two past publications and is eager to join our group to provide insight accumulated from several European laboratories.

Industry/Knowledge Users

- **Richard Jonkers PhD** and **Cheryl Lambert** Clinical Research Specialist, Medtronic of Canada Ltd, provided input on standardization of CGM use and creating input on sharing CGM data among multiple sites using Medtronic software. They provided insight on the potential to apply to Medtronic for a pilot study using the *Canadian E-PAraDiGM Protocol*.

APPENDIX B

Data Collection Forms

PHONE SCREENING

Persons interested in taking part in the study are to complete this form with the Study Coordinator by phone. Start by asking if they have any questions about the study?

1. Have been diagnosed with type 2 diabetes? Y N

   If yes: How long ago? _____ (include if more than 6 months)

2. Are you between 30-90 years of age Y N

   If yes: What is your date of birth? (mm/dd/yr) _____

3. Do you currently take insulin? Y N

4. (Circle: Male Female)
   If female: When was your last menstrual cycle? _____

5. Are you able to walk for 45 minutes, continuously? Y N

6. Would you be able to visit our lab (state location) for 2 hours at dinner time on two separate occasions within 3 days of each other? Y N

7. To the best of your knowledge, have you ever suffered from any serious medical problems other than type 2 diabetes? (For example, heart attack or stroke) Y N

   If so, please specify.

If participants meet the above criteria:

Name: _____

Best way to contact them: _____

Instructions:

-Provide participant with directions to lab for *full screening* and/or *baseline assessment.*
-Ask if they have any other questions about the study or if they would like to receive an information sheet by e-mail before the next visit.
-Remind participant to bring results with recent A1C and Lipids (HDL-C, LDL-C Total-C, TG) within last 6 months and serum creatinine within last year to the *Baseline Assessment* visit. --- Remind participants that they will be asked about their medications (some have lists they can bring). Could bring walking shoes to practice walking on treadmill at about 5 kph (i.e., 3mph).

Participant Information and Consent Form

Title: Exercise-Physical Activity Diabetes Glucose Monitoring (E-PAraDiGM) Protocol

Principal Investigator: **Normand Boulé PhD**
(nboule@ualberta.ca)
(780) 492-4695

Study Coordinator: **Jordan Rees** (rees@ualberta.ca)
(780) 492-8079

INVITATION.

You are being asked to participate in a research study on exercise. This study is looking at how glucose levels change over time when people with type 2 diabetes exercise.

Before you make a decision one of the researchers will go over this form with you. You are encouraged to ask questions if you feel anything needs to be made clearer. You will be given a copy of this form for your records.

WHO IS CONDUCTING THE STUDY?

The study is being conducted by Dr. Normand Boulé, from the Faculty of Physical Education and Recreation at the University of Alberta. This study is part of a larger multi-site study. It is one out of seven sites completing the same study protocol called: the **Exercise-Physical Activity Diabetes Glucose Monitoring (E-PAraDiGM) Protocol.** Several professors from Universities across Canada are involved with this Protocol.

BACKGROUND.

Exercise is recommended for people with type 2 diabetes. Often in exercise studies, researchers rely on single measures to describe the effects of exercise on blood glucose (sugar) control. For example, they measure glucose from a blood sample before and after exercise. More recently, **continuous glucose monitors (CGM)** have given researchers a wider lens to examine different aspects of glucose control. People wear these small devices to measure glucose in the body every 5 minutes. The CGM can be worn for many days in a row. CGM studies are often small due to their demanding nature. Also, it is very difficult to make comparisons among CGM and exercise studies due to the lack of similarities between the studies.

This study provides a standardized approach to assist in comparisons among studies. This protocol will allow researchers to combine results from different sites. It will lead to a better understanding of why some people have greater improvements in glucose than others after exercise.

Figure: A continuous glucose monitor.

Note: Only the flexible filament is inserted under the skin. →

PURPOSE.

This study seeks to examine the effect of a single bout of walking on glucose levels in people with type 2 diabetes.

WHO CAN PARTICIPATE IN THE STUDY?

You may be able to participate in this study if:

- You are between the age of 30-90 years.
- You have been diagnosed with type 2 diabetes for at least 6 months.
- You have an A1C less than 9%.
- You have never been diagnosed with heart disease, stroke, kidney disease (or any other chronic condition that may impact your ability to exercise).
- You are able to understand and comply with study requirements (e.g., attend visits during the day and eat the meals that will be provided to you).

WHAT DOES THE STUDY INVOLVE?

This study involves at total of 5 visits to the laboratory at the University of Alberta. Each visit will last 1-2 hours and will take place over a 1 week period.

<u>Visit 1</u>. Determining your eligibility (1-hour lab visit).

- **Initial Meeting.** You will come to the Physical Activity and Diabetes Laboratory (**PADL**) on the University of Alberta main campus. PADL is accessible by LRT. We will discuss any questions or concerns you may have about the study. If you agree to participate, we ask that you sign this consent form before any study procedures are done. Then you will complete an eligibility questionnaire. Questions include general information about your health, medications, and ability to exercise. We will also measure your blood pressure and heart rate.
- **Treadmill Familiarization.** If your blood pressure is normal, you will complete 15 minutes of walking on a treadmill. This is to help you get comfortable with our suggested walking speeds and make changes if necessary. You will walk at 5 km/hr (i.e., 3.1 miles per hour.) under the supervision of an exercise professional.

<u>Visit 2</u>. Inserting the continuous glucose monitor (CGM) (30-minute lab visit).

You will be asked to come to PADL for a CGM insertion by a trained individual. CGM is a small device that measures your blood glucose every 5 minutes. You will wear the CGM for the length of the study (6 days). The small CGM sensor will be placed on the skin of your abdomen by a person trained by the CGM manufacturer. The CGM sensor has a small filament that is inserted under your skin with a small needle (less than 1 cm long). The needle is then removed and only the flexible filament remains under your skin. Tape will be placed over the CGM to hold it in place. The CGM insertion should take no more than 5 minutes. During this lab visit you will be given a small booklet containing important information about the CGM, pedometer, and food log. You will also be given standardized meals for the following 2 days.

<u>Visit 3 & 4</u>. Test days 1 & 2 (2-hour lab visit for each testing day).

For each of the test days you will arrive at the laboratory at your scheduled appointment time (3-5 hours after lunch). You will complete a bout of walking or remain seated for 50 minutes. The order of these conditions (i.e., walking vs sitting) will be random. Visit 3 must take place within 24 hours of Visit 2. Visit 4 must take place exactly 3 days after Visit 3.

Walking Condition. You will complete 50 minutes of walking at 5.0km/hr. The speed can be adjusted to allow you to finish comfortably. Your heart rate will be monitored during exercise. Blood pressure and capillary blood glucose will be measured before and after exercise.

Seated Control. You will sit quietly and be allowed to read, work on a computer, and/or watch a video for 50 minutes. Capillary blood glucose and blood pressure will be monitored before and after the 50 minutes of sitting.

<u>Visit 5</u>. CGM Drop Off (5-minute lab visit).

You will drop off your CGM at the PADL laboratory at a time convenient to you within 5 days of Visit 4.

Summary of Visits and Timeline.

Before	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**After (within 5 days)**
Visit 1: Eligibility	**Visit 2**: CGM insertion	Standardized meals & **Visit 3**: (walking or sitting)	Standardized meals, no lab visit	No standardized meals, no lab visit	Standardized meals & **Visit 4**: (walking or sitting)	Standardized meals, no lab visit	**Visit 5**: CGM return

CGM= Continuous glucose monitor

You are being asked to:

1) Show up to the lab at your scheduled appointment times.
2) Consume all standardized meals to the best of your ability.
3) Record any changes that you make to your meals in your food log.
4) Check your capillary glucose 4 times per day (i.e., finger prick glucose; supplies provided).
5) Report any noticeable changes in your health status (e.g., sickness, cold).

WHAT ARE THE POSSIBLE BENEFITS?

We hope this study will help us better understand how exercise affects glucose levels. You will receive information on how your blood glucose responds to exercise. You are not expected to receive any other benefits from participating in this study.

WHAT ARE THE POSSIBLE RISKS?

Continuous glucose monitoring. There is a small risk of infection and a very low risk of bruising from the insertion of the sensor. These could last for up to a few days. The insertion of the CGM is done under sterile conditions by a trained researcher. The skin of some individuals is sensitive to the medical tape and can get red or itchy when the CGM is attached. There may be redness in the area where the tape was applied but this will usually disappear after a few days. The CGM also requires calibration from four capillary glucose measures per day. We will provide the capillary glucose monitor and strips. There is discomfort and a small risk of infection with these measures as well.

Adverse event during exercise. Exercise has many health benefits but it is possible that exercise will cause light headedness, muscle cramps, fatigue, nausea, and joint pain. The risks are minimized by supervision while you are walking on the treadmill. You are free to stop exercising if you feel any discomfort or do not wish to continue exercising. A questionnaire completed during screening will also ensure there is a low risk of an adverse event (e.g. heart attack) occurring with walking.

Low blood sugar. There may be a small risk of low blood sugar during or after exercise. We will assess your capillary glucose before and after exercise. This will help us make sure the appropriate steps are taken if your glucose is too low. In our experience with exercise studies in type 2 diabetes, we have not seen a hypoglycemia following moderate intensity exercise.

It is not possible to know all of the risks that may happen in a study. The researchers have taken all reasonable safeguards to minimize any known risks to a study participant.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study.

CONFIDENTIALITY.

During the study we will be collecting data about you. We will do everything we can to make sure that this data is kept private. No data relating to this study that includes your name will be released outside of the study investigator's office or published by the researchers. Sometimes, by law, we may have to release your information with your name so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your information is kept private.

During research studies it is important that the data we get is accurate. For this reason your data, including your name, may be looked at by people from: the University of Alberta auditors and members of the Research Ethics Board. By signing this consent form you are giving permission for the study investigator/staff to collect, use and disclose information about you as described above. After the study is done, we will still need to securely store your data that was

collected as part of the study. At the University of Alberta, we keep data stored for 5 years after the end of the study.

COMPENSATION FOR INJURY.

If you become ill or injured as a result of this study, you will receive necessary medical treatment at no additional cost to you. By signing this consent form you are not releasing the investigator(s) and/or institution(s) from their legal and professional responsibilities. Inform the study personnel if you have been injured. They will help provide immediate aid and refer you to appropriate follow-up treatment.

REIMBURSEMENT OF EXPENSES.

Parking fees up to $15 per visit will be reimbursed. Public transportation fees (e.g. bus or LRT) will also be reimbursed up to $15 per visit.

CONTACT NAMES AND TELEPHONE NUMBER.

If you have any concerns about your rights as a study participant, please contact the Research Ethics Office at the University of Alberta: (780) 492-2615.This office is independent of the study investigators.

If you have any concerns or questions about the study, please contact the study coordinator (Jordan Rees (780) 492-8079, rees@ualberta.ca), or the principal investigator (Normand Boulé (780) 492-4695, nboule@ualberta.ca)

Title of Project: **Exercise-Physical Activity Diabetes Glucose Monitoring (E-PAraDiGM) Protocol**

Principal Investigator	**Phone Number**
Dr. Normand Boulé PhD	(780) 492-4695
Research Coordinator	**Phone Number**
Jordan Rees	(780) 492-8079

To be completed by the research participants:	Yes	No
Do you understand that you have been asked to be in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to withdraw from the study at any time?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand who will have access to your information?	☐	☐
Do you want the investigator(s) to inform your family doctor that you are participating in this research study? If so, give his/her name _____	☐	☐

Who explained this study to you? _____

Signature of Research Participant _____

(Print Name) _____

Date: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee _____ Date _____

FUTURE RESEARCH.

We would like your permission to contact you in the future to participate in other research studies related to diabetes and exercise. You can mark your choice below. Agreeing to be

contacted does not mean you are agreeing to participate in future studies. You do not have to agree to be contacted for future research and your decision will not impact your participation in this study.

I agree to be contacted for future research: YES:____ NO:___

Form 2a
PARTICIPANT SCREENING AND MEDICAL INFORMATION FORM

Persons interested in taking part in the study are to complete this form with the Study Coordinator.

8. Have been diagnosed with type 2 diabetes? Y N
   If yes: How long ago? _____ (include if more than 6 months)

9. Are you between 30-90 years of age Y N
   If yes: What is your date of birth? (mm/dd/yr) _____

10. Do you currently take insulin? Y N

11. (Circle: Male Female)
    If female: When was your last menstrual cycle? _____

12. Are you able to walk for 45 minutes, continuously? Y N

13. Has your body weight changed by more than 5 lbs (~2.5 kg) within the last 3 months?
    Y N
    (Note: If weight is not stable, we could wait for stability before including)

14. Has your diabetes medication been changed in the last 3 months? Y N
    (Note: If medication not stable, we could wait for stability before including)

15. Please list any medications you are taking for diabetes, blood pressure, or cholesterol?

    _____

    _____

    _____

    _____

16. Please list other medications if applicable?
    If so, please specify. (Note: this includes Valium®, aspirin, antacids, vitamins)

    _____

    _____

    _____

    _____

    (Note: treatment with corticosteroids are not eligible for this study)

17. To the best of your knowledge, have you ever suffered from any serious medical problems other than type 2 diabetes? (For example, heart attack or stroke) Y N
    If so, please specify.

    _____

    _____

    _____

    (Although not reasons for exclusion, particular note should be made of polycystic ovary syndrome (PCOS), Cushing's syndrome, musculoskeletal limitations, chronic obstructive pulmonary disease, heart disease such as possible cardiac deficiencies including arrhythmia and other heart conditions, high blood pressure, epilepsy, glaucoma, Parkinson's, hypo/hyperthyroidism and blood disorders such as anemia.)

18. Do you have a pacemaker for your heart or other implantable electronic devices? Y N

19. Are you aware of any allergies to drugs (e.g., to aspirin, penicillin, sulfonamides, phenothiazines or antihistamines)? Y N
    If so, please specify.

    _____

    _____

    _____

20. Do you have any other known allergies, including to certain foods? Y N
    If so, please specify.

    _____

    _____

    _____

21. Do you have any other dietary restrictions?
    If so, please specify.

    _____

    _____

    _____

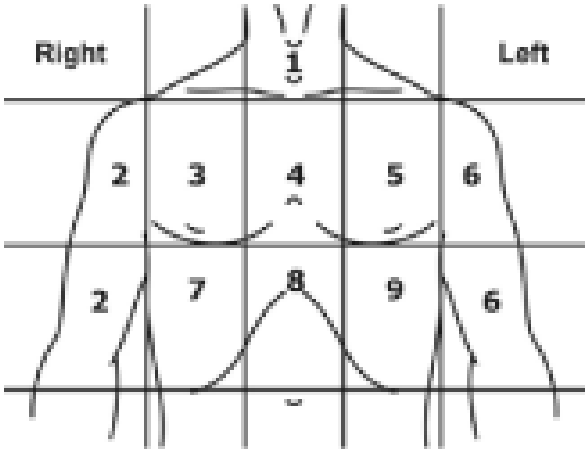
22. Do you smoke more than one cigarette (cigar or other) per day?
    Y N
23. Do you consume alcohol? Y N
    If so, how many drinks do you consume per week on average? _____
24. Within the last 12 months, have you experienced alcohol or substance abuse?
    Y N

Rose Angina Questionnaire

Please circle the appropriate response to the following questions:

1) Do you ever experience any pain or discomfort in your chest? **Yes / No**

2) Where do you get this pain or discomfort? Please mark '**X**' on the appropriate places in the diagram.



3) When you walk at an ordinary pace on level ground, does this produce the pain? **Yes / No**

4) When you walk uphill or hurry, does this produce the pain? **Yes / No**

5) When you get any pain or discomfort in your chest on walking, what do you do?

Stop / Slow down / Continue at same pace / Not Applicable

6) Does the pain or discomfort in your chest go away if you stand still? **Yes / No**

7) How long does it take to go away? **10 minutes or less / More than 10 minutes**

Definite Rose Angina is defined as chest pain or discomfort (yes to question 1) that fulfilled all of the following criteria:

(a) was brought on by exertion (yes to either question 3 or 4)

(b) was situated in the central or left anterior chest (site 4, 5, or 8 on diagram in question 2)

(c) forced the subject to slow down or stop (question 5)

(d) was relieved if the subject did so (yes to question 6)

(e) was relieved within 10 minutes (question 7)

This definition is further subcategorised into severe (grade II) if the exertional chest pain comes on when walking on level ground (yes to question 3) and not severe (grade I) if the exertional chest pain only comes on when hurrying or walking up hill (no to question 3 and yes to question 4).

*Lawler et al. J Epidemiol Community Health 2003;**57**:538-541*

<u>If participants meet the above criteria:</u>

Name: _____ **E-mail _____**

Assign participant ID (local ID#, centre, mth, yr): _____ (e.g., 01-UofA-06-15)

Phone numbers:

Home: _____ **Cell: _____** **Work: _____**

Emergency contact: _____

*** Remind participant to bring results with recent A1C (within last 6 months) and serum creatinine within last year) to the *Baseline Assessment* visit.

Form 3a
Baseline Assessment

Participant ID: _____ **Date (dd-mm-yr):_____**
1. Body Mass: 1st ___ ___ ___ . ___ Kg 3rd (if Δ>0.1kg) ___ ___ ___ . ___ Kg 2nd ___ ___ ___ . ___ Kg **Average: ___ ___ ___ . ___Kg**
2. Standing Height: 1st ___ ___ ___ . ___ cm 3rd (if Δ>0.5cm) ___ ___ ___ . ___ cm 2nd ___ ___ ___ . ___ cm **Average: ___ ___ ___ . ___cm**
3. Waist Circ.: 1st ___ ___ ___ . ___ cm 3rd (if Δ>0.5cm) ___ ___ ___ . ___ cm 2nd ___ ___ ___ . ___ cm **Average: ___ ___ ___ . ___cm**
4. Hip Circ.: 1st ___ ___ ___ . ___ cm 3rd (if Δ>0.5cm) ___ ___ ___ . ___ cm 2nd ___ ___ ___ . ___ cm **Average: ___ ___ ___ . ___cm**
5. Heart rate and Blood pressure. Cuff Size = _____ Systolic BP (SBP): Average of two consecutive measures with 5 mmHg? (1)___ ___ ___mm Hg (2)___ ___ ___mm Hg (3)___ ___ ___mm Hg (4)___ ___ ___mm Hg (5) ___ ___ ___mm Hg **Average SBP: ___ ___ ___mm Hg** Diastolic BP (DBP): Average of two consecutive measures with 5 mmHg? (1)___ ___ ___mm Hg (2)___ ___ ___mm Hg (3)___ ___ ___mm Hg (4)___ ___ ___mm Hg (5)___ ___ ___mm Hg **Average DBP: ___ ___ ___mm Hg** Resting Heart Rate (HR): Average of two consecutive measures with 5 bpm? (1) ___ ___ ___bpm (2)___ ___ ___bpm (3)___ ___ ___bpm (4)___ ___ ___bpm (5)___ ___ ___bpm **Average HR: ___ ___ ___bpm** *Blood pressure must be ≤ 160/100 mmHg to be eligible*

6. A1C within last 6 months _____%
7. A1C taken in lab _____%

8. Within last 6 months: HDL-C _____mmol/L LDL-C _____mmol/L
   Total-Chol _____mmol/L TG _____mmol/L

9. Serum Creatinine within last year _____ µmol/L

10. For women
    a. Do you take hormone replacement therapy or contraceptives? Y N
    b. If so, please describe: _____
    _____
    c. When was your last menstrual cycle? _____

    Note: The testing sessions should take place during the follicular phase. About ten days following first day of menstrual period in most women (may be prolonged with some contraceptives).

11. Practice/short exercise session.

    Predicted Max HR = 220- age = _____

    Predicted 70% of max HR = _____

    Gradually/safely bring the speed up to 5 kph or 3.1 mph with a 0% grade. Participants can hold rails for balance at the beginning, but try to minimize this.

	Speed (mph)	Grade (%)	HR	RPE
5 min				
10 min				
15 min				

    Ask participant: Do you think you would be able to continue at this speed for another 30 minutes? _____
    _____

12. Schedule date and time for CGM insertion

    Date: _____ Time:_____

Form 3b
Godin Leisure-Time Exercise Questionnaire

1. During a typical 7-Day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

Times Per Week

a) STRENUOUS EXERCISE (HEART BEATS RAPIDLY) _____
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

b) MODERATE EXERCISE (NOT EXHAUSTING) _____
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

c) MILD EXERCISE (MINIMAL EFFORT) _____
(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

2. During a typical 7-Day period (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

OFTEN	SOMETIMES	NEVER/RARELY
1.	2.	3.

INSTRUCTIONS

In this excerpt from the Godin Leisure-Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

CALCULATIONS

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 × Strenuous) + (5 × Moderate) + (3 × Light)

The second question is used to calculate the frequency of weekly leisure-time activities pursued “long enough to work up a sweat“ (see questionnaire).

EXAMPLE

Strenuous = 3 times/wk
Moderate = 6 times/wk
Light = 14 times/wk

Total leisure activity score = (9 × 3) + (5 × 6) + (3 × 14) = 27 + 30 + 42 = 99 Godin, G., *Shephard, R. J.. (1997) MSSE. June Suppl: S36-S38.*

Form 3c
Patient Health Questionnaire-8 (PHQ-8)

Over the last 2 weeks, how often have you been bothered by any of the following problems? Read each item carefully, and indicate your response with a check mark.	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
a. Little interest or pleasure in doing things				
b. Feeling down, depressed, or hopeless				
c. Trouble falling asleep, staying asleep, or sleeping too much				
d. Feeling tired or having little energy				
e. Poor appetite or overeating				
f. Feeling bad about yourself, feeling that you are a failure, or feeling that you have let yourself or your family down				
g. Trouble concentrating on things such as reading the newspaper or watching television				
h. Moving or speaking so slowly that other people could have noticed. Or being so fidgety or restless that you have been moving around a lot more than usual				

If you checked off any problem on this questionnaire so far, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not Difficult at All **Somewhat Difficult** **Very Difficult** **Extremely Difficult**

Form 3d
The Pittsburgh Sleep Quality Index (PSQI)

Instructions:
The following questions relate to your usual sleep habits during the past month *only*. Your answers should indicate the most accurate reply for the *majority* of days and nights in the past month. Please answer all the questions.

1. During the past month, when have you usually gone to bed at night?

usual bed time_____

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

number of minutes_____

3. During the past month, when have you usually got up in the morning?

usual getting up time_____

4. During the past month, how many hours of *actual* sleep did you get at night? (This may be different than the number of hours you spend in bed).

hours of sleep per night_____

5. For each of the questions, check the one best response. Please answer *all* questions.

During the past month, how often have you had trouble sleeping because you…	**Not during the past month**	**Less then once a week**	**Once or twice a week**	**Three or more times a week**
(a) Cannot get to sleep within 30 minutes				
(b) Wake up in the middle of the night or early morning				
(c) Have to get up to use the bathroom				
(d) Cannot breathe comfortably				
(e) Cough or snore loudly				
(f) Feel too cold				
(g) Feel too hot				

(h) Had bad dreams				
(i) Have pain				
(j) Other reason(s), please describe_____ _____ _____				
How often during the past month have you had trouble sleeping because of this? Please circle your response. Not during the past month / Less than once a week / Once or twice a week / Three or more times a week				
6. During the past month, how would you rate your sleep quality overall? Please circle your response. Very good / Fairly good / Fairly bad / Very bad				
7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	Not during the past month	Less then once a week	Once or twice a week	Three or more times a week
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
10. Do you have a bed partner or roommate?	No bed partner or roommate	Partner/roommate in other room	Partner in same room, but not same bed	Partner in same bed
11. How often do you feel tired during the following times during the day?	most days	often	occasionally	never
Morning				
Afternoon				
Evening				

Form 5b - Exercise Session

Date:_____

Pre Exercise Blood Glucose:_____

Pre Exercise Blood Pressure:_____

Pre Exercise Step Count:_____

Note: The Standard speed and grade for the E-PAraDiGM protocol are written in the upper right corner of the cells. These speed and grades can be adapted as described in the manual (section 4).
Conversions: 5 km/h = 3.1 mph, 4.5 km/h = 2.8 mph, 4 km/h = 2.5 mph, 3.5 km/hr = 2.2 mph

Time (mins)	Speed (km/h)	Grade (%)	Heart Rate (bpm)	RPE	Indirect Calorimetry
0 - 5	3.5	0%			
5 - 10	5	0.5%			
10 - 15	5	0.5%			
15 - 20	5	0.5%			
20 - 25	5	0.5%			
25 - 30	5	0.5%			
30 - 35	5	0.5%			
35 - 40	5	0.5%			
40 - 45	5	0.5%			
45 - 50	3.5	0%			

Post Exercise Blood Glucose:_____

Post Exercise Blood Pressure:_____

Post Exercise Step Count:_____

Time of Dinner:_____
Additional Comments:_____

Seated Control Session

Date:_____

Time of Arrival:_____

Pre Seated Control Blood Glucose:_____

Pre Seated Control Blood Pressure:_____

50 Minute Seated Control Start Time:

Time (mins)	Indirect Calorimetry
0 - 5	
5 - 10	
10 - 15	
15 - 20	
20 - 25	
25 - 30	
30 - 35	
35 - 40	
40 - 45	
45 - 50	

Post Seated Control Blood Glucose:_____

Post Seated Control Blood Pressure:_____

Time of Dinner:_____
Additional Comments:

APPENDIX C

E-PAraDiGM Protocols

E-PAraDiGM Protocols for Baseline Testing

Prior to coming into the lab for baseline assessment and intervention sessions, please let the participants know the following information:

1) Avoid caffeine within 6 hours of entering the lab
2) Avoid alcohol for 24 hours prior to entering the lab
3) Avoid strenuous exercise for 12 hours prior to entering the lab
4) Come prepared to exercise (comfortable clothing and running shoes)
5) Make sure that you are feeling healthy and well to do exercise

Height – CSEP protocol

- Ensure tape is vertical against a wall
- Have participant stand erect, without footwear, against a wall
- Feet together, heels against the wall, arms at sides, and head is level (looking straight ahead)
- Place the set square or stadiometer on the head, making firm contact and taking the measurements are taken on a deep breath in
- Record the distance from the floor to the mark to the nearest 0.5cm
- Repeat measurement. Both measures should be within 0.5 cm. If > then 0.5 cm, repeat measurement a third time

Weight (body mass) – CSEP protocol

- Have participant stand on scale (e.g., calibrated beam scaled), without footwear
- Record weight in kilograms to the nearest 0.1kg
- Repeat measurement. Second measure should be within 0.1 kg. If > then 0.1 kg, repeat measurement a third time

Waist Circumference – CSEP protocol

- Clear the participant's abdomen of clothing and accessories (e.g. belt)
- Participant is standing with feet shoulder width apart and arms crossed over the chest in a relaxed manner
- Kneel to one side of participants body when taking the measurement
- Measurement is taken at the superior edge of the iliac crest
- To find this landmark, palpate the upper hipbone on the participant until you locate the uppermost lateral border of the iliac crest
- Position the tape directly around the abdomen so that the inferior edge of the tape is at the level of the landmarked point

- Use a cross-handed technique to bring the zero line of the tape in line with the measuring aspect of the tape
- Ensure the tape is positioned in a horizontal plane around the abdomen and apply tension to the tape so that it is snug
- At the end of a normal expiration, take the measurement to the nearest 0.5 cm
- Repeat measurement. Both measures should be within 0.5 cm. If > then 0.5 cm, repeat measurement a third time

Hip Circumference

- As for the waist circumference measurement, except
- Position the tape around the hips at the level of the symphysis pubis and the greatest gluteal protuberance
- Record the measurement to the nearest 0.05 cm
- Repeat measurement. Both measures should be within 0.5 cm. If > then 0.5 cm, repeat measurement a third time

Resting Heart Rate – CSEP protocol

- Have participant sit and rest quietly for at least 5 minutes before measuring heart rate
- The participant should have feet flat on the floor and arms should be supported (chair rests or table)
- Using your index and middle fingers, palpate the radial artery on the left side, applying gentle pressure
- Using a 15 second count, start the stopwatch simultaneously with a 'beat' counting the first beat as '0'
- Multiply this number by 4 to get beats per minute and record
- Repeat the measurement. If the second measure yields a difference > then 5 bpm, repeat measure a third time

Resting Blood Pressure– CSEP protocol

- Have participant sit and rest quietly for at least 5 minutes before measuring blood pressure
- The participant should have feet flat on the floor and arms should be supported (chair rests or table)
- Place the cuff on the bare left arm, 2-3 cm above the anticubital space, with the lower edge of the cuff level with the heart. Cuff should be tight enough so that two fingertips can be slipped under the top edge of the cuff.
- You may want to palpate the brachial pulse and mark it (for placement of the diaphragm of the stethoscope)
- While taking radial pulse with one hand, inflate the cuff until you no longer feel a radial pulse, then continue to inflate 30-40 mmHg above this point.

- Place the diaphragm of the stethoscope over the brachial artery, applying minimal pressure (the diaphragm should be in complete contact with the skin and should not be touching the cuff or tubing
- Release the cuff pressure at a rate of about 2 mmHg/second
- The first perception of a distinct tapping sound (1st Korotkoff sound) is the systolic blood pressure
- Diastolic blood pressure is determined when the sounds cease to be tapping and become fully muted of muffled (4th Korotkoff sound)
- Record your measurements, and repeat measurement after 5 minutes of rest
- Consecutive measurement of systolic and diastolic must be within 4 mmHg of each other. If > then 4 mmHg, repeat measurement a third time.

APPENDIX D

Participant Sample Menus

Participant Sample Menu, Example 1:

Height: 141.5 cm
Weight: 44.6 kg
Age: 64
Sex: Female
Harris Benedict Calculation: 665.0955 + 9.5634 (44.6) + 1.8496 (141.5) – 4.6756(64)
= 1054.10314 calories
Physical Activity: 1054.10314 calories x 1.4
=1475.744369 calories
Daily Caloric Target: 1476 (±74) calories

	CALORIES	CHO (g)	FAT (g)	PRO (g)	CHO (%)	FAT (%)	PRO (%)	TOTAL (%)	FIBRE	SUGAR
BREAKFAST										
Banana, med	110	29	0	1	98%	0%	4%	102%	4	21
2% milk (237ml)	102	12.18	2.37	8.22	38%	21%	32%	91%	5	12
PC Blue Menu, Omega-3 Regular Whol Grain Instant Oatmeal (45g)	170	31	3.5	6	67%	19%	14%	100%	5	0
TOTALS:	**382**	**72.18**	**5.87**	**15.22**	**68%**	**13%**	**17%**	**98%**	**14**	**33**
SNACKS										
Oikos 2% Vanilla Yogourt, 100g	100	13	1.5	8	52%	14%	32%	98%	0	10
Roasted Almonds (~10)	85	2.75	7.5	3.15	5%	79%	15%	99%	3.3	1.4
TOTALS:	**185**	**15.75**	**9**	**11.15**	**29%**	**47%**	**23%**	**99%**	**3.3**	**11.4**
LUNCH										
6" Subway, Turkey Beast & Ham (with cheese & mayo)	450	48	20	20	40%	40%	18%	98%	5	7
Apple, med	80	22	0	0	98%	0%	0%	98%	5	16
TOTALS:	**530**	**70**	**20**	**20**	**69%**	**20%**	**9%**	**98%**	**10**	**23**
SNACKS										
Carrots, chopped, 1 cup	41	9.58	0.24	0.93	79%	5%	9%	93%	3	4.54
TOTALS:	**41**	**9.58**	**0.24**	**0.93**	**79%**	**5%**	**9%**	**93%**	**3**	**4.54**
DINNER										
PC Blue Menu, Shepards Pie (275g)	300	28	9	28	35%	27%	37%	100%	3	6
Salad (mixed greens)	20	3	0	2	20%	0%	40%	60%	4	5
Dressing (Renee's Balsamic vinaigrette) 2tbsp	60	2	6	0.3	13%	90%	2%	105%	0	0
TOTALS:	**380**	**33**	**15**	**30.3**	**23%**	**39%**	**26%**	**88%**	**7**	**11**
DAILY TOTALS	**1518**	**200.51**	**50.11**	**77.6**	**50%**	**27%**	**18%**	**95%**	**37.3**	**82.94**

Participant Sample Menu, Example 2:

Height: 172.5 cm
Weight: 111.6 kg
Age: 59
Sex: Male
Harris Benedict Calculation: 66.4730 + 13.7516(111.6) + 5.0033(172.5) - 6.7750(59)
=2064.49581 calories
Physical Activity: 2064.49581 calories x 1.4
=2890.294134 calories
Daily Caloric Target: 2890 (±145) calories

	CALORIES	CHO (g)	FAT (g)	PRO (g)	CHO (%)	FAT (%)	(%)	TOTAL (%)	FIBRE	SUGAR
BREAKFAST										
Banana, med	110	29	0	1	98%	0%	4%	102%	4	21
PC Blue Menu, Omega-3 Regular Whole Grain	170	31	3.5	6	67%	19%	14%	100%	5	0
2% milk (237ml)	102	12.18	2.37	8.22	38%	21%	32%	91%	5	12
Oikos 2% Vanilla Yogourt, 100	100	13	1.5	8	52%	14%	32%	98%	0	10
TOTALS	**482**	**85.18**	**7.37**	**23.22**	**64%**	**13%**	**20%**	**98%**	**14**	**43**
SNACKS										
Nature Valley Peanut Granola Bar	220	28	11	5	48%	45%	9%	102%	3	9
Roasted Almonds (~20)	170	5.5	15	6.3	9%	79%	15%	103%	3.3	1.4
Ensure, Vanilla	220	32	6	9	58%	25%	16%	99%	0	15
TOTALS	**610**	**65.5**	**32**	**20.3**	**38%**	**50%**	**13%**	**102%**	**6.3**	**25.4**
LUNCH										
6" Subway, Turkey Beast & Ham (with	450	48	20	20	40%	40%	18%	98%	5	7
Apple, med	80	22	0	0	98%	0%	0%	98%	5	16
TOTALS	**530**	**70**	**20**	**20**	**69%**	**20%**	**9%**	**98%**	**10**	**23**
SNACKS										
Ensure, Milk Chocolate	220	33	6	9	59%	25%	16%	100%	1	15
Nature Valley Oats & Honey	210	31	9	4	56%	39%	8%	102%	3	12
TOTALS	**430**	**64**	**15**	**13**	**58%**	**32%**	**12%**	**101%**	**4**	**27**
DINNER										
PC Blue Menu, Italian Lasagna (283g)	280	36	7	18	49%	23%	26%	98%	3	7
Subway Turkey Breast Salad w/ Olive (	230	13	16	11	19%	63%	19%	101%	4	5
Chocolate covered almonds (12)	230	20	16	4	32%	60%	9%	101%	1	12
TOTALS	**740**	**69**	**39**	**33**	**33%**	**48%**	**18%**	**100%**	**8**	**24**
DAILY TOTALS	**2792**	**353.68**	**113.37**	**109.52**	**52%**	**32%**	**16%**	**99%**	**42.3**	**142.4**

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