

University of Massachusetts Amherst

ScholarWorks@UMass Amherst

---

Doctoral Dissertations

Dissertations and Theses

---

March 2017

# Interfacing Continuous Measurement of Glucose and Physical Activity to Predict Glycemic Control in Individuals with Type 2 Diabetes

Jennifer Blankenship

*University of Massachusetts Amherst*

Follow this and additional works at: [https://scholarworks.umass.edu/dissertations\\_2](https://scholarworks.umass.edu/dissertations_2)



Part of the [Exercise Science Commons](#)

---

## Recommended Citation

Blankenship, Jennifer, "Interfacing Continuous Measurement of Glucose and Physical Activity to Predict Glycemic Control in Individuals with Type 2 Diabetes" (2017). *Doctoral Dissertations*. 865.  
<https://doi.org/10.7275/9421286.0> [https://scholarworks.umass.edu/dissertations\\_2/865](https://scholarworks.umass.edu/dissertations_2/865)

This Open Access Dissertation is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact [scholarworks@library.umass.edu](mailto:scholarworks@library.umass.edu).

INTERFACING CONTINUOUS MEASUREMENT OF GLUCOSE AND PHYSICAL  
ACTIVITY TO PREDICT GLYCEMIC CONTROL IN INDIVIDUALS WITH  
TYPE 2 DIABETES

A Dissertation Presented

by

JENNIFER M. BLANKENSHIP

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 2017

Department of Kinesiology

© Copyright by Jennifer M. Blankenship 2017

All Rights Reserved

INTERFACING CONTINUOUS MEASUREMENT OF GLUCOSE AND PHYSICAL  
ACTIVITY TO PREDICT GLYCEMIC CONTROL IN INDIVIDUALS WITH  
TYPE 2 DIABETES

A Dissertation Presented

by

JENNIFER M. BLANKENSHIP

Approved as to style and content by:

---

Patty S. Freedson, Chair

---

Barry Braun, Member

---

Stuart Chipkin, Member

---

John Staudenmayer, Member

---

Catrine Tudor-Locke, Department Chair  
Department of Kinesiology

## ACKNOWLEDGMENTS

I am so thankful for all of the people that have come into my life over the past 6 years to get me to this point. Thank you to all of the faculty and staff members in the Kinesiology department, my fellow graduate students, friends and family for all of the support, encouragement and joy that you have given me over the years as a student at UMass.

I would like to graciously thank my committee members, for without their contributions, this dissertation would not have been a success. Barry, even though we've spent the last 2 years 1,881 miles apart, you were always there when I really needed you. The distance has been difficult, but I'm glad we stuck through it. I couldn't have asked for a more supportive advisor to get me through the last 6 years. Thank you for always giving me the confidence and encouragement to get back on my broomstick and *defy gravity* time and time again. Patty, thank you for always making time for me, whether it was to talk science or what to do with my life after graduate school, you always had a 6<sup>th</sup> sense of knowing when I needed some extra guidance. I am so grateful for the many opportunities that you have given me over the years. John, thank you for limitless patience as I fumbled through learning and attaining competency in the R. I owe the confidence in my own abilities to appropriately analyze and interpret my statistical models 100% to you. Stu, words cannot describe my appreciation for the many hours we spent together talking about my data and writing. Thank you for always cutting time out of your very busy

day, night and weekend for me. I am so blessed to have someone so dedicated to student's success on my committee.

To my former labmates- Kirsten, Hannah, and Steve, thank you all for your support, critiques and most of all, friendship. *I know I'm who I am today, because I knew you...* Rich, it's been just us two for a while now and I'm still surprised that we didn't kill each other after all this time. Thanks for letting me sing and dance in the lab with my headphones on (even when I didn't know you were right behind me). I can genuinely say that I've enjoyed our time in room 3 together.

To my parents (Patricia and Diz, that includes you too!)- you've all been there, every step of the way. You been there for my successes and failures, for every graduation and every presentation. Your dedication to supporting me is unwavering and makes me proud to be your daughter. I hope I've made you proud.

Finally, I would like to thank and acknowledge my husband, Thomas. I owe my sanity and happiness in life to you. Thank you for being my rock and for always reminding me of the important things in life.



subjects are most important because  
they are people  
then cats  
then grants  
grants are not people or cats

## **ABSTRACT**

### **INTERFACING CONTINUOUS MEASUREMENT OF GLUCOSE AND PHYSICAL ACTIVITY TO PREDICT GLYCEMIC CONTROL IN INDIVIDUALS WITH TYPE 2 DIABETES**

**FEBRUARY 2017**

**JENNIFER M. BLANKENSHIP, B.A., UNIVERSITY OF MASSACHUSETTS AMHERST**

**M.S., UNIVERSITY OF MASSACHUSETTS AMHERST**

**Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST**

**Directed by: Professor Barry Braun**

Physical activity is a cornerstone in the management of hyperglycemia and risk of cardiovascular disease in type 2 diabetes (T2D). However, the dose response relationship between physical activity and glucose regulation is not well defined. The overall goal of this dissertation was to assess the magnitude and timing of changes of daily glucose concentrations in response to continuous and intermittent light physical activity in T2D. Through utilizing continuous glucose monitors (CGM) and physical activity monitoring concurrently, we were able to assess the glycemic impact of physical activity and sedentary behavior in the free-living environment.

Study 1 aimed to examine the effect of regularly interrupting 7-h of prolonged sitting (SIT) with brief bouts of light walking (LW) or simple resistance activities (SRA) on 22-h glucose homeostasis in adults with T2D. Twenty-four individuals with T2D completed 3 conditions (SIT, LW and SRA) in the laboratory. A CGM was worn during the laboratory conditions and in the free-living environment through next morning. Compared to SIT, both LW and SRA reduced mean 22-h glucose concentrations (SIT:  $11.5 \pm 0.3$ , LW:  $8.7 \pm 0.3$  and SRA:  $8.8 \pm 0.3$  mmol·L<sup>-1</sup>), daily

duration of hyperglycemia (SIT:  $14.7 \pm 0.9$ , LW:  $6.3 \pm 0.8$  and SRA:  $6.3 \pm 0.9$  hours), and mean glucose concentrations through to the next morning.

Study 2 compared the effect of increasing physical activity by breaking up sitting time after meals (BR) or by a continuous bout of morning walking (EX) on daily and postprandial glucose (PPG) concentrations (measured by CGM). Thirty individuals with T2D completed EX, BR and a control condition (normal behavior [CON]) in their free-living environment over 1 week. Participants increased their total physical activity in EX and BR by 20, 40 or 60 minutes. Overall, EX was the only condition to significantly lower duration of postprandial glycemia ( $\downarrow 11.4 \pm 4.0\%$ ) and the 40-minute dose of activity lowered mean PPG. In a subset of participants with high postprandial hyperglycemia at CON (n=9): (1) both EX and BR significantly shortened duration of hyperglycemia and (2) the 40 and 60-minute doses of activity significantly lowered mean PPG.

Study 3 evaluated the sex differences in the glucose response to the EX and BR conditions described in Study 2. We found that men had a significant glucose lowering effect of EX and BR compared to control, whereas women's level of glycemia was unchanged with the activity conditions. This sex difference was driven by higher levels of hyperglycemia in men during the CON condition.

This dissertation utilized CGM and physical activity monitors to identify effective interventions to manage hyperglycemia in T2D. The combination of studies performed in the laboratory and free-living environment in this dissertation have potential to better inform physical activity guidelines for the management of T2D.



## TABLE OF CONTENTS

	Page
<b>ACKNOWLEDGMENTS .....</b>	<b>iv</b>
<b>ABSTRACT .....</b>	<b>vi</b>
<b>LIST OF TABLES .....</b>	<b>xii</b>
<b>LIST OF FIGURES .....</b>	<b>xiii</b>
 <b>CHAPTER</b>	
 <b>1. INTRODUCTION.....</b>	<b>1</b>
Statement of the Problem.....	1
Diabetes and Cardiovascular Disease Relationships.....	2
Managing Cardiovascular Disease Risk in Diabetes with Physical Activity .....	2
Comparing Exercise and Breaks from Sitting.....	4
Objectives and Significance .....	5
 <b>2. REVIEW OF LITERATURE .....</b>	<b>7</b>
The Importance of Glycemic Control for Diabetes and Cardiovascular Disease.....	7
Glycemic Response to Exercise.....	9
Measuring Glycemic Control .....	9
Comparing Interstitial Glucose with Blood Glucose .....	10
Glycemic Effects of Exercise .....	12
Consequences of Sedentary Behavior .....	14
Using Continuous Glucose Monitors to Investigate Variability of Daily Glucose .....	21
Exercise Prescription .....	23
Translating Prescription to Practice.....	25
Physical activity monitors.....	26
Influence of Other Factors on Glycemic Control.....	28
Dietary Considerations.....	28
Sleep and Glucose Metabolism .....	30
Sex Differences .....	30
Summary and Future Directions .....	32

Specific Aims .....	33
Study 1 .....	33
Study 2 .....	33
Study 3 .....	33
Tables.....	34
<b>3. INTERRUPTING PROLONGED SITTING IN TYPE 2 DIABETES: NOCTURNAL PERSISTENCE OF IMPROVED GLYCEMIA .....</b>	<b>35</b>
Introduction .....	35
Methods.....	37
Participants.....	37
Study design .....	37
Experimental protocol and laboratory conditions .....	38
Standardization of diet, medications and physical activity .....	39
Continuous glucose monitoring.....	40
Physical Activity Monitors Data Handling.....	41
Continuous Glucose Monitor Data Handling.....	41
Results .....	43
Participant characteristics .....	43
Postural allocation and meal/sleep periods.....	43
22-h glucose homeostasis and glycemic variability.....	44
Postprandial glycemic control.....	44
Nocturnal glycemic control.....	45
Discussion .....	45
Figures .....	52
Tables.....	54
<b>4. MANAGING FREE-LIVING HYPERGLYCEMIA WITH EXERCISE OR INTERRUPTED SITTING IN TYPE 2 DIABETES: AN ECOLABICAL APPROACH.....</b>	<b>57</b>
Introduction .....	57
Methods.....	61
Participants.....	61

Experimental Design .....	62
Resting Metabolic Rate .....	63
Body Composition.....	64
Dietary and Medication Control .....	64
Continuous Glucose Monitoring .....	65
Physical Activity Monitoring.....	67
Statistical Analysis .....	68
Results .....	70
Participant Characteristics.....	70
Medications.....	70
Physical Activity .....	70
Differences in Glycemia During Conditions.....	71
24-hour Glucose Control.....	71
Postprandial Glycemic Control.....	71
Meal Specific Effects.....	72
Dose-Responses within Physical Activity Conditions .....	72
24-hour Glucose Control.....	73
Postprandial Glucose Control .....	73
Reallocating Physical Activity and Sedentary Behaviors.....	74
Discussion .....	74
Effects of Physical Activity .....	76
Unexpected Dose-Response Relationships.....	78
Figures .....	84
Tables.....	91
<b>5. SEX DIFFERENCES IN POSTPRANDIAL GLUCOSE RESPONSES TO PHYSICAL ACTIVITY AFTER MEALS .....</b>	<b>93</b>
Introduction .....	93
Methods.....	96
Continuous Glucose Monitor Data .....	97
Physical Activity Data.....	98
Statistical Analysis .....	99
Results .....	99
Participant Characteristics.....	99

Medications.....	99
Physical Activity .....	100
Postprandial Glucose Responses: Effect of Conditions.....	100
Postprandial Glucose Responses: Dose-Response Relationships .....	101
Discussion .....	102
Figures .....	106
Tables.....	109
<b>6. SUMMARY AND CONCLUSION .....</b>	<b>110</b>
<b>Study 1 .....</b>	<b>110</b>
<b>Study 2 and 3 .....</b>	<b>112</b>
Conclusions.....	115
<b>REFERENCES.....</b>	<b>117</b>

## LIST OF TABLES

	Page
Table 2.1: Timing of Exercise and Subsequent Measurement of Glucose.....	34
Table 3.1: Participant Characteristics .....	54
Table 3.2: Physical Activity During the Laboratory Condition and Evening Period after Condition .....	55
Table 3.3: Glycemic Control Over 22-h and Nocturnal Glycemia .....	55
Table 3.4: Glycemic Variability Over 22-h.....	56
Table 4.1: Description of Experimental Conditions within each Activity Volume Group.....	91
Table 4.2: Relative Macronutrient Composition by Meal (mean $\pm$ SD).....	91
Table 4.3: Percent of Complete Continuous Glucose Monitor Data.....	91
Table 4.4: Participant Characteristics (mean $\pm$ SD).....	91
Table 4.5: Participant Characteristics: Activity Volume Group (mean $\pm$ SD) .....	92
Table 4.6: Total Daily Physical Activity (mean $\pm$ SD).....	92
Table 5.1: Participant Characteristics .....	109

## LIST OF FIGURES

	Page
Figure 3.1: 22-h glucose profiles over 22 hours during experimental condition and free-living environment .....	52
Figure 3.2: Change in Postprandial Glycemia from Control Condition .....	53
Figure 4.1: Overall Study Design.....	84
Figure 4.2: Hypoglycemic medications combinations in participants .....	84
Figure 4.3: Daily Physical Activity During Experimental Conditions .....	85
Figure 4.4: Stepping Time after Breakfast.....	85
Figure 4.5: Postprandial Continuous Glucose Monitor Data .....	86
Figure 4.6: Postprandial Duration of Hyperglycemia in People with High Duration Hyperglycemia at Control.....	87
Figure 4.7: Beta Coefficients from Mixed Model Regressions.....	88
Figure 4.8: Dose Response for People with High and Low Glucose at Control.....	89
Figure 4.9: Dose-Response Between Change in Steps and Change in Postprandial Glucose.....	90
Figure 5.1: Medications by Sex.....	106
Figure 5.2: Physical Activity During Normal Activity Days: Sex Differences....	106
Figure 5.3: Change in Physical Activity from Control: Sex Differences.....	107
Figure 5.4: Sex differences in the Response to Exercise and Post-Meal Breaks from Sitting .....	107
Figure 5.5: Sex Differences in Dose Response Relationship .....	108

## CHAPTER 1

### INTRODUCTION

#### **Statement of the Problem**

It is well established that physical activity is beneficial for the treatment and prevention of type 2 diabetes (T2D). Exercise is an effective strategy for managing daily hyperglycemia (high blood glucose) and for improving long-term glycemic control in T2D (162, 188). Recently, breaking up sitting time with short bouts of walking and standing have been reported to lower glucose concentrations in laboratory settings (52, 55, 62, 136, 173). It is unknown whether the glucose lowering benefits of light physical activity breaks in sitting time are comparable to that of traditional exercise. To date, no study has performed a direct comparison of the glucose lowering effects of continuous exercise and physical activity breaks in sitting time in an ecological setting. Further, the dose-response relationship between physical activity, sedentary behavior and glucose concentrations is not well defined. To address these knowledge gaps, laboratory interventions must be applied to a free-living environment to understand the real world impact of physical activity. Additionally, a range of physical activity doses need to be systematically compared to determine the minimum amount of physical activity required to lower blood glucose concentrations in individuals with T2D. Further, establishing a dose-response relationship would also provide evidence of a true effect of physical activity that illustrates systematic change in glucose outcomes with incremental exposures.

## **Diabetes and Cardiovascular Disease Relationships**

The prevalence of diabetes is widespread and a major public health concern. Currently, 1 in 10 Americans have diabetes, and it is projected that by 2050, 1 in 3 Americans will have diabetes (29, 40). The key to treatment of diabetes is managing blood glucose in a narrow range. Deviations above normal glucose concentrations (>5-8 mmol/L) result in negative health outcomes (e.g. increased production of oxidative stress, activation of inflammatory molecules). Over time, high glucose concentrations (hyperglycemia) cause systemic damage to the microvasculature. The profound impact of hyperglycemia on the vasculature partly explains why cardiovascular disease (CVD) is the number one cause of death among individuals with diabetes (83, 84, 167). One large clinical trial (Look AHEAD) assessed the impact of an intensive lifestyle intervention on reducing the incidence of cardiovascular events. While the Look AHEAD trial successfully induced weight loss, lowered HbA1c and improved other measures of cardiovascular risk (112), there was no reduction in the incidence of cardiovascular events (113). While lowering glucose does not result in changes in cardiovascular events, there is a reduction in the risk of complications in the microvasculature (e.g. nephropathy, neuropathy) (82). Therefore, maintaining glucose in a narrow range and limiting episodes of hyperglycemia is important for the prevention of complications in T2D.

## **Managing Cardiovascular Disease Risk in Diabetes with Physical Activity**

There is a wealth of evidence that physical activity confers a multitude of benefits (79). Many studies have shown that exercise before or after a meal lowers postprandial glucose concentrations in individuals with diabetes (47, 49, 96, 105,



132, 133, 139, 153). These reductions in postprandial glycemia have been shown to result in reduced risk of microvascular and macrovascular complications in diabetes (114, 167, 177). Therefore, the glucose lowering effect of physical activity is critical managing the risk of complications in T2D including neuropathy, nephropathy, and amputations (82). While exercise is an effective strategy to manage hyperglycemia, most people do not exercise (119). Instead, the majority of waking hours are spent in sedentary behaviors (e.g. watching TV, desk work) (120). Sedentary behavior is known to have a detrimental effect on cardiovascular and metabolic (cardiometabolic) health (61, 134).

Recent evidence has shown that breaking up prolonged periods of sitting time with short bouts of light physical activity lowers the postprandial glucose response (62, 106). The majority of these studies have been performed in the laboratory, or under strictly controlled conditions, which limits the translation of these findings. Several studies have demonstrated that light walking and cycling breaks from sitting improve postprandial glycemia compared to an all sitting condition (10, 52, 55, 62). In contrast, the glucose lowering effects of interrupting sitting time with standing are equivocal (144, 173). Therefore, the composition of a break from sitting to effectively lower postprandial glucose has not been well established. Additionally, it is unknown how long the glucose lowering effects of breaks from sitting will last. The few studies that have investigated the duration of action of breaks from sitting have been performed in the laboratory (52, 106). It is unclear, however whether results found in the laboratory will translate to the free-living environment.

### **Comparing Exercise and Breaks from Sitting**

Before light physical activity breaks from sitting can be recommended as an alternative behavioral strategy to manage hyperglycemia in T2D, a direct comparison to traditional exercise is required. Further, to maximize the translation of these results, it is necessary to compare effective laboratory interventions (e.g. walking breaks from sitting vs. single walking exercise) in a real world setting. A major challenge of performing physical activity research studies in the free-living environment is the variability in human behaviors (e.g. dietary intake, sleep durations). Without controlling for some of these key variables, interpreting the glucose effects of physical activity becomes very difficult. Combining aspects of a laboratory study within an ecological context allows for maximum generalizability to real world scenarios. This approach, which we term *ecolabical*, takes place in the free-living environment and controls for important confounding variables (e.g. diet) essential to the interpretation of changes in glucose.

Because of its glucose lowering effects, physical activity has the potential to be prescribed like a medication. Unlike pharmaceutical medications, exercise is economical and has many systemic benefits that cannot be mimicked with a pharmaceutical medication (79, 87). However, current physical activity and health guidelines do not have specific recommendations for individuals with T2D. The lack of specific guidelines is due to the limited understanding of the dose-response relationship between exercise and specific health outcomes/disease states (138). Before pharmaceutical medications are prescribed to patients, dose-response studies must be performed to determine the effectiveness of that medication. This

systematic approach to testing many doses of a medication allows for physicians to effectively prescribe medications to improve their patients' health. More dose-response studies between physical activity and specific health outcomes are needed to prescribe exercise like a pharmaceutical medication.

By studying the glycemic impact of both traditional exercise and breaks from sitting, we are uniquely positioned to determine the minimum amount of physical activity and the type of activity required to meaningfully reduce blood glucose. Results from these studies will add to our understanding of the impact of structured exercise and the independent effects of daily physical activity and sedentary behavior on glucose control. This dissertation has the potential to impact millions of individuals trying to manage daily blood glucose concentrations with exercise.

### **Objectives and Significance**

The main goal of this dissertation was to examine the effect of physical activity and sedentary behavior on the magnitude and timing of changes in daily glucose concentrations. We integrated data from continuous glucose monitors with physical activity monitors in a series of 3 studies performed in the laboratory and free-living environments to understand the impact of physical activity and sedentary behavior on glucose responses.

**Study 1 investigated how short breaks from sitting affected glucose responses over the course of a day in the laboratory and free-living environment.** Participants performed either 7 hours of either uninterrupted sitting or interrupted sitting time with short bouts of physical activity in the laboratory. We compared the glucose lowering effects of two different types of physical activity

breaks from sitting (walking and simple resistance activities) and investigated whether those benefits were sustained in a free-living environment through the next day. Study 1 provides important laboratory based evidence comparing walking and resistance breaks from sitting during and after a laboratory intervention.

**Study 2 determined the comparative effectiveness of a bout of continuous morning walking and post-meal activity breaks from sitting on 24-hour and postprandial glycemia measures.** We investigated the dose-response relationship between bouts of activity and postprandial glucose regulation. Participants performed all experimental conditions in their own free-living environment. Physical activity was added to their normal behavior in the form of either a continuous walk after breakfast or physical activity breaks within sitting time periods. We compared the glycemic effects of these physical activity interventions to participant normal physical activity behavior. These data provide the first direct comparison of traditional exercise and breaks from sitting in a real world setting.

**Study 3 evaluated sex differences in the response to a continuous morning walk and post-meal breaks from sitting on postprandial glycemia.** We determined any sex differences in the dose response to physical activity. This study utilized the same participants and methods as Study 2. These data add to the limited evidence available on sex differences in the metabolic response to physical activity.

## CHAPTER 2

### REVIEW OF LITERATURE

#### **The Importance of Glycemic Control for Diabetes and Cardiovascular Disease**

Glucose is critical for normal body functioning and is required to provide energy for the brain and central nervous system as well as providing fuel for exercise tasks. Maintaining glucose concentrations in a narrow range is essential to avoid the harmful effects of hypoglycemia and hyperglycemia on the body. In a healthy individual, fasting blood glucose concentration is  $\sim 5\text{mM/L}$  to provide a steady flow of glucose to the brain and central nervous system (122). There are many tissues that rely on blood glucose at rest including the kidneys, gut and muscle, but these tissues consume a smaller proportion of glucose at rest than the brain. If blood glucose concentrations fall below normal resting values, brain and other tissue functions can become severely compromised. In healthy individuals, a reduction in blood glucose signals counter-regulatory hormones to increase glucose production and release by the liver that raise blood glucose concentrations. If these countermeasures are not functioning appropriately, as occurs in diabetes, hypoglycemia can be problematic. Severe hypoglycemia results in a lack of glucose available for the brain and can eventually cause comas and even death.

On the other hand, hyperglycemia poses a different set of issues and strains on the body. It is impossible to eliminate hyperglycemia entirely since blood glucose rises in response to a meal. The rise in blood glucose is necessary to replenish glycogen stores that are depleted in efforts to maintain normal fasting glucose concentrations or after a bout of exercise. In type 2 diabetes (T2D), individuals

cannot produce enough insulin to compensate for the prevailing elevated blood glucose, which results in prolonged periods of hyperglycemia. This hyperglycemia causes damage to cells including the vasculature by increasing oxidative stress and reducing the vasodilatory capacity of blood vessels (42, 43). These effects of hyperglycemia on the vasculature can be observed during postprandial hyperglycemia. Over time, in response to chronic hyperglycemia, blood vessels become less compliant and vascular complications can result, including nephropathy, retinopathy and CVD.

Epidemiological data clearly indicates that diabetes and CVD are closely related. Diabetes doubles the likelihood that an individual will develop CVD (167). Further, the number one cause of death among individuals with diabetes is CVD (83, 84, 167). The negative effects of hyperglycemia on the vasculature partly explain these close connections, making glycemic control central to both diabetes and CVD. There are many pharmaceutical therapies that are used to minimize hyperglycemia including biguanides (e.g. metformin), DPP4 inhibitors, GLP-1 agonists and SGLT-2 inhibitors (27). These medications vary in their mechanism of action, but are all used to treat diabetes and reduce the prevalence of hyperglycemia. As with any pharmaceutical therapy, these medications are associated with a host of side effects (e.g. weight loss or gain, hypoglycemia, gastrointestinal issues and water retention), some of which are counterproductive to the treatment of diabetes. In contrast, exercise has been shown to result in reductions in blood glucose along with many whole body improvements including increased insulin sensitivity and muscle mass and weight stability or maintenance of weight loss (36). With the capacity to confer

multiple health benefits in a variety of tissues, exercise can be thought of as a medication to treat and prevent a number of diseases including diabetes and CVD.

### **Glycemic Response to Exercise**

#### **Measuring Glycemic Control**

Many methods have been used to assess the effects of exercise on glycemic control. Traditionally, glycemic control has been characterized using static blood measures including fasting glucose and hemoglobin A1c (HbA1c), however, neither measure is very responsive to changes in activity and do not indicate how an individual responds to a glucose challenge (e.g. a meal) (115). Postprandial glycemia is more predictive of future cardiovascular events than fasting glucose or hemoglobin A1c (HbA1c) (37, 108) and for this reason postprandial glycemic responses (e.g. 2 hour glucose, postprandial area under the curve) are commonly used as measures of glycemic control. However, examining the postprandial glucose response can be inadequate as a large portion of the day is unaccounted for (e.g. time between meals).

Continuous glucose monitors allow for the investigation of changes in glucose over the course of a 24-hour period. Over the last 10 years, use of continuous glucose monitors has increased in research. The first continuous glucose monitor, GlucoWatch was approved for use in 1999. Since then, there have been 4 devices used in both the clinical and research environments (26). Continuous glucose monitors measure interstitial glucose using glucose oxidase based electrochemical methods. Capillary blood glucose is measured several times over the course of the day to calibrate the interstitial glucose readings from the

continuous glucose monitors. Mathematical models then integrate the interstitial glucose data and capillary blood glucose to provide estimates of blood glucose (39). Depending on whether the continuous glucose monitor is used for research purposes or monitoring at home, these monitors can display glucose concentrations in real time or store the data to be downloaded at a later date.

Continuous glucose monitors measure interstitial glucose concentrations every 5 minutes in the free-living environment for up to 7 consecutive days. With data collected so frequently, there are many different ways that glycemic control can be characterized using continuous glucose monitors. Most commonly, researchers will calculate 24-hour mean glucose, total area under the curve and duration of hyperglycemia (glucose concentration > 10 mmol/dL). Using a combination of these methods, the effect of exercise, and to a lesser extent, sedentary behavior, on glycemic control have been investigated.

### **Comparing Interstitial Glucose with Blood Glucose**

It must be made clear that continuous glucose monitors measure glucose in the interstitial fluid and not in the blood. There are important differences to note between blood and interstitial glucose. Interstitial glucose concentrations are lower in magnitude than blood glucose but there is a strong correlation between interstitial and blood glucose ( $r = 0.77-0.82$ ) (12, 86). Glucose enters the interstitial fluid by simple diffusion across a concentration gradient from the capillary endothelium to the interstitial fluid (39). Because there are no active transporters moving glucose into the interstitial fluid there is a lag time from when glucose appears in the blood to when glucose reaches the interstitial fluid.



The physiological lag time has been measured directly using glucose tracers infused into the blood and measured in the interstitial fluid by microdialysis. From these studies, Basu *et al.* have approximated that there is a 6-10 minute delay for glucose to appear in the interstitial fluid after it has reached the blood. Interestingly, lag time was inversely correlated with waist to hip ratio ( $r = -0.31$ ), a gross measure of central obesity (19). While this correlation was not significant, it suggests that body fat may modulate the physiological lag time of glucose appearance in the interstitial fluid. These data are corroborated by a recent study correlating percent body fat (measured by bioelectrical impedance) and time to peak glucose (64). Together, these studies indicate that controlling for body fat and investigating lag time may be important factors to consider when investigating the dynamics of the glucose response to a stimulus (e.g. exercise, meals).

In addition to the physiological lag, there is a lag at the level of the sensor that accounts for a significant amount of time. There is some variation in the sensor lag time between brands, but in general the total lag time from appearance of glucose in the blood to glucose being detected by the continuous glucose monitor is 15-20 minutes (64, 86). The additional lag time added by the sensor is due to the calibration algorithms that run within the device to determine interstitial glucose concentrations (146, 184). These issues related to lag time are only apparent when glucose concentrations are rapidly changing (e.g. after a meal). Some researchers have suggested that the lag times may change depending on whether the rise or fall in glucose is being measured (5). The potential differences in lag time on the rising and falling end of the glucose curve may be related to what glucose is used for after

it appears in the blood (e.g. stored as fat or taken up by muscle to produce ATP).

More studies are needed in this area to understand the physiological mechanisms responsible for differences in lag time.

Using a combination of static (fasting glucose, HbA1c) and dynamic (postprandial glucose responses, 24 hour glucose changes) measures of glycemic control, research investigating the relationship between physical activity and changes in glucose responses is available. While most researchers have used blood glucose to characterize changes in glycemic control after exercise, some have examined the prolonged effects of exercise using continuous glucose monitors. Recent studies have been designed specifically to investigate the unique changes that occur with sedentary behavior. The following section will review the literature demonstrating the glycemic lowering effects of exercise and the deleterious impact of sedentary behavior on glucose metabolism.

### **Glycemic Effects of Exercise**

Many researchers have investigated the effects of continuous exercise performed after a meal or oral glucose load. Continuous exercise ranging from light to vigorous intensity blunts the rise in postprandial glucose (47, 49, 96, 105, 132, 133, 139, 153), reduces 2 hour postprandial glucose concentrations and area under the curve compared to no exercise (105, 132, 133, 153). The glucose lowering effect of continuous exercise is apparent in individuals with and without diabetes during the immediate period following exercise (47, 49, 96, 139). Overall, exercise has a glucose lowering effect, but the magnitude of the effect and the duration of action (the length of time that the effect lasts for) are not clearly understood.

Intensity has a variable impact on the magnitude of the glucose lowering effect of exercise. In a study of 18 men and women with prediabetes, participants performed either a moderate or high intensity exercise, matched for total energy expenditure (~200 kcals). Despite similar energy expenditure, high intensity exercise resulted in a greater reduction in postprandial glucose concentrations during an oral glucose tolerance test than the moderate intensity exercise (153). These data suggest that high intensity is better than moderate intensity exercise to reduce blood glucose, however, data from Manders and colleagues do not support this idea. In a group of patients with T2D, 60 minutes of low intensity exercise reduced the 24-hour duration of hyperglycemia by  $49.7 \pm 4.4\%$ , whereas the energy expenditure matched high intensity exercise bout only reduced hyperglycemia by  $18.6 \pm 8.8\%$  compared to a sedentary control (116).

The difference in responses between these two studies may be due the population that was studied. Compared to healthy controls, patients with T2D secrete greater concentrations of glucagon and epinephrine after high intensity exercise that subsequently results in a period of elevated glucose (102). Thus the response to a bout of exercise among individuals with T2D will likely be different than the response to healthy individuals or people with prediabetes. Additionally, the way that the glucose response was measured and the amount of time it was measured for differed between studies. Immediate changes in glycemic control may not match what happens over extended periods of time (24-48 hours after exercise). In both studies of exercise intensity and glycemic control, total energy expenditure was held constant, so it is clear that intensity plays a role in the glycemic response

to exercise, independent of total energy expended. The discrepancy between these studies illustrates an important point: the glycemic effect of exercise may be different depending on the population or the method used to quantify the glucose response.

Some studies have investigated distributing activity over the course of the entire day, rather than one concentrated bout. DiPietro *et al.* compared glycemic control of one 45-minute walk (performed in the morning or afternoon) to three 15-minute bouts performed after every meal. Superior improvements in glycemic control measured by 24-hour mean glucose were found when exercise was distributed throughout the day compared to one concentrated bout of continuous exercise performed either in the morning or afternoon (59). Similar findings in favor of distributing activity during the day have been shown with short high intensity bouts of cycling in terms of 3-hour postprandial glycemia and average 24-hour mean glucose (70). The glycemic lowering effects of short high intensity bouts of cycling were maintained the next day whereas the continuous exercise bout did not show any lasting reductions in 24-hour mean glucose the day following exercise.

### **Consequences of Sedentary Behavior**

In modern day society, sedentary behavior has become a major part of daily life. Sedentary behavior, like sitting at a computer or driving, require a very low amount of energy and is performed in a seated or reclined position. The MET levels (multiple of resting metabolic rate) for these activities range from 1.0-1.5 METs and have been implicated in many negative health outcomes. Since the 1950s, sedentary behavior has been recognized as an important determinant of cardiovascular and

metabolic health (129). Early experimental work in the area of sedentary behavior used extended periods of bed rest and found that there are significant declines in insulin sensitivity and exaggerated postprandial glucose responses (23, 109, 123, 163, 168). In the free-living environment, people spend a lot of time sitting and accumulate a significant amount of sedentary behavior, but it is uncommon to have an individual completely bed bound. For this reason, bed rest is not representative of the way that sedentary behavior is accrued over the course of the day. Recently, researchers have used reduced activity models of sedentary behavior that involve regulating parameters of activity (e.g. reducing daily steps, increasing total time sitting) to determine the cardiometabolic effects of sedentary behavior. Mikus and colleagues reduced daily stepping time of young healthy adults from 10,000 to approximately 5,000 steps per day. They found that when participants decreased daily stepping time, there was a reduction in glycemic control, indicated by higher rate of change in peak postprandial glycemia (124). Even though, sitting time was not specifically measured, it is likely that the stepping time was replaced with increased sitting time, suggesting that sedentary behaviors led to poorer glycemic control. Other researchers have shown that prolonged sitting exaggerates postprandial glucose responses (62, 136) which has led to an area of research focused on countering the deleterious effects of sedentary behavior.

In 2008, Healy *et al.* showed that breaks from sedentary time were associated with lower 2-hour postprandial glucose concentrations and smaller waist circumferences (89). Many other epidemiological studies have showed similar positive associations with breaks in sitting time and positive cardiometabolic health

outcomes (15, 51, 90). As a result of epidemiological evidence, there has been an emphasis on investigating the metabolic effects of breaking up long periods of sedentary behavior with short (< 5 minutes) light intensity bouts of activity. Experimental evidence that link breaks from sitting with improvements in cardiometabolic health is limited. Some of the first experimental evidence came from Dunstan and colleagues who showed that short (3 minutes) light walking bouts reduced postprandial glucose and insulin compared to a sedentary control in overweight to obese adults (62). These data emphasize the need for activity during the postprandial period to reduce postprandial glucose concentrations.

Other researchers have built on the work of Dunstan *et al.* to determine how taking short activity breaks throughout the day compares with a structured bout of exercise. Studies in this area focus on decreasing prolonged and overall sitting time by systematically distributing short bouts of low to moderate intensity activity throughout the day. Similar to the findings of DiPietro *et al.*, performing activity regularly during the day and breaking up prolonged sitting time resulted in improvements that were at least as good and sometimes better than an energy-matched bout of continuous exercise (63, 95, 104). It is important to note the activity accrued throughout the day in these studies was less than the physical activity guidelines recommend as each bout of physical activity was less than 10 minutes long. These data indicate that improvements in glycemic control can be achieved without actually meeting the physical activity and health guidelines. While the mechanisms of action are still being investigated, improvements in glycemic control due to distributing activity over time may be related to frequent muscle

activation and contraction mediated glucose uptake that persists throughout the day (22). Alternatively, changes in posture and subsequently increases in blood flow repeatedly over the day may also result in improved glycemic control. Collectively, these data demonstrate that activity and sedentary behavior have distinct effects on glycemic control and the way that activity is accumulated can modulate the changes in daily glucose profiles.

It is essential to understand the timing of the changes in glucose in order to fully comprehend the glycemic lowering effect of exercise. Physicians prescribe medications with the knowledge of the duration of action (how long one dose of a medication will last). This information is essential to effectively prescribe medications to treat patients with elevated glucose. In order to determine the duration of action of a bout of physical activity, changes in blood glucose need to be tracked for many hours and days after exercise. van Dijk *et al.* performed a study aimed to determine whether daily exercise was required to maintain the glucose lowering effects of a bout of exercise. To do this, glucose was measured continuously for 2 days to determine daily prevalence of hyperglycemia (glucose concentrations > 10 mmol/dL) in response to 2 different exercise protocols. Patients with T2D performed either daily exercise (30 minutes of moderate exercise) or every other day exercise (60 minutes of moderate exercise performed only on the first day). They found that both daily and every other day exercise reduced the prevalence of hyperglycemia on both monitored days by approximately 30% (181). These data suggest that there is a volume of exercise required to reduce

hyperglycemia and if the volume of exercise is large enough, the effect can be maintained over a 24-hour period.

While examining 24-hour glucose concentrations is a good place to start to understand the timing of the glucose lowering response, using a summary value of the entire day does not indicate when the effects of exercise on blood glucose peak and dissipate. Some studies have measured the glycemic response to several meals after a bout of exercise. For example, Holmstrup *et al.* compared the time course of changes in glucose after one continuous bout of morning exercise or intermittent exercise distributed over the course of the entire day. Participants consumed 6 equally spaced meal replacement beverages (239 kcals each) over 12 hours. Postprandial glucose responses were summarized as 2-hour area under the curve and were determined for each of the meals. There were no significant differences between the continuous or intermittent exercise in the response to any of the meals at any of the time points. Overall, however, there was a glucose lowering effect as evidenced by significantly lower 12-hour glucose incremental area under the curve in the intermittent exercise group compared to the morning exercise and sedentary control condition. The small meals consumed, however, may have limited the ability to detect a large glucose lowering effect. While there were no significant reductions to glucose responses within any particular meal, these data support distributing small amounts of exercise over the day to reduce overall daily glucose concentrations (95).

In a similarly designed study, Oberlin *et al.* tracked the timing of the changes in glucose over a 48-hour period after 60 minutes of moderate intensity morning



exercise (133). Instead of 6 small meals, sedentary individuals with T2D were fed 3 meals per day matched on total energy intake and macronutrient composition. Using continuous glucose monitors, the glycemic response was characterized using the postprandial glucose responses to each meal for 48 hours. Average glucose was lowered in the first 24 hours after exercise, but significant postprandial glucose reductions were only evident at the lunchtime meal (the second meal consumed after the exercise). The glucose lowering effect was not sustained the following day. The volume of exercise in this study was equivalent to that of the study by van Dijk *et al.* (181), but there were no lasting improvements in glycemic control on the second day as observed by van Dijk. The lack of a sustained effect may have been due to a difference in glycemic control of the patients in the different studies. Participants in the van Dijk *et al.* study had an average HbA1c of  $7.0 \pm 0.2\%$  and a fasting glucose of  $8.4 \pm 0.5$  mmol/L while the Oberlin *et al.* study participants' HbA1c was  $6.3 \pm 0.2\%$  and fasting glucose was  $6.5 \pm 0.6$  mmol/L. Since participants in the van Dijk study started with a higher baseline level of glycemia, they may have had a larger and more prolonged effect of a single bout of exercise. The conflicting data in this area emphasize the need for more research to determine the time course of changes in glucose in response to structured bouts of physical activity.

As discussed, there are many ways that the glycemic response to exercise can be represented. Many studies simplify the effects of exercise to the response of just one meal consumed either before or after exercise. As seen in the study by Oberlin *et al.*, the glycemic response to the second meal eaten 6 hours after the exercise, was significantly reduced by 15%, whereas the meal eaten immediately after exercise

was not significantly lower than the control (no exercise) condition (133). Since the glycemic lowering effects may not become apparent until several hours after exercise, it is important to track meal responses for multiple meals after exercise. As illustrated in Table 2.1, the way studies define the glycemic response (e.g. area under the curve, peak postprandial glucose) is not consistent within the literature. The lack of consistency may partly explain contradicting findings regarding the glycemic lowering effects of similar exercise doses. One way to deal with this shortcoming of previously published data is to collect glucose data frequently for an extended period of time after exercise so that the immediate and prolonged glycemic effects of exercise can be captured. By collecting data more frequently, the glycemic response of meals after exercise can be described in greater detail to better understand the effects of exercise on glucose outcomes.

One novel aspect of glycemic control that has been investigated using continuous glucose monitoring is glycemic variability. It has been suggested recently that daily patterns of glucose impact cardiometabolic health. The investigations into glycemic variability and cardiometabolic health are based on the findings of a study by Ceriello *et al.* in 2008. In this study, blood glucose concentrations were manipulated by infusing glucose intravenously in different patterns to better understand the vascular implications. Blood glucose was either sustained at 10 mmol/L for 24 hours or was oscillated between 5 mmol/L and 15 mmol/L every 6 hours for 24 hours. Mean blood glucose in both conditions was 10 mmol/L. Endothelial function was measured every 6 hours using flow mediated dilation, which is a well-accepted measure of vascular function (172). Interestingly,

endothelial function was significantly compromised in the oscillating glucose condition compared to the sustained hyperglycemia condition even though the mean glucose in both conditions was the same (42). This compromised endothelial function was coupled with a significant increase in nitrotyrosine (a measure of oxidative stress). Other researchers have shown similar effects of higher oxidative stress with oscillating glycemia compared to sustained hyperglycemia in *in vitro* models (77, 156, 157) and in patients with T2D (41, 128).

### **Using Continuous Glucose Monitors to Investigate Variability of Daily Glucose**

There are many different ways that glycemic variability can be calculated. Continuous glucose monitors provide frequently measured glucose data that can be used to examine different indices of glycemic variability. Standard deviation is a popular gross measure of variability but other researchers have developed different indices of variability that can be calculated with continuous glucose monitor data. MAGE, mean amplitude of glycemic excursions, calculates the mean of the glucose excursions greater than 1 standard deviation above mean glucose (160). One limitation of MAGE is that it excludes glucose excursions that are not above a specified threshold. These smaller excursions may be just as important to consider for vascular health, but are excluded in the calculation. Others have used McDonnell's CONGA calculation to describe the glycemic variability of an individual (121). CONGA, continuous overlapping net glucose action, calculates the difference between glucose values at set intervals (e.g. 1 hour, 2 hours, 4 hours) and then applies those differences to the CONGA formula. McDonnell *et al.* developed CONGA specifically for continuous glucose monitor data and it has been used as an index of

variability in many published studies (25, 58, 155). There are many other indices of glycemic variability that can be calculated and have been reviewed elsewhere (94). Automated algorithms to calculate glycemic variability have made it easy to summarize continuous glucose monitor data (13, 94), however, the clinical significance and value of these measures is still unclear.

A recent systematic review examined whether glycemic variability has a real impact on CVD risk among individuals with T2D (130). Among the 10 studies reviewed, 9 showed a positive association between glycemic variability and negative cardiovascular outcomes, including diabetic retinopathy, myocardial infarction and cardiovascular mortality. Initial findings suggest that glycemic variability is related to cardiovascular health and that efforts should be made to minimize variability of glucose in the treatment of T2D. More long term studies are needed that intervene to change these indices of variability and measure clinical outcomes to understand the value in calculating glycemic variability.

Continuous glucose monitoring allows researchers to examine aspects of glucose regulation throughout the day and night, but very few studies even report nocturnal glycemia (25, 52, 116). There is much potential for new analyses and novel techniques to analyze continuous glucose monitor datasets, but researchers have yet to utilize these data to their full capacity. There have been many studies that have used continuous glucose monitors to investigate the effect of activity and sedentary behavior on glycemic control (59, 70, 78, 95, 101, 104, 110, 111, 116, 124, 133, 141, 181), however the majority of research condenses these rich datasets into simple summary statistics (e.g. area under the curve, time spent above or below a

cut-point, change in postprandial glucose). It should be noted that our knowledge of the impact of changing glycemic variability on actual cardiovascular function and cardiometabolic disease risk is limited. However, it is clear that reducing blood glucose concentrations is beneficial for cardiometabolic health.

### **Exercise Prescription**

Exercise is widely recommended to a variety of populations to improve health and prevent disease. The US Department of Health and Human Services released the first set of evidence-based physical activity guidelines to the public in 2008. In these guidelines, it is recommended that adults accumulate at least 150 minutes of moderate or 75 minutes of vigorous physical activity in bouts greater than 10 minutes per day on most days of the week. While there are no specific recommendations for glycemic control, a joint position statement from the American College of Sports Medicine and the American Diabetes Association supports these recommendations. The joint position statement specifies that individuals with diabetes should not allow more than 2 consecutive days between bouts of physical activity (48).

Because of the limited evidence available, there are no recommendations for sedentary time or breaking up sitting time with light physical activity for glycemic control. It is clear that sedentary behavior is detrimental for metabolic health and glycemic control. Preliminary evidence suggests that interrupting prolonged periods of sedentary behavior with light physical activity may be an effective alternative to traditional exercise to manage hyperglycemia in T2D. Direct comparisons of breaking up sitting time with exercises known to reduce blood glucose

concentrations (e.g. continuous walking) are necessary to determine whether using breaks from sitting to reduce hyperglycemia is an acceptable alternative to traditional exercise.

The lack of disease-specific physical guidelines limits the specificity of exercise prescriptions. When a physician prescribes a medication for a condition there is a very specific dose and instructions on how that medicine should be taken (e.g. with a meal, before breakfast). Physical activity prescriptions are not treated the same way in practice. Even when government guidelines are followed, the prescriptions are quite loose with no discussion of exercising around meal times or frequency of exercise. If pharmaceutical medications were prescribed the same way that physical activity is prescribed, patients would be instructed to take “some” medication most days of the week.

Before physical activity prescriptions specific for glycemic control can be made, there are 2 important pieces of information that must be understood. First, the dose-response relationship between an acute bout of activity and the time course, magnitude and direction of change in glucose must be clearly determined. As discussed previously, some studies have described the glycemic effects of exercise, but there has yet to be a study that systematically tests the glycemic effects of varying doses of a specific physical activity to understand the dose-response relationship. Subsequently, it must be determined whether the acute changes in glycemic control result in an improvement of CVD risk factors. Research in this area has been limited to large epidemiological studies investigating the relationship between simple measures of glycemic control including HbA1c, fasting glucose or 2-

hour postprandial glucose. HbA1c is a measure of the average blood glucose concentrations over the last 3 months and is an indicator of long-term glycemic control (74). In large epidemiological studies, it has been shown that HbA1c is positively correlated with risk of CVD (7, 28, 60, 114). It is important to note that HbA1c consolidates both fasting and postprandial glucose concentrations into one value. Just examining HbA1c is insufficient to determine whether changes in fasting or postprandial glucose are driving the increased risk of CVD. Studies have shown that postprandial glucose concentrations are more predictive of future cardiovascular events than fasting glucose or HbA1c (17, 18, 37, 38, 54, 73). While the impact of reducing the prevalence of hyperglycemia or mean 24-hour glucose on cardiovascular outcomes are not yet clear, current research indicates that reducing glucose exposure reduces the prevalence of microvascular complications (e.g. amputations, retinopathy and neuropathies) in T2D (82).

### **Translating Prescription to Practice**

According to the National Health Interview Survey, in 2010, 32.4% of adults who had seen a medical professional were recommended to participate in more physical activity. In 10 years, there has been a 10% increase in the number of physicians recommending exercise to their patients (16). While this is a step in the right direction, the majority of Americans still do not participate in regular exercise. The most common reason cited for not exercising is lack of time. In response to this, work must be done to determine the minimum physical activity required to gain health benefits and to investigate alternatives to traditional exercise that result in similar health improvements. Optimizing physical activity to specific health

outcomes is essential to gain the greatest benefits from a single bout of activity. In order to get to the point that clinicians can prescribe specific exercises to improve glycemic control, the dose-response relationship between physical activity and glucose needs to be better characterized. Understanding the characteristics of physical activity (e.g. duration, frequency performed, intensity) that yield a lasting glycemic lowering effect and those activities that work best to immediately lower glucose quickly will be helpful in developing personally tailored exercise prescriptions to match the needs of the individual.

### **Physical activity monitors**

It is clear that physical activity and sedentary behavior play an important role in glucose regulation. Both of these human behaviors can be captured using small wearable monitors. There is a great potential for continuous glucose monitor data to be integrated with physical activity and sedentary behavior data collected with one activity monitor. Using physical activity monitors in conjunction with continuous glucose monitors is the next step required to define the dose-response relationship between activity and glucose.

A major advantage to using physical activity monitors is that human behavior can be estimated in the free-living environment. Like continuous glucose monitors, physical activity monitors collect time-stamped data on a very frequent basis (as often as every second) and can be used to examine patterns of physical activity and sedentary behavior throughout the course of the day. Physical activity monitors are relatively inexpensive and do not pose significant participant burden, which make them the optimal tool to assess physical activity in the free-living environment.



There are many different types of physical activity monitors. The majority of these monitors estimate physical activity using accelerometers. Acceleration is measured in 1, 2 or 3 axes, depending on the monitor. Acceleration signals are then converted to counts by the accelerometer using proprietary algorithms. Researchers can use counts to estimate measures of physical activity (e.g. energy expenditure, absolute activity intensity) that can be related to health outcomes. The ActiGraph is an example of a commonly used accelerometer worn on the hip or wrist that measures accelerations in 3 axes. These tools are very useful in quantifying physical activity, but have some limitations. There are some physical activities that are not accurately measured using hip mounted devices. Activities that rely on upper body movement or do not involve much movement at the hip, like bicycling, or weight lifting, are not accurately characterized using hip mounted accelerometers. Some researchers have tried to address this issue by moving the monitor to the ankle or wrist, however, many of the prediction models used to convert counts to meaningful measures of physical activity, were developed using hip mounted accelerometers. As a result, prediction models validated for use on the hip have limitations when accelerometers are worn on a different part of the body.

Another major limitation of accelerometers is the ability to assess sedentary behavior. As illustrated by the wealth of knowledge implicating sedentary behavior with poor cardiometabolic health, measuring sedentary behavior is very important in physical activity research. Acceleration signals that come from standing and sedentary behaviors (e.g. sitting/lying) are very similar, which makes hip mounted accelerometers not ideal for estimating sedentary behaviors. The activPAL is a

different kind of physical activity monitor specifically designed to assess sedentary behavior. With a built in inclinometer, the activPAL detects changes in posture. It is worn on the front side of the thigh that can distinguish from seated and standing positions. Studies have shown that the activPAL is a valid and precise tool to measure sedentary behavior in the free-living environment (81, 103). Given the importance of understanding the distinct impact of physical activity and sedentary behavior on cardiometabolic health, several studies have implemented a combination of accelerometers and inclinometers to assess physical activity and sedentary behavior.

Some studies have used physical activity monitors in conjunction with continuous glucose monitors. Within these studies, physical activity monitors are generally used to quantify total physical activity (31, 63, 124, 133). In these studies, physical activity is measured to account for total energy expenditure as a potential confounding variable, and as a result an entire day's worth of physical activity data is condensed into a single value. Rather than summarizing total sitting time in a day, future studies can examine the effect of one, or multiple, bouts of sitting on measures of health, more specifically glucose concentrations.

### **Influence of Other Factors on Glycemic Control**

#### **Dietary Considerations**

It is established that physical activity has a major impact on glucose concentrations, however, there are many other key factors that modulate blood glucose throughout the day. One of the most influential factors is energy intake. There are many characteristics of energy intake that must be considered including

total carbohydrate intake, composition of meals and distribution of carbohydrates. These dietary characteristics are briefly reviewed below.

The composition and timing of meals consumed within the day has a major impact on the glucose responses. Total carbohydrate content influences blood glucose concentrations. Nutritionists encourage their patients with diabetes to count the grams of carbohydrate they consume to minimize blood glucose excursions. It is clear that as the total amount of carbohydrate in a meal increases, the blood glucose response becomes exaggerated, but other nutrients that are eaten with carbohydrate can change the meal response dramatically (99, 140). When protein is consumed with carbohydrate, more insulin is secreted compared to carbohydrate alone, resulting in an overall lower glucose response (76). While fat slows the emptying of food from the stomach, it does not alter the glucose response to carbohydrate intake significantly (66, 75). Fiber has a dramatic impact on postprandial glucose concentrations and causes a slower rise and lower peak in postprandial blood glucose (150). Fiber is especially important because the amount of fiber in dietary carbohydrate generally dictates whether it is a high or low glycemic index food. There is evidence that low glycemic index diets are beneficial for minimizing glucose excursions and overnight glycemia in overweight and obese non-diabetic individuals (30, 33).

When evaluating blood glucose responses, it is critical to consider the distribution of carbohydrate over the course of the day. Pearce *et al.* found that when carbohydrate consumption was loaded in the middle of the day at lunchtime, duration of hyperglycemia (glucose concentrations > 12 mmol/L), max glucose

concentrations, and total glucose area under the curve were significantly lower compared to evenly distributing carbohydrates over breakfast, lunch and dinner (135). It is important to note that in this study, the total amount of carbohydrate was the same on both days, but the distribution was different. These data indicate that the timing of carbohydrate consumption has a large impact on blood glucose regulation and should be a factor that is not ignored.

### **Sleep and Glucose Metabolism**

Recently, sleep duration and quality have become important factors to consider in the context of glycemic control. Several epidemiological studies have demonstrated a U-shaped relationship between sleep duration and glycemic control (measured by HbA1c and fasting glucose) indicating that very low and high amounts of sleep are associated with high glucose concentrations and greater risk of T2D (35, 161). A causative role has been established through some experimental studies in which sleep was restricted to 4-5 hours per night for several days up to 2 weeks. In these studies, after sleep restriction, insulin sensitivity is reduced and postprandial glucose concentrations are increased (131, 158). Interestingly, the onset of abnormal glucose metabolism occurs rapidly, making it an important factor to consider when investigating glucose regulation.

### **Sex Differences**

There have not been many studies that have specifically investigated sex differences in the metabolic effects of exercise. More studies need to consider the potential for sex differences because women with diabetes have a significantly higher incidence of CVD than men with diabetes (6, 117, 125). There are many

factors that could be driving the sex differences among individuals with diabetes. In general, individuals with diabetes do not exercise regularly but women tend to participate in significantly less physical activity than men (125). It has been shown that the impairment in exercise capacity that occurs with diabetes is significantly greater in women compared to men (147, 149). Additionally, in a secondary analysis of participants in the US Diabetes Prevention Program, men had greater reductions in 2-hour glucose and insulin concentrations and greater improvements in insulin sensitivity compared to women (137). These differences indicate that men and women exposed to the same physical activity intervention may have different metabolic improvements. Finally, there is evidence that women may need a greater volume of exercise to attain the same benefits as men. A large observational study of walking behavior and CVD mortality in diabetes, Sadarangani and colleagues (154) compared dose-response relationships between men and women. In this study, men showed a consistent dose-response pattern between walking and risk of all-cause mortality, however only women who walked above the median walking group had a significant reduction in risk of mortality. Understanding whether women require a larger dose of activity to gain the same benefits for glycemic control would have significant public health implications. Therefore, investigating potential sex differences in the glycemic lowering effects of exercise is an important area of research.

## **Summary and Future Directions**

Understanding the role of physical activity in glucose control is a challenging task. While a structured bout of exercise lowers blood glucose concentrations, the other habitual activities that occur throughout the day can modulate the magnitude of that effect. Sitting time and interruptions in sedentary behavior have been shown to have a measurable impact on blood glucose in the laboratory but the duration of action of breaks from sitting is not well defined. **Study 1 evaluated the impact of a interrupting sitting time with light physical activity in the laboratory on free-living glycemia in the evening and morning after the intervention.**

Interruptions in sitting time may be an effective alternative to manage hyperglycemia in T2D, but the effects must be compared to established treatment options (e.g. continuous exercise). **Study 2 compared the effect of increasing daily physical activity through a continuous morning walk or post-meal breaks from sitting on daily glycemic control in the free-living environment.** Controlling the dietary intake allowed us to compare different activity interventions in a more ecologically relevant environment.

Finally, considering different factors that may modulate the response to a physical activity intervention is critical in moving towards individually tailored physical activity recommendations. There is evidence that men and women may have different cardiometabolic responses to a bout of physical activity, but the research in this area is limited. **Study 3 investigated sex differences in response to adding physical activity in the form of continuous walking or post-meal**

**breaks from sitting.** Before specific exercise prescriptions can be developed, many studies investigating sex differences in are necessary.

### **Specific Aims**

To address the gaps in knowledge highlighted by this literature review, we proposed the following 3 specific aims:

#### **Study 1**

To determine how 2 different types of breaks from sitting (walking vs. simple resistance activities) affect glucose responses during and after a laboratory intervention.

- We compared daily and postprandial glucose responses during the breaks from sitting conditions to an all sedentary control condition.

#### **Study 2**

To compare the effect of a bout of continuous morning walking and post-meal breaks from sitting on glucose responses in the free-living adults with T2D.

- We compared daily and postprandial glucose measures in the active conditions to participants' normal sedentary behavior
- We assessed the dose-response relationship between 3 different durations of activity (20, 40 and 60 minutes)

#### **Study 3**

To evaluate sex differences in the glucose response to continuous morning walking and post-meal breaks from sitting in free-living adults with T2D.

- We compared the effect of conditions and the dose response relationships investigated in Study 2 between men and women.

## Tables

**Table 2.1: Timing of Exercise and Subsequent Measurement of Glucose**

<i>Reference</i>	<i>Timing of Exercise (relative to meal)</i>	<i>Timing of Glucose Measurement</i>	<i>Glycemic Effects</i>
Rynders <i>et al.</i> , 2014	1h pre-meal	3h after exercise recovery	↓ 3h glucose AUC
Oberlin <i>et al.</i> , 2014	Pre-meal (morning)	0-48h post exercise	↓ 24h mean glucose ↓↓↓ 2h glucose at all meals
Colberg <i>et al.</i> , 2009	Immediately pre/post-meal	During/2h post exercise	↓↓↓↓ Glucose 90m post-meal
Hostmark <i>et al.</i> , 2006	Immediately post-meal	During/2h post exercise	↓↓↓↓ Peak glucose Blunted rise in glucose
Nygaard <i>et al.</i> , 2009	Immediately post-meal	During/2h post exercise	↓ Peak glucose ↓ 2h AUC
Poirier <i>et al.</i> , 2001	Immediately post-meal	During/90m post exercise	↓↓↓↓ 60-90m glucose (vs. fasted EX)
Colberg <i>et al.</i> , 2014	30 min post-meal	During/2.5h post exercise	↓↓↓↓ 90m post-meal
Larsen <i>et al.</i> , 1997	45 min post-meal	During/4h post exercise and after 2 <sup>nd</sup> meal	↓↓↓ 4h AUC and blunted rise in glucose No effect of 2 <sup>nd</sup> meal

↓: 0-9%; ↓↓: 10-19%; ↓↓↓: 20-40%



## CHAPTER 3

### INTERRUPTING PROLONGED SITTING IN TYPE 2 DIABETES: NOCTURNAL

#### PERSISTENCE OF IMPROVED GLYCEMIA

*Dempsey PC, Blankenship JM, Larsen RN, Sacre JW, Sethi P, Straznicky NE, Cohen ND, Cerin E, Lambert GW, Owen N, Kingwell BA, Dunstan DW. Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycemia. [Submitted to Diabetologia]*

#### Introduction

Postprandial hyperglycemia is linked to an increased risk of micro- and macro-vascular complications, particularly in individuals with type 2 diabetes (T2D) (2, 37). Even with anti-hyperglycemic medications, adults with T2D can spend between 25 and 40% of the day, and approximately 2-h on average nocturnally, in a state of hyperglycemia (blood glucose >10 mmol/l) (179). Further, the frequency and magnitude of glucose fluctuations and oscillations throughout the day (glycemic variability) may also increase the risk of diabetic and cardiovascular complications independently of overall glycemia (42, 58, 128). Therefore, identifying safe and effective ways to manage postprandial glucose homeostasis is imperative within T2D management.

Lifestyle modification, including physical activity, remains a key cornerstone in T2D management. A 30-60 min continuous bout of exercise has been shown to significantly improve glycemic control and insulin sensitivity for up to 72-h post-exercise bout (175, 182). Current T2D guidelines recommend that individuals engage in a minimum of 150 min of moderate-vigorous physical activity a week, in bouts of at least 10 min (46). However, despite the multitude of benefits, many adults with T2D do not meet physical activity recommendations (189). Indeed,

population studies demonstrate that adults can spend over 65% of their waking hours in sedentary behaviors (involving prolonged sitting), while only ~5% of waking hours are spent in moderate-vigorous physical activity (56, 120). These large volumes of sedentary time are associated with higher T2D risk, even after controlling for leisure-time moderate-vigorous physical activity (24, 185).

Recent experimental evidence suggests that reducing and interrupting prolonged sitting with brief bouts (<5 min) of standing or light ambulation has acute beneficial effects on postprandial glucose and insulin concentrations in healthy, overweight/obese adults and in those with prediabetes (44, 57, 92). We recently expanded upon these findings, providing the first laboratory evidence in patients with T2D that regular brief interruptions to high amounts of prolonged sitting (7-h) with light-activities (3 min bouts every 30 min) significantly improved concurrent postprandial glucose, insulin and C-peptide responses following standardized mixed-meals (166). Further, in healthy-active young adults, a day of light-intensity physical activity and minimal sitting (<6 hours) improved whole body insulin action the following morning, compared to a day of prolonged (16 hours) sitting (55). Altogether, these studies highlight the detrimental effects of prolonged sitting and benefits of interrupting and reducing overall sitting time. However, it remains unclear whether benefits in T2D patients: 1) persist beyond the immediate 7-h intervention period (i.e. nocturnally through until waking the subsequent morning); and 2) extend to reductions in glycaemic variability.

Utilizing continuous glucose monitoring (CGM) technology to better understand meal-to-meal and temporal glucose homeostasis, glycaemic variability

and potential carryover effects beyond the controlled laboratory setting (55), we compared the impact of 7-h prolonged sitting to sitting interrupted with brief bouts of light-intensity walking (LW) or simple resistance activities (SRA) on 22-h glucose homeostasis in adults with T2D. We hypothesized that 7-h of interrupting prolonged sitting time would lower postprandial glucose responses, 22-h hyperglycaemia and glycaemic variability, and that improvements in glycaemic control would be sustained nocturnally through to the morning following the intervention.

## **Methods**

### **Participants**

As previously reported, non-smoking men and women [body mass index (BMI) 25-40 kg/m<sup>2</sup>] aged 35-75 years with T2D [diet or Metformin-controlled, ≥3 months duration, based on American Diabetes Association diagnostic criteria (11)] were recruited. Participants were excluded if they self-reported sitting <5h/day and/or were meeting physical activity guidelines (≥150 min/week of moderate-intensity exercise). The study was approved by the Institutional Human Research Ethics Committee and all participants provided written informed consent.

### **Study design**

This randomized crossover trial was undertaken at the Baker IDI Heart & Diabetes Institute between October 2013 and November 2014. Detailed screening and testing procedures have been described previously (55). In brief, participants attended the laboratory on five separate occasions: 1) medical screening visit; 2) familiarization visit; and 3-5) three acute 8-h trial condition visits in a randomized

order, each separated by 6-14 days. Trial condition order was randomized by a third party (block-randomization and balanced block sizes) and stratified by sex.

### **Experimental protocol and laboratory conditions**

On trial condition days, participants arrived at the laboratory at ~0715h after a 12h fast. For 48h prior to condition days, participants were asked to abstain from caffeine, alcohol, and structured moderate-vigorous physical activities (i.e., no physical activity beyond activities of daily living). Each laboratory condition was 8-h total duration (~0800-1600h; see *Figure 3.1*) and commenced with a 60 min 'steady-state' period (-1h to 0h), after which participants consumed standardized breakfast (0h) and lunch (3.5h) meals, with the time taken to consume (<20 min per meal) replicated in subsequent conditions. Participants began the following experimental protocols after the breakfast meal: A) SIT: uninterrupted sitting; B) LW: sitting interrupted with 3 min bouts of light-intensity walking ( $3.2 \text{ km}\cdot\text{h}^{-1}$ ) every 30 min; and, C) SRA: sitting interrupted with 3 min bouts of simple resistance activities every 30 min (comprising 20 s body weight half-squats, 20 s calf raises, 20 s gluteal contractions and knee raises; repeated 3 times in sequential order while mimicking a standardized video recording).

Participants sat upright in a comfortable chair throughout each 8-h laboratory condition and were instructed to minimize excessive movement, only rising from the chair to void. Standardized lavatory visits incorporated into the protocol minimized unscheduled physical activity; however, additional lavatory visits were permitted. Participants complied with the respective laboratory 8-h condition protocols under direct supervision from research staff.

At the end of each 8-h laboratory visit (~1600h), participants returned home and were asked to consume their standardized evening meal between 1900-2000h that evening and sleep at their usual time, keeping these timings as consistent as possible for subsequent conditions. Participants were asked to keep this timing as consistent as possible between trial conditions. As per the 48h lead-in to each trial condition, participants were asked to abstain from caffeine and alcohol.

### **Standardization of diet, medications and physical activity**

To minimize any potential diet-induced variability during testing periods, meals were standardized between conditions and were individualized to meet 33% of daily estimated energy requirements using the Schofield equation and a physical activity factor of 1.5 (159). The target macronutrient profile was 12-15% energy from protein, 55-58% from carbohydrate and 29-31% from fat. Evening meal packs were provided for participants to consume (between 1900- 2000h) at home on the evening of, and prior to, each experimental condition. Prescribed medications were continued throughout the study.

Participants were instructed to maintain their normal physical activities after leaving the laboratory, but refrain from any structured moderate-vigorous physical activity until after the removal of the CGM the following morning. To objectively measure any possible postural compensatory behaviour during the evening of the test day that may have occurred as a result of the trial condition, an activPAL3™ tri-axial physical activity monitor (PAL-technologies Ltd, Glasgow, Scotland) was worn on the right thigh during each condition for 22-h for objective measurements of time spent sitting, standing, and stepping while both inside and outside the

laboratory (81). As previously described (55), anthropometric, biochemical, dietary and accelerometer-derived physical activity data 48h before each of the respective trial conditions were not significantly different.

### **Continuous glucose monitoring**

A continuous glucose monitor [iPro2 CGM with Enlite® sensors (Medtronic, Northridge, CA, USA)] was inserted immediately upon arrival at the laboratory (0700-0715h) by trained research personnel into the subcutaneous fat in the lumbar region and secured using a thin clear film according to the manufacturer's instructions. Once inserted, the CGM recorded interstitial fluid glucose concentrations every 5 min for 22-h (data collection period from 9am on trial day until 7am the following morning). For subsequent conditions, new sensors were inserted within approximately two centimeters of the initial insertion site. To calibrate the CGM, capillary (finger-stick) blood glucose samples were collected at six standardized times across the 22-h period (three in the laboratory and three at home) according to the manufacturer's instructions using a commercial, time-stamped glucometer (Abbott Freestyle Optium, Witney, OX, UK). Participants were provided with verbal and written instructions on how to collect the three capillary measurements at home, the times of which were later confirmed in the laboratory using the glucometers stored memory function. Validation studies have demonstrated good agreement between individual glucose measurements derived via Enlite® sensors and venous blood (14, 53), along with test-retest reliability (171).

### **Physical Activity Monitors Data Handling**

Physical activity monitor data (*activPAL* events files) were processed in SAS 9.4 (SAS Institute Inc., Cary NC) to generate time spent sitting, standing, and stepping for both the trial condition (laboratory) and the post-trial-condition until bedtime (evening) periods. A modified algorithm was used to identify participant sleep time as 20+ minutes of continuous sitting/lying occurring at or following self-reported bedtime (91). Invalid/non-wear days were identified as containing <10h of waking wear,  $\geq 95\%$  of waking wear time spent in any one activity or <500 steps (65).

### **Continuous Glucose Monitor Data Handling**

CGM data were analyzed using R-statistical software package, version 3.1.2 (R-Foundation for Statistical Computing, Vienna, Austria). Data were summarized into 3 different time periods: overall (waking and nocturnal hours over 22-h), meal times and nocturnal. To summarize the overall CGM data, we calculated 22-h mean glucose and total area under the curve ( $AUC_{total}$ ) using the trapezoidal method from a baseline concentration of zero. Time in hyperglycemia was quantified as time spent with glucose  $>10$  mmol/l. Glycaemic variability over 22 h was calculated using the following indices: percent coefficient of variance (%CV), standard deviation of glucose ( $SD_{glucose}$ ), mean amplitude of glycaemic excursion (MAGE) and continuous overall net glycaemic action (CONGA). An automated algorithm in R developed by Baghurst and colleagues (13) was used to calculate  $SD_{glucose}$ , MAGE and  $CONGA_1$ . Finally, we determined %CV by dividing the  $SD_{glucose}$  by mean 22 h glucose.

Meal times for breakfast, lunch and dinner were defined as 15 min before the meal through to 3-h after the end of the meal. The time that participants were eating the meal was excluded. We calculated the baseline glucose concentration before each meal (mean of glucose during the 15 min before meal). To summarize each meal response we calculated the net incremental area under the curve (iAUC) because it has been shown to be more reflective of the glucose response to a meal than  $AUC_{total}$  (9). Net iAUC was calculated for each meal as all incremental area below the curve, subtracting the area below each pre-meal baseline glucose concentration from that above. Finally, time in hyperglycemia was calculated for all meal periods.

Nocturnal glycemia was defined as the period beginning with *activPAL*-derived sleep time through to self-reported wake time the next day. Nocturnal glycemia was quantified by mean glucose,  $AUC_{total}$  and time in hyperglycemia. Waking glucose was defined as the average of the final 15 min of the 22-h CGM period for all participants.

Generalized linear mixed-models with random intercepts were used to evaluate the differential effects of the experimental conditions on all summary outcome variables using Stata 12 (StataCorp LP). Residuals were examined for serial correlation, heteroscedasticity and normality. Substantial departures from model assumptions were not observed. A two-tail probability level of 0.05 was adopted. Data are expressed as mean $\pm$ SEM in text unless otherwise stated. All models were adjusted for potential covariates explaining residual outcome variance (age, BMI and sex), including pre-prandial values and period effects (treatment order) for



glucose outcomes. Glycemic variability measures were additionally adjusted for mean glucose concentrations. Meal-by-condition, sex-by-condition and BMI-by-condition interaction tests were also performed for mean glucose, iAUC and time in hyperglycemia.

## **Results**

### **Participant characteristics**

Twenty-four participants were randomized and completed all trial conditions (see *Table 3.1*). Aside from BMI (men 31.5 versus women 35.2 kg·m<sup>-2</sup>,  $p=0.005$ ), there were no significant differences in sex-related baseline parameters or medications (55).

### **Postural allocation and meal/sleep periods**

Data from the *activPAL* are shown in *Table 3.2*. By design, the LW and SRA conditions saw greater proportions of the laboratory period spent standing or stepping (versus SIT). In turn, LW and SRA were characterized by greater allocations of time to stepping and standing, respectively. During the evening period, there were no significant differences in time spent seated, standing or stepping between trial conditions.

Recorded dinner, bedtime and waking times were between 1815-2030h (mean=1915h), 2038-0221h (mean=2228h) and 0545-0920h (mean=0701h) respectively. Mean ( $\pm$ SD) within-participant differences in dinnertime ( $23\pm 20$  min), bedtime ( $48\pm 32$  min) and waking time ( $24\pm 16$  min) were not significantly different between trial conditions, nor were mean sleep durations (SIT: 8h 12 min $\pm$ 55 min, LW: 7h 55 min $\pm$ 57 min, SRA: 8h 13 min $\pm$ 56 min) (all  $p>0.1$ ).

## **22-h glucose homeostasis and glycemic variability**

An overview of the 22-h glycemic profiles is presented in *Figure 3.1*. Over the entire 22-h period, mean glucose concentrations, cumulative AUC<sub>total</sub> and time spent in hyperglycemia (>10 mmol/l) were all significantly reduced during the LW and SRA conditions compared to SIT (*Table 3.3*). Measures of glycemic variability (MAGE, CONGA<sub>1</sub> and SD<sub>glucose</sub> glycemia) were significantly reduced for the LW and SRA conditions compared to SIT when adjusting for baseline glucose levels and other covariates, but not after additionally adjusting for mean 22-h glucose levels (*Table 3.4*). Similarly, %CV was not significantly different between conditions. No significant differences were observed between LW and SRA for any glycemic outcomes. No hypoglycemic episodes (i.e. glucose <3.9 mmol/l) were observed during any of the trial conditions.

### **Postprandial glycemic control**

Mean glucose, iAUC, and time spent in hyperglycemia were all significantly lower for the LW and SRA conditions compared to SIT for each meal (see *Figure 3.2* and *Table 3.3*). A significant meal-by-condition interaction effect was observed for mean glucose and glucose iAUC responses (*Figure 3.2a* and *3.2b*), but not time in hyperglycemia (*Figure 3.2c*). Both LW and SRA reduced glucose concentrations for each meal period compared to SIT. The largest reductions in mean glucose and glucose iAUC were observed during breakfast compared to both lunch and dinner (*Figure 2a* and *2b*;  $p < 0.05$ ). Further, mean glucose and iAUC reductions, following the dinner meal, were significantly greater for the SRA condition compared to both

LW and SIT (*Figure 3.2a and 3.2b*;  $p<0.05$ ). No significant sex-by-condition or BMI-by-condition interaction effects were observed for any of the glycemic variables.

### **Nocturnal glycemic control**

Mean glucose concentrations, AUC<sub>total</sub> and time spent in hyperglycemia were all significantly reduced during the sleeping period (see *Table 3.3*). Mean glucose concentrations were significantly lower the morning following the intervention for both LW and SRA (both  $-2.7\pm0.4$  mmol/l;  $p<0.001$ ) compared to SIT (*Table 3.3*). No significant differences were observed between LW and SRA.

### **Discussion**

The novel finding in this study is that interrupting high levels of prolonged sitting (7-h) with brief bouts of LW and SRA (3 min every 30 min) significantly lowered 22-h hyperglycemia, including nocturnal hyperglycemia, in inactive overweight/obese adults with T2D. Importantly, while reductions in postprandial glucose were observed during the 7-h controlled laboratory period, improved glycemic control persisted into the subsequent free-living evening and sleeping periods until the following morning. An average waking glucose reduction of 2.7 mmol/l was observed for both the LW and SRA conditions compared to prolonged sitting.

These findings provide unique insights beyond our primary experimental observations in the controlled laboratory setting (55) and those of others (116, 133, 141, 180, 183) in T2D patients, demonstrating that brief bouts of LW and SRA compared to prolonged sitting over 7-h elicit persistent and clinically meaningful improvements in postprandial hyperglycemia over 22-h. Our results are supportive

of the broader hypotheses postulated in epidemiological studies (89, 91), and emerging experimental evidence (20), suggesting that reducing and frequently interrupting very high levels of prolonged sitting time may represent important clinical and public health interventions in T2D management.

The use of CGM facilitated the tracking of glucose homeostasis both within the laboratory conditions and during subsequent free-living and nocturnal periods outside the laboratory. Exposure to postprandial hyperglycemia ( $>10$  mmol/l) was highly prevalent during the observed 22-h period. Indeed, for the prolonged sitting condition, participants spent 57% more time in hyperglycemia over the 22-h compared to both active conditions. For perspective, this duration of time in hyperglycemia equates to approximately twice that previously reported in individuals with T2D on standardized diets while observed in a free-living environment (142, 179, 180). In addition, time spent in nocturnal hyperglycemia was more than 60% longer for prolonged sitting compared to the activity conditions.

These differences in glycemic control observed between the prolonged sitting and activity interruption conditions highlight both the persistent and detrimental nature of very high levels (i.e. 7-h) of prolonged sitting in T2D patients, but also the beneficial effects of regular brief interruptions in prolonged sitting. Recent studies have reported similar reductions in 24-h time in hyperglycemia with a single bout of aerobic or resistance exercise compared to a non-exercise condition (180, 183), and interruptions in prolonged sitting more effectively reduced nocturnal glycemia compared to a 30 min bout of pre-lunch moderate-intensity

walking in overweight/obese adults (25). Whether the improvements in glycemic control induced by interruptions in sitting time intervention are similar to that of a continuous 30-45 min bout of exercise in T2D remains to be determined.

The most marked reductions in postprandial glucose responses were observed following breakfast, likely due to overall glucose responses being highest for this meal. Glucose responses across all conditions were lower following the lunch and dinner meals compared to breakfast, which is concordant with the second-meal phenomenon (98). The reduction in time spent in hyperglycemia was slightly lower for the dinner meal (though not statistically significant) compared to breakfast and lunch, which could be a consequence of the breakfast and lunch meals being closer together than lunch and dinner, allowing less time for glucose clearance. Interestingly, while postprandial glucose excursions were improved with both LW and SRA compared to SIT for each of the three post-meal periods, the postprandial glucose responses following the dinner meal were significantly lower for SRA compared to LW. While the reasons for this are unknown, it could be related to the nature of the activity-break intervention (55) and/or differential effects on hepatic glucose output or peripheral insulin sensitivity (151, 175). However, such factors would not fully explain why glucose concentrations were generally similar between LW and SRA during both the laboratory and sleeping periods.

This is the first study to report data on glycemic variability when comparing a bout of prolonged sitting to sitting frequently interrupted with brief bouts of activity in T2D. Although the prognostic value of glycemic variability in T2D remains controversial (126), largely due to the relatively recent advent of CGM technology

and a lack of prospective data, there is evidence to suggest that greater glycemic variability is adversely associated with endothelial dysfunction, oxidative stress and diabetic complications (42, 58, 128). In the absence of a gold-standard measure to assess glycemic variability, we computed a range of commonly used variability measures. While significant reductions in MAGE, CONGA<sub>1</sub> and SD<sub>glucose</sub> were observed with LW and SRA compared to SIT, these effects were not apparent following statistical adjustment for mean glucose levels. These findings – together with the lack of between-condition differences in %CV (which directly normalizes for mean glucose) – point to a similar *relative* magnitude of glucose fluctuations around its lower “set point” in the LW and SRA conditions, rather than less instability *per se*.

The measures used in this study do not permit conclusions on the putative mechanisms responsible for the improvements in glycemic control. However, in the same participants, we previously reported concurrent attenuations in both venous glucose, insulin and C-peptide during the LW and SRA conditions relative to the prolonged sitting condition (55). The lower C-peptide supports reduced endogenous insulin secretion – suggesting either enhanced insulin sensitivity or a greater reliance on insulin-independent contraction mediated glucose disposal, or both (22, 175). Indeed, recent investigations suggest that the skeletal muscle contraction-mediated glucose uptake pathway may be particularly important during acute one day interventions examining frequent ambulatory interruptions in sitting time (22).

A key strength of this study is the randomized cross-over design, which incorporated both controlled-laboratory, free-living and nocturnal elements.

Participants were their own controls, which enhanced both the internal validity and reliability of our data and permitted a smaller sample size. The laboratory trial and subsequent free-living phases were examined with the use of objective, posture-discriminating devices, while participants consumed a standardized, ecologically valid, Western-type diet (3). The continuous activity measurements, alongside CGM, enabled us to account for these key activity and dietary behaviors, thereby increasing the experimental rigor of our findings.

We acknowledge that both the prescribed activity/sedentary behaviour during the laboratory phase and the dietary profiles (e.g. macronutrient profile, glycaemic index, meal frequency and size) may not reflect habitual behaviours in sedentary individuals in real-world settings and could have exaggerated the glycaemic differences we observed between trial conditions. Although it was important to first establish ‘proof-of-concept’ in a controlled laboratory setting and to accurately describe dose-response parameters, 7-h prolonged sitting with only 1-2 toilet breaks – while plausible under some circumstances (e.g. extended automobile/plane journeys or those who may be required to carry out uninterrupted desk work to meet deadlines) – is likely to be an extreme scenario for much of the population.

It will be important to establish the efficacy of these interventions in T2D patients with more advanced disease, particularly as such patients are more likely to have poorer glycemic control, are more likely to experience hypoglycemic episodes, and may be less responsive to exercise-mediated glucose-lowering (164). Nevertheless, our findings have relevance to a majority of those with T2D (~80-

85%), who are not treated with insulin or insulin combined with other oral hypoglycaemic agents (40). Further, it was also encouraging that no hypoglycaemic events were observed despite marked reductions in postprandial hyperglycaemia during our sitting-breaks conditions.

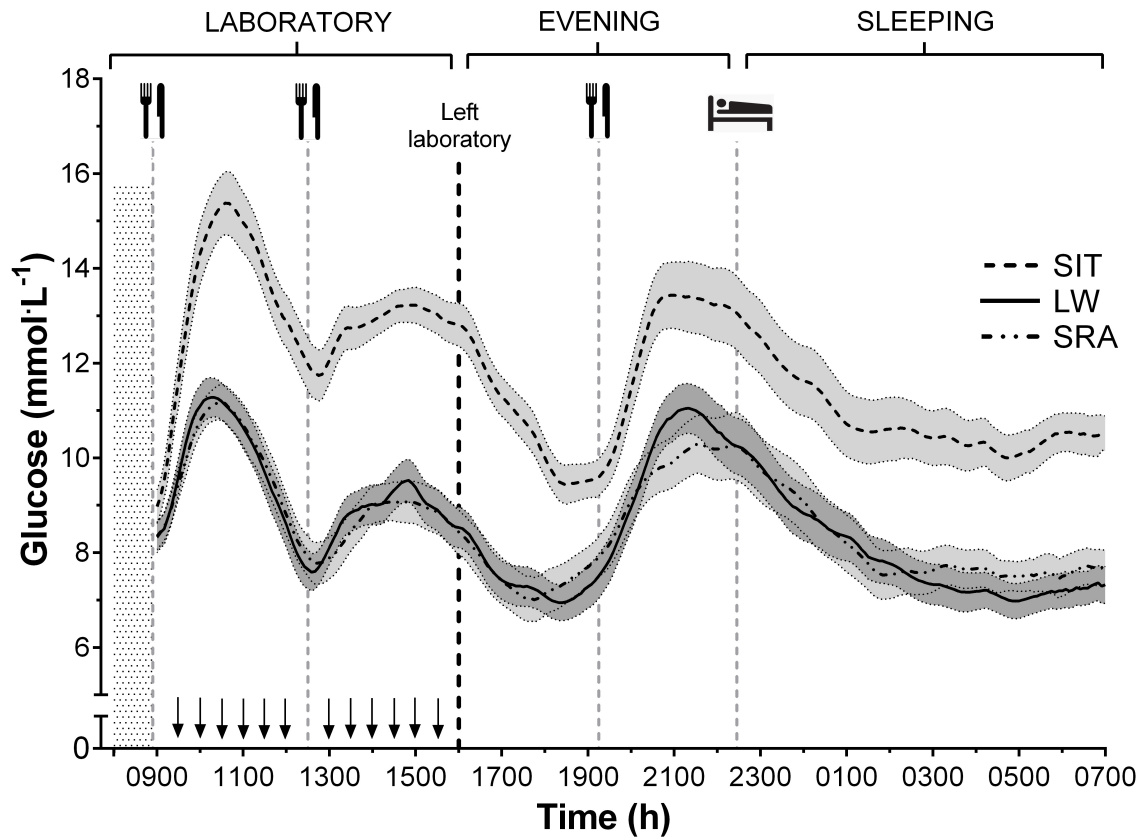
Future studies should consider examining the glycemic effects of interrupting prolonged sitting interventions in more ecologically relevant, free-living and workplace environments that are more reflective of habitual sitting patterns. It will also be important to determine the effects over longer time-periods (i.e. multiple days or weeks) and the specific mechanisms by which different light-intensity activities improve glycemic control. In the interest of optimizing 24-h and postprandial glycemia for T2D management, it would be relevant to compare and combine strategically placed frequent interruptions in sitting with a structured bout of exercise to determine whether or not timing and/or dose-dependent (additive) relationships exist. In these contexts, a further consideration will be the impact of energy balance, which was not strictly controlled in the current study.

In conclusion, this study demonstrates that interrupting high levels of prolonged sitting with brief light walking or simple resistance activity bouts over 7-h reduces sequential postprandial glucose responses in adults with T2D, with glycaemic improvements persisting until the next morning. Although longer term efficacy, practicality and suitability for the workplace and home environment still need to be established, there is the potential for interrupting prolonged sitting time to be an effective intervention for relatively well- controlled T2D patients living or working in environments that demand or encourage high levels of prolonged sitting



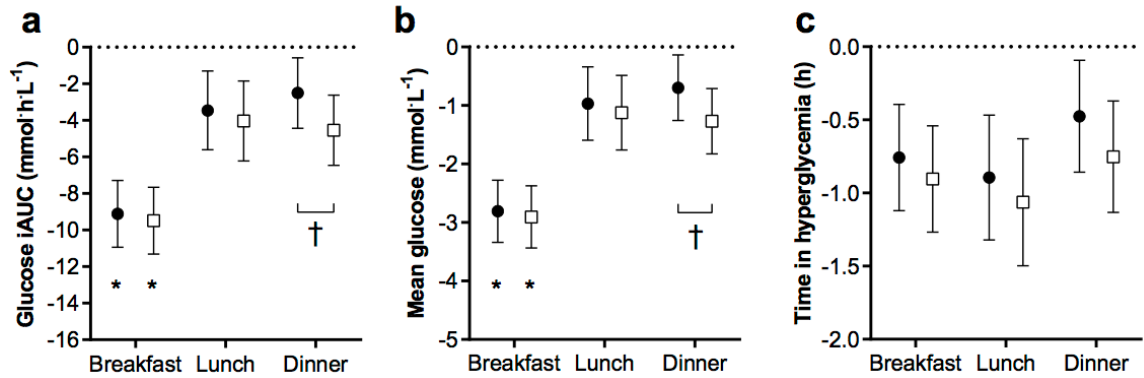
time. This strategy, in parallel with a whole-day approach to increasing unstructured physical activities, may be a particularly helpful adjunct in T2D management for those who are sedentary, de-conditioned, or unable or unmotivated to engage in structured moderate-vigorous exercise.

## Figures



**Figure 3.1: 22-h glucose profiles over 22 hours during experimental condition and free-living environment**

Mean  $\pm$  SEM glucose profiles over 22-h during and following each trial condition. SIT, Uninterrupted sitting. LW, sitting + light-intensity walking bouts. SRA, sitting + simple resistance activity bouts. The shaded area (prior to 0900 h) denotes the 1-h sitting steady-state prior to commencing each trial condition. Intervals for the 3 min activity bouts every 30 min during the LW and SRA interventions are illustrated by the arrows. As denoted by the grey vertical dashed lines, standardized meals in the laboratory were consumed at 0900 h and 1230 h, while mean dinnertime and bedtimes were at 1915 h and 2228 h respectively. The thick black vertical dashed line denotes when participants left the laboratory at ~1600 h. The continuous grey horizontal line at 10 mmol/L represents the hyperglycemic threshold. See text and Table 3.2 for further details and statistical comparisons of glycemic measures.



**Figure 3.2: Change in Postprandial Glycemia from Control Condition**

Difference relative to uninterrupted sitting in (a) mean glucose, (b) glucose net incremental area under the curve (iAUC), and (c) time in hyperglycemia by meal for light- intensity walking bouts (black circles; LW-SIT) and simple resistance activity bouts (white squares; SRA-SIT). \*Differences in LW-SIT and SRA-SIT significantly larger for breakfast meal compared to lunch and dinner meals ( $P < 0.001$ ). †SRA-SIT significantly different from LW-SIT ( $P < 0.05$ ). Data are expressed as mean (95% CI).

## Tables

**Table 3.1: Participant Characteristics**

Demographics	
Sex (male/female)	14/10
Age (y)	62 ± 6
BMI (kg/m <sup>2</sup> )	33.0 ± 3.4
Waist circumference (cm)	112.6 ± 9.7
Diabetes duration (y)	6.8 ± 5.1
Ethnicity	
European	20 (83%)
Asian	4 (17%)
Medications, n (%)	
Metformin	23 (96%)
Statin	15 (63%)
Anti-hypertensive	16 (67%)
Metabolic and cardiovascular risk factors	
HbA <sub>1c</sub> (%) <sup>b</sup>	7.2 ± 0.7
HbA <sub>1c</sub> (mmol/mol) <sup>b</sup>	55.1 ± 8.0
eGFR (ml·min <sup>-1</sup> ·1.73m <sup>-2</sup> ) <sup>b</sup>	86.7 ± 8.1
Fasting glucose (mmol/l) <sup>a</sup>	8.2 ± 1.4
Fasting insulin (pmol/l) <sup>a</sup>	85.9 ± 54.7
Fasting triacylglycerol (mmol/l) <sup>a</sup>	1.9 ± 1.0
Fasting total cholesterol (mmol/l) <sup>a</sup>	4.4 ± 0.8
Fasting LDL-cholesterol (mmol/l) <sup>a</sup>	2.5 ± 0.8
Fasting HDL-cholesterol (mmol/l) <sup>a</sup>	1.1 ± 0.3
Systolic blood pressure (mmHg) <sup>b</sup>	123 ± 14
Diastolic blood pressure (mmHg) <sup>b</sup>	77 ± 9

Data are expressed as mean ± SD or number (%) where specified. <sup>a</sup> Measured at the screening visit. <sup>b</sup> Measured at the beginning of the first trial condition.

**Table 3.2: Physical Activity During the Laboratory Condition and Evening Period after Condition**

	<b>Total time (min)</b>	<b>SIT</b>	<b>LW</b>	<b>SRA</b>
Laboratory	Wear time	504 ± 3	504 ± 3	502 ± 3
	Sitting	499 ± 3	449 ± 3*	453 ± 3*
	Standing	4 ± 1	9 ± 1*	30 ± 1*†
	Stepping	2 ± 1	46 ± 1*	19 ± 1*†
Evening	Wear time	417 ± 24	418 ± 25	375 ± 24
	Sitting	279 ± 11	265 ± 11	262 ± 11
	Standing	108 ± 21	119 ± 22	79 ± 21
	Stepping	30 ± 2	34.6 ± 3	35 ± 2
Laboratory+Evening	Wear time	919 ± 29	900 ± 30	839 ± 30*
	Sitting	777 ± 17	700 ± 18*	682 ± 17*
	Standing	110 ± 21	123 ± 22	105 ± 21
	Stepping	32 ± 3	79 ± 3*	52 ± 3*†

Data are expressed as mean ± SEM. SIT, Uninterrupted sitting. LW, sitting + light-intensity walking bouts. SRA, sitting + simple resistance activity bouts. \*significantly different from SIT ( $p < 0.05$ ); †significantly different from LW ( $p < 0.05$ ).

**Table 3.3: Glycemic Control Over 22-h and Nocturnal Glycemia**

		<b>SIT</b>	<b>LW</b>	<b>SRA</b>
22-h	Mean glucose (mmol·L <sup>-1</sup> )	11.6 ± 0.3	8.9 ± 0.3*	8.7 ± 0.3*
	AUC <sub>total</sub> (mmol·h·L <sup>-1</sup> )	254.9 ± 6.7	194.7 ± 6.6*	191.5 ± 6.6*
	Time in hyperglycemia (h)	14.7 ± 0.9	6.3 ± 0.8*	6.3 ± 0.9*
Sleeping	Mean glucose (mmol·L <sup>-1</sup> )	10.6 ± 0.4	8.1 ± 0.4*	8.3 ± 0.4*
	AUC <sub>total</sub> (mmol·h·L <sup>-1</sup> )	86.9 ± 3.7	64.6 ± 3.6*	68.0 ± 3.7*
	Time in hyperglycemia (h)	4.7 ± 0.4	1.4 ± 0.4*	1.8 ± 0.4*
	Waking glucose (mmol·L <sup>-1</sup> )	10.3 ± 0.3	7.6 ± 0.3*	7.7 ± 0.3*

Data are expressed as mean±SEM. SIT, Uninterrupted sitting. LW, sitting + light-intensity walking bouts. SRA, sitting + simple resistance activity bouts. Sleeping is the time-period from bedtime until end of the 22-h period. AUC<sub>total</sub>, total area under the curve. \*significantly different from SIT ( $p < 0.05$ ).

**Table 3.4: Glycemic Variability Over 22-h**

		<b>SIT</b>	<b>LW</b>	<b>SRA</b>
MODEL 1	% CV	19.8 ± 1.2	21.8 ± 1.2	20.7 ± 1.2
	SD <sub>glucose</sub> (mmol/L)	2.3 ± 0.1	1.9 ± 0.1*	1.8 ± 0.1*
	MAGE (mmol/L)	5.9 ± 0.3	4.6 ± 0.3*	4.3 ± 0.3*
	CONGA <sub>1</sub> (mmol/L)	2.0 ± 0.1	1.6 ± 0.1*	1.5 ± 0.1*
MODEL 2	SD <sub>glucose</sub> (mmol/L)	2.1 ± 0.1	2.0 ± 0.1	1.8 ± 0.1
	MAGE (mmol/L)	5.2 ± 0.4	5.0 ± 0.3	4.7 ± 0.3
	CONGA1 (mmol/L)	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1

Data are expressed as mean ± SEM.

Model 1: adjusted for baseline glucose, treatment order, BMI, age and sex.

Model 2: additional adjustment for 22-h mean glucose concentrations.

SIT, uninterrupted sitting. LW, sitting + light intensity walking bouts. SRA, sitting + simple resistance bouts. \*significantly different from SIT (p<0.05).

## **CHAPTER 4**

### **MANAGING FREE-LIVING HYPERGLYCEMIA WITH EXERCISE OR INTERRUPTED**

#### **SITTING IN TYPE 2 DIABETES: AN ECOLABICAL APPROACH**

##### **Introduction**

Type 2 diabetes (T2D) has become an epidemic worldwide. Since 1980, the World Health Organization estimated that the prevalence of diabetes has quadrupled, affecting 422 million adults worldwide (4). Characterized by high circulating blood glucose (hyperglycemia), T2D is associated with a number of comorbidities including obesity, hypertension, and dyslipidemia (97). Further, diabetes increases the risk of cardiovascular disease (CVD) by up to 4 times compared to normoglycemic individuals (69, 100). The causes of the increased CVD risk in diabetes are multifaceted and include hyperglycemia, insulin resistance, inflammation and oxidative stress (118). The variability in glucose values throughout the day may also represent a particular risk for CVD (43).

Several large epidemiological studies have shown strong associations between glucose concentrations after a meal (postprandial) and CVD mortality in diabetes (37, 38). Carefully controlled studies in humans have shown that postprandial glycemia interferes with the blood vessels ability to vasodilate (41, 42). The exact mechanisms responsible for the interaction between high blood glucose and vascular function have not been well defined but likely include oxidative stress or inflammation caused by high postprandial glucose (118). Regardless of the mechanism, postprandial glucose concentrations are central to the strong relationship between diabetes and CVD. Most individuals with T2D spend 25-

40% of each day in hyperglycemia (glucose >10 mmol/L), (179, 180). Further, postprandial hyperglycemia is the major contributor to the overall level of hyperglycemia among individuals with an HbA1c < 7.3 (127). Since postprandial hyperglycemia is the major driver of total hyperglycemia in mild to moderate diabetes, managing glucose after meals in T2D is a top priority among health care providers.

Sedentary behavior, like prolonged sitting, has been associated with high blood glucose 2 hours after a meal (88, 93). Fritschi and colleagues (72) objectively measured sedentary behavior and free-living glucose concentrations in a large group of adults with T2D. Not only did participants in this study accumulate a large amount of sedentary time during waking hours (> 8 hours/day) but sedentary time was significantly associated with the total time in hyperglycemia (glucose >10 mmol/L). Adults spend more than 60% of waking time engaged in sedentary behaviors (120, 176, 178) and less than 5% of waking hours in moderate to vigorous physical activity (119). Further, there is evidence that individuals with T2D are even more sedentary than people without diabetes (45). Because sedentary time dominates most waking hours and is strongly related to hyperglycemia in T2D, reducing sedentary behavior might be an effective strategy to lower overall hyperglycemia.

There is a wealth of evidence documenting the glucose lowering effects of continuous and intermittent physical activity at a range of intensities in people with diabetes (47, 49, 96, 105, 132, 133, 139, 153). While broad concepts are known, the exact dose-response relationship and timing of activity induced changes in glucose



remain less clear. A single bout of exercise results in immediate reductions in daily glucose (116, 180) and, after several months of exercise training, improves long term glycemic control (48, 71, 115). For example, van Dijk and colleagues demonstrated that a single bout of aerobic or resistance exercise lowers the daily duration of hyperglycemia by 35% (180). Despite the well-recognized benefits of exercise for managing hyperglycemia in T2D, the majority of adults do not exercise (119). Short light physical activity interruptions in sitting time minimize the exaggerated glucose responses caused by prolonged sitting. Several groups have observed lower postprandial glucose concentrations and a shorter duration of hyperglycemia when sitting time is interrupted with light physical activity (55, 62). Because most adults don't exercise, performing light physical activity might be beneficial to lower blood glucose and may represent a clinically important strategy to manage hyperglycemia in T2D.

In free-living settings, normal behavior dictates that people get up from their chairs and take breaks from sitting throughout the day (e.g. to prepare meals, use the bathroom). While important from a laboratory control perspective, having people sit continuously for 8-15 hours is not representative of normal human behavior (62, 136, 166). Performing a study in a real world relevant (ecological) setting has high generalizability but there are many sources of variability (diet, sleep). Without controlling some of these key sources of variability, namely diet, interpretation of blood glucose data is almost impossible. To improve upon ecological models, we've created a hybrid approach which we call *ecolabical* that

provides the benefits of the real world setting while retaining control of specific experimental variables.

It is unknown whether it is better to deliver physical activity as one continuous bout versus an equivalent dose delivered in smaller amounts for hyperglycemia in diabetes. Using our *ecolabical* approach, the primary aim of this study was to assess the comparative effectiveness of increasing total physical activity through continuous walking after breakfast (EX) or light physical activity breaks in sedentary time after meals (BR) on postprandial glycemic control during a day. Targeting post-meal periods, when glucose is high, as a time to insert breaks from sitting may be a more acceptable alternative to taking regular breaks during every waking hour of the day (62). We hypothesized that by performing EX after breakfast, EX would result in lower postprandial glucose compared to BR following breakfast. BR would result in better overall postprandial glycemic because of regular muscle contractions and postprandial glucose uptake throughout the day. Since the assigned doses of physical activity (20, 40 or 60 minutes of additional physical activity) were the same between EX and BR, we hypothesized that 24-hour glycemic control would be equivalent in the active conditions (EX and BR) and both would be better when compared with a sedentary condition (CON).

A secondary aim of this study was to compare three volumes of activity to determine whether a dose-response relationship exists between postprandial physical activity and postprandial glycemic control. Participants in the BR and EX conditions were assigned to perform an additional 20, 40 or 60 minutes of physical activity during the day. Because there will be more muscle contractions in the

highest activity dose, the greatest improvement in postprandial glycemic control was predicted to be seen with 60 minutes of physical activity, followed by 40 minutes and then 20 minutes.

Finally, as an exploratory aim, we determined the impact of reallocating sedentary and physical activity behaviors on glycemic control throughout the day. We used isothermal substitution analysis, which takes into account that time is not infinite. If an individual decreases time in sedentary behavior, time spent in an active behavior (e.g. standing, stepping) must increase. Isothermal substitution analysis has been used previously in cross-sectional studies investigating the health effects of substituting sedentary behavior for moderate to vigorous physical activity (32, 186). To date, these analyses have not been applied to physical activity and continuous glucose monitoring data. We sought to investigate the utility of applying this statistical method to continuously measured physical activity and glucose data.

## **Methods**

### **Participants**

Thirty sedentary individuals with T2D (BMI range: 22.4-41.9 kg/m<sup>2</sup>) participated in this study. Participants were between the ages of 39-74 years old and had diabetes diagnosed by a physician for at least 6 months and were not taking insulin to manage their diabetes. Individuals taking insulin were excluded from the study to minimize the risk of exercise induced hypoglycemia. Other medications not known to interfere with blood glucose control (e.g. blood pressure, cholesterol lowering medication) were permitted in the study. At the time of enrollment, participants self-reported that they were not meeting the physical activity and

health guidelines of at least 150 minutes moderate to vigorous physical activity per week. The Institutional Review Board at the University of Massachusetts approved this study protocol. All participants gave written and verbal informed consent before beginning the study.

### **Experimental Design**

This study included 4 visits to the Energy Metabolism laboratory and a 1-week free-living environment period. An overall schematic of the study design is found in *Figure 4.1*. During the first visit, participants completed a health history questionnaire to assess factors related to their health (e.g. duration of diabetes diagnosis, list of medications). Afterwards, resting metabolic rate and body composition were measured in each participant. Participants then returned to the laboratory on a separate visit to pick up their physical activity monitor (activPAL, PAL Technologies Ltd, Glasgow, Scotland), and food for the free-living environment period. During this visit, a trained researcher inserted a continuous glucose monitor (iPro2, Medtronic, Northridge, CA, USA) into the subcutaneous fat of the participant's abdomen. Participants were instructed to wear the activPAL and continuous glucose monitor during the entire week.

During the free-living environment period, participants performed 3 experimental conditions: morning walk after breakfast (EX), post-meal breaks from sitting (BR), and sedentary control (CON). Each condition was separated by one washout day of normal activity and was performed in a counter-balanced order. To minimize the impact of diet variability on daily glycemic control, meals were provided to participants during each condition. Depending on the activity volume

group they were assigned to (low, moderate, high), participants were instructed to increase their physical activity in the EX and BR conditions by 20 minutes (low activity volume group), 40 minutes (moderate activity volume group) or 60 minutes (high activity volume group). In the EX condition, participants went on a brisk walk after breakfast for their designated amount of time within 30 to 60 minutes after breakfast. During the BR condition, participants performed short physical activity breaks every 30 minutes for 2 hours after breakfast, lunch and dinner (12 total bouts of activity spread across 3 postprandial periods). The duration of each break from sitting was 1.67, 3.33 or 5 minutes long to correspond to a total 20, 40 or 60 minutes of added daily physical activity. A description of conditions is depicted in *Table 4.1*. The goal was to match the duration of total physical activity in the BR condition to the EX condition. In the CON condition, participants followed the same dietary control and were asked to maintain their habitual physical activity behaviors.

After the free-living environment period was over, participants returned to the laboratory for the final visit and returned the physical activity monitors. The continuous glucose monitor was removed by a trained researcher and all data were downloaded from the monitors.

### **Resting Metabolic Rate**

Resting metabolic rate was measured using open circuit spirometry (TrueMax2400 Metabolic Measurement System, Parvomedics, Salt Lake City, UT). Participants entered the laboratory fasted for at least 12 hours and laid supine for a period of 10 minutes. Afterwards, resting energy expenditure was measured for 15

minutes. Resting metabolic rate was calculated by taking the average of the last 10 minutes of energy expenditure data.

### **Body Composition**

To assess body composition (fat free mass and fat mass), dual energy x-ray absorptiometry, DEXA, (Lunar, Madison, WI) was used. Participants laid on a bed and a moveable arm passed over them while emitting low level x-rays of two different photon energies. The x-ray beams pass through the body and are absorbed by the bones, fat tissue and lean tissue. The DEXA machine measured energy from the x-ray beams that was not absorbed by the body. Based on known x-ray absorption rates of bone, fat tissue and lean tissue, a report was generated, quantifying bone density and total fat free mass and fat mass (21).

### **Dietary and Medication Control**

Participants were provided all food for each of the conditions. Resting metabolic rate was multiplied by an activity factor of 1.4 to determine daily caloric needs for a sedentary individual and to ensure that participants were approximately in energy balance during all conditions (8, 68). A combination of solid foods and non-caffeinated beverages were given to participants to consume in 3 discrete meals. Each meal was designed to contain the same number of total calories to have the same relative contribution from fat, carbohydrate and protein (*Table 4.2*). Participants were instructed to consume each meal within 30 minutes and to separate meals by at least 4 hours. The timing of the meals was chosen by participants and recorded in a meal timing log. Participants used the meal timing log

to replicate meal times during each condition. During the washout days, participants recorded their energy intake using food logs.

Throughout the study, participants recorded any medications or supplements that they took on the provided medication log.

### **Continuous Glucose Monitoring**

We used an iPro2 continuous glucose monitor (Medtronic, Northridge, CA, USA) to measure interstitial glucose concentrations. A trained researcher inserted a small disposable glucose sensor into the participant's subcutaneous fat of the abdominal area using sterile techniques. The glucose sensor measured glucose concentrations in the interstitial fluid using glucose oxidase based electrochemical methods (39). Participants were instructed to measure capillary glucose concentrations 3-4 times per day around meal times and before going to bed to calibrate the interstitial glucose signal to blood glucose concentrations.

At the end of the free-living environment period, data were downloaded from the monitor using the web-based iPro Carelink Software. Before analyzing data from continuous glucose monitors, the data were examined for completeness. Participants with less than 75% of 24 hour CGM data during a condition were excluded from final analysis (n=4).

The daily glucose response was characterized by the following measures:

- *Daily mean glucose concentrations*: mean glucose of 24-hour data
- *Total area under the curve*: trapezoidal area under the curve of 24-hour data

- *Mean amplitude of glycemic action (MAGE)*: index of glycemic variability calculated using automated algorithms published by Baghurst and colleagues (13)
- *Continuous overlapping net glycemic action (CONGA<sub>4</sub>)*: index of glycemic calculated using automated algorithms published by Baghurst and colleagues (13)
- Standard deviation (SD): standard deviation of 24-hour glucose data
- Daily duration of hyperglycemia: daily duration of time glucose > 10 mmol/L

Using the meal logs, continuous glucose monitor data was separated by postprandial periods. A postprandial period was defined as the 3 hours after the end of a meal. During this time, the postprandial response was characterized by the following measures:

- *Pre-meal glucose concentration*: mean glucose of the 15 minute interval prior to beginning of the meal
- *Incremental area under the curve (iAUC)*: trapezoidal area under the curve minus area under pre-meal glucose concentration
- *Peak postprandial glucose (PPG)*: peak glucose
- *Time to peak glucose*: duration to peak glucose concentration
- *Rate of change to PPG*: peak glucose concentration divided by time to peak glucose
- *Rate of decline to 150 minutes*: rate of change from peak glucose to glucose concentration at 150 minutes



- *Postprandial duration of hyperglycemia*: duration of time glucose >10mmol/L

All CGM data were examined for completeness. We excluded CGM data that was less than 75% complete for each period of interest (24 hour and postprandial periods). The percent of complete CGM data are reported for each condition in *Table 4.3*.

### **Physical Activity Monitoring**

Participants wore an activPAL on the midline of the right thigh to assess physical activity and sedentary behavior. The activPAL uses accelerometer-derived information about thigh position to estimate time spent in different body positions (i.e., sitting/lying, standing & stepping) with high level of accuracy (80, 103, 145, 169). Non-wear time was eliminated prior to analysis of the activPAL data. The activPAL generated event and 15 second epoch files were processed using SAS 9.4 (SAS Institute Inc., Cary NC). Physical activity and sedentary behavior was characterized using the following measures:

- Time spent sitting (total minutes and % of wear time)
- Time spent standing (total minutes and % of wear time)
- Time spent stepping (total minutes and % of wear time)
- Duration of prolonged sitting (time spent sitting in bouts of >30 minutes)
- Number of breaks from sitting (transition from sitting to standing)

Daily physical activity and sedentary behavior was quantified for the entire day during each of the conditions (EX, BR and CON) beginning with the self-reported wake time on the day of the condition and lasting through the self-reported wake

time the following morning. We also quantified physical activity and sedentary behavior during the non-study days (normal activity day). Data with less than 10 hours per day were considered invalid and were eliminated from the dataset. Participants had a range of 1-4 valid days during non-study days (mean: 2.8, SD: 0.8). The average of the valid days was determined to represent their normal levels of physical activity and sedentary behavior.

Physical activity and sedentary behavior was also characterized during each meal period (start time of meal through 3 hours after end of meal) using the same measures described above. This process was repeated for all meals consumed during each of the study days.

### **Statistical Analysis**

All statistical analyses were performed using the statistics package and computing language, R (R Foundation for Statistical Computing, Vienna, Austria, 2008; [www.R-project.org](http://www.R-project.org)). Significance for all statistical tests was set at  $p < 0.05$ . All data are expressed as mean  $\pm$  standard deviation (SD) unless otherwise noted. Differences in participant characteristics between men and women were assessed by analysis of variance (ANOVA) and Tukey HSD post hoc testing. Linear mixed models were used to evaluate differences between conditions for measures of physical activity, sedentary behavior and glucose control (24-hour and postprandial). Interactions between postprandial glucose control and meals were also performed. In a subgroup analysis we determined the effect of EX and BR among individuals with high postprandial glucose at CON (greater than 50% of meal spent in hyperglycemia).

To assess the dose-response relationship between glycemia and physical activity, we subset the glucose data to only include the active conditions (EX and BR). Linear mixed models were then used to assess whether there were differences in glycemia (24-hour and postprandial) by volume of activity of activity performed. Volume of activity was quantified as the activity volume group participants were assigned to (low, moderate, high) and by the percent of time spent stepping. Separate linear mixed models were run with either the categorical variable (activity volume group) as the dependent variable or the continuous variable (percent time spent stepping). In all models, fit linear regression models to evaluate the relationship between change in physical activity and change in glycemia. Additionally, we tested for an interaction between condition (EX and BR) and volume of activity. We performed the same subgroup analysis to determine the dose response relationship among individuals with high levels of hyperglycemia at CON.

Finally, we used isothermal substitution modeling to assess the impact of substituting sedentary behavior for active behaviors (e.g. standing and stepping) on 24-hour and postprandial glucose control. Because there was not a wide range of stepping and standing in this study, we combined standing and stepping to represent active behaviors. Each model contained 1 of the 2 behaviors (sitting, active behavior) as independent variables and one of the indices of glucose control. Isothermal substitution models were performed for each of the measures of glucose control (e.g. mean, duration hyperglycemia).

## **Results**

### **Participant Characteristics**

Thirty individuals (14 men and 16 women) participated in this study. There were no significant differences between men and women for age, body mass index or years since diabetes diagnosis. Men had significantly higher RMR ( $p<0.01$ ), TDEE ( $p<0.001$ ), and percent body fat ( $p<0.0001$ ) compared to women (*Table 4.4*). All women enrolled were postmenopausal.

There were no significant differences in any of the participant characteristics between the assigned activity volume groups (*Table 4.5*). Fat mass was significantly higher in men compared to women in the moderate activity volume group ( $p<0.0001$ ).

### **Medications**

Participants took a variety of glucose lowering medications. There were 15 individuals who only took biguanides (metformin). Eight participants took biguanides in combinations with other glucose lowering agents. Nine individuals were taking sulfonylureas either exclusively ( $n=2$ ) or in combination with other glucose lowering agents. The full medication breakdown is illustrated in *Figure 4.2*.

### **Physical Activity**

Mean wear time during condition days was not significantly different between any of the conditions (overall mean:  $906.9 \pm 137.6$  minutes). None of the physical activity summary measures were different between CON and normal activity days.

Total stepping time was significantly higher in EX and BR compared to control (*Figure 4.3, Figure 4.4*), and there were no differences in stepping time or step count between EX and BR (*Table 4.6*). Number of breaks from sitting (sit to stand transitions) were significantly lower in EX compared to CON. While participants did not increase breaks from sitting in BR, the duration of prolonged sitting was significantly lower in BR compared to CON. During physical activity conditions (EX and BR), sitting and standing time were not different from CON.

### **Differences in Glycemia During Conditions**

#### **24-hour Glucose Control**

Baseline glucose was significantly lower in BR compared to CON by 12.9 mg/dL ( $p=0.02$ ). Mean and peak glucose were normalized to baseline glucose concentrations. Using the baseline adjusted glucose measures, there were no significant differences in mean glucose, peak glucose or area under the curve between any of the conditions. Daily duration of hyperglycemia was lower by 1.8 hours in EX compared to CON ( $p=0.06$ ), but EX was not significantly different from BR.

#### **Postprandial Glycemic Control**

Mean continuous postprandial glucose is depicted in *Figure 4.5*. Postprandial glucose was significantly lower in EX vs. CON for the following summary measures: duration of hyperglycemia (reduced by  $11.4 \pm 4.0\%$ ,  $p=0.005$ ) and rate of decline from peak glucose to 150-minute glucose ( $p=0.04$ ). There were no significant differences between EX vs. CON for the following measures: baseline adjusted mean,

peak and 150-minute glucose, incremental area under the curve, and rate of increase from baseline to peak glucose.

Postprandial duration of hyperglycemia was lower in BR and CON (reduced by  $7.5 \pm 4.1\%$   $p>0.1$ ) but there were no significant differences in any of the postprandial glucose measures between BR compared to CON. There were also no significant differences between EX compared to BR.

In contrast, individuals with a high postprandial hyperglycemia at CON had a significantly shorter duration of hyperglycemia in both EX and BR and there were no differences between the two active conditions (*Figure 4.6*). Duration of hyperglycemia was not different between CON, EX or BR among individuals with low postprandial hyperglycemia at CON.

### **Meal Specific Effects**

There was no interaction between condition and meal for any of the postprandial glycemia measures. Overall, compared to breakfast, baseline adjusted mean glucose and peak was lower and daily duration of hyperglycemia was shorter at lunch during all conditions ( $p<0.01$ ). The rate of increase to peak glucose was highest at dinner (significantly different from breakfast,  $p<0.0001$ ) and was lowest at lunch (significantly different from breakfast and dinner  $p<0.05$ ). Finally, the rate of decline from peak glucose to 150-minute glucose was significantly higher in lunch and dinner compared to breakfast ( $p<0.05$ ).

### **Dose-Responses within Physical Activity Conditions**

There was no significant interaction between condition and activity volume for either the 24-hour glucose control or postprandial glucose summary measures.

Because there was no interaction, the data from EX and BR were combined to investigate differences across activity volume groups (low, moderate and high). We only included data from the active conditions (EX and BR) to assess the dose response relationship between activity volume and glycemic control.

### **24-hour Glucose Control**

There were no significant differences in 24-hour glucose control between activity volume groups. Additionally, there was no significant associations between change in stepping time and change in any measure of 24-hour glucose control.

### **Postprandial Glucose Control**

Baseline adjusted mean (*Figure 4.7*), 150-minute glucose and incremental area under the curve were significantly lower in the moderate compared to the low activity volume group ( $p<0.05$ ). Postprandial duration of hyperglycemia, baseline adjusted peak glucose, and the rate of increase or decrease were lower but not significantly different between the moderate and low activity volume group. Overall, the measures of postprandial glycemia in the high activity volume group were lower than the low activity volume group, but these differences were also not statistically significant. Finally, there were no differences between the moderate and high activity volume groups for any of the postprandial glucose measures.

A similar dose response relationship was found among individuals with a high duration of hyperglycemia at CON. People with a high duration of hyperglycemia at CON had significantly lower mean postprandial glucose in the moderate and high activity volume group compared to the low activity volume group (*Figure 4.8*). We did not observe any significant differences between the

moderate and high activity volume groups. Finally, there were no significant differences between any of the activity volume groups among individuals with low postprandial glycemia during CON.

Finally, there was no significant association between change in daily steps and change in postprandial glucose (*Figure 4.9*) or any of the postprandial glucose control measures.

### **Reallocating Physical Activity and Sedentary Behaviors**

There was no significant effect of reallocating sitting with standing or stepping on any of the measures of 24-hour glucose control.

The isothermal substitution models yielded 3 significant results for postprandial glucose control. Reallocating 30 minutes of sitting with an additional 30 minutes of non-sitting behaviors (e.g. standing or stepping) was associated with a reduction in postprandial incremental area under the curve (14.1% difference (CI:-1.0-29.2);  $p=0.06$ ) and a reduction in 3 hour postprandial glucose (40.6% difference (CI: 1.8-79.4);  $p=0.04$ ). Additionally, this substitution was also associated with a higher rate of increase to peak glucose (14.0% difference (CI: 2.8-25.2);  $p=0.02$ ).

### **Discussion**

The primary purpose of this study was to determine the comparative effectiveness of increasing physical activity by continuous walking (EX) or by breaking up sitting time after meals (BR) on daily and postprandial glucose control in a free-living environment. While we found that there were no significant differences between EX and BR, EX was the only condition to significantly shorten



daily and postprandial duration of hyperglycemia for all volumes of activity. We also sought to determine the dose-response relationship between physical activity and glycemic control and found that the moderate dose of activity (40 minutes) resulted in the most favorable postprandial glucose responses for the EX and BR conditions. Interestingly, in our subgroup analysis, both EX and BR significantly lowered duration of hyperglycemia in individuals with a high duration of postprandial duration of hyperglycemia. Additionally, the moderate and high dose of activity lowered mean postprandial glucose concentrations in these individuals.

An essential element to the design of this study was participant compliance to study conditions. According to our objectively monitored data, the study conditions were executed well by participants without researcher supervision. Steps were increased in the EX and BR conditions compared to CON and were not significantly different from each other, indicating that participants increased their total physical activity similarly in both active conditions. Prolonged sitting (duration of sitting in bouts greater than 30 minutes) was lower in the BR condition, but participants did not increase their daily frequency of breaks from sitting in a day. It is likely that participants performed their physical activity breaks from sitting at the same time as their activities of daily living (doing laundry, meal preparation, bathroom breaks). Importantly, because physical activity in CON was not significantly different from the normal activity days, we can be confident that the control condition is representative of our participant's habitual behavior. *Since we carefully controlled dietary intake during the study days, we are confident that the*

changes in glycemic control are due to differences in physical activity and sedentary behavior in our participants.

### **Effects of Physical Activity**

Overall, we observed modest reductions in postprandial glycemic control in the EX condition. Because the magnitude of the effect during exercise was so small, we were unable to detect differences between the EX and BR. While there were no significant differences between EX and BR, BR was not significantly lower than the CON condition. There are 2 major reasons that may explain the modest effects of the current intervention: total and prolonged sitting had a greater negative impact on glycemic control and exercise/physical activity stimulus was not high enough.

This study was designed to decrease the amount of prolonged sitting (defined as > 30 minutes). While our participants had a shorter duration of daily prolonged sitting, the intervention did not change total daily sitting or increase the frequency of breaks from sitting. Previous studies that have found significant glucose lowering effect of taking breaks from sitting either: (1) increased the number of breaks from sitting time relative to control (56, 62, 136) or (2) dramatically decreased total sitting time (63, 166, 173). It is well established that sitting time has a negative impact on glucose regulation and insulin sensitivity (166). Regularly interrupting sitting time has been proposed to negate some of the hazards of prolonged sitting time (57). However, based on our results in the BR condition, simply reducing the total duration of prolonged sitting is not enough to make a meaningful impact on postprandial glycemia in the free-living environment.

It is not clear whether increasing the total number of breaks from sitting per day would significantly impact glycemia.

On the other hand, the increased prolonged sitting in the EX condition may have counteracted some of the benefits of continuous exercise. The negative impact of sitting could potentially explain our observation of a modest benefit of walking after breakfast. Our previously published work (25) as well as others (63) have shown that high amounts of sitting during a day with a bout of exercise can minimize the benefits of that exercise bout on glucose and insulin. Duvivier *et al.* have also shown that replacing 1 hour of sitting with vigorous exercise was not enough to overcome the insulin resistance induced by sitting (63). Taken together, the negative effects of long durations of sitting in our subjects may have trumped the glucose lowering benefits of light physical activity and also minified the glucose benefits of continuous exercise.

The characteristics of our exercise and physical activity bouts (e.g. intensity and duration) may also explain the modest benefits we observed in daily and postprandial duration of hyperglycemia. In contrast to previous studies, we did not observe any differences in daily mean glucose or 24-hour area under the curve with an exercise bout. Van Dijk and colleagues (180) found that a 45-minute bout of either aerobic or resistance exercise reduced mean daily glucose from 9.6 to 8.6 mmol/L. Additionally, duration of hyperglycemia was reduced by  $33 \pm 11\%$  and  $35 \pm 7\%$ , respectively. Our intervention shortened the duration of hyperglycemia only by  $11.4 \pm 4.0\%$  and did not affect mean glycemia. Participants in our study were unsupervised whereas the exercise bout in the above mentioned study was

performed in a laboratory at a set intensity (50% of max watts). To simulate a real world environment, our participants were given general instructions on the intensity of their exercise (e.g. “walk at a brisk pace” “you should be able to easily carry on a conversation with someone else as you walk”). Because we did not give strict exercise intensity limits, the exercise intensity was likely not very high and as a result, we may have only observed modest reductions in daily and postprandial glucose.

In our subgroup analysis, we found that the glucose lowering effect of EX and BR were both significant in the individuals with high glycemia in the CON condition. Terada *et al.* demonstrated that the strongest predictor of the capillary blood glucose in response to exercise is the pre-exercise blood glucose concentration (170). Our findings support the idea that largest glucose lowering benefits occur among individuals with the highest initial glucose concentrations in the CON condition. Further, among these individuals, a continuous bout of exercise or purposeful physical activity breaks from sitting are equally effective to lower postprandial glucose concentrations. Thus, whether a continuous bout of exercise or light physical activity breaks from sitting is recommended to manage postprandial glycemia may depend on the baseline levels of postprandial glycemia.

### **Unexpected Dose-Response Relationships**

Our secondary aim was to determine whether a dose-response relationship exists between postprandial physical activity and postprandial glycemic control. After analyzing the dose of activity by the assigned activity volume group we identified that the moderate dose resulted in the greatest reductions in postprandial

glycemia. Surprisingly, the highest dose (60 minutes of additional physical activity) did not significantly lower postprandial glucose compared to the low group (20 minutes) and it was not significantly different compared to the moderate dose either. Only in our subgroup of individuals with high levels of postprandial hyperglycemia at CON were the moderate and high activity volume group both significantly lower than the low volume group. Individuals with low durations of hyperglycemia showed no significant lowering of postprandial hyperglycemia between any of the activity volume groups. Previous studies have shown significant reductions in postprandial glycemia with 60 minutes of continuous exercise (181). Based on the data from the activPAL, participants complied to study conditions. While it is puzzling that 60 minutes did not improve glycemia in all of our participants, it is encouraging that 60 minutes of physical activity did improve the individuals with high levels of hyperglycemia.

We did not find a significant association between daily steps and postprandial glucose concentrations. Since the activPAL does not reliably discriminate between activity intensities (34), we were unable to determine whether there were significant differences in activity intensity between the 3 volume groups which may have masked a relationship. More likely, expecting that one metric of physical activity, change in daily stepping time, will predict changes in glucose may be an overly simplistic view. The impact of stepping may depend on the behavior being replaced. For example, replacing 30 minutes of standing with 30 minutes of stepping may have a different health impact than replacing 30 minutes of sitting with stepping.

To better evaluate different combinations of behaviors on 24-hour and postprandial glycemic control we used the statistical method of isothermal substitution modeling. Our simple isothermal substitution models showed that replacing sitting time with active behaviors (e.g. standing and/or stepping) was associated with lower incremental area under the curve as well as 3-hour glucose concentrations. This result would be expected given the benefits of physical activity for lowering postprandial glucose. Based on the simple isothermal model, replacing sitting time with active behaviors was associated with a higher rate of increase to peak glucose. We would have predicted that physical activity would decrease the rate of change to peak glucose due to the contraction mediated glucose uptake during the time that glucose is rising. Reducing the rate of change to peak glucose is thought to also be beneficial for lowering the risk of CVD (43) because high rates of change are associated with increased production of oxidative stress and lower endothelial function. It is perplexing that replacing sitting time with active behaviors was associated with an increase in the rate of change to peak glucose. These contradictory findings may be a result of the variable nature of an *ecolabical* environment. However, because the clinical relevance of the rate of change of glucose concentrations is unknown, these results should be interpreted with caution.

Applying isothermal substitution modeling to continuously measured physical activity and glucose data has the potential to inform physical activity recommendations for individuals with T2D. This type of analysis can be very useful not only in identifying behaviors to improve glycemia, but also to avoid promoting

ineffective behaviors. Future research should work to generate larger datasets with diverse samples of individuals with a range of physical activity and sedentary behaviors. Promoting and supporting collaborations in this area will be critical in developing science driven and individually tailored physical activity recommendation to the public on a large scale.

Although our focus in the present study was glycemic control, it would be interesting to know the impact of these interventions on insulin sensitivity. It is possible that even though the BR condition did not improve glycemic control in all participants, there was a benefit in improved insulin sensitivity. In a previous study in our laboratory, we improved insulin sensitivity without a change in glycemia during an all standing day compared to an energy balance matched all sitting day in healthy young volunteers (166). Therefore future studies investigating both glycemic control and insulin sensitivity would yield a more complete picture of the health effects of exercise compared to breaks from sitting interventions.

This was the first study to investigate the glycemic impact of manipulating physical activity and sedentary behavior in the free-living environment in a clinically relevant population. A major strength of this study was using participants' normal behavior as the control condition. Previous studies in this area have compared physical activity interventions to extreme sedentary control conditions (63, 183). While these study designs are valuable in investigating mechanisms and informing future research, the generalizability to everyday life is limited. Future work investigating the health effects of breaks in sitting time interventions should consider adopting more ecologically relevant control conditions.

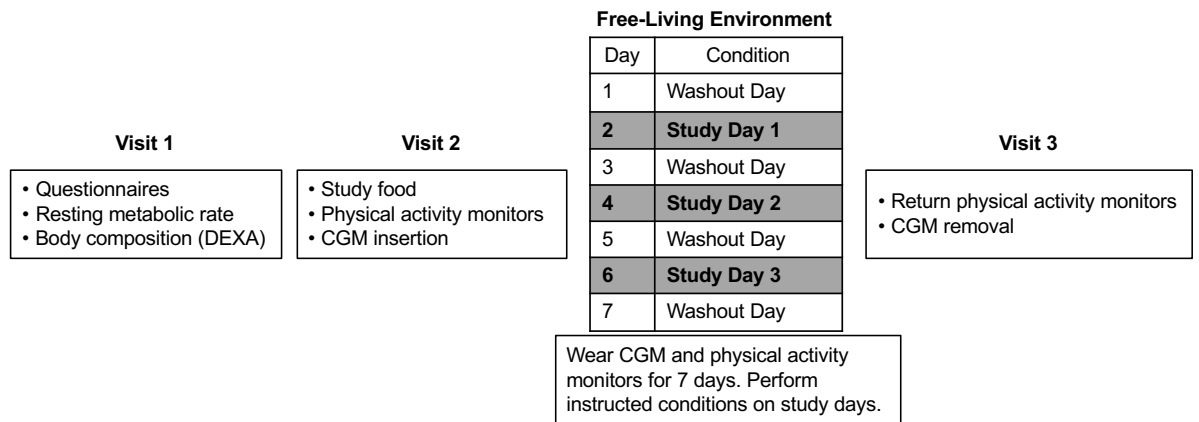
The results from the present study have major public health ramifications. We have shown that distributing 20-60 minutes of physical activity as light activity breaks from sitting after meals only results in a significant reduction in daily glycemic control among individuals with high levels of postprandial hyperglycemia. For the individuals in this study who had a less robust response, the dose of physical activity breaks in sitting may need to be much higher than it was in the present study. Others who have shown reductions in postprandial glycemia with breaks from sitting either dramatically reduce daily sitting time or increases in standing/stepping time. These strategies are difficult to implement in the real world. Light physical activity has been used effectively in the free-living to lower glucose concentrations in individuals with impaired glucose tolerance (59) and T2D (183). The common thread between these studies is that the light physical activity is performed as a continuous short bout (15 minutes) after meals. Based on current data available, using breaks from sitting as a means to manage high glucose concentrations is likely only effective for individuals who already have high postprandial glucose concentration and should be recommended after an assessment of free-living duration of hyperglycemia. Future studies investigating the benefits and effect of regular short walks ("exercise snacks" (136)) after meals as a means to manage postprandial hyperglycemia in diabetes may have a large public health impact.

In summary, we showed that a continuous bout of walking after breakfast confers a modest improvement in postprandial glycemia in the free-living environment. The differential glucose lowering impact of physical activity breaks



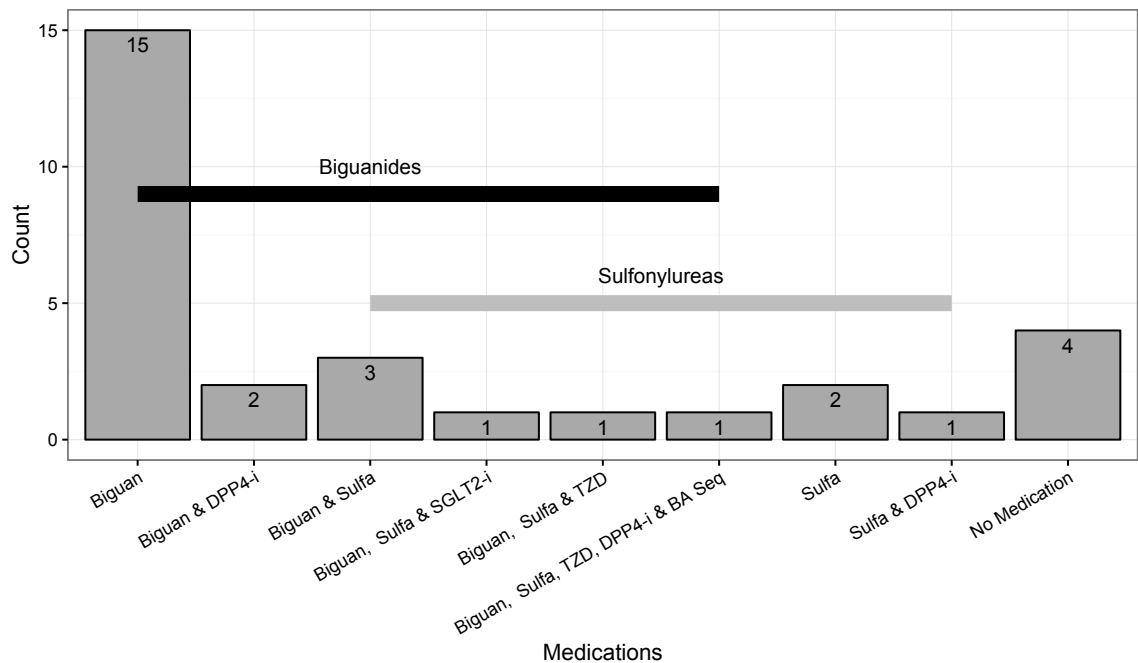
from sitting in the present study highlights the importance of applying interventions performed into the laboratory in the free-living environment. Future research studies should consider utilizing this *ecolabical* approach to determine the real world impact of these interventions on the health of individuals.

## Figures



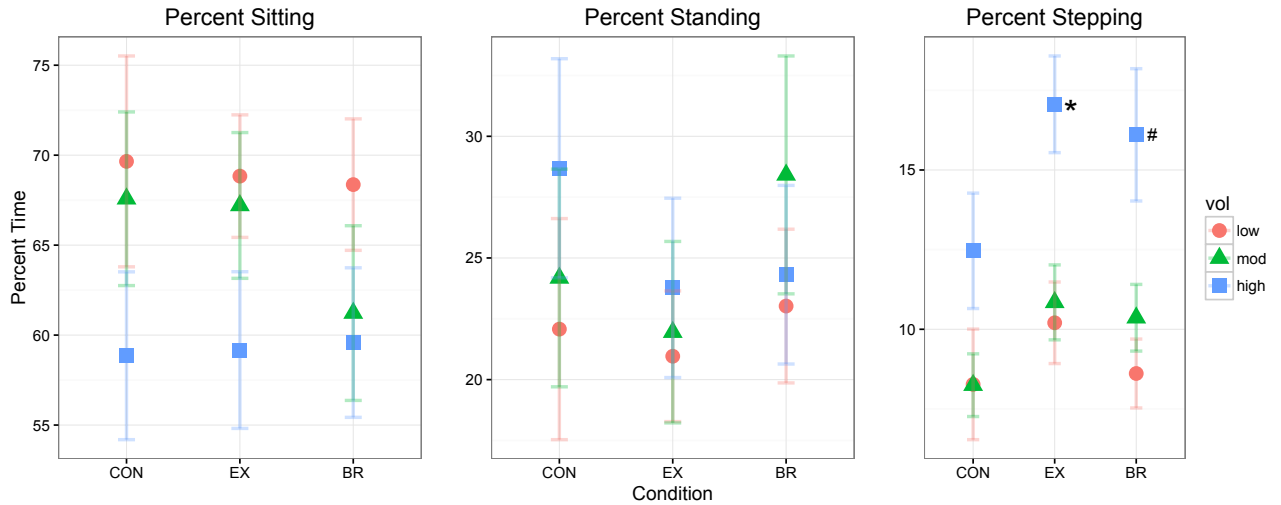
**Figure 4.1: Overall Study Design**

Participants performed the conditions (CON, EX and BR) on Study Day 1, 2 and 3. DEXA= dual energy x-ray absorptiometry, CGM= continuous glucose monitor



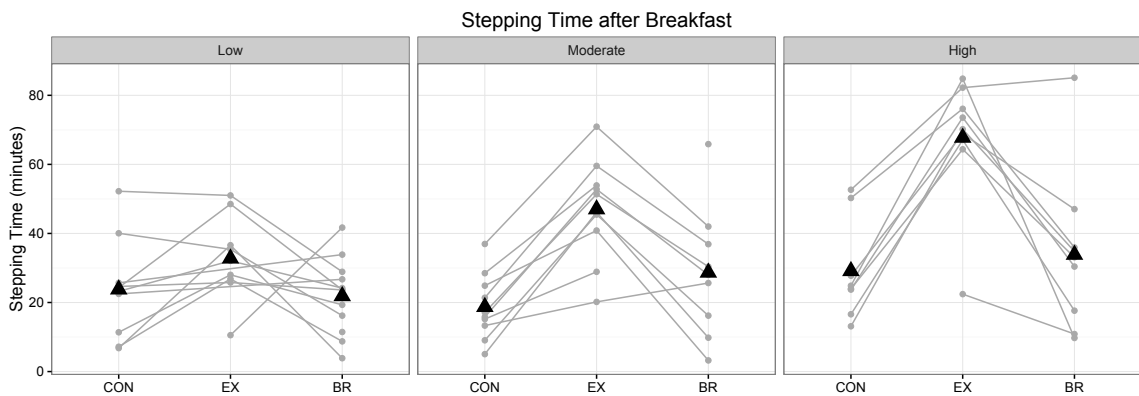
**Figure 4.2: Hypoglycemic medications combinations in participants**

Biguanides: Metformin; Sulfonylureas: Glipazide, Glimepiride, Glyburide; Thiazolidinediones: Pioglitazone; DPP4-inhibitors: Lingalipatin, Sitagliptin; SGLT2-inhibitors: Canagliflozin; Bile Acid Sequestrants: Colesevelam



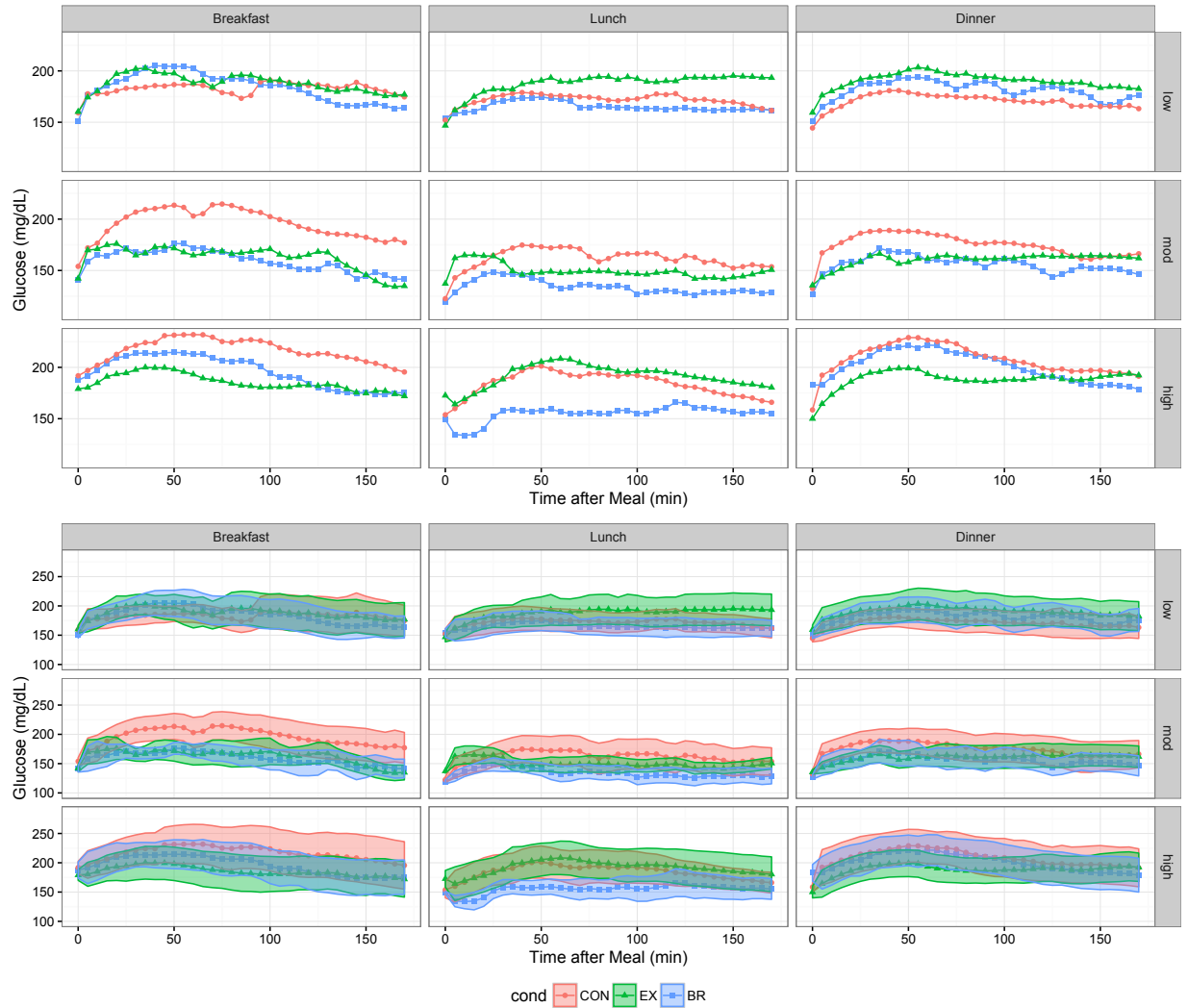
**Figure 4.3: Daily Physical Activity During Experimental Conditions**

Time spent sitting, standing and stepping expressed as a percent of total wear time in experimental conditions. Data are presented as mean  $\pm$  95% confidence interval. # compared to low volume CON condition  $p=0.03$ ; \* compared to low volume CON condition  $p=0.10$



**Figure 4.4: Stepping Time after Breakfast**

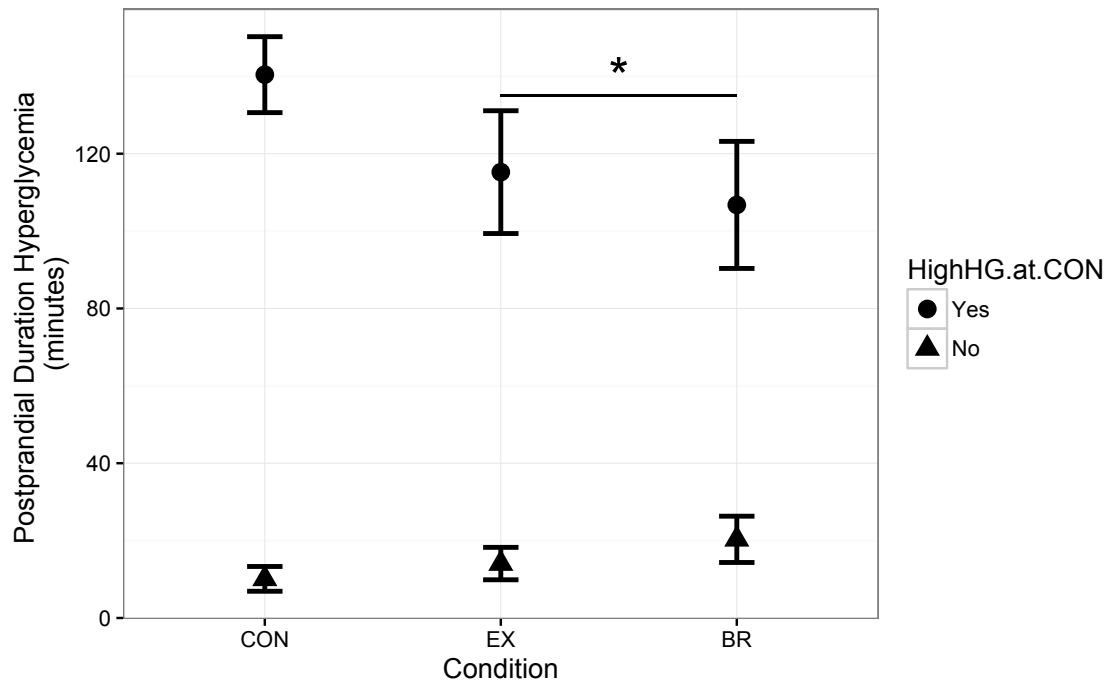
Time spent stepping in minutes after breakfast in all participants separated by activity volume group. Individual data points are plotted as filled grey circles and lines. Mean stepping for each activity volume group by condition is shown by black triangles.



**Figure 4.5: Postprandial Continuous Glucose Monitor Data**

*Top:* Continuous tracings of mean interstitial glucose separated by meal and activity volume group (low, moderate, high). CON (red circles), EX (green triangles), BR (blue squares).

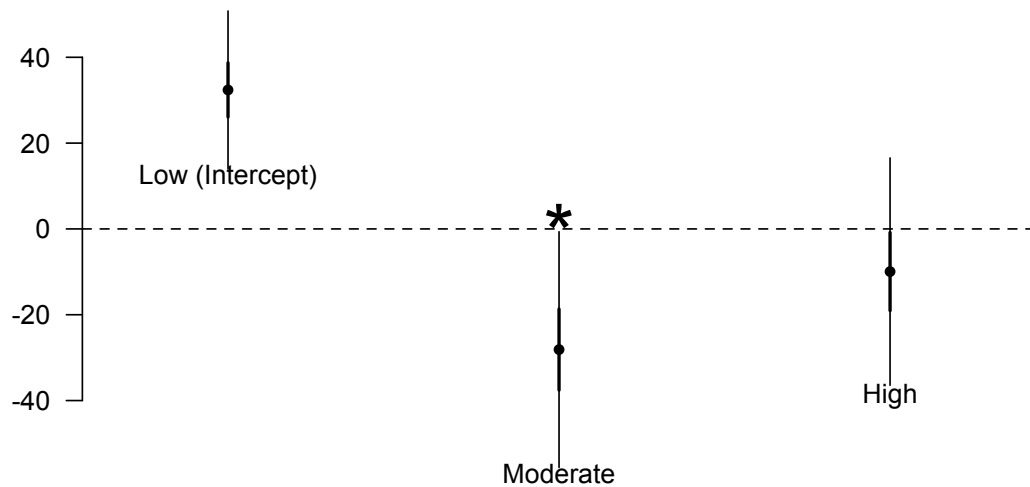
*Bottom:* Continuous tracings of mean interstitial glucose  $\pm$  standard error of the mean (SEM) separated by meal and activity volume group. CON (red circles), EX (green triangles), BR (blue squares).



**Figure 4.6: Postprandial Duration of Hyperglycemia in People with High Duration Hyperglycemia at Control**

Postprandial duration of hyperglycemia during CON, EX and BR in people who spent more than 50% of meal in hyperglycemia (black circles). Individuals who spent less than 50% of meal in hyperglycemia (black triangles). Data are presented as mean  $\pm$  SEM. \* significantly different from control ( $p < 0.05$ ).

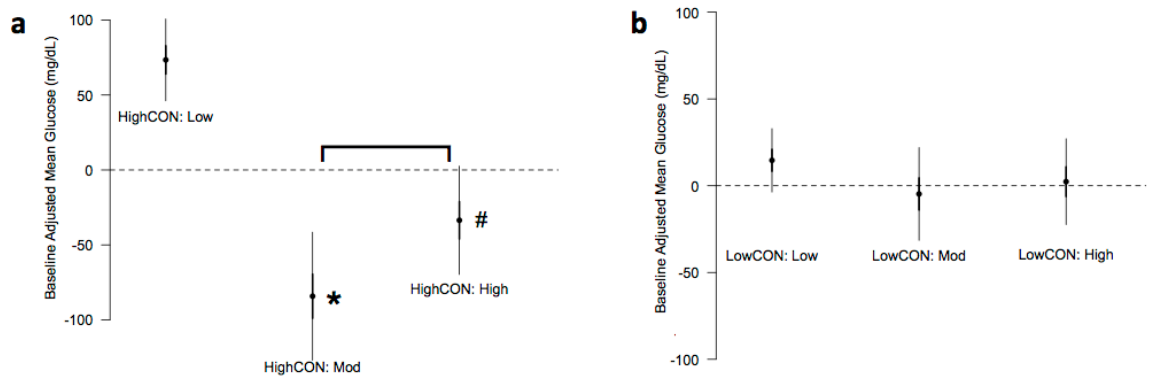
### Activity Volume Groups and Mean Postprandial Glucose Coefficients from Mixed Model



**Figure 4.7: Beta Coefficients from Mixed Model Regressions**

Beta estimates with 95% confidence intervals from mixed model regression plotted relative to intercept (low activity volume group). Actual estimated means for Moderate and High can be determined by adding the estimate from Low to the estimated mean for the respective group. \* significantly different from low activity volume group  $p < 0.05$ .

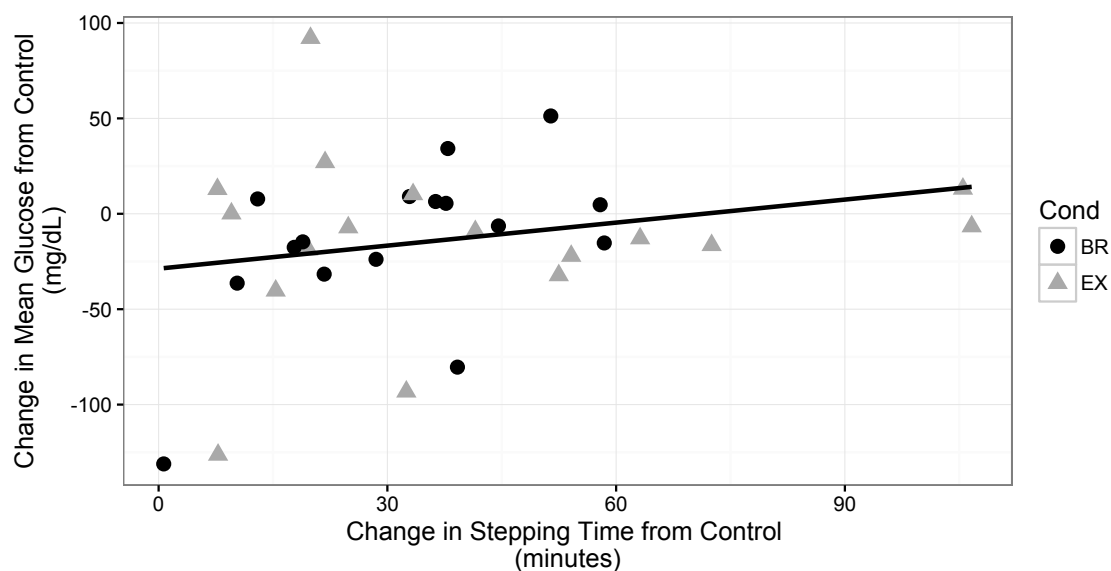
### High Glucose at Control: Dose Response Relationship



### Figure 4.8: Dose Response for People with High and Low Glucose at Control

Beta estimates with 95% confidence intervals from mixed model regression plotted relative to intercept (low activity volume group). Actual estimated means for Moderate and High can be determined by adding the estimate from Low to the estimated mean for the respective group.

- Results from linear mixed model including individuals with high duration of hyperglycemia at control \* significantly different from low activity volume group ( $p < 0.001$ ); # significantly different from moderate volume group ( $p = 0.01$ ), compared to low ( $p = 0.07$ )
- Results from linear mixed model including individuals with low duration of hyperglycemia at control.



**Figure 4.9: Dose-Response Between Change in Steps and Change in Postprandial Glucose**

Association between difference in change in daily steps and change in mean postprandial glucose from control (CON). Individual data points plotted from BR (black circles) and EX (grey triangles) conditions.



## Tables

**Table 4.1: Description of Experimental Conditions within each Activity Volume Group**

	Activity Volume Group			
	<i>Low</i>		<i>Moderate</i>	<i>High</i>
	<b>CON</b>	No additional activity	No additional activity	No additional activity
<b>Condition</b>	<b>EX</b>	1 bout of 20 minutes after breakfast	1 bout of 40 minutes after breakfast	1 bout of 60 minutes after breakfast
	<b>BR</b>	4 bouts of 1.67 minutes over 2 hours after breakfast, lunch and dinner	4 bouts of 3.33 minutes over 2 hours after breakfast, lunch and dinner	4 bouts of 5 minutes over 2 hours after breakfast, lunch and dinner

**Table 4.2: Relative Macronutrient Composition by Meal (mean  $\pm$  SD)**

	<i>Carbohydrate (%)</i>	<i>Fat (%)</i>	<i>Protein (%)</i>
<b>Breakfast</b>	55.8	30.0	15.4
<b>Lunch</b>	54.7	28.4	16.6
<b>Dinner</b>	55.9	29.2	15.4

**Table 4.3: Percent of Complete Continuous Glucose Monitor Data**

	<i>24-hour</i>	<i>Breakfast</i>	<i>Lunch</i>	<i>Dinner</i>
<b>CON</b>	80% (n=24)	77% (n= 23)	77% (n=23)	87% (n=26)
<b>EX</b>	93% (n=28)	73% (n=22)	87% (n=26)	93% (n=28)
<b>BR</b>	90% (n=27)	67% (n= 20)	83% (n=25)	93% (n=28)

Percent of individuals with >75% continuous glucose monitor data for each defined period.

**Table 4.4: Participant Characteristics (mean  $\pm$  SD)**

<i>Men/Women</i>	14/16
<i>Age</i>	64 $\pm$ 8.2
<i>Weight (kg)</i>	197.2 $\pm$ 36.9
<i>BMI (kg/m<sup>2</sup>)</i>	31.7 $\pm$ 5.4
<i>Years since menopause (women only)</i>	13.3 $\pm$ 8.7
<i>Years since diabetes</i>	10.0 $\pm$ 7.8
<i>RMR</i>	1766.9 $\pm$ 388.9
<i>TDEE</i>	2425.9 $\pm$ 561.7
<i>Body fat (%)</i>	38.6 $\pm$ 10.0
<i>HbA1c (%)</i>	7.4 $\pm$ 1.1

HbA1c= Hemoglobin A1c, BMI= Body Mass Index

**Table 4.5: Participant Characteristics: Activity Volume Group (mean  $\pm$  SD)**

	<i>Low (n=11)</i>	<i>Mod (n=10)</i>	<i>High (n=9)</i>
<i>Age (yrs)</i>	67.9 $\pm$ 5.8	61.2 $\pm$ 9.8	62.3 $\pm$ 7.7
<i>Weight (kg)</i>	89.4 $\pm$ 17.2	95.16 $\pm$ 16.2	83.75 $\pm$ 16.2
<i>BMI (kg/m<sup>2</sup>)</i>	32.1 $\pm$ 4.6	32.9 $\pm$ 5.8	30.1 $\pm$ 6.1
<i>Years since menopause</i>	15.3 $\pm$ 10.8	9.3 $\pm$ 6.2	14.2 $\pm$ 8.1
<i>Years since diabetes</i>	10.7 $\pm$ 6.0	7.7 $\pm$ 5.1	12.0 $\pm$ 12.0
<i>RMR</i>	1593.1 $\pm$ 337.8	1970.8 $\pm$ 412.9	1751 $\pm$ 340
<i>TDEE</i>	2253.8 $\pm$ 460.8	2606.8 $\pm$ 705.8	2436.5 $\pm$ 474.1
<i>Body fat (%)</i>	41.7 $\pm$ 6.9	37.3 $\pm$ 12.2	36.3 $\pm$ 10.6
<i>HbA1c (%)</i>	7.5 $\pm$ 1.3	6.9 $\pm$ 0.5	7.7 $\pm$ 1.2

HbA1c= Hemoglobin A1c, BMI= Body Mass Index

**Table 4.6: Total Daily Physical Activity (mean  $\pm$  SD)**

	<i>Control</i>	<i>Exercise</i>	<i>Breaks</i>	<i>Normal Activity</i>
<i>Sitting time (min)</i>	591.0 $\pm$ 144.8	586.5 $\pm$ 121.3	566.8 $\pm$ 114.5	582.7 $\pm$ 151.0
<i>Standing time(min)</i>	225.4 $\pm$ 130.0	201.5 $\pm$ 98.2	230.3 $\pm$ 122	252.8 $\pm$ 105.1
<i>Stepping time (min)</i>	85.4 $\pm$ 42.6	114.1 $\pm$ 46.1*	103.7 $\pm$ 48.4*	97.1 $\pm$ 54.3
<i>Number of steps</i>	3157 $\pm$ 1597.0	4810 $\pm$ 2125.7*	4055 $\pm$ 2431.2*	3657 $\pm$ 2317.4
<i>Sit to stand transitions</i>	52.7 $\pm$ 24.5	43.8 $\pm$ 12.5*	53.8 $\pm$ 18.3	50.0 $\pm$ 20.4
<i>Duration of prolonged sitting (&gt;30 min)</i>	316.1 $\pm$ 183.2	357.0 $\pm$ 132.4	233.7 $\pm$ 129.5*	317.0 $\pm$ 182.3
<i>Wear Time (minutes)</i>	901.7 $\pm$ 68.2	902.1 $\pm$ 91.3	900.8 $\pm$ 76.6	932.6 $\pm$ 95.7

\* significantly different from control (p<0.05)

## **CHAPTER 5**

### **SEX DIFFERENCES IN POSTPRANDIAL GLUCOSE RESPONSES TO PHYSICAL ACTIVITY AFTER MEALS**

#### **Introduction**

The prevalence of diabetes is widespread and affects 15.5 million men and 13.1 million women in the United States (143). Despite roughly equal prevalence of diabetes, the risk of cardiovascular disease (CVD) events in women with diabetes is significantly higher compared to men (148). While not well understood, the causes of this health disparity are multifactorial, including sex differences in the underlying physiology of women (e.g. impact of hormones) and management of CVD risk factors (125, 148). Previous studies have shown that men and women can respond differently to therapies that reduce CVD risk, such as aspirin. Women taking low dose aspirin have a reduced risk of stroke but no change in the risk of myocardial infarction (152). Men, however, have the exact opposite response. Aspirin reduces the risk of myocardial infarction and has no effect on the occurrence of stroke in men (1). This comparison highlights the existence of sex differences in treatments to reduce the risk of CVD in diabetes. It is critical to investigate sex differences in other treatments for diabetes to effectively manage CVD risk in women. Exercise is used as a primary treatment strategy in the prevention of CVD in diabetes, but men and women may respond differently to a given bout of physical activity.

There are very few studies that have investigated sex differences in the metabolic response to physical activity. Coon and colleagues performed a meta-analysis of self-management strategies for people with type 2 diabetes (T2D) (50).

Interestingly, women who exercised were less likely to show improvements in Hemoglobin A1c (HbA1c) than exercising men. The authors speculated that women exercise for shorter durations or at lower intensities. While women do tend to participate in less moderate to vigorous physical activity than men (67), there is some evidence that the metabolic effects of exercise are blunted in women (137, 154). In a secondary analysis of the US Diabetes Prevention Program, men had greater reductions in 2-hour glucose and insulin concentrations as well as significantly improved insulin sensitivity compared to women (137). Both men and women had the same relative weight loss, but the metabolic health benefits were not comparable. Further, others have shown that women with diabetes may need to walk more than men to gain the same reductions in all-cause mortality risk (154). Therefore, the activity stimulus required to induce metabolic health benefits may be larger for women compared to men.

Numerous studies have demonstrated the cardiovascular and metabolic (cardiometabolic) consequences of sedentary behavior (e.g. higher postprandial glycemia, reduced insulin sensitivity) (61, 62, 166). Overall, individuals with T2D are more sedentary than individuals without diabetes (45). While women tend to be more sedentary than men from adolescence through adulthood, older women sit less than older men (120). Few studies have investigated the sex specific effects of sitting time and cardiometabolic health. Staiano and colleagues (165) showed that sitting time was significantly associated with fasting triglycerides and 2-hour glucose in men but not in women. In contrast, a study of British adults found a significant association between self-reported daily sitting time and fasting insulin

only in women (187). Taken together, these two studies suggest that there may be sex differences in the response to sedentary behavior, but the exact relationships are unclear at this point.

Recent experimental evidence has shown that interrupting sitting time with light physical activity reduces postprandial (after a meal) glucose and insulin concentrations. Postprandial glycemia is strongly associated with CVD risk and mortality in men and women with T2D (37, 38). Therefore, lowering glucose concentrations after meals is vital in managing the risk of CVD. Little attention has been given to investigating sex differences in the response to interrupting sedentary time with physical activity. Given the sex differences in the cardiometabolic responses to physical activity and other treatments to reduce CVD risk, it is plausible to expect there will be sex differences in the response to light physical activity interruptions in sedentary time. If women do have a blunted metabolic response to physical activity compared to men, there could be significant implications for managing CVD risk factors with physical activity. Therefore, the purpose of this study was to explore sex differences in the glucose response to adding physical activity in the form of a continuous bout of morning walking or short bouts of activity after meals. We compared the glucose response to equal volumes of physical activity across sexes to determine if there were differences in the postprandial glycemic effect of activity when time in physical activity is matched between men and women. Further, we investigated the dose-response relationship between physical activity and postprandial glycemia in men and women.

## **Methods**

The methods for this study have been previously described in Chapter IV. Briefly, we collected data on 30 sedentary individuals with T2D (14 men, 16 women). Glucose lowering medications, with the exception of insulin, were permitted in this study. The Institutional Review Board at the University of Massachusetts Amherst approved this study. Prior to performing any study procedures, participants gave their verbal and written informed consent.

This study took place in both the laboratory and free-living environment. Participants visited the Energy Metabolism Laboratory in the beginning of the study to have their resting metabolic rate (RMR) and body composition measured (by dual energy x-ray absorptiometry, DEXA). Participants returned to the laboratory on a separate occasion to pick up their physical activity monitor (activPAL, PAL Technologies Ltd, Glasgow, Scotland) and continuous glucose monitor (iPro2, Medtronic, Northridge, CA, USA). Both monitors were worn for one week in their free-living environment where they were asked to perform 3 experimental conditions: CON, EX and BR. During CON, participants maintained their normal physical activity behaviors. In the EX condition, participants were asked to go on a morning walk after breakfast and otherwise, maintain their normal behavior. The BR condition asked participants to perform 4 short bouts of physical activity during the 2-hour postprandial period after each meal. Participants were asked to increase the total duration of physical activity in EX and BR by a low (20 minutes), moderate (40 minutes) or high (60 minutes) volume. The active conditions (EX and BR) were designed to be matched on total time within each activity volume group (low,

moderate, high). At the conclusion of the 1-week free-living environment period, participants returned the physical activity monitor and continuous glucose monitor.

Dietary intake was strictly controlled during all experimental condition days (CON, EX, BR). Resting metabolic rate was multiplied by an activity factor of 1.4 to determine total daily energy intake for all participants. We attempted to keep participants energy balance to avoid the confounding variables of energy deficit/surplus (8, 68, 85, 166).

### **Continuous Glucose Monitor Data**

Throughout the experimental conditions, participants kept a log of the timing of breakfast, lunch and dinner. Using a customized program in R (R Foundation for Statistical Computing, Vienna, Austria, 2008; [www.R-project.org](http://www.R-project.org)), we separated the continuous glucose monitor data by postprandial periods. Each postprandial period was defined as 3 hours after the self-reported end time of that meal. Postprandial periods with less than 75% of data were excluded. We calculated several summary measures of postprandial glycemia:

- *Pre-meal glucose concentration*: mean glucose of the 15-minute interval prior to beginning of the meal
- *Incremental area under the curve (iAUC)*: trapezoidal area under the curve minus area under pre-meal glucose concentration
- *Peak postprandial glucose (PPG)*: peak glucose concentration
- *Time to peak glucose*: duration to peak glucose concentration
- *Rate of change to PPG*: peak glucose concentration divided by time to peak glucose

- *Rate of decline to 150 minutes*: rate of change from peak glucose to glucose concentration at 150 minutes
- *Postprandial duration of hyperglycemia (DH)*: duration glucose >10mmol/L

To compare postprandial glucose responses between EX and BR, we calculated the daily postprandial glucose responses for each of the glucose summary measures. We calculated the mean of each postprandial glucose response measure (e.g. iAUC, duration of hyperglycemia) of all of the meals within a condition.

### **Physical Activity Data**

Using the activPAL, we were able to assess free-living physical activity and sedentary behavior with a high degree of accuracy (80, 103, 145, 169). The activPAL data were processed using SAS 9.4 (SAS Institute Inc., Cary NC). We eliminated non-wear time prior to analyzing the activPAL data. The activPAL generated event and 15-second epoch files were used to determine the following summary measures of physical activity and sedentary behavior:

- Time spent sitting (total minutes and % of wear time)
- Time spent standing (total minutes and % of wear time)
- Time spent stepping (total minutes and % of wear time)
- Duration of prolonged sitting (time spent sitting in bouts of >30 minutes)
- Number of breaks from sitting (transition from sitting to standing)

We quantified daily physical activity and sedentary behavior during each of the experimental conditions and on all other days participants were not required to



follow any study conditions (NORM). In order for data to be counted, participants were required to wear the activPAL for at least 10 hours per day. Invalid days were eliminated from the dataset.

### **Statistical Analysis**

We performed all statistical analyses using the R-software package. Significance levels were set at  $p < 0.05$ . All data are expressed as mean (95% confidence interval) unless otherwise noted. Differences in participant characteristics between men and women were assessed by analysis of variance (ANOVA) and Tukey HSD post hoc testing. We used linear mixed models with repeated measures to determine sex differences in mean postprandial glucose responses and differences in physical activity during the control condition. Finally, we investigated the interaction of sex in the dose response relationship between physical activity and postprandial glycemia.

### **Results**

#### **Participant Characteristics**

As previously reported, men had a significantly higher RMR and total daily energy expenditure and a lower percent body fat than women. There were no other significant differences in participant characteristics (*Table 5.1*). Finally, all women who participated in this study were postmenopausal.

#### **Medications**

Overall, the proportion of individuals taking biguanides was equivalent between men ( $n=7$ ) and women ( $n=8$ ). There were more women ( $n=3$ ) who were

not taking any glucose lowering medications than men (n=1). A complete depiction of medications can be found in *Figure 5.1*.

### **Physical Activity**

There were significant differences between men and women in physical activity and sedentary behavior during the NORM days. Women spent less time sitting per day (106.5 minutes (CI: 24.4-188.6),  $p=0.01$ ) and more time standing per day than men (86.1 minutes (CI: 29.6-142.7,  $p=0.002$ ). Finally, there were no differences in daily stepping time between men and women during the NORM days (*Figure 5.2*). There were no significant differences in physical activity or sedentary behavior between the NORM days and CON. There were also no differences in wear time between men and women.

There were no sex differences in the change in physical activity or sedentary behavior between conditions. Both men and women significantly increased their daily stepping time in EX and BR compared to CON (*Figure 5.3*) and there were no differences in stepping time between EX and BR. Additionally, there total sitting or standing time were not different between any of the conditions. Prolonged sitting (bouts of sitting >30 minutes) was significantly lower in BR, but participants did not increase the frequency of breaks from sitting. Finally, the number of breaks from sitting was lower in EX compared to CON.

### **Postprandial Glucose Responses: Effect of Conditions**

Postprandial duration of hyperglycemia was the only postprandial glucose summary measure that was significantly different between men and women.

Women had a significantly shorter duration of hyperglycemia compared to men at

control. Further, there was no effect of EX or BR compared to CON among the women in this study. On the contrary, the duration of hyperglycemia among men was significantly shorter in EX and BR compared to control (*Figure 5.4*). There were no significant differences between EX and BR within the men. As previously reported in Chapter IV, individuals with a long duration of hyperglycemia at CON had a shorter duration of hyperglycemia in the EX and BR conditions independent, of sex. However, individuals with low postprandial hyperglycemia at CON also showed no significant effect of EX or BR compared to CON.

### **Postprandial Glucose Responses: Dose-Response Relationships**

We investigated sex differences in the dose response relationship between physical activity volume groups and postprandial duration of hyperglycemia. There was a significant interaction effect of sex in our dose-response models for postprandial duration of hyperglycemia. (*Figure 5.5*). Among the men, the duration of hyperglycemia got progressively shorter in the moderate and high activity volume groups, but only the high volume group was significantly different from the low volume group ( $p < 0.05$ ). Again, there were no significant differences between the activity volume groups in the women. A similar relationship was found in individuals with a high duration of hyperglycemia at CON ( $n=9$ ). People with a high duration of hyperglycemia had a shorter duration postprandial glucose in the moderate and high activity volume group compared to the low activity volume group ( $p=0.08$ ) and there were no significant differences between the moderate and high activity volume groups. Finally, there were no significant differences between

activity volume groups among individuals with low postprandial glycemia during CON.

### **Discussion**

The primary purpose of this study was to evaluate any sex differences in the glucose response to 2 different physical activity interventions (continuous walking and post-meal physical activity breaks from sitting). Men and women similarly increased stepping time in the active conditions (EX and BR) and there were no significant changes to sitting or standing time in any of the conditions. Despite men and women's similar increases in physical activity, their glucose responses were not the same. We found that men had a robust response to both active conditions and showed a predictable dose-response relationship. On the contrary, postprandial glycemia in women did not change in the active conditions and no significant dose response relationship was observed. These sex differences appear to be primarily driven by the high duration of hyperglycemia in the CON condition among men.

Despite no sex differences in HbA1c, the duration of hyperglycemia during the control condition was higher in men compared to women. This sex difference in postprandial duration of hyperglycemia may be due to the differences in their habitual sitting and standing time. We are confident that physical activity and sedentary behavior in the CON condition is representative of habitual behavior due to the lack of difference between NORM days and the CON condition. We found that women in the present study sat less and stood more than the men in their habitual state. These behavioral sex differences in daily sitting time are supported in a representative sample of US older adults (120). Sitting is well established to

negatively impact postprandial glucose regulation (61). The less time that women spent sitting may have maintained lower durations of hyperglycemia during CON. However, it is still surprising that we observed differences in postprandial glycemia given that there were no sex differences in HbA1c (overall average HbA1c:  $7.4 \pm 1.1$ ). While the variations in HbA1c concentrations less than 7.3 are primarily due to postprandial hyperglycemia, (127) it is possible that the women recruited for our study had more issues with fasting glucose regulation. It is clear based on these results that using HbA1c as a criteria for matching participants on postprandial glycemic control is inadequate.

Overall, the sex differences observed between conditions and in the dose-response relationship in this study can be primarily explained by the discordant levels of glycemia between men and women in the CON condition. Terada *et al.* demonstrated that the strongest predictor of capillary blood glucose in response to exercise is the pre-exercise blood glucose concentration (170). Our findings further the idea that the individuals who have the largest glucose lowering benefits are the ones who have the highest initial glucose concentrations in their normal behavioral state. In our study, the majority of individuals with a high duration of hyperglycemia in CON were men, which explain the robust glucose lowering effects we observed in the men. In order to conclusively determine the influence of sex in the glucose response to exercise, a large number of men and women with varying levels hyperglycemia are needed.

It is interesting that we observed a dose response relationship between activity volume and duration of hyperglycemia in the men and not in the women.

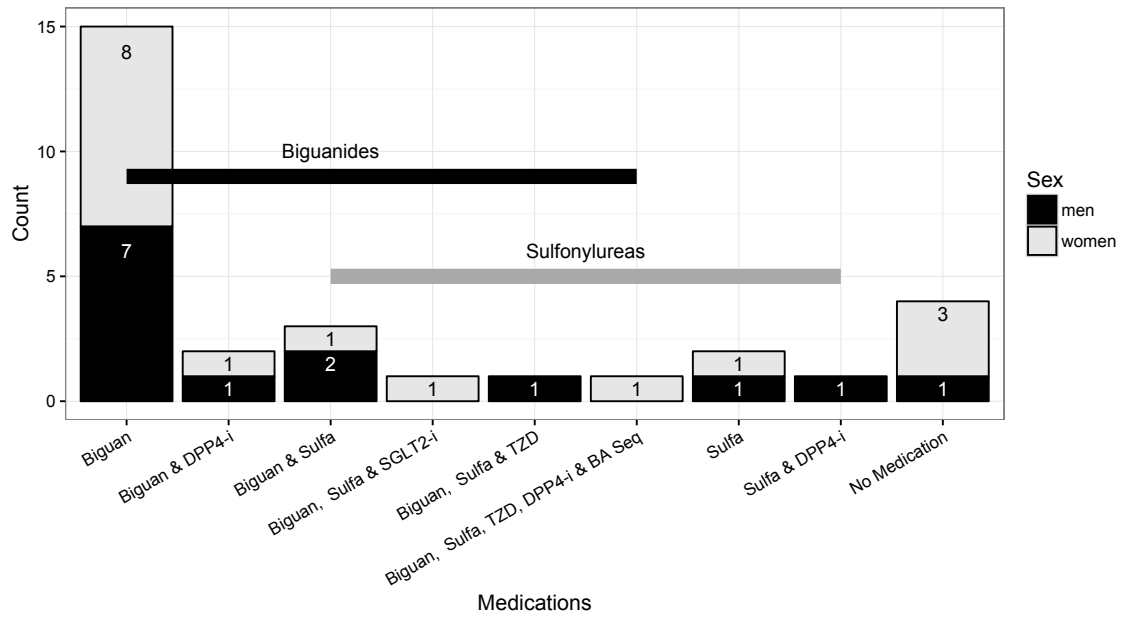
These data are in agreement with previous studies that have found that a bout of exercise is less effective to improve metabolic responses in women compared to men (137, 154). In our participants, it is possible that the women may have required either a longer duration or higher intensity of activity to attain the same benefits as the men. However, these results should be interpreted with caution since the duration of hyperglycemia between men and women was significantly different in the CON condition.

The use of a clinically relevant participant population and real world setting are unique strengths to this study. The standardization of energy intake during the conditions allowed for the effective comparison of different methods to increase physical activity. This approach that we term *ecolabical*, combined essential elements of a laboratory controlled study to an ecologically relevant setting. We utilized a minimally invasive tool to measure glucose in the free-living environment to better understand the real world impact of different physical activity interventions on daily glucose concentrations. Finally, the men and women in our study were well matched on many participant characteristics (e.g. age, BMI and HbA1c). However, the significant differences in postprandial glycemia during CON condition between men and women limited our ability to investigate the effect of sex on postprandial glycemia. To ensure an even distribution of high and low postprandial glycemia, future studies would benefit from prescreening postprandial glucose concentrations prior to enrolling participants in their study. Additionally, while men and women were matched on age, all of the women enrolled in this study were postmenopausal women. To completely understand the potential sex

differences in the glucose response to exercise, future studies will need to include men and women of varying ages and hormonal levels.

In this comparison of continuous exercise and post-meal breaks from sitting, we observed a significant glucose lowering effect of activity in men but not women. This sex difference was likely driven by the men's high duration of hyperglycemia during the CON condition. Our results highlight the importance of matching men and women on daily glycemia in order to effectively investigate potential sex differences. Future studies with higher statistical power are required to provide evidence demonstrating whether physical activity benefits men and women equally for the management of hyperglycemia.

## Figures

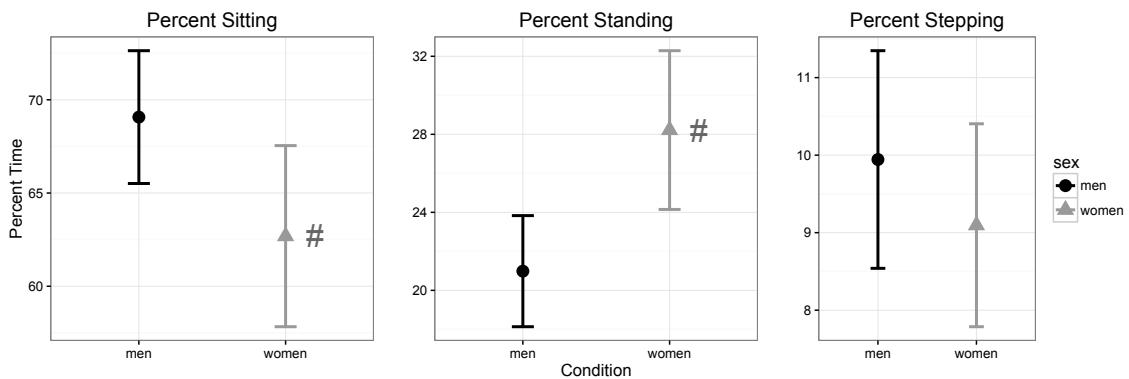


**Figure 5.1: Medications by Sex**

Count of each medicate on combination divided by men (black) and women (grey).

Biguanides: Metformin; Sulfonyureas: Glipazide, Glimepiride, Glyburide;

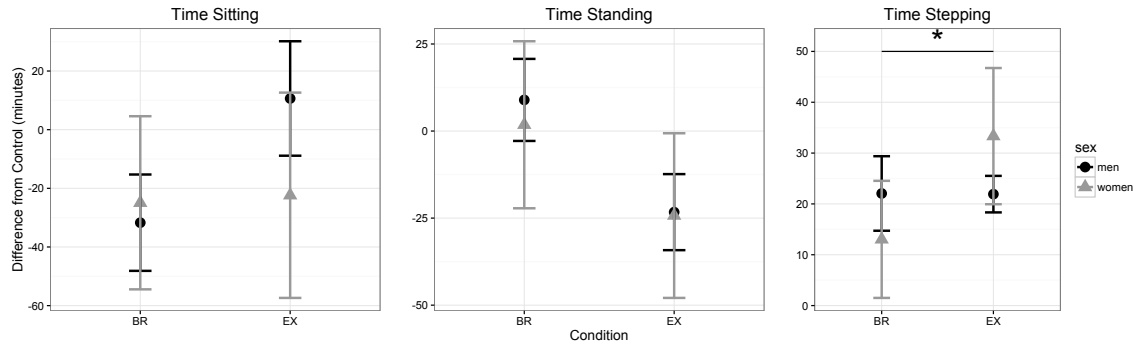
Thiazolidinediones: Pioglitazone; DPP4-inhibitors: Lingalipitin, Sitagliptin; SGLT2-inhibitors: Canagliflozin; Bile Acid Sequestrants: Colesevelam



**Figure 5.2: Physical Activity During Normal Activity Days: Sex Differences**

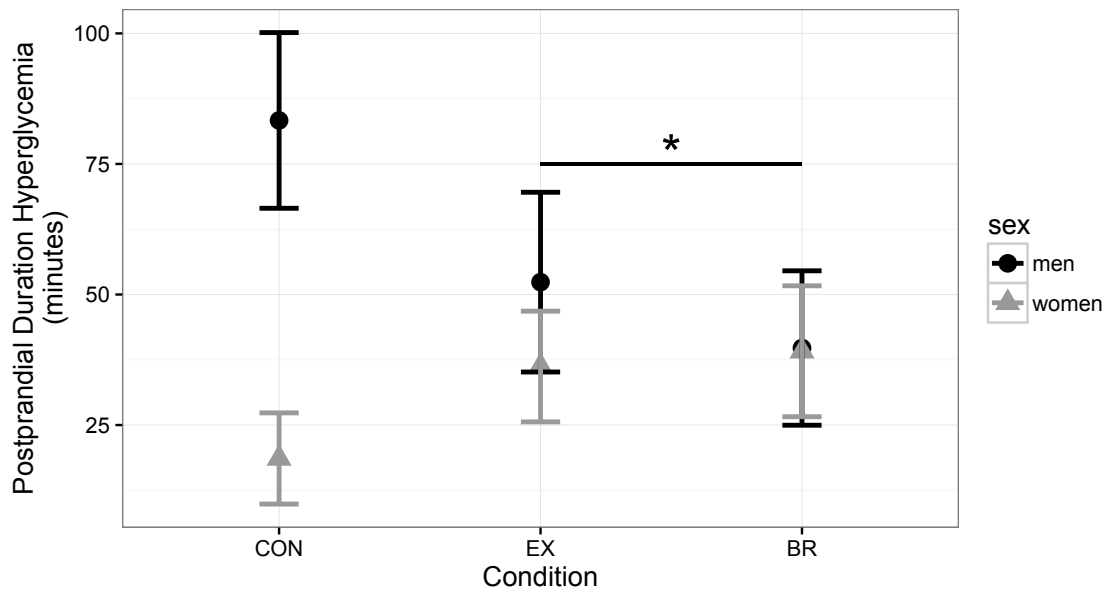
Percent time spent sitting, standing and stepping during the normal activity days (NORM) in men (black circles) and women (grey triangles). Data are presented as mean  $\pm$  SEM. # significantly different from men ( $p < 0.05$ )





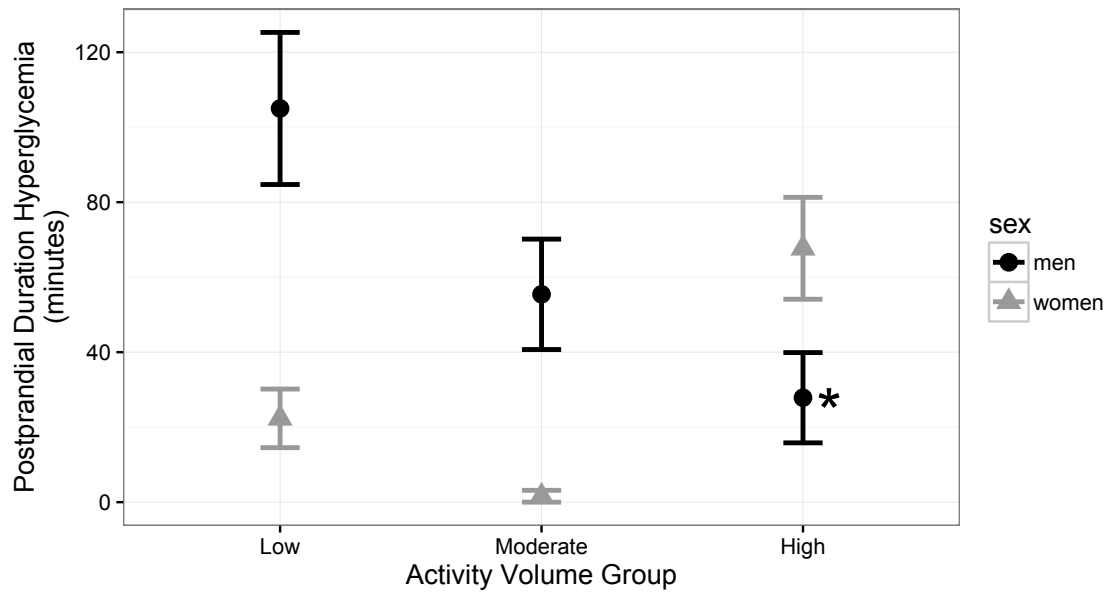
**Figure 5.3: Change in Physical Activity from Control: Sex Differences**

Change in percent time spent sitting, standing and stepping from CON to active conditions (BR and EX) in men (black circles) and women (grey triangles). Data are presented as mean  $\pm$  SEM. \* significantly different from control condition in both sexes ( $p < 0.01$ )



**Figure 5.4: Sex differences in the Response to Exercise and Post-Meal Breaks from Sitting**

Duration of postprandial hyperglycemia (minutes) during each condition in men (black circles) and women (grey triangles). Data are presented as mean  $\pm$  SEM  
\* significantly different from CON in men  $p < 0.05$



**Figure 5.5: Sex Differences in Dose Response Relationship**

Dose response relationship between duration of hyperglycemia and activity volume group in men (black circle) and women (grey triangles). Data presented as mean  $\pm$  SEM. \* significantly different from low activity volume group in men ( $p < 0.05$ ).

## Tables

**Table 5.1: Participant Characteristics**

	Men (n=14)	Women (n=16)
Age (yrs)	60.9 ± 10.1	66.8 ± 4.8
Weight (kg)	94.7 ± 16.1	85.2 ± 16.6
BMI (kg/m <sup>2</sup> )	31.6 ± 5.4	32.2 ± 5.6
Years since menopause	NA	13.3 ± 8.7
Years since diabetes	12.5 ± 9.5	8.0 ± 5.5
RMR	2002.1 ± 354.9 *	1594.5 ± 308.9
TDEE	2816.8 ± 446.4 **	2128.9 ± 442.6
Body fat (%)	30.9 ± 7.0 ***	45.4 ± 6.8
HbA1c (%)	7.4 ± 1.1	7.3 ± 1.2

HbA1c= Hemoglobin A1c, BMI= Body Mass Index

\* p = 0.01

\*\* p=0.001

\*\*\* p ≤ 0.0001

## **CHAPTER 6**

### **SUMMARY AND CONCLUSION**

Managing daily and postprandial hyperglycemia is critical in the treatment of type 2 diabetes (T2D). Exercise is generally accepted to improve glycemic control, but most Americans do not meet current physical activity recommendations. In fact spend less than 5% of waking hours in moderate to vigorous physical activity and the majority of the day (60-70%) is spent in sedentary behavior (120, 176).

Sedentary behavior has a detrimental impact on postprandial glycemia. Recent laboratory based studies have shown that interrupting sedentary time with light physical activity lowers postprandial glucose concentrations (62). However, it is unknown whether the robust benefits demonstrated in the laboratory translate to the free-living environment. Further, it is still unclear whether the potential benefits of breaks from sitting in a free-living environment are similar to the established glucose-lowering effects of continuous moderate to vigorous exercise. This dissertation directly addressed these gaps in knowledge by continuously measuring glucose concentrations during and after a laboratory intervention of breaks from sitting in T2D (study 1). Further we compared the effect of 2 different physical activity interventions (continuous exercise and breaks from sitting) to lower glucose concentrations in free-living men and women with T2D (study 2 and 3).

#### **Study 1**

In 2008, Healy and colleagues published the first epidemiological study showing that breaks from sitting was associated with better cardiometabolic health (89). Since then, there has been an explosion of laboratory based studies

investigating the merits of interrupting sitting time with short bouts of walking, cycling and standing (10, 62, 92, 106, 107, 173). Chapter III provides the first study in T2D that investigated the glycemic effects during and after a laboratory intervention of breaks from sitting (either walking or light resistance activities). There are 2 major contributions from this study: (1) walking or resistance activity breaks from sitting similarly lowered daily and postprandial glycemia compared to an all sedentary control and (2) these glucose lowering effects are maintained in the free living environment through the next morning. Previous studies using regular walking breaks from sitting have been criticized because this type of activity requires participants to stop what they are doing (e.g. work, watching TV). Standing breaks from sitting allow individuals to continue the task at hand, but provide modest to negligible glucose lowering effects (144, 173). This study provides an effective stationary activity (light resistance activity) to lower daily glucose concentrations.

The use of continuous glucose monitoring allowed us to investigate how long the glycemic effect of the laboratory intervention lasted in the free-living environment. The concurrent use of physical activity monitors enabled us to account for physical activity and sedentary behavior in the free-living environment. We observed that the glucose lowering effects of both types of physical activity breaks from sitting persisted through the morning of the next day. This finding can be interpreted as either (1) breaks from sitting have a lasting glucose lowering effect or (2) the detrimental effects of prolonged sitting continue for many hours after a bout of sitting ends. Like most laboratory studies to date, our participants in

the control condition sat uninterrupted for a prolonged period of time. It is clear that 7 hours of sustained sitting is not representative of most free-living sedentary behavior. In order to understand the real world impact of interrupting sitting time with light physical activity, a control condition more representative of the free-living sedentary behavior is needed.

### **Study 2 and 3**

To address the limitations of study 1 and previous breaks from sitting studies in the literature, we conducted study 2 and 3 in free-living individuals with T2D. We used, what we term, an *ecolabical* approach to maximize the translation of our findings. This approach applied the essential elements of laboratory controlled studies (standardized meals) in an ecologically relevant setting. The control condition, therefore, was each participant's sedentary behavior, which included the regular interruptions in sedentary time that occur naturally throughout the day (e.g. bathroom breaks, meal preparation). Instead of removing physical activity in the control condition, our participants added physical activity to a normal day in the form of a continuous walk after breakfast or light physical activity breaks from sitting after meals. Results from Chapter III indicated that both walking and stationary resistance activity are equally effective to reduce postprandial glycemia. We used this evidence as a rationale to allow participants to walk or perform stationary resistance activities during their breaks from sitting.

Chapter IV provides evidence that in free-living individuals with T2D, a bout of continuous walking after breakfast is more effective to lower postprandial glucose concentrations than post-meal breaks from sitting. However, in a subgroup

of individuals (n=9) with a high duration of hyperglycemia in the control condition, we found that walking after breakfast and post-meal breaks from sitting were equally effective to reduce postprandial duration of hyperglycemia.

The influence of baseline levels of hyperglycemia on the glucose lowering effects of physical activity is an important contribution with real clinical implications. For individuals with high postprandial hyperglycemia, breaks from sitting may be an effective alternative to traditional exercise for daily glucose concentrations. It is important to consider that there may be other health benefits of taking physical activity breaks from sitting. To date, the majority of studies have focused on the glucose lowering effects. There have been a few studies that have investigated the impact of breaks from sitting on blood pressure and vascular function (106, 107, 174). Before recommending breaks from sitting as a global strategy to manage T2D and prevent future complications, more studies with comprehensive assessments of cardiometabolic health are needed. Therefore, with the current evidence available, we would not recommend breaks from sitting as a global strategy to improve the health of individuals with T2D.

For those with lower durations of hyperglycemia, the dose of breaks from sitting likely needs to be much higher in order to yield significant glucose lowering benefits. Previous studies that have shown a benefit of breaks from sitting either by dramatically reduced sitting time or increased the frequency of breaks from sitting. This higher dose of breaks from sitting may not be readily adopted by sedentary individuals with T2D. Exercise, on the other hand, is a well-established treatment option to improve CVD risk factors in diabetes (112). Encouraging continuous

exercise to manage CVD risk in those individuals with both high and low durations of hyperglycemia is, without a doubt, an important message.

We also determined that adding a moderate amount of physical activity (40 minutes) resulted in the lowest mean postprandial glucose concentrations in our participants. Interestingly, only in the subgroup of individuals with high levels of postprandial glycemia did the 60-minute dose of activity provide a significant glucose lowering effect. Again, the finding that the initial level of hyperglycemia predicts the change in response to physical activity is an important clinically relevant contribution.

Finally, chapter IV provides a new application for isothermal substitution modeling to identify the impact of substituting different behaviors during the day (e.g. replace 30 minutes of sitting with 30 minutes of standing). Previous epidemiological studies have used this method in large cross-sectional datasets to understand the impact of replacing sedentary time with physical activity on general measures of glucose and insulin metabolism (e.g. 2-hour glucose concentrations, insulin sensitivity) (32). Isothermal substitution modeling been used to analyze to datasets that have concurrent continuous measures of an exposure (physical activity) and health outcome (daily glucose). Applying isothermal substitution modeling to these data provide a unique opportunity to determine behavior substitutions that translate to meaningful health benefits.

The major contribution from Chapter V (Study 3) is our consideration that men and women with T2D may respond differently to the same physical activity intervention. We found that the glucose lowering effect of exercise and physical



activity breaks were equivalent in men, whereas women did not respond to the activity conditions. We also found that 60 minutes of additional physical activity lowered glucose concentrations in the men but no dose of activity lowered glucose concentrations in the women. As previously noted, the sex differences observed in this study were driven by the high glucose concentrations in men during the control condition. Interestingly, men and women were matched on glycemic control (i.e. no differences in HbA1c) but had dramatically different postprandial glucose concentrations. This study highlights the importance of screening participants for varying levels of postprandial hyperglycemia. The results from Chapter V make an important contribution to the limited research in sex differences in the metabolic response to physical activity. Because women with diabetes have a disproportionately higher risk of CVD than their male counterparts, more systematic studies of sex differences in the response to physical activity are needed.

### **Conclusions**

This dissertation has the potential to significantly influence physical activity recommendations for individuals with T2D. Studies 1, 2 and 3 provided evidence to better our understanding of the magnitude and timing of changes in glycemia induced by physical activity breaks from sitting or continuous exercise in the free-living environment. Study 1 demonstrated that both walking and resistance activities result in lasting reductions in glucose concentrations compared to 7 hours of uninterrupted sitting. To our knowledge, Study 2 provided the first direct comparison of breaks from sitting and continuous exercise on daily and postprandial glycemia in the free-living environment. Our analysis of sex differences

in Study 3 indicated that individuals with high postprandial glucose concentrations (which were primarily men in this study) will respond equally well to continuous exercise or physical activity breaks from sitting.

Applying our *ecolabical* approach to future studies has the potential to enhance our understanding of the dose response relationships between physical activity and daily glycemic control. Results from studies, like the ones presented in this dissertation, will inform future physical activity guidelines and allow for specific recommendations to manage glycemia in diabetes with physical activity.

## REFERENCES

1. Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. *The New England journal of medicine*. 1989;321(3):129-35.
2. The Diabetes Control and Complications Trial (DCCT) Research Group. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes*. 1995;44(8):968-83.
3. *Nutrient reference values for Australia and New Zealand, including recommended dietary intakes*. Canberra: National Health and Medical Research Council. Commonwealth of Australia. Department of Health and Ageing 2006. Available from: National Health and Medical Research Council. Commonwealth of Australia. Department of Health and Ageing.
4. World Health Statistics 2016. *Geneva: World Health Organization*. 2016.
5. Aalders AL, Schmidt FJ, Schoonen AJ, Broek IR, Maessen AG, Doorenbos H. Development of a wearable glucose sensor; studies in healthy volunteers and in diabetic patients. *The International journal of artificial organs*. 1991;14(2):102-8.
6. Abbate R, Mannucci E, Cioni G, Fatini C, Marcucci R. Diabetes and sex: from pathophysiology to personalized medicine. *Internal and emergency medicine*. 2012;7 Suppl 3:S215-9.
7. Action to Control Cardiovascular Risk in Diabetes Study G, Gerstein HC, Miller ME et al. Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine*. 2008;358(24):2545-59.
8. Alfonso-Gonzalez G, Doucet E, Almeras N, Bouchard C, Tremblay A. Estimation of daily energy needs with the FAO/WHO/UNU 1985 procedures in adults: comparison to whole-body indirect calorimetry measurements. *European journal of clinical nutrition*. 2004;58(8):1125-31.
9. Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in diabetes research. *Diabetes care*. 1995;18(2):245-50.
10. Altenburg TM, Rotteveel J, Dunstan DW, Salmon J, Chinapaw MJ. The effect of interrupting prolonged sitting time with short, hourly, moderate-intensity cycling bouts on cardiometabolic risk factors in healthy, young adults. *Journal of applied physiology*. 2013;115(12):1751-6.
11. American Diabetes A. Standards of medical care in diabetes--2013. *Diabetes care*. 2013;36 Suppl 1:S11-66.
12. Baek YH, Jin HY, Lee KA et al. The Correlation and Accuracy of Glucose Levels between Interstitial Fluid and Venous Plasma by Continuous Glucose Monitoring System. *Korean diabetes journal*. 2010;34(6):350-8.
13. Baghurst PA. Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data: an automated algorithm. *Diabetes technology & therapeutics*. 2011;13(3):296-302.
14. Bailey TS, Ahmann A, Brazg R et al. Accuracy and acceptability of the 6-day Enlite continuous subcutaneous glucose sensor. *Diabetes technology & therapeutics*. 2014;16(5):277-83.

15. Bankoski A, Harris TB, McClain JJ et al. Sedentary activity associated with metabolic syndrome independent of physical activity. *Diabetes care*. 2011;34(2):497-503.
16. Barnes PM, Schoenborn CA. Trends in adults receiving a recommendation for exercise or other physical activity from a physician or other health professional. *NCHS data brief*. 2012;(86):1-8.
17. Barr EL, Boyko EJ, Zimmet PZ, Wolfe R, Tonkin AM, Shaw JE. Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study. *Diabetologia*. 2009;52(3):415-24.
18. Barr EL, Zimmet PZ, Welborn TA et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation*. 2007;116(2):151-7.
19. Basu A, Dube S, Slama M et al. Time lag of glucose from intravascular to interstitial compartment in humans. *Diabetes*. 2013;62(12):4083-7.
20. Benatti FB, Ried-Larsen M. The effects of breaking up prolonged sitting time: a review of experimental studies. *Medicine and science in sports and exercise*. 2015;47(10):2053-61.
21. Berger A. Bone mineral density scans. *Bmj*. 2002;325(7362):484.
22. Bergouignan A, Latouche C, Heywood S et al. Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: Ancillary analysis from randomized clinical trials. *Scientific reports*. 2016;6:32044.
23. Bergouignan A, Rudwill F, Simon C, Blanc S. Physical inactivity as the culprit of metabolic inflexibility: evidence from bed-rest studies. *Journal of applied physiology*. 2011;111(4):1201-10.
24. Biswas A, Oh PI, Faulkner GE et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Ann Intern Med*. 2015;162(2):123-32.
25. Blankenship JM, Granados K, Braun B. Effects of subtracting sitting versus adding exercise on glycemic control and variability in sedentary office workers. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2014;39(11):1286-93.
26. Blevins TC, Bode BW, Garg SK et al. Statement by the American Association of Clinical Endocrinologists Consensus Panel on continuous glucose monitoring. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*. 2010;16(5):730-45.
27. Bolen S, Tseng E, Hutfless S et al. *Diabetes Medications for Adults With Type 2 Diabetes: An Update*. Rockville (MD)2016.
28. Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease in Type II diabetes: the epidemiological evidence. *Diabetologia*. 2001;44(12):2107-14.

29. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Population health metrics*. 2010;8:29.
30. Brynes AE, Adamson J, Dornhorst A, Frost GS. The beneficial effect of a diet with low glycaemic index on 24 h glucose profiles in healthy young people as assessed by continuous glucose monitoring. *The British journal of nutrition*. 2005;93(2):179-82.
31. Buckley JP, Mellor DD, Morris M, Joseph F. Standing-based office work shows encouraging signs of attenuating post-prandial glycaemic excursion. *Occupational and environmental medicine*. 2014;71(2):109-11.
32. Buman MP, Winkler EA, Kurka JM et al. Reallocating time to sleep, sedentary behaviors, or active behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005-2006. *American journal of epidemiology*. 2014;179(3):323-34.
33. Buscemi S, Cosentino L, Rosafio G et al. Effects of hypocaloric diets with different glycemic indexes on endothelial function and glycemic variability in overweight and in obese adult patients at increased cardiovascular risk. *Clinical nutrition*. 2013;32(3):346-52.
34. Calabro MA, Lee JM, Saint-Maurice PF, Yoo H, Welk GJ. Validity of physical activity monitors for assessing lower intensity activity in adults. *The international journal of behavioral nutrition and physical activity*. 2014;11:119.
35. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes care*. 2010;33(2):414-20.
36. Catenacci VA, Grunwald GK, Ingebrigtsen JP et al. Physical activity patterns using accelerometry in the National Weight Control Registry. *Obesity*. 2011;19(6):1163-70.
37. Cavalot F, Pagliarino A, Valle M et al. Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. *Diabetes care*. 2011;34(10):2237-43.
38. Cavalot F, Petrelli A, Traversa M et al. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *The Journal of clinical endocrinology and metabolism*. 2006;91(3):813-9.
39. Cengiz E, Tamborlane WV. A tale of two compartments: interstitial versus blood glucose monitoring. *Diabetes technology & therapeutics*. 2009;11 Suppl 1:S11-6.
40. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States. Atlanta, GA: U.S. Department of Health and Human Services. 2014.

41. Ceriello A, Esposito K, Piconi L et al. Glucose "peak" and glucose "spike": Impact on endothelial function and oxidative stress. *Diabetes research and clinical practice*. 2008;82(2):262-7.
42. Ceriello A, Esposito K, Piconi L et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. 2008;57(5):1349-54.
43. Ceriello A, Kilpatrick ES. Glycemic variability: both sides of the story. *Diabetes care*. 2013;36 Suppl 2:S272-5.
44. Chastin SF, Egerton T, Leask C, Stamatakis E. Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. *Obesity (Silver Spring)*. 2015;23(9):1800-10.
45. Cichosz SL, Fleischer J, Hoeyem P et al. Objective measurements of activity patterns in people with newly diagnosed Type 2 diabetes demonstrate a sedentary lifestyle. *Diabetic medicine : a journal of the British Diabetic Association*. 2013;30(9):1063-6.
46. Colberg SR, Albright AL, Blissmer BJ et al. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Medicine and science in sports and exercise*. 2010;42(12):2282-303.
47. Colberg SR, Grieco CR, Somma CT. Exercise effects on postprandial glycemia, mood, and sympathovagal balance in type 2 diabetes. *Journal of the American Medical Directors Association*. 2014;15(4):261-6.
48. Colberg SR, Sigal RJ, Fernhall B et al. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes care*. 2010;33(12):2692-6.
49. Colberg SR, Zarrabi L, Bennington L et al. Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. *Journal of the American Medical Directors Association*. 2009;10(6):394-7.
50. Conn VS, Hafdahl AR, Mehr DR, LeMaster JW, Brown SA, Nielsen PJ. Metabolic effects of interventions to increase exercise in adults with type 2 diabetes. *Diabetologia*. 2007;50(5):913-21.
51. Cooper AR, Sebire S, Montgomery AA et al. Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia*. 2012;55(3):589-99.
52. Crespo NC, Mullane SL, Zeigler ZS, Buman MP, Gaesser GA. Effects of Standing and Light-Intensity Walking and Cycling on 24-h Glucose. *Medicine and science in sports and exercise*. 2016.
53. Damiano ER, McKeon K, El-Khatib FH, Zheng H, Nathan DM, Russell SJ. A comparative effectiveness analysis of three continuous glucose monitors: the Navigator, G4 Platinum, and Enlite. *Journal of diabetes science and technology*. 2014;8(4):699-708.
54. Decode Study Group tDEEG. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. *Archives of internal medicine*. 2001;161(3):397-405.

55. Dempsey PC, Larsen RN, Sethi P et al. Benefits for Type 2 Diabetes of Interrupting Prolonged Sitting With Brief Bouts of Light Walking or Simple Resistance Activities. *Diabetes care*. 2016;39(6):964-72.
56. Dempsey PC, Owen N, Biddle SJ, Dunstan DW. Managing sedentary behavior to reduce the risk of diabetes and cardiovascular disease. *Curr Diab Rep*. 2014;14(9):522.
57. Dempsey PC, Owen N, Yates TE, Kingwell BA, Dunstan DW. Sitting Less and Moving More: Improved Glycaemic Control for Type 2 Diabetes Prevention and Management. *Curr Diab Rep*. 2016;16(11):114.
58. Di Flaviani A, Picconi F, Di Stefano P et al. Impact of glycemic and blood pressure variability on surrogate measures of cardiovascular outcomes in type 2 diabetic patients. *Diabetes care*. 2011;34(7):1605-9.
59. DiPietro L, Gribok A, Stevens MS, Hamm LF, Rumpler W. Three 15-min bouts of moderate postmeal walking significantly improves 24-h glycemic control in older people at risk for impaired glucose tolerance. *Diabetes care*. 2013;36(10):3262-8.
60. Duckworth W, Abaira C, Moritz T et al. Glucose control and vascular complications in veterans with type 2 diabetes. *The New England journal of medicine*. 2009;360(2):129-39.
61. Dunstan DW, Howard B, Healy GN, Owen N. Too much sitting--a health hazard. *Diabetes research and clinical practice*. 2012;97(3):368-76.
62. Dunstan DW, Kingwell BA, Larsen R et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes care*. 2012;35(5):976-83.
63. Duvivier BM, Schaper NC, Bremers MA et al. Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. *PloS one*. 2013;8(2):e55542.
64. Dye L, Mansfield M, Lasikiewicz N et al. Correspondence of continuous interstitial glucose measurement against arterialised and capillary glucose following an oral glucose tolerance test in healthy volunteers. *The British journal of nutrition*. 2010;103(1):134-40.
65. Edwardson CL, Winkler EAH, Bodicoat DH et al. Considerations when using the activPAL monitor in field-based research with adult populations. *J Sport Health Sci*. 2016;In press.
66. Ercan N, Gannon MC, Nuttall FQ. Effect of added fat on the plasma glucose and insulin response to ingested potato given in various combinations as two meals in normal individuals. *Diabetes care*. 1994;17(12):1453-9.
67. Evenson KR, Buchner DM, Morland KB. Objective measurement of physical activity and sedentary behavior among US adults aged 60 years or older. *Prev Chronic Dis*. 2012;9:E26.
68. Ferro-Luzzi A. The conceptual framework for estimating food energy requirement. *Public Health Nutr*. 2005;8(7A):940-52.

69. Fox CS. Cardiovascular disease risk factors, type 2 diabetes mellitus, and the Framingham Heart Study. *Trends in cardiovascular medicine*. 2010;20(3):90-5.
70. Francois ME, Baldi JC, Manning PJ et al. 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia*. 2014;57(7):1437-45.
71. Franks P, Griffin S. Review: exercise training reduces HbA1c levels but not body mass in type 2 diabetes mellitus. *ACP J Club*. 2002;136(3):100.
72. Fritschi C, Park H, Richardson A et al. Association Between Daily Time Spent in Sedentary Behavior and Duration of Hyperglycemia in Type 2 Diabetes. *Biol Res Nurs*. 2016;18(2):160-6.
73. Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet*. 1980;1(8183):1373-6.
74. Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM. Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus. *The Journal of clinical endocrinology and metabolism*. 1977;44(5):859-64.
75. Gannon MC, Ercan N, Westphal SA, Nuttall FQ. Effect of added fat on plasma glucose and insulin response to ingested potato in individuals with NIDDM. *Diabetes care*. 1993;16(6):874-80.
76. Gannon MC, Nuttall FQ, Neil BJ, Westphal SA. The insulin and glucose responses to meals of glucose plus various proteins in type II diabetic subjects. *Metabolism: clinical and experimental*. 1988;37(11):1081-8.
77. Ge QM, Dong Y, Zhang HM, Su Q. Effects of intermittent high glucose on oxidative stress in endothelial cells. *Acta diabetologica*. 2010;47 Suppl 1:97-103.
78. Gillen JB, Little JP, Punthakee Z, Tarnopolsky MA, Riddell MC, Gibala MJ. Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes, obesity & metabolism*. 2012;14(6):575-7.
79. Goodyear LJ. The exercise pill--too good to be true? *The New England journal of medicine*. 2008;359(17):1842-4.
80. Grant PM, Dall PM, Mitchell SL, Granat MH. Activity-monitor accuracy in measuring step number and cadence in community-dwelling older adults. *J Aging Phys Act*. 2008;16(2):201-14.
81. Grant PM, Ryan CG, Tigbe WW, Granat MH. The validation of a novel activity monitor in the measurement of posture and motion during everyday activities. *Br J Sports Med*. 2006;40(12):992-7.
82. Gregg EW, Li Y, Wang J et al. Changes in diabetes-related complications in the United States, 1990-2010. *The New England journal of medicine*. 2014;370(16):1514-23.
83. Gu K, Cowie CC, Harris MI. Mortality in adults with and without diabetes in a national cohort of the U.S. population, 1971-1993. *Diabetes care*. 1998;21(7):1138-45.



84. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *The New England journal of medicine*. 1998;339(4):229-34.
85. Hagobian TA, Braun B. Interactions between energy surplus and short-term exercise on glucose and insulin responses in healthy people with induced, mild insulin insensitivity. *Metabolism: clinical and experimental*. 2006;55(3):402-8.
86. Hasson RE, Freedson PS, Braun B. Use of continuous glucose monitoring in normoglycemic, insulin-resistant women. *European journal of applied physiology*. 2010;108(6):1181-7.
87. Hawley JA, Hargreaves M, Joyner MJ, Zierath JR. Integrative biology of exercise. *Cell*. 2014;159(4):738-49.
88. Healy GN, Dunstan DW, Salmon J et al. Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. *Diabetes care*. 2007;30(6):1384-9.
89. Healy GN, Dunstan DW, Salmon J et al. Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes care*. 2008;31(4):661-6.
90. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *European heart journal*. 2011;32(5):590-7.
91. Healy GN, Winkler EA, Owen N, Anuradha S, Dunstan DW. Replacing sitting time with standing or stepping: associations with cardio-metabolic risk biomarkers. *European heart journal*. 2015;36(39):2643-9.
92. Henson J, Davies MJ, Bodicoat DH et al. Breaking up prolonged sitting with standing or walking attenuates the postprandial metabolic response in postmenopausal women: a randomized acute study. *Diabetes Care*. 2016;39(1):130-8.
93. Henson J, Yates T, Biddle SJ et al. Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health. *Diabetologia*. 2013;56(5):1012-20.
94. Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR. Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes technology & therapeutics*. 2011;13(9):921-8.
95. Holmstrup M, Fairchild T, Keslacy S, Weinstock R, Kanaley J. Multiple short bouts of exercise over 12-h period reduce glucose excursions more than an energy-matched single bout of exercise. *Metabolism: clinical and experimental*. 2014;63(4):510-9.
96. Hostmark AT, Ekeland GS, Beckstrom AC, Meen HD. Postprandial light physical activity blunts the blood glucose increase. *Preventive medicine*. 2006;42(5):369-71.
97. Iglay K, Hannachi H, Joseph Howie P et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. *Curr Med Res Opin*. 2016;32(7):1243-52.

98. Jovanovic A, Gerrard J, Taylor R. The second-meal phenomenon in type 2 diabetes. *Diabetes care*. 2009;32(7):1199-201.
99. Kang X, Wang C, Lifang L et al. Effects of different proportion of carbohydrate in breakfast on postprandial glucose excursion in normal glucose tolerance and impaired glucose regulation subjects. *Diabetes technology & therapeutics*. 2013;15(7):569-74.
100. Kannel WB. Diabetes and Cardiovascular Disease. *JAMA*. 1979;241(19):2035.
101. Karstoft K, Christensen CS, Pedersen BK, Solomon TP. The acute effects of interval- vs. continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a cross-over, controlled study. *The Journal of clinical endocrinology and metabolism*. 2014;jc20141837.
102. Kjaer M, Hollenbeck CB, Frey-Hewitt B, Galbo H, Haskell W, Reaven GM. Glucoregulation and hormonal responses to maximal exercise in non-insulin-dependent diabetes. *Journal of applied physiology*. 1990;68(5):2067-74.
103. Kozey-Keadle S, Libertine A, Lyden K, Staudenmayer J, Freedson PS. Validation of wearable monitors for assessing sedentary behavior. *Medicine and science in sports and exercise*. 2011;43(8):1561-7.
104. Krogh-Madsen R, Pedersen M, Solomon TP et al. Normal physical activity obliterates the deleterious effects of a high-caloric intake. *Journal of applied physiology*. 2014;116(3):231-9.
105. Larsen JJ, Dela F, Kjaer M, Galbo H. The effect of moderate exercise on postprandial glucose homeostasis in NIDDM patients. *Diabetologia*. 1997;40(4):447-53.
106. Larsen RN, Kingwell BA, Robinson C et al. Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults. *Clinical science*. 2015.
107. Larsen RN, Kingwell BA, Sethi P, Cerin E, Owen N, Dunstan DW. Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2014.
108. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. *Archives of internal medicine*. 2004;164(19):2147-55.
109. Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq FR, Schnure JJ. Glucose intolerance during decreased physical activity in man. *Diabetes*. 1972;21(2):101-7.
110. Little JP, Gillen JB, Percival ME et al. Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of applied physiology*. 2011;111(6):1554-60.
111. Little JP, Jung ME, Wright AE, Wright W, Manders RJ. Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2014.

112. Look ARG, Wing RR. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. *Archives of internal medicine*. 2010;170(17):1566-75.
113. Look ARG, Wing RR, Bolin P et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *The New England journal of medicine*. 2013;369(2):145-54.
114. Macisaac RJ, Jerums G. Intensive glucose control and cardiovascular outcomes in type 2 diabetes. *Heart, lung & circulation*. 2011;20(10):647-54.
115. MacLeod SF, Terada T, Chahal BS, Boule NG. Exercise lowers postprandial glucose but not fasting glucose in type 2 diabetes: a meta-analysis of studies using continuous glucose monitoring. *Diabetes/metabolism research and reviews*. 2013;29(8):593-603.
116. Manders RJ, Van Dijk JW, van Loon LJ. Low-intensity exercise reduces the prevalence of hyperglycemia in type 2 diabetes. *Medicine and science in sports and exercise*. 2010;42(2):219-25.
117. Mascarenhas-Melo F, Marado D, Palavra F et al. Diabetes abrogates sex differences and aggravates cardiometabolic risk in postmenopausal women. *Cardiovascular diabetology*. 2013;12:61.
118. Matheus AS, Tannus LR, Cobas RA, Palma CC, Negrato CA, Gomes MB. Impact of diabetes on cardiovascular disease: an update. *Int J Hypertens*. 2013;2013:653789.
119. Matthews CE. Physical activity in the United States measured by accelerometer: comment. *Medicine and science in sports and exercise*. 2008;40(6):1188; author reply 9.
120. Matthews CE, Chen KY, Freedson PS et al. Amount of time spent in sedentary behaviors in the United States, 2003-2004. *American journal of epidemiology*. 2008;167(7):875-81.
121. McDonnell CM, Donath SM, Vidmar SI, Werther GA, Cameron FJ. A novel approach to continuous glucose analysis utilizing glycemic variation. *Diabetes technology & therapeutics*. 2005;7(2):253-63.
122. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends in neurosciences*. 2013;36(10):587-97.
123. Mikines KJ, Richter EA, Dela F, Galbo H. Seven days of bed rest decrease insulin action on glucose uptake in leg and whole body. *Journal of applied physiology*. 1991;70(3):1245-54.
124. Mikus CR, Oberlin DJ, Libla JL, Taylor AM, Booth FW, Thyfault JP. Lowering physical activity impairs glycemic control in healthy volunteers. *Medicine and science in sports and exercise*. 2012;44(2):225-31.
125. Miller TM, Gilligan S, Herlache LL, Regensteiner JG. Sex differences in cardiovascular disease risk and exercise in type 2 diabetes. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*. 2012;60(4):664-70.
126. Monnier L, Colette C. Glycemic variability: can we bridge the divide between controversies? *Diabetes care*. 2011;34(4):1058-9.

127. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes care*. 2003;26(3):881-5.
128. Monnier L, Mas E, Ginnet C et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*. 2006;295(14):1681-7.
129. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical activity of work. *Lancet*. 1953;265(6796):9.
130. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes, obesity & metabolism*. 2010;12(4):288-98.
131. Nedeltcheva AV, Kessler L, Imperial J, Penev PD. Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. *The Journal of clinical endocrinology and metabolism*. 2009;94(9):3242-50.
132. Nygaard H, Tomten SE, Hostmark AT. Slow postmeal walking reduces postprandial glycemia in middle-aged women. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2009;34(6):1087-92.
133. Oberlin DJ, Mikus CR, Kearney ML et al. One bout of exercise alters free-living postprandial glycemia in type 2 diabetes. *Medicine and science in sports and exercise*. 2014;46(2):232-8.
134. Owen N, Healy GN, Matthews CE, Dunstan DW. Too much sitting: the population health science of sedentary behavior. *Exercise and sport sciences reviews*. 2010;38(3):105-13.
135. Pearce KL, Noakes M, Keogh J, Clifton PM. Effect of carbohydrate distribution on postprandial glucose peaks with the use of continuous glucose monitoring in type 2 diabetes. *The American journal of clinical nutrition*. 2008;87(3):638-44.
136. Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *The American journal of clinical nutrition*. 2013;98(2):358-66.
137. Perreault L, Ma Y, Dagogo-Jack S et al. Sex differences in diabetes risk and the effect of intensive lifestyle modification in the Diabetes Prevention Program. *Diabetes care*. 2008;31(7):1416-21.
138. Physical Activity Guidelines Advisory Committee. Physical Activity Guidelines Advisory Committee Report, 2008. *Washington, DC; U.S. Department of Health and Human Services*. 2008.
139. Poirier P, Mawhinney S, Grondin L et al. Prior meal enhances the plasma glucose lowering effect of exercise in type 2 diabetes. *Medicine and science in sports and exercise*. 2001;33(8):1259-64.

140. Powers MA, Cuddihy RM, Wesley D, Morgan B. Continuous glucose monitoring reveals different glycemic responses of moderate- vs high-carbohydrate lunch meals in people with type 2 diabetes. *Journal of the American Dietetic Association*. 2010;110(12):1912-5.
141. Praet SF, Manders RJ, Lieveve AG et al. Influence of acute exercise on hyperglycemia in insulin-treated type 2 diabetes. *Medicine and science in sports and exercise*. 2006;38(12):2037-44.
142. Praet SF, Manders RJ, Meex RC et al. Glycaemic instability is an underestimated problem in Type II diabetes. *Clin Sci (Lond)*. 2006;111(2):119-26.
143. Prevention CfDCA. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. *U.S. Department of Health and Human Services*. 2014;Atlanta, GA.
144. Pulsford RM, Blackwell J, Hillsdon M, Kos K. Intermittent walking, but not standing, improves postprandial insulin and glucose relative to sustained sitting: A randomised cross-over study in inactive middle-aged men. *Journal of science and medicine in sport / Sports Medicine Australia*. 2016.
145. RDC. R: A language and environment for statistical computing. In: 2008.
146. Rebrin K, Sheppard NF, Jr., Steil GM. Use of subcutaneous interstitial fluid glucose to estimate blood glucose: revisiting delay and sensor offset. *Journal of diabetes science and technology*. 2010;4(5):1087-98.
147. Regensteiner JG, Bauer TA, Huebschmann AG et al. Sex differences in the effects of type 2 diabetes on exercise performance. *Medicine and science in sports and exercise*. 2015;47(1):58-65.
148. Regensteiner JG, Golden S, Huebschmann AG et al. Sex Differences in the Cardiovascular Consequences of Diabetes Mellitus: A Scientific Statement From the American Heart Association. *Circulation*. 2015;132(25):2424-47.
149. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Medicine and science in sports and exercise*. 1995;27(6):875-81.
150. Riccardi G, Rivellese AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. *Diabetes care*. 1991;14(12):1115-25.
151. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev*. 2013;93(3):993-1017.
152. Ridker PM, Cook NR, Lee IM et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *The New England journal of medicine*. 2005;352(13):1293-304.
153. Rynders CA, Weltman JY, Jiang B et al. Effects of exercise intensity on postprandial improvement in glucose disposal and insulin sensitivity in prediabetic adults. *The Journal of clinical endocrinology and metabolism*. 2014;99(1):220-8.
154. Sadarangani KP, Hamer M, Mindell JS, Coombs NA, Stamatakis E. Physical activity and risk of all-cause and cardiovascular disease mortality in diabetic adults from Great Britain: pooled analysis of 10 population-based cohorts. *Diabetes care*. 2014;37(4):1016-23.

155. Salkind SJ, Huizenga R, Fonda SJ, Walker MS, Vigersky RA. Glycemic Variability in Nondiabetic Morbidly Obese Persons: Results of an Observational Study and Review of the Literature. *Journal of diabetes science and technology*. 2014.
156. Santilli F, Formoso G, Sbraccia P et al. Postprandial hyperglycemia is a determinant of platelet activation in early type 2 diabetes mellitus. *Journal of thrombosis and haemostasis : JTH*. 2010;8(4):828-37.
157. Schisano B, Tripathi G, McGee K, McTernan PG, Ceriello A. Glucose oscillations, more than constant high glucose, induce p53 activation and a metabolic memory in human endothelial cells. *Diabetologia*. 2011;54(5):1219-26.
158. Schmid SM, Hallschmid M, Jauch-Chara K et al. Disturbed glucoregulatory response to food intake after moderate sleep restriction. *Sleep*. 2011;34(3):371-7.
159. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Human nutrition. Clinical nutrition*. 1985;39 Suppl 1:5-41.
160. Service FJ, Nelson RL. Characteristics of glycemic stability *Diabetes care*. 1980;3(1):4.
161. Shan Z, Ma H, Xie M et al. Sleep Duration and Risk of Type 2 Diabetes: A Meta-analysis of Prospective Studies. *Diabetes care*. 2015;38(3):529-37.
162. Sigal RJ, Kenny GP, Boule NG et al. Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Annals of internal medicine*. 2007;147(6):357-69.
163. Smorawinski J, Kaciuba-Uscilko H, Nazar K, Kaminska E, Korszun P, Greenleaf JE. Comparison of changes in glucose tolerance and insulin secretion induced by three-day bed rest in sedentary subjects and endurance or strength trained athletes. *Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology*. 1998;5(1):P103-4.
164. Solomon TP, Malin SK, Karstoft K, Haus JM, Kirwan JP. The influence of hyperglycemia on the therapeutic effect of exercise on glycemic control in patients with type 2 diabetes mellitus. *JAMA Intern Med*. 2013;173(19):1834-6.
165. Staiano AE, Harrington DM, Barreira TV, Katzmarzyk PT. Sitting time and cardiometabolic risk in US adults: associations by sex, race, socioeconomic status and activity level. *Br J Sports Med*. 2014;48(3):213-9.
166. Stephens BR, Granados K, Zderic TW, Hamilton MT, Braun B. Effects of 1 day of inactivity on insulin action in healthy men and women: interaction with energy intake. *Metabolism: clinical and experimental*. 2011;60(7):941-9.
167. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Bmj*. 2000;321(7258):405-12.
168. Stuart CA, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR. Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism: clinical and experimental*. 1988;37(8):802-6.

169. Taraldsen K, Askim T, Sletvold O et al. Evaluation of a body-worn sensor system to measure physical activity in older people with impaired function. *Phys Ther.* 2011;91(2):277-85.
170. Terada T, Friesen A, Chahal BS, Bell GJ, McCargar LJ, Boule NG. Exploring the variability in acute glycemic responses to exercise in type 2 diabetes. *J Diabetes Res.* 2013;2013:591574.
171. Terada T, Loehr S, Guigard E et al. Test-retest reliability of a continuous glucose monitoring system in individuals with type 2 diabetes. *Diabetes technology & therapeutics.* 2014;16(8):491-8.
172. Thijssen DH, Black MA, Pyke KE et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American journal of physiology. Heart and circulatory physiology.* 2011;300(1):H2-12.
173. Thorp AA, Kingwell BA, Sethi P, Hammond L, Owen N, Dunstan DW. Alternating Bouts of Sitting and Standing Attenuates Postprandial Glucose Responses. *Medicine and science in sports and exercise.* 2014.
174. Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of Prolonged Sitting and Breaks in Sitting Time on Endothelial Function. *Medicine and science in sports and exercise.* 2014.
175. Thyfault JP. Setting the stage: possible mechanisms by which acute contraction restores insulin sensitivity in muscle. *Am J Physiol Regul Integr Comp Physiol.* 2008;294(4):R1103-10.
176. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. *Medicine and science in sports and exercise.* 2008;40(1):181-8.
177. UK Prospective Diabetes Study Group U. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352(9131):837-53.
178. van der Berg JD, Stehouwer CD, Bosma H et al. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: The Maastricht Study. *Diabetologia.* 2016;59(4):709-18.
179. van Dijk JW, Manders RJ, Hartgens F, Stehouwer CD, Praet SF, van Loon LJ. Postprandial hyperglycemia is highly prevalent throughout the day in type 2 diabetes patients. *Diabetes research and clinical practice.* 2011;93(1):31-7.
180. van Dijk JW, Manders RJ, Tummers K et al. Both resistance- and endurance-type exercise reduce the prevalence of hyperglycaemia in individuals with impaired glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic patients. *Diabetologia.* 2012;55(5):1273-82.
181. van Dijk JW, Tummers K, Stehouwer CD, Hartgens F, van Loon LJ. Exercise therapy in type 2 diabetes: is daily exercise required to optimize glycemic control? *Diabetes care.* 2012;35(5):948-54.
182. van Dijk JW, van Loon LJ. Exercise strategies to optimize glycemic control in type 2 diabetes: a continuing glucose monitoring perspective. *Diabetes spectrum : a publication of the American Diabetes Association.* 2015;28(1):24-31.

183. van Dijk JW, Venema M, van Mechelen W, Stehouwer CD, Hartgens F, van Loon LJ. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. *Diabetes care*. 2013;36(11):3448-53.
184. Voskanyan G, Barry Keenan D, Mastrototaro JJ, Steil GM. Putative delays in interstitial fluid (ISF) glucose kinetics can be attributed to the glucose sensing systems used to measure them rather than the delay in ISF glucose itself. *Journal of diabetes science and technology*. 2007;1(5):639-44.
185. Wilmot EG, Edwardson CL, Achana FA et al. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia*. 2012;55(11):2895-905.
186. Yates T, Henson J, Edwardson C et al. Objectively measured sedentary time and associations with insulin sensitivity: Importance of reallocating sedentary time to physical activity. *Preventive medicine*. 2015;76:79-83.
187. Yates T, Khunti K, Wilmot EG et al. Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity. *American journal of preventive medicine*. 2012;42(1):1-7.
188. Zanuso S, Jimenez A, Pugliese G, Corigliano G, Balducci S. Exercise for the management of type 2 diabetes: a review of the evidence. *Acta diabetologica*. 2010;47(1):15-22.
189. Zhao G, Ford ES, Li C, Mokdad AH. Compliance with physical activity recommendations in US adults with diabetes. *Diabetic medicine : a journal of the British Diabetic Association*. 2008;25(2):221-7.