Acute Glycemic Response to Different Strategies of Breaking Up Sedentary Time

by

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ABSTRACT

Most studies that explored the health benefits of interrupting sitting time focused on using different modalities (i.e., comparing walking vs standing breaks)[33,36,59]. However, experimental studies that directly compare patterns of interrupting sitting time through standing only are needed to advance the field. This study aimed to (i) determine if there is a difference in glucose response between continuous sitting (CS) and two intermittent standing regimes (high frequency, low duration breaks (HFLD) and low frequency, high duration breaks (LFHD)) and (ii) to determine if there is a difference in glucose response between the two strategies (HFLD vs. LFHD).

Ten sedentary employees (mean±SD age 46.8±10.6 years; 70% female) with impaired fasting glucose (mean glucose= 109.0±9.8 mg/dL) participated. Eligible participants were invited to three 7.5 hour laboratory visits where they were randomized to perform each study conditions: (i) CS, (ii) HFLD and (iii) LFHD. Standardized meals (breakfast and lunch) were given with each meal providing 33% of the participant's total daily caloric needs following a typical American diet (50-60% carbohydrates, 25-30% fat, and 10-20% protein). Participants wore an activPAL device to measure compliance with the sit-stand condition and a continuous glucose monitor to measure post-prandial glucose response. Post-prandial mean glucose, incremental area under the curve and mean amplitude glycemic excursion between conditions were evaluated using linear mixed models.

Participants demonstrated high compliance with the study condition. The results indicated that the mean glucose of the HFLD condition were significantly lower ($p < .01$)

than the CS condition with mean difference of -7.70 (-11.98, -3.42) mg/dL·3.5h and -5.76 (-9.50, -2.03) mg/dL·7h for lunch and total time, respectively. Furthermore, the mean post-prandial glucose during lunch and total time were significantly lower in the HFLD condition compared to the LFHD condition with mean difference of -9.94 (-14.13, -5.74) mg/dL·3.5h and -6.23 (-9.93, -2.52) mg/dL·7h, respectively. No differences were found between the CS and LFHD conditions.

This study provides evidence favoring the use of frequent interruptions in sitting time to improve glycemic control of prediabetic individuals. In contrast, less frequent, although longer bouts of standing resulted in similar post-prandial glucose profile to that of the continuous sitting condition despite total standing time being equal to the LFHD condition.

DEDICATION

This dissertation is dedicated to my family and friends who have continuously been in my side, supporting and encouraging me throughout this process. Your constant support helped me realize my potential and succeed in my endeavors. Without you, this journey would have been dull and dreary.

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CHAPTER 1

INTRODUCTION

A. Background of the Study

Sedentary behavior, defined as any sitting or reclining activities that require a low level of energy expenditure (<1.5 METS)[111], has received substantial attention in the past decade[81,98]. Previous large scale epidemiological data have shown that the average American spends about 7.7 hours per day being sedentary[82]. This is concerning considering that subsequent studies suggest that this set of behaviors significantly increase all-cause mortality, cardiovascular disease incidence and mortality, and type 2 diabetes incidence[10,11,51,54,98]. Although these studies also indicated that the negative effects of sedentary behavior can be attenuated by high levels of moderate-to-vigorous physical activity, the majority of Americans do not accumulate the levels of moderate-to-vigorous physical activity that are adequate to protect against excess sedentary behavior. Furthermore, there are preliminary studies suggesting that sedentary behavior may have unique mechanistic pathways on health outcomes, particularly glucose metabolism, that operate independently from moderate-to-vigorous physical activity[44,46].

Early evidence on the potential mechanisms at which sedentary behavior exerts its negative impact on health came from studies looking at animal models[44,45]. Studies using animal models showed significant reductions in activity of lipoprotein lipase, an enzyme responsible for breaking down triglycerides[67], after subjecting mice to an acute bout of inactivity[44]. This change came without any significant change in lipoprotein lipase mRNA. More recently, experimental studies on human subjects provided evidence on the

benefits of interrupting sitting time on various cardiometabolic risk factors (i.e., post-prandial glucose and insulin level, insulin sensitivity and triglycerides)[28,33,59,105]. A study in 2016 provided some insights on the potential mechanism of which interruptions in sitting time benefits health. Their result showed that acute interruptions to sitting time over one day with light intensity activity stimulate the contraction-mediated glucose uptake pathway while only interruptions using moderate activity modulates the insulin-signaling pathway through increased capacity for glucose transport[13]. Overall, these results suggest that there is a potent regulatory process at the lower end of the physical activity continuum controlling these mechanisms, independent from physical activity.

Since a typical American spends at least eight hours of their day at work, the workplace has emerged as a popular setting for interventions aiming to reduce sedentary behavior[20,25,30,56]. A promising strategy to reduce sedentary behavior in the workplace is the use of environmental changes (i.e., use of sit-stand workstations) to promote breaks in sitting time without significantly affecting work productivity. This strategy complements traditional-evidence-based interventions that provide sustainable interventions that explicitly target sitting time by increasing light physical activity. Two large cluster-randomized trials that studied the efficacy of using such interventions reported significant reductions in sitting time[30,53]. However, these reductions in sitting time failed to result in cardiometabolic improvements equal to those observed in the acute, laboratory-based trials. This was especially true for glucose parameters[53]. In addition, several trials have evaluated different types of interventions such as sit-stand workstations, implementing walking breaks, providing information and counseling, and combinations of these interventions. However, a meta-analysis of these studies concluded only low-quality

evidence as to the efficacy of these interventions in lowering sitting behaviors at work[113]. This is primarily due to various design-related problems of studies that examined this topic: (1) no or inconsistent effects of these interventions, (2) insufficient sample size, (3) lack of studies that examined long-term effects of these interventions, and (4) lack of cluster-randomized studies that are sufficiently sampled[113]. Clearly, more studies are needed to understand the nature of this behavior and inform the development of novel interventions that can address this public health problem.

Health-related behaviors are determined by an interplay of personal, behavioral, and environmental factors[9]. These factors can be both static (e.g., personality trait, built environment, sex, race, income status, educational attainment, etc.) and dynamic (e.g., mood and affect, physical states) in nature. Thus, an effective and efficient behavior change intervention requires a full understanding of the complex and dynamic relationship between the behavior and the factors surrounding it to deliver an intervention that is adaptive and responsive to these dynamic processes. An emerging and innovative approach in the behavioral science community is the concept of an adaptive intervention. [93]. An adaptive intervention aims to provide the right intervention, at the right time, by adapting to an individual's changing internal and contextual state. Unlike most of the previously tested interventions that only focused on providing a static intervention, adaptive interventions offers an innovative approach to efficiently and effectively reduce sitting behavior by accounting for the dynamic nature of the behavior and the factors that could potentially lead to it. As such, adaptive interventions provides a novel framework that directly address the dynamic and multi-factorial aspects of sedentary behavior. However, the lack of current studies that explores the dynamic in-the-moment

relationships between sitting behavior significantly delays the development of these adaptive interventions.

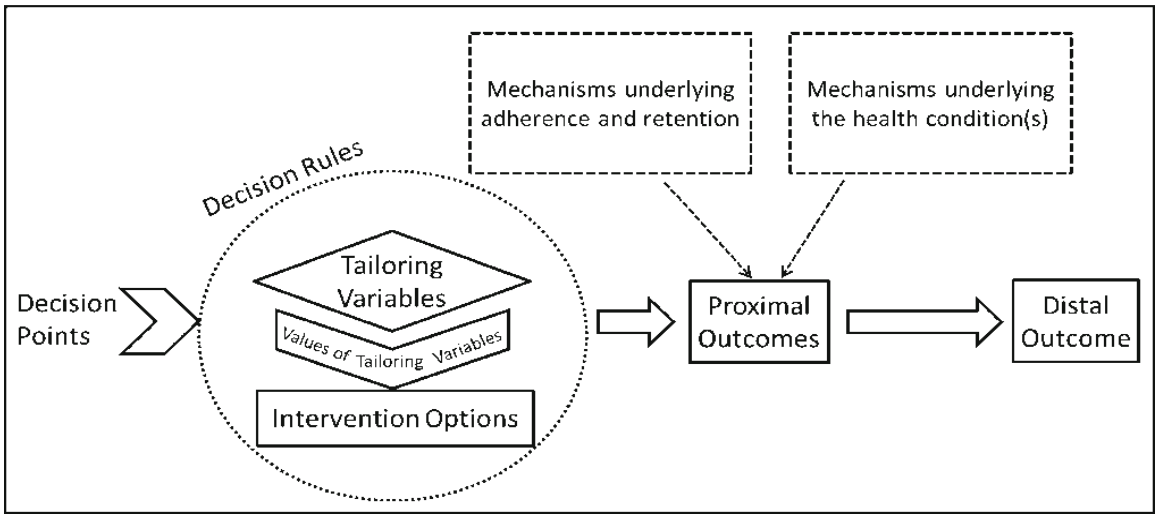

Figure 1. Conceptual framework for developing a just-in-time intervention for sedentary behavior. Adapted from Muller et al[92].

Figure 1 shows the conceptual framework and key elements (i.e., distal outcome, proximal outcome, tailoring variables, intervention options, decision rules and decision points) for developing a just-in-time adaptive intervention (JITAI)[93]. In brief, a robust JITAI should integrate existing theoretical and empirical evidence into a conceptual framework that clearly delineate potential decision points, decision rules, tailoring variables, and the different intervention options. All of these factors would dictate what, when, where and how much of an intervention should be delivered to maximize the potential of eliciting a change in the proximal outcome of interest and consequently affecting a long-term distal outcome. This study aimed to contribute to this conceptual framework by providing evidence to support the use of different strategies to interrupt sitting to reduce total sitting time. A potential proximal behavioral outcome that can be

targeted by JITAI is the patterns at which sitting time is being reduced (i.e., variations in frequency and duration of standing bouts). A recent cross-sectional study that explored the association between objectively measured sitting accumulation patterns and cardiometabolic risk in a sample of 678 adults (mean±SD: 58±10 years old) revealed significant variations in how participants accumulate sitting time[12]. Furthermore, the authors concluded that patterns with frequently interrupted sitting behavior, compared to patterns with relatively fewer interruptions in sitting behavior, were significantly associated with fasting glucose and 2-hour post-load glucose independent of total sitting time. Although there had been studies that explored cardiometabolic responses to interrupting sitting time, these studies have only compared different modalities (e.g., walking vs standing breaks) and not the pattern of interruptions[33,36,59]. Thus, experimental studies that directly compare patterns of interrupting sedentary time through standing are needed.

In this project, we aimed to determine if there is a significant difference in acute glucose response between two different patterns to reduce sitting time to inform future JITAI of the most optimal strategy to improve glycemic control.

B. Statement of the Problem and Hypotheses

In this study, we aim to identify effective strategies for reducing sedentary time to improve health. Specifically, we seek to address the following aims:

1. To determine if there is a significant difference in acute post-prandial glucose response between continuous sitting and two intermittent standing regimes (high frequency, low duration breaks (HFLD) and low frequency, high duration breaks (LFHD)).

Ha1: We hypothesize that intermittent standing (combined HFLD breaks and LFHD breaks regimes) will result in lower overall acute post-prandial glucose compared to continuous sitting.

2. To determine if there is a significant difference in acute post-prandial glucose response between two strategies to reduce sitting with standing (HFLD standing breaks vs. LFHD standing breaks).

Ha2: We hypothesize that the HFLD breaks condition will elicit lower acute post-prandial glucose level compared to the LFHD breaks condition.

C. Significance of the Study

Sedentary behavior has received substantial attention in the scientific community in recent years[33,50,54,81,97]. It is estimated that adults spend 7.7 hours per day in sedentary behavior[82]. This is problematic considering that studies indicate that an hour increment in time spent in subjectively measured total sitting time or watching TV, a surrogate measure of sitting behavior, is associated with an 18% increase risk in cardiovascular disease mortality[97]. Results from epidemiological and small clinical trials suggest that accumulating large amount of sitting time can lead to adverse health consequences including cardiometabolic diseases, cancer and premature mortality. In addition, meta-analysis and systematic reviews have indicated that the association between glucose and sedentary behavior exist consistently across gender and different ethnic backgrounds[42,62,71]. Studies have also shown that this association exist in both children

and adults, independent of adiposity and physical activity level. Furthermore, several lab-based RCTs have demonstrated that breaking up prolonged sitting through low-intensity activity (i.e., standing or walking) resulted in lower glycemic excursions compared to an uninterrupted sitting bout[33,59]. Collectively, these studies support the notion that reducing sedentary behavior can have a significant beneficial impact on glycemic control.

Since Americans spend about 70-80% of their work time sitting at their desk[87], the worksite has received considerable attention as a venue for interventions aiming to improve overall health[20,30,53,61]. Studies in this area have shown success in increasing moderate-to-vigorous physical activity through worksite level interventions[61]. However, implementing a worksite intervention that improve moderate-vigorous physical activity alone do not directly address the problem with sitting time accumulation. A recent meta-analysis that analyzed data from a large sample revealed that moderate-vigorous physical activity alone does not completely negate the negative effects of sitting time[37]. Thus, there is still a need for studies that evaluate the effectiveness of interventions that directly target sitting behavior.

A promising strategy to reduce sedentary behavior in the workplace is the use of environmental changes (i.e., use of sit-stand workstations) to promote breaks in sitting time without significantly affecting work productivity. This strategy complements evidence-based interventions that provide sustainable interventions that explicitly target sitting behavior by increasing light physical activity. Two large cluster-randomized trials that studied the efficacy of using such interventions reported significant reductions in sitting behavior[30,53]. However, these reductions in sitting time failed to result into meaningful improvement in cardiometabolic risk parameters, especially glucose[53]. A

potential explanation to these confusing results is the different patterns at which sitting time is being reduced (i.e., frequent short bouts of standing vs non-frequent longer bouts of standing). A recent study that explored the association between objectively measured sitting accumulation patterns and cardiometabolic risk in a sample of 678 adults (mean±SD 58±10 years old) showed significant variations in how participants accumulates sittingy time[12]. Furthermore, the authors also concluded that patterns with frequently interrupted sitting time, compared to patterns with relatively fewer interruptions, were significantly associated with fasting glucose and 2-hour post-load glucose independent of total sitting time. Although there had been studies that explored cardiometabolic responses of breaking sitting time using different patterns, these studies have only compared different modality of breaks[33,36,59]. Thus, experimental studies that directly compare patterns of breaking sitting time are needed.

The proposed research is expected to contribute to the field of sedentary behavior research by exploring the differences in acute glucose response from two distinct patterns of reducing sitting time. In this study, we employed research design features to control for the effect of total time spent seated and total time spent standing to deepen our current understanding of how sedentary behavior affects glucose metabolism. Using these strategies, we aimed to isolate the effects of breaks in sitting through standing from the effects of engaging in other higher intensity activities. This can provide future researchers with relevant information needed to develop efficient strategies to improve cardiometabolic health through reductions in sedentary behavior. Overall, these contributions will be significant because it is expected to inform the development of efficient strategies to decrease cardiometabolic risk through a reduction in sitting time.

Such strategies would have broad translational importance in the prevention of chronic diseases and health promotion.

D. Definition of Terms

For clarity, the following commonly used terms were defined conceptually and operationally:

1. Sedentary behavior: The Sedentary Behavior Research Network defines sedentary behavior as any waking behavior characterized by an energy expenditure ≤ 1.5 metabolic equivalents (METs), while in a sitting, reclining, or lying posture (Tremblay et al., 2017). In this study, we operationalize sedentary behavior as any bouts of sitting measured by the activPAL device.
2. Sedentary breaks: Breaks in sedentary behavior are often characterize as an interruption to a continuous bout of sedentary behavior. Sedentary breaks in this study were defined as any change in posture from a sitting position. We interrupted sedentary time with (1) a high-frequency, low-duration bout of standing and (2) a low-frequency, high duration bout of standing (see chapter 3 for a full description of these sedentary breaks).
3. Impaired fasting glucose: Impaired fasting glucose in this study was defined as having a fasting blood sugar level between 100-125 mg/dL.
4. Glucose level: Glucose level in this study was operationalized as any glucose value measured continuously (every 15 minutes) by the LibrePro continuous glucose monitor. The LibrePro measures interstitial glucose level which are then used to estimate actual plasma glucose level. The device has been evaluated to be valid and accurate in previous studies[8].

5. Standardized meals: Standardized meals were defined as meals served to the participants during each study visit. The total caloric content of each meal was estimated using Schofield's equation with a 1.5 physical activity factor. Furthermore, meals were prepared following the macronutrient content of a typical American diet (50-60% carbohydrates, 25-35% fat, and 10-20% protein).

E. Scope, Delimitations and Limitations

In brief, the study aimed to determine if there was a significant difference in acute glucose response between two different patterns of reducing sitting time to inform future interventions of the most optimal strategy to reduce sitting time and improveglycemic profile. The study focused on standing as a medium to interrupt sitting time. This enabled us to tease out the effect of interrupting sitting time from the effect of doing moderate-vigorous physical activity. Participants include full-time employees in the metropolitan Phoenix area. The study was conducted in Arizona Biomedical Collaborative from August 2018 to May 2019.

Because of constraints related to study resources, the study focused on studying individuals with impaired fasting glucose. This study focused on the acute benefits of breaking up sedentary time but there are other studies that suggest positive benefits on other risk outcomes (e.g., insulin sensitivity, triglycerides and high density lipoprotein level) which should be explored in future studies. To minimize variability, individuals with normal fasting glucose levels were excluded since previous studies have shown that glucose response to similar interventions were minimal among those participants. Participants diagnosed with diabetes were also excluded to eliminate any bias that can occur from taking diabetic medications and/or other comorbidities. In addition, we chose

to delimit our participants to only those who are overweight or obese to help focus recruitment resources and exclude any bias from any unknown underlying metabolic problem. To render results relevant to the workplace setting, we recruited full-time employees with sedentary jobs. Unlike previous studies, standing breaks were primarily used to interrupt sitting time to isolate the beneficial effects of reducing sedentary behavior from the benefits of engaging in more active behaviors such as walking.

Despite best effort to design the study to produce valid and unbiased results, there are some factors that should be considered when interpreting the results. Standardized meals were provided on each visit to control for effects of food between visits. However, the amount of food consumed can vary by visit and cause differences in the total calorie intake between study visits. To minimize this problem, participants were encouraged to consume 100% of the provided meals or the same amount of the meal for all visits. In addition, each food item was carefully weighed before and after each meal to document the amount of variations in consumption. Adherence to the sitting/standing protocol also varied between participants. Although the protocol was carefully explained before the start of each visit, there were circumstances that caused participants to deviate from the protocol (e.g., use of restrooms). Nevertheless, all participants wore the activPAL device on each visit to accurately determine how closely they followed the protocol. Any inconsistencies in adherence were noted by research staff.

CHAPTER 2

REVIEW OF LITERATURE

The *Sedentary Behavior Research Network* defined sedentary behavior as any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture[123]. The field of sedentary behavior research has expanded rapidly in the last few years. Subsequently, the scientific evidence as to the negative effects of sedentary behavior on our health is building up and our understanding on its effect on our health is increasing. For example, the *2018 Physical Activity Guidelines Advisory Committee* recently published their scientific report[1] where they reviewed nine published meta-analysis that included 20 original research articles that looked at the relationship between sedentary behavior and all-cause-mortality. In the report, they concluded that there is strong evidence demonstrating a significant relationship between greater time spent in sedentary behavior and a higher mortality rate from all-causes and from cardiovascular diseases. In the following section, we will review original articles, systematic reviews, and meta-analysis of studies that explored sedentary behavior, the different factors associated with it, the current evidence on its effects on health, and interventions specifically designed to reduce exposure to this behavior.

A. Sedentary Behavior and Health

In retrospect, the earliest scientific evidence on the relationship between sedentary behavior and risk for cardiovascular disease was demonstrated by Jeremy Morris when he showed that bus drivers, who were engaged in less active jobs, had a higher incidence of

coronary heart disease compared to their more active counterparts (i.e., bus conductors) [91]. Interestingly, sedentary behavior has been mostly ignored in the scientific community until it was referenced by Owen in a paper on the environmental determinants of physical activity and sedentary behavior[99]. This was followed by a number of epidemiological studies that examined the impact of sitting time on health. However, these studies had mostly relied on self-report measures and have only examined TV viewing as a proxy for the sitting time. These earlier studies examined the data collected from the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study, a longitudinal population-based study aimed at examining the natural history of diabetes, pre-diabetes, heart disease and kidney disease. Results from these cross-sectional studies have showed that self-reported TV viewing time was positively associated with undiagnosed abnormal glucose metabolism[35] and metabolic syndrome[34] even after accounting for the time spent in moderate-vigorous physical activity. Since then, research on sedentary behavior and its effects on health have proliferated.

All-Cause Mortality. To date, there are a number of systematic reviews and meta-analyses that examined the overall relationship between sedentary behavior and all-cause mortality[17,24,37,42,104,107,118,120,124]. The most recent was a systematic review by Biddle et al[15] in 2016 where they analyzed data from eight meta-analyses to examine evidence supporting this relationship using Bradford Hill's causal criteria[60]. The review included studies that evaluated TV viewing, screen time, or total sedentary (sitting) time as an outcome. The study concluded that overall, current studies examining the causal relationship between sitting time and all-cause mortality showed strong evidence for consistency and temporality, and some evidence for strength of association. The meta-

analysis of Biswas et al[17] looked at 13 prospective cohort studies of self-report sitting time and reported an overall hazard ratio of 1.22 (95% confidence interval (CI): 1.09-1.41) for the relationship between sitting time and all-cause mortality, adjusted for levels of physical activity. Furthermore, results from large epidemiological studies that used objective measures of physical activity and sitting behavior (primarily using the NHANES data) also showed similar significant relationship between sitting time and all-cause mortality[40,41,68,76,78,83,109,110]. The above-mentioned studies also support the notion of a dose-response relationship between sitting time and all-cause mortality. Chau et al[24] found that a spline model of best fit had a hazard ration of 1.0 (95% CI: 0.98-1.03), 1.02 (95% CI: 0.99-1.05), and 1.05 (95% CI 1.02-1.08) for every hour increase in daily sitting time intervals between 0 to 3, more than 3 to 7, and more than 7 hours per day of total sitting time, respectively. Similarly, Sun et al[118] also reported that TV viewing was statistically significantly associated with all-cause mortality risk in a curvilinear manner. The newer studies (published from 2014 to 2017) looked at by the *2018 Physical Activity Guidelines Advisory committee* scientific report also showed that there is a significant dose response relationship (see figure 2) between sitting time and all-cause mortality.

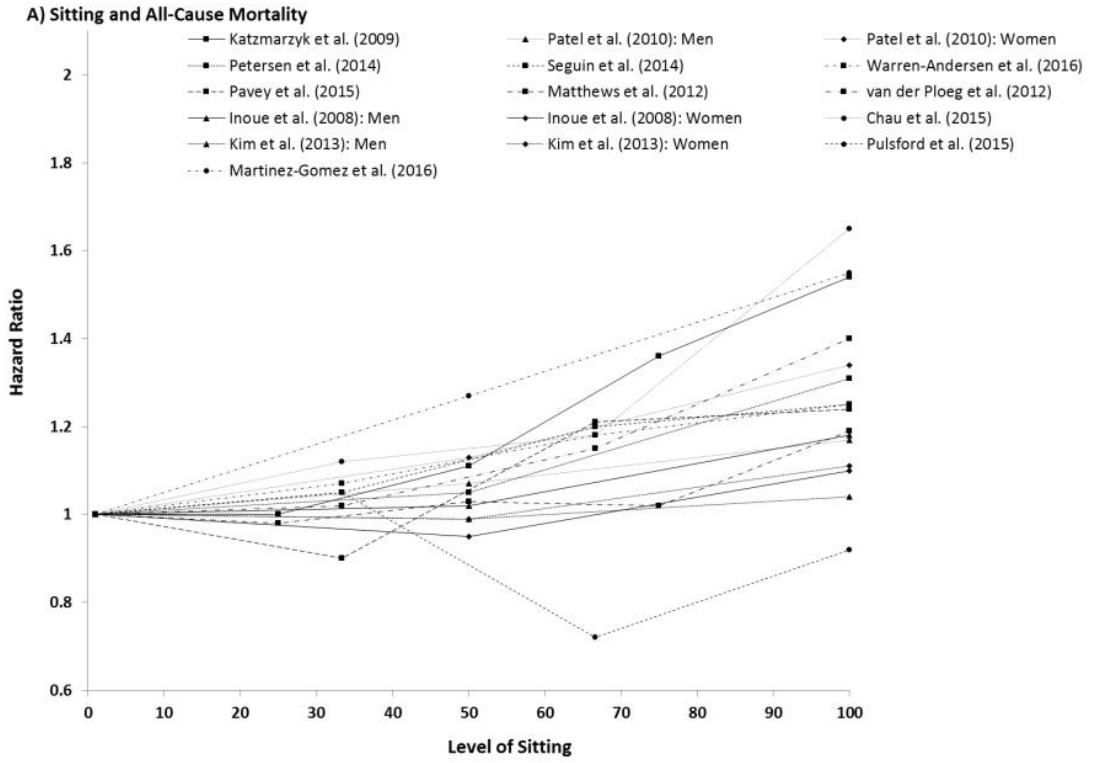

Figure 2. Dose response relationship between sedentary behavior and all-cause mortality. The figure report hazard ratio for each category of sitting assigned as the referent at the zero on the X-axis and the highest value assigned at 100. The original categories of sitting from the studies (tertiles, quartiles, quintiles, etc) have been rescaled from 0 to 100 using an ordinal scale (e.g., for a study with three categories, the points were plotted at 0, 50 and 100). Source: Adapted from the *2018 Physical Activity Guidelines Advisory Committee Scientific Report.*

Cardiovascular Disease Mortality. Most of the studies described above also provided strong evidence as to the relationship between sitting time and cardiovascular disease. Biswas et al[17] analyzed seven prospective cohort studies and found a hazard ratio of 1.15 (95% CI: 1.11-1.20) for the relationship between sitting time and cardiovascular disease mortality. Further, another meta-analysis by Wilmot et al[124] reported a relative risk of 1.90 (95% CI: 1.36-2.66) for the same relationship. Although these two studies showed significant risk estimates for the relationship between sitting time and cardiovascular disease mortality, the summary estimates between the two studies are slightly different,

with Wilmot et al reporting higher magnitude of risk. This is mainly just from the differences in studies included in each review and differences in the exposure categories and types of sedentary behavior among the included studies[1]. Several newer studies that also examined this relationship showed a significant positive relationship between time spent in sedentary behavior and cardiovascular disease mortality. These studies represent several population cohorts that apply broadly to the U.S. population and the results are consistent in direction and the size of the effect. There is also strong evidence that demonstrates a dose-response relationship between sedentary behavior and cardiovascular disease mortality. The harmonized meta-analysis of 11 prospective cohort studies by Ekelund et al[38] demonstrated that the associations among sitting time, moderate-to-vigorous physical activity, and cardiovascular disease mortality were similar to those observed for all-cause mortality.

Cardiovascular Disease Morbidity. In addition to being associated with mortality from cardiovascular disease, there are also studies that associate higher sedentary time to higher risk for cardiovascular disease incidence. Grontved and Hu[43] reported a pooled relative risk of 1.15 (95% CI: 1.06-1.23) per 2 hours of TV viewing per day. In addition, the study by Biswas et al[17] and Pandey et al[100] reported summary hazard ratios of 1.14 (95% CI: 1.00-1.30) and 1.14 (95% CI: 1.09- 1.19), respectively, for high versus low sitting time and incident cardiovascular disease. Finally, Wilmot et al[124] reported a significant summary relative risk for cardiovascular events of 2.47 (95% CI: 1.44-4.24). All of these meta-analyses indicate that sitting time is significantly associated with incidence cardiovascular disease risk. Newer research studies[18,26,90,101,125] published between 2014 and 2017 also found a significant association between sitting time and

incident cardiovascular disease. The study by Petersen et al[101] reported that total daily time spent sitting was significantly associated with incident myocardial infarction although not with incident coronary heart disease. Another recent study by Young et al[125] also reported a significant association between sedentary time and incident heart failure in U.S. men. Lastly, the study by Borodulin et al[18] showed a significant association between daily sitting time and incident fatal and nonfatal cardiovascular disease among Finnish adults. There are also evidence that hints to the possible dose-response in the association between sitting time and incident cardiovascular disease[43]. The meta-analysis by Grontved and Hu[42] showed a significant linear dose-response association between TV viewing and incident fatal and nonfatal cardiovascular disease.

Type II Diabetes Mellitus. Several meta-analyses addressed the issue of sedentary behavior and the incidence of type 2 diabetes, with all of them reporting significant association between incidence of type 2 diabetes and sitting time. The meta-analysis of Grontved and Hu et al[43] reported a pooled relative risk of 1.20 (95% CI: 1.14-1.27) per 2 hours of TV viewing per day among four original papers analyzed in the study. The summary relative risk (from five cross-sectional and five prospective studies) for type 2 diabetes reported by Wilmot et al[124] was 2.12 (95% CI: 1.61-2.78) for highest versus lowest sedentary time. Lastly, the Biswas et al[17] reported a summary hazard ratio of 1.91 (95% CI: 1.64-2.22) for type 2 diabetes from five studies included in their analysis. In addition to these meta-analyses of previous studies, newer studies published between 2014 to 2017 also showed a significant association between higher levels of sitting time and a higher risk of type 2 diabetes[5,64,80] in a fully adjusted model (i.e., adjusted for possible covariates such as age, sex, BMI, and physical activity). In three additional

studies[6,102,114], it was reported that the significant effects of sitting time on risk of type 2 diabetes in minimally adjusted models (e.g., age, sex) were attenuated to null when additional covariates, including BMI, were added to the models. Similar results were reported by the meta-analysis of Grontved and Hu[43] where they reported a pooled relative risk per 2 hours of TV viewing per day on risk of type 2 diabetes (1.20 (95% CI: 1.14-1.27) was attenuated to a relative risk of 1.13 (95% CI: 1.08-1.18) when calculated from models that included BMI or another obesity measure. These results suggest that effects of sitting time on risk of type 2 diabetes may be operating, in part, through its association with BMI. There is limited evidence that suggests a graded, positive association between sitting time and incident type 2 diabetes. The meta-analysis of Grontved and Hu[43] reported a significant, positive linear dose-response association between TV viewing and type 2 diabetes.

Overall, these studies demonstrated a significant relationship between sedentary behavior and all-cause mortality risk, mortality risk and incidence of cardiovascular disease and incidence of type 2 diabetes. The relationship between sedentary behavior and all-cause mortality seems to be moderated by the individual's level of physical activity where the hazardous effects of sedentary behavior are higher in inactive individuals. Furthermore, there are evidence pointing to physical activity as a moderator for the relationship between sedentary behavior and incidence of type two diabetes. All of these studies support the notion that sedentary behavior is a unique modifiable factor that needs to be addressed to achieve better overall health.

B. Measurement of Sedentary Behavior

In the previous section, we discussed studies that showed the negative impact of sedentary behavior on health. As such, generating an effective intervention to reduce sedentary behavior is a must to address this problem. The development of a valid assessment tool for this behavior is important to advance the research on this area. Although there are a number of instruments available to accurately assess physical activity, most of these subjective and objective measures were not designed to assess sedentary behavior. This section reviews the different methods and issues encountered with sedentary behavior measurement in population- and intervention-based studies.

Researchers studying specific domain of these behaviors (i.e., leisure-time, occupational, or transportation) still rely on self-report to isolate the behaviors that occur in each of these domains. Distinguishing which domains these behaviors occur is necessary in developing and evaluating targeted intervention to modify these domain-specific behaviors [23]. Thus, self-report remains an important method of measurement for physical activity and sedentary behaviors. Various types of physical activity questionnaires have been developed and were initially used in sedentary behavior research, ranging from global questionnaires to detailed quantitative history. Strath et al[117] classified physical activity questionnaires into three broad categories (i.e. global, short recalls, and quantitative history). Global physical activity questionnaires are usually short (2 to 4 items) and provide an overview of an individual's overall activity level. They are primarily used to identify whether individuals meet the physical activity standard or classify individuals according to their physical activity levels (e.g. active vs. inactive). In contrast, short recalls provide a measure of an individual's physical activity

level as classified by the dimension of intensity level or domain. Quantitative history questionnaires are detailed measures that are used to understand the types and intensity of physical activities that contribute to mortality or morbidity. A systematic review of studies that evaluate the reliability and objective-criterion-related validity of new and existing physical activity questionnaires[58] examined 65 studies that looked at a total of 96 physical activity questionnaires. Their results revealed poor to moderate validity, with median validity coefficients ranging from 0.30-0.39 for existing, and from 0.25-0.41 for new physical activity questionnaires. However, although other studies have shown that although these questionnaires show acceptable agreement for structured vigorous intensity physical activities, they are less accurate for more prevalent lower intensity activities[3,63,85,116]. Unlike physical activity that are mostly structured and purposive, sedentary behaviors are ubiquitous and appear throughout a person's day. This characteristic significantly increases the cognitive load associated with recall of this type of behavior which ultimately leads to inaccurate reporting on questionnaires[49]. Current studies that evaluated existing sedentary behavior questionnaires showed similar pattern of accuracy and reliability as any other self-report measures for physical activity. A review of newly developed self-report measures of sedentary behaviors revealed a median validity coefficient (Spearman ρ) of 0.23 for sedentary behavior[58]. In addition, studies also showed that habitual domain-specific sedentary behaviors tend to have higher correlations with criterion measures than overall sedentary time (0.14-0.83 vs. 0.07-0.61)[49]. This pattern is mainly because of the high cognitive demands associated with reporting usual daily activities[84].

Several advancement in technology has led to devices that can objectively quantify sedentary behavior[79]. Accelerometers, which have been widely used to measure free-living physical activity, were the obvious choice. Traditionally, accelerometers have been used to measure physical activity by measuring the body's acceleration. The absence or a very low level of it is often considered an indication that the individual is engaging in a sedentary activity. However, although accelerometers provided a means for objective measurement of sedentary behavior, determining the appropriate cut point to distinguish sedentary behavior from higher intensity activities had been difficult. Results from previous studies that have been conducted to determine this optimal cut point vary and depended on multiple factors such as device placement (hip vs. wrist), demographics of target population (i.e., sex, age, BMI status), epoch length, the accelerometer parameter being used (vertical axis vs. vector magnitude), and even the context/domain[27] of sedentary behavior. Studies have suggested various cut points from a low 50 counts/minute depending on day of the week[29,57] to 200 counts/minute for overweight/obese adults[69,77]. In addition, a review of studies on these cut points suggest a vector magnitude of <200 counts/minute as cut point for sedentary behavior in older adults[57]. In adults, the most common cut point for sedentary behavior is 100 counts/minute. Several epidemiologic studies on sedentary behavior[19,55,97], specifically those that used the NHANES dataset, used the 100 counts/minute cut point for hip-worn accelerometers to derived associations between time spent in sedentary behavior and other key cardiometabolic parameters. However, a common shortcoming of using accelerometers in measuring sedentary behavior is that they can only measure body

acceleration and are unable to distinguish the posture at which an individual is engaging in (i.e., lying, sitting, or standing).

A novel device that has been popular for sedentary behavior researchers is the activPAL, a thigh-worn device that measures posture. The device has been shown to be both valid and reliable in measuring sedentary and physical activity[4,32,108]. In addition, the activPAL device has been shown to be valid in detecting sitting time with r^2= .94 and mean bias of -2.8% (95% CI = -4.7% to 0.9%) against direct observation[70]. Furthermore, the device was also validated to be accurate at classifying and estimating time spent at higher-intensity activities, mean bias= -2.6 (-5.8, 0.7) min, RMSE= 8.4, ICC= 0.98 (95% CI= 0.95 to 0.99)[79]. This is an important aspect considering that the latest consensus statement from the sedentary behavior research network defined sedentary behavior by both energy expenditure and posture[123]. Although limited evidence exists as to the benefits of standing, it still an important behavioral target that needs to be explored. Downsides are that these activity monitors can be costly, difficult to operate, and do not provide information on the context at which these behaviors are performed[117].

C. Determinants of Sedentary Behavior

Another important aspect of developing an efficient intervention to reduce sedentary behavior is studying the factors that lead to the behavior. Years of physical activity promotion research has clearly demonstrated which factors predict physical activity engagement. Unfortunately, these same factors are not predictive of sedentary behavior. In this section, we will discuss several key studies that investigated the different predictors of sedentary behavior at different levels of the socio-ecological model (see Figure 3).

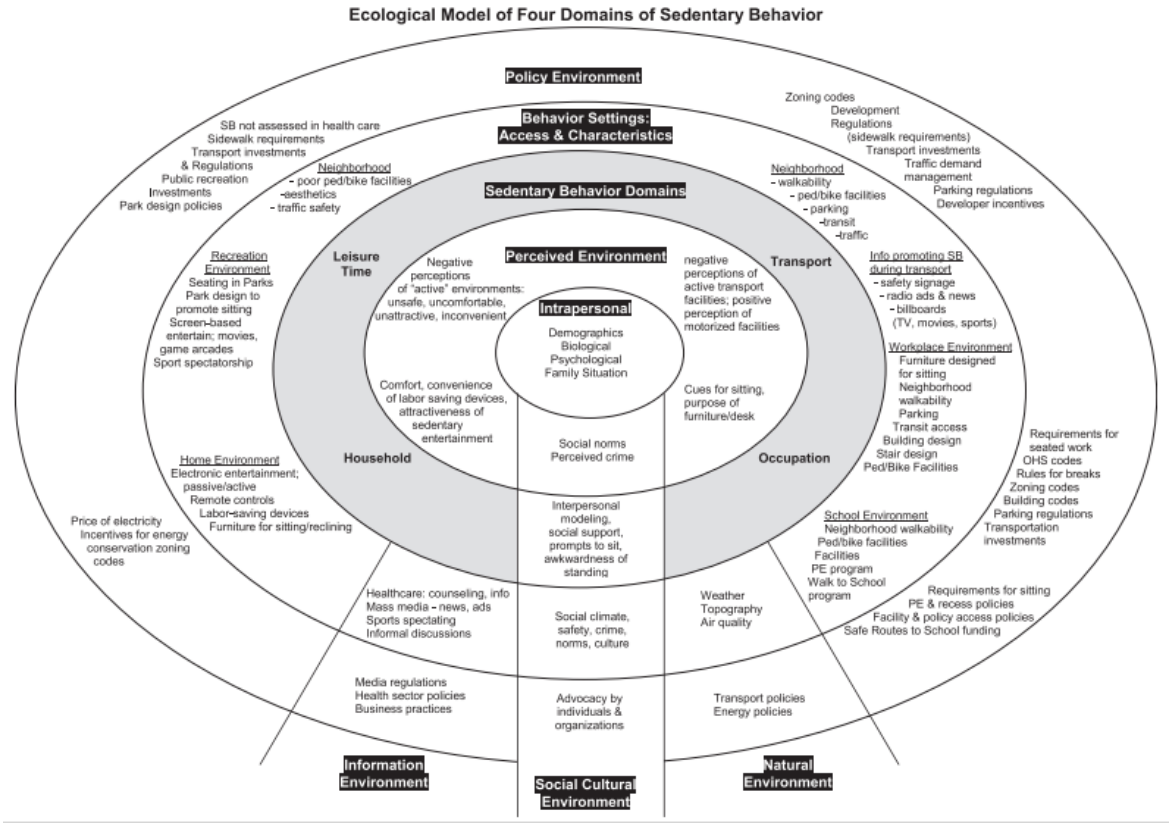

Figure 3. Socio-ecological model of four domains of sedentary behavior. Source: Adapted from Prince et al[103].

There are three[21,95,115] published systematic reviews that examined this topic and all are part of the Determinants of Diet and Physical Activity (DEDIPAC) joint action of

the European Joint Programming Initiative "A Healthy Diet for a Healthy Life"[72]. These reviews separately discussed predictors of sedentary behavior on youth[115], adults[95], and older adults[21].

Chastin et al[21] reviewed papers that investigated key determinants of sitting time in adults ages 18-65 that were published from the year 2000 to 2015. Their review included a total of 74 studies (sample ranges from 10 to 246,920 adults) in this topic: 71 observational studies, 2 qualitative, and 1 experimental. Most of these studies measured sedentary behavior through self-report (screen leisure time and total sitting time) and only 15 studies reported measuring sedentary behavior objectively. Their results indicated that individual level factors such as age, physical activity levels, body mass index, socio-economic status and mood were significantly associated with sedentary behavior. They also identified several environmental correlates such as proximity of green space, neighborhood walkability and safety, and weather.

Stierlin et al[115] reviewed 37 longitudinal, experimental and observational studies among youth (<18 years of age; participants ranged from toddlers to pre-adolescents) with sample size varying from 19 to 18,900 youths. All cross-sectional studies were excluded in the review. Overall, their results showed that sitting time tends to be positively associated with age, weight status and baseline screen time. In addition, they also found that a higher playground density and a higher availability of play and sports equipment at school were consistently related to a higher sedentary time. They also reported evidence as to the presence of safe places to crossroads and lengthening morning and lunch breaks as being associated with less total sitting time.

Lastly, the review by Chastin et al[21] focused on older adults and included 22 studies in their review with sample size ranging from nine in small qualitative study to 460,000 from a large cross-sectional study. Because of the limited studies on this population, almost all studies included were cross-sectional or observational in design. Like other reviews, their results showed strong evidence associating age with sitting time (i.e., older and retired elderly tend to sit more). In addition, employment status was also found to be predictive of sitting time were unemployed participants were more sedentary. Unfortunately, there is lack of studies that explored possible modifiable determinants of sedentary behavior in this population such as functional capacity, housing and transportation options, perceived safety, and determinants related to policy.

Overall, the current available evidence on studies that looked at the correlates of sedentary behavior is predominantly based on studies conducted in Europe, the United States, and Australia. Most of the studies in these reviews specifically looked at TV viewing, and not necessarily total sedentary time as their primary dependent variable and that most of them also relied on self-reported measure of sedentary behavior. Furthermore, the correlates explored in these studies were primarily those that are non-modifiable (e.g., sex, age, employment status, and socio-economic status). Thus, these reviews generally tell more about who engages in sedentary behavior but less so on why they engaged in it. Information on these non-modifiable factors is still important in terms of deciding the population that is most at risk. However, in order to inform future interventions, more insights on modifiable and dynamic correlates of sedentary behavior are needed.

D. Breaking Up Sedentary Time

Sedentary behavior research over the past couple of years have proliferated and made a significant contribution to our current understanding of sedentary behavior. In fact, the current physical activity guidelines now recommend reducing the amount of sitting time that a person engage in. A seminal paper by Healy[50] that cross-sectionally examined the associations between objectively-measured sedentary time with cardiometabolic risk markers in 168 participants from the 2004-2005 AusDiab study demonstrated that increased breaks in sedentary time is significantly associated with lower cardiometabolic risk profile, specifically waist circumference (β = −0.16, 95% CI −0.31 to −0.02), BMI (β = −0.19, −0.35 to −0.02), triglycerides (β = −0.18, −0.34 to −0.02), and 2-h plasma glucose (β = −0.18, −0.34 to −0.02). Another study by Evenson[40] provided some epidemiological evidence on the differential effect of different patterns of accumulating sedentary time on cardiometabolic risk. Using latent class analysis, the authors showed that participants who accumulated sitting time in longer bouts are more at risk for dying of all causes compared to those who accumulates their sedentary time by smaller bouts (adjusted hazard ratio= 2.10 95% CI= 1.11 to 3.97). All of these epidemiological evidences suggest that the pattern at which we accumulate our sedentary bouts can influence the risk of developing cardiometabolic disease.

A randomized controlled study in 19 overweight/obese adults (mean±SD age of 53.8±4.9 years, 42% female) was the first study that experimentally showed the benefits of breaking up sedentary time[33]. The results showed that breaking up sitting time every 20 minutes with 2 minutes of light (i.e., walking at 3.2 km/hour) or moderate (i.e., walking at 5.8-6.4 km/hour) intensity activity can significantly lower 5-hour glucose

iAUC [5.2 (4.1, 6.6) mmol/L and 4.9 (3.8, 6.1) mmol/L, respectively) compared to continuous sitting [6.9 (5.5, 8.7) mmol/L]. Previous studies that experimentally tested the effects of breaking up sedentary time on post-prandial glucose in various population is summarized in Appendix F. To summarize, results from these studies suggest that breaking up sitting time, regardless of modality (i.e., with LPA, MVPA, or resistance exercise) can significantly attenuate postprandial glucose compared with continuous sitting. Interestingly, most studies also show that the benefits of breaking up sedentary time on post-prandial glucose through LPA breaks and MVPA breaks were not statistically significant, suggesting that intensity of breaks have little consequence. On the other hand, most studies[7,28,48,106] that utilized standing as a medium to break up sedentary time did not significantly change postprandial glucose, as compared with uninterrupted sitting. Notably, only three studies[28,59,119] indicated a significance effect of breaking up sitting time through standing (5-30% reduction in glucose iAUC). However, this study particularly looked at individuals with impaired glucose metabolism, a more at-risk group as opposed to healthy individuals from other studies. Despite all of these accumulated evidences, there are still a lot to learn.

One area that has not been fully explored yet is the effect of different combinations of bouts and frequency of sitting time. Unfortunately, there is very little experimental evidence that look at this specific problem. Most experimental studies[7,28,31,33,47,48,59,89,106,119] that look at the effect of breaking up sedentary time have relied on using light physical activity or moderate-vigorous physical activity to break up sedentary time, thus it is still unclear whether the benefits of breaking sedentary time was due to the physical activity or reduction in sitting time. Although understanding which

modality can result in greater benefits in terms of improving glycemic control, it is equally valuable to understand how frequency and duration of breaks can impact this specific outcome. Gaining a full understanding on how different patterns of breaking up sedentary time can contribute to the development of efficient interventions (such as JITAI) to reduce sitting time especially in settings where individuals are limited to the type of activity that they can engage in (i.e., office employees). This study aimed to answer these questions by demonstrating the effect of using different patterns of breaking up sedentary time on post-prandial glucose.

To summarize, these studies have demonstrated the benefits of engaging in more active behaviors, and it seems like more active behaviors (LPA and MVPA) and not standing can significantly reduce the negative effects of sedentary behavior. It must be noted, however, that most of these studies have explored this problem in healthy populations. Considering that the body has a very complex method of maintaining homeostatis and the potentially small effect of sedentary behavior on cardiometabolic risk, it is imperative that we study these relationships in a more at-risk sample (pre-diabetic and diabetic). In addition, previous studies also focuses on the intensity of the breaks in sedentary time so they are also not able to provide any insights to whether different patterns of breaks (i.e., different combination of bouts) have significant effects on the detrimental effects of sedentary behavior. Thus, further studies should focus on the complex interactions between different patterns of accumulating sedentary time and on how it can negatively impact an individual's health.

E. Interventions to Reduce Sedentary Behavior

The increasing evidence from multiple observational and randomized controlled studies had demonstrated the negative effects of higher levels of sitting time. These studies show that sedentary behavior is a unique public health problem that should be targeted by lifestyle interventions. This section discusses the different studies that evaluated the efficacy of different interventions to reduce sitting time in both the workplace and outside the workplace setting.

Workplace Interventions. Since Americans spend about 70-80% of their work time sitting at their desk[87], the worksite has received considerable attention as a venue for interventions aiming to reduce sitting time. Over the past years, multiple worksite interventions have been evaluated in their efficacy in reducing sitting time in the workplace. The recently published work by Strestha et al[113] reviewed a total of 20 experimental studies that evaluated different worksite intervention strategies to reduce sitting time. Worksite interventions to reduce sitting time include physical workplace changes (e.g., sit-stand workstations and treadmill desks), policy changes (e.g., encouraging walking meetings), providing information and counselling, and combinations of these strategies.

Perhaps the most popular workplace intervention is the addition of the sit-stand workstation. However, initial studies that examined this intervention only provided low quality evidence as to the efficacy of this intervention[113]. Nevertheless, these past studies showed that sit-stand desks alone decreased workplace sitting with about half an hour to two hours per day. The study by Neuhaus et al[94] compared a sit-stand desk only with a sit-stand desk plus counselling and with no intervention. Healy et al[52] compared a sit-

stand desk plus counselling with no intervention. The pooled effect estimates of the three study arms showed a reduction of 52 minutes per eight-hour workday (95% CI -79 to -26) in sitting episodes lasting 30 minutes or more in the intervention group[113]. Analysis of the subgroup of sit-stand desks combined with counselling resulted in a mean reduction of 63 minutes per eight-hour workday (95% CI -93 to -33). In two studies counselling decreased sitting time with 28 minutes and in another study mindfulness training did not have any effect on sitting at work. There was no considerable increase in work engagement with counselling. Computer prompting software did not reduce sitting time in two studies. In another study computer prompts reduced sitting time with 55 minutes compared to no intervention. One study found that prompts to stand reduced sitting 14 minutes more than prompts to step. They also showed that computer prompts did not change the number of sitting episodes that last 30 minutes or longer. When multiple categories of interventions were combined to decrease sitting, there was reduction in workplace sitting time at 12 weeks' and six months' follow-up but there was no considerable difference between intervention and control group at 12 months' follow-up.

The recently published results of two cluster-randomized trials that evaluated the efficacy of combining these strategies reported a significant reduction in sedentary time[30,53]. Danquah et al[30], they tested if a multi-component work-based intervention can reduce prolonged sitting periods. The study involved four worksites with 19 offices and a total of 317 workers. Their intervention included managerial support, local worksite ambassadors, environmental changes, and information sessions through lectures and workshops. Their results showed that in their one and three month follow-up, their total

sitting time was 71 (p<0.001) and 48 (p<0.001) minutes lower per 8-hour workday in the intervention group compared to the control group. In addition, the study by Healy et al[53] also evaluated similar strategy on 14 office worksites (a total of 231 full-time participants). They found that workplace sitting time was significantly reduced in the intervention group compared with the control at 3 months (-99.1 [95% confidence interval = -116.3 to -81.8] min per 8-h workday) and 12 months (-45.4 [-64.6 to -26.2] min per 8-h workday). All of these evidence showed the effectiveness of combining multiple interventions into a multi-component intervention to reduce sitting behavior at work.

Non-Worksite Interventions. Most of the published studies that tested the efficacy of interventions at reducing sitting time have been mostly focused on the worksite[121]. To date, there are limited studies that actually evaluated different strategies primarily aimed at reducing sedentary time outside of work. Otten et al[96] in 2009 conducted a 3 week randomized controlled trial (n= 36; mean age 42.6±13.3, 69% females) where they utilized an electronic lock out system to reduce TV viewing time. Their results indicated a significant difference in daily sitting time between the intervention and control group, mean change (95% CI)= -3.8% (-6.3 to -1.3) vs 1.1% (-3.2 to 5.4), p< 0.04. Several studies have also utilized theory-based interventions such as self-monitoring tool and motivational calls to reduce sitting time. The randomized controlled trial involving 166 participants (mean age 52.0±14.1, 53% females) conducted by Aadahl et al[2] found no significant difference in objectively measured sedentary time between participants that received motivational counselling versus those that did not. Another similar study by Biddle et al[16] tested the efficacy of a combination of educational workshops, motivational

calls and a self-monitoring tool to reduce sitting time on 187 overweight adults (mean age 32.8±13.5, 83% females). Their results also showed no significant difference in sedentary time between the intervention and control group after 12 months of intervention. Another strategy that has been evaluated are the use of point-of-choice prompts. In a study by Lang et al[73], the effectiveness of point-of-choice prompts were tested for 819 conference attendees. The researchers randomly selected conference sessions at which they read a prompt to encourage standing to attendees at the beginning of the session. The number of participants who stood during the sessions were counted and compared to sessions where they did not read the prompt. Their results indicated that larger proportions of individuals in the intervention group stood during the session compared to those in the control group (17±2% vs 11±2%). Lastly, Kerr et al[66] evaluated the use of a combination of education, goal setting, and tools according to participant's preference (i.e., smartphone or PC app, timers, watches, haptic feedback, standing desks, etc) to reduce sitting time in 30 non-working adults (mean age 60.4±5.9, 73% females). Interestingly, their results indicated that participants randomized to the sitting time reduction group had a decrease (-130 min/day) in daily sitting time but no difference in sit-stand transitions. Additionally, those that were randomized to interventions to increase sit-stand transitions increased their sit-stand transitions (13 transitions/day) but did not change their total sedentary time. Overall, these studies provide preliminary evidence as to the efficacy of interventions designed to decrease overall sedentary time outside of work. However, the studies that explored the efficacy of these interventions are limited and of low quality. Sufficiently powered studies that evaluate the efficacy of each of these interventions, or a combination of these are needed.

CHAPTER 3

METHODOLOGY

A. Study Participants and Recruitment

The target participants were sedentary office employees with impaired fasting glucose level. Participants were recruited through a study flyer (appendix A) via various recruitment channels (i.e., ASU faculty website, social media, word of mouth). The study flyer contained a link to a Qualtrics survey to pre-screen (appendix B) interested participants. Inclusion criteria were: (i) ages 35-65 years, (ii) sedentary work habits, (iii) presence of impaired fasting glucose (fasting glucose level of 100-125 mg/dL), (iv) willing to engage in three 7.5 hour lab visit, (v) willing to wear the activPAL and continuous glucose monitor, (vii) current sit-stand workstation owner, and (viii) BMI 25-45 kg/m^2. Participants were excluded when they had at least one of the following: (i) chronic mobility limitations, such as moderate-to-severe arthritis and (ii) psychiatric disorders, (iii) cardiometabolic abnormality, (iv) food allergy/restriction, or (v) BMI>45 kg/m2. All eligible participants were scheduled for a 30-minute screening visit to assess fasting glucose level. A total of 15 sedentary and inactive employees (indicated by >6 hrs of workplace sedentary time assessed by the activPAL device during a one-week screening period) were enrolled to participate in a fully randomized crossover trial. All study procedures were approved by the institutional review board and written consent was obtained from each participant prior to participation.

B. Study Design

The study design was a crossover randomized trial with three conditions: (i) uninterrupted sitting, (ii) high-frequency and low duration (HFLD) standing breaks, and (iii) low-frequency and high duration (LFHD) standing breaks. In the interrupted sitting conditions (i.e., HFLD and LFHD), total sitting and standing time were designed to be equal in both groups (see table 2). The only difference between the two conditions was on the pattern to accumulate sitting time. A common threshold for prolonged sitting time in epidemiological studies of sedentary behavior is 30 minutes of continuous sitting[25,53,54,97]. In the HFLD condition, we used half of this threshold and asked participants to interrupt their sitting time every 15 minutes using a 2.5-minute standing break. In contrast, participants performed twice this threshold in the LFHD standing breaks where they completed a 10-minute standing break every hour of sitting.

All possible sequence of condition were determined and organized into blocks (a total of six blocks). Each eligible participant was randomly assigned to a block to determine the sequence that they would perform the conditions. Randomization process involved a separate research staff preparing 40 sealed enveloped that contained a block number randomly determined using a computer-generated random sequence. These envelopes were then kept in a secure cabinet by another research staff not directly involved in the project. Each participant was blinded to the condition that they were to perform during the visit until after their first standardized meal.

Participants were invited to three 450-minute (7.5-hour) laboratory visits where they were provided with a private room, a sit-stand workstation, and a desktop computer. During each laboratory visit, participants performed their usual desk-based work

activities. All visits were scheduled one week apart. Participants were instructed to fast overnight, and standardized breakfast and lunch meals were provided to control for any dietary effects on glucose level. In addition, each participant was instructed to avoid any moderate-vigorous physical activities for at least two days and smoking cigarettes and consuming alcoholic beverages at least three days prior to each visit. Upon arriving to the lab for their first visit, participants completed a dietary log of their last meal the previous day. They were then instructed to replicate this meal the night prior to each visit.

C. Study Protocol

All eligible and consented participants were subjected to all three study conditions. A full description of the study conditions is summarized in Table 1 and Figure 4. After consent was acquired, participants were scheduled for the first lab visit. All three lab visits were scheduled on the same day of the week for each participant and occurred on a typical work week. A day before the first visit, the participants were invited for a 30-minute lab visit to insert the CGM device and attach the activPAL device. The CGM sensor needs to be attached for at least 12 hours to ensure accurate glucose reading. During the actual visit, participants were instructed to fast for at least 10 hours. An initial CGM reading is performed to ensure that the CGM sensor was accurately collecting glucose data. A standardized breakfast meal was then provided and participants were instructed to consume the meal within a 15-minute period. Following breakfast, participants were asked to perform their usual desk-based work activities. Participants were to sit or stand still and avoid any light movement (e.g., swaying, fidgeting, or squatting). They were instructed on how to use a sit-stand workstation. Depending on their visit day, participants were prompted when to sit/stand-up using a smartphone

(using a slide presentation with a timer). A 15-min break was provided to the participants after the 210-minute mark where they consumed their standardized lunch meal. They were also allowed to use the restroom during this period. All moving activities were limited during the testing period. Start and end time of each visit and meal periods were recorded. At the end of each visit, participants were asked to record the time and details of their meals for the next three days using a paper log.

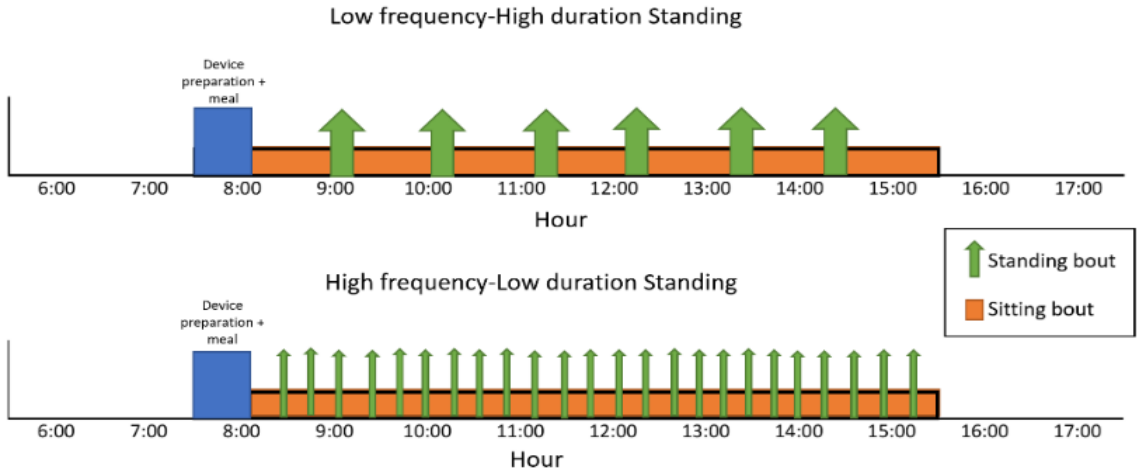

Figure 4. Laboratory visit protocol for low-frequency, high-duration vs. high-frequency, low-duration standing break conditions.

Table 1. Experimental conditions.

	CS	HFLD	LFHD
Total sitting time (min)	420	360	360
Total sitting bouts	1	24	6
Sitting bout duration (min)	420	15	60
Total standing time (min)	0	60	60
Total standing bouts	0	24	6
Standing bout duration (min)	0	2.5	10

CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition.

D. Outcome Measures

Continuous Glucose Monitors. A day prior to the first visit, participants were fitted with a Freestyle Libre Pro (Abbott Laboratories, Chicago, IL) continuous glucose monitor by a trained researcher. The sensor (Figure 5) was attached to the back part of the participant's non-dominant arm and programmed to measure interstitial glucose at 15-minute intervals. The device is designed to be worn continuously for 14 days and is waterproof, lightweight (roughly the size of a quarter) and minimally obtrusive. The sensors were attached using proper aseptic procedures and in accordance with the manufacturer's instructions (see appendix C). Lastly, each participant was given instructions on how to care for the device (see appendix D).

Figure 5. Freestyle Libre Pro continuous glucose monitoring system.

At the end of the last visit, data from each sensor were acquired using the Libre Pro reader and uploaded to an online patient repository (LibreView). Data were then

processed, and 15-minute epoch data were downloaded into a local secure drive for further processing. Continuous glucose data corresponding to each visit date and time were isolated using the paper logs and inspected for completeness and quality of data. Files with less than 80% of valid observation were excluded from the succeeding analyses. Glucose incremental area under the curve (iAUC) was calculated using the trapezoidal method along with other metrics for variability (i.e., MAGE: mean amplitude of glycemic excursions).

activPAL Device. Objective measures of sitting, standing and moving time were derived from the activPAL micro accelerometer worn on the midline of the right thigh. Participants wore the device on two occasions: (i) for 7 consecutive days during the baseline period and (ii) for 14 consecutive days during the study. The validity and reliability of the activPAL in measuring sedentary and physical activity behaviors has been previously reported [4,32,108]. Collected data during were processed into events of sitting, standing, or moving (i.e., stepping) using the activPAL software version 7.2.32 (PAL Technologies Ltd, Scotland, UK).

For baseline data, sleep intervals were self-reported using an electronic daily log. The consensus definition of sedentary behavior as seated/lying positions with low energy expenditure was used for this study[111]; therefore, all wake time measured by the activPAL as lying/seated was considered sedentary. The remaining wake time periods were then classified as either standing or moving events by the activPAL device. All sitting, standing, and moving behaviors were summed to obtain total time spent in that respective activity and expressed in minutes/day. Times excluded from analysis included 1) continuous sittings or standing behavior in excess of six hours as indicated by the

activPAL, as these were considered non-wear time; 2) all days with ≤10 hours of valid wear time; and 3) participants with only one valid day of activPAL wear.

Furthermore, data specific to each visit were isolated using the paper logs to correspond with the glucose data. All observation measured by the activPAL as lying/seated was considered sedentary. The remaining observations were then classified as either standing or moving events by the activPAL device. All sitting, standing, and moving behaviors were summed to obtain total time spent in that respective activity and expressed in total minutes for that visit.

Standard Meals. Standardized meals (breakfast and lunch) were provided in each lab visit to control for any dietary influence. Each meal was designed to provide 33% of the participant's total daily caloric needs following a typical American diet (50-60% carbohydrates, 25-35% fat, and 10-20% protein). Basal metabolic rate was calculated using Schofield's equation using a 1.5 activity factor. After calculating the required caloric content of each meal, a meal with the closest caloric value was chosen from a list of meal plans (see appendix F). A typical breakfast was composed of a croissant, ham, cheddar cheese, cereals with milk, fruit cup, and orange juice while lunch items consisted of a ciabatta ham and cheese sandwich and orange juice. The same meal was provided during all follow-up visits.

E. Statistical Analysis

Participant characteristics were described through frequencies and means (SD). Outcome variables with non-normal distributions were transformed to assume a normal distribution. All data processing and statistical analysis will be performed in SAS (SAS v9.4, Cary, NC) using an alpha of 0.05.

To estimate the sample size needed, we based our effect size estimates from previous studies[33,59] that evaluated the effect of interrupting prolonged sitting on glucose iAUC. These studies reported a 20-30% decrease in post-prandial glucose iAUC level in the interventions group. In this study, we compared two interventions that utilized similar modalities, so we used a conservative estimate of 15% difference between the two intervention conditions and the all-day siting condition with a 1% population estimate of standard deviation. Using G*Power software (v3.1.9.2) we estimated a required sample of 12 participants allowing for 0.5 correlation coefficients between repeated measurements and an alpha of 0.05 to obtain a power of 80%. Considering a 20% attrition rate, we planned to recruit a total of 15 participants.

To address our specific aims and hypotheses, we utilized a linear mixed model analyses[88,112] with experimental conditions, sequence (order of conditions performed), and time period as fixed factors and an unstructured covariance structure for the three repeated measurements per person. Incremental area under the curve (iAUC), mean glucose and MAGE on post-prandial periods were evaluated as outcomes. The HFLD and LFHD conditions were jointly compared to the all-day sitting conditions to address specific aim 1. To answer specific aim 2, data from uninterrupted sitting group were ignored and comparison between HFLD and LFHD were conducted. All data from randomized participants were included in the analysis in accordance to the intent-to-treat principles.

CHAPTER 4

RESULTS

A. Recruitment and Baseline Characteristics

Figure 6 illustrates the shortened version of the consort diagram to highlight the study recruitment and data collection flow. Please refer to Appendix G for the full version of the consort diagram. A total of 57 participants were invited to the laboratory for fasting blood glucose screening and 15 participants consented to participate in the study. Four participants were then excluded from the study due to unresponsiveness (2) or not being interested in participating. Overall, 10 participants completed the entire study protocol and were included in the analysis. The baseline participant characteristics are summarized at Table 2. The participants were mainly middle-aged adults. They were highly sedentary with an average of 626.9±135.7 minutes/day of sedentary time.

Table 2. Participant characteristics (N= 10).

Demographic variable	Mean±SD or Percentage (n/total)
Age	46.8±10.6
Sex	
Male	30.0% (3/10)
Female	70.0%(7/10)
BMI (kg/m^2)	34.6±5.4
Fasting glucose (mg/dL)	109.0±9.8
Sedentary (min/day)	626.9±135.7
Standing (min/day)	213.8±97.8
Stepping (min/day)	89.6±44.4

BMI: Body mass index. Fasting glucose were measured via finger-stick method after 10 hours of fasting. Sedentary, standing and stepping time were objectively measure by the activPAL.

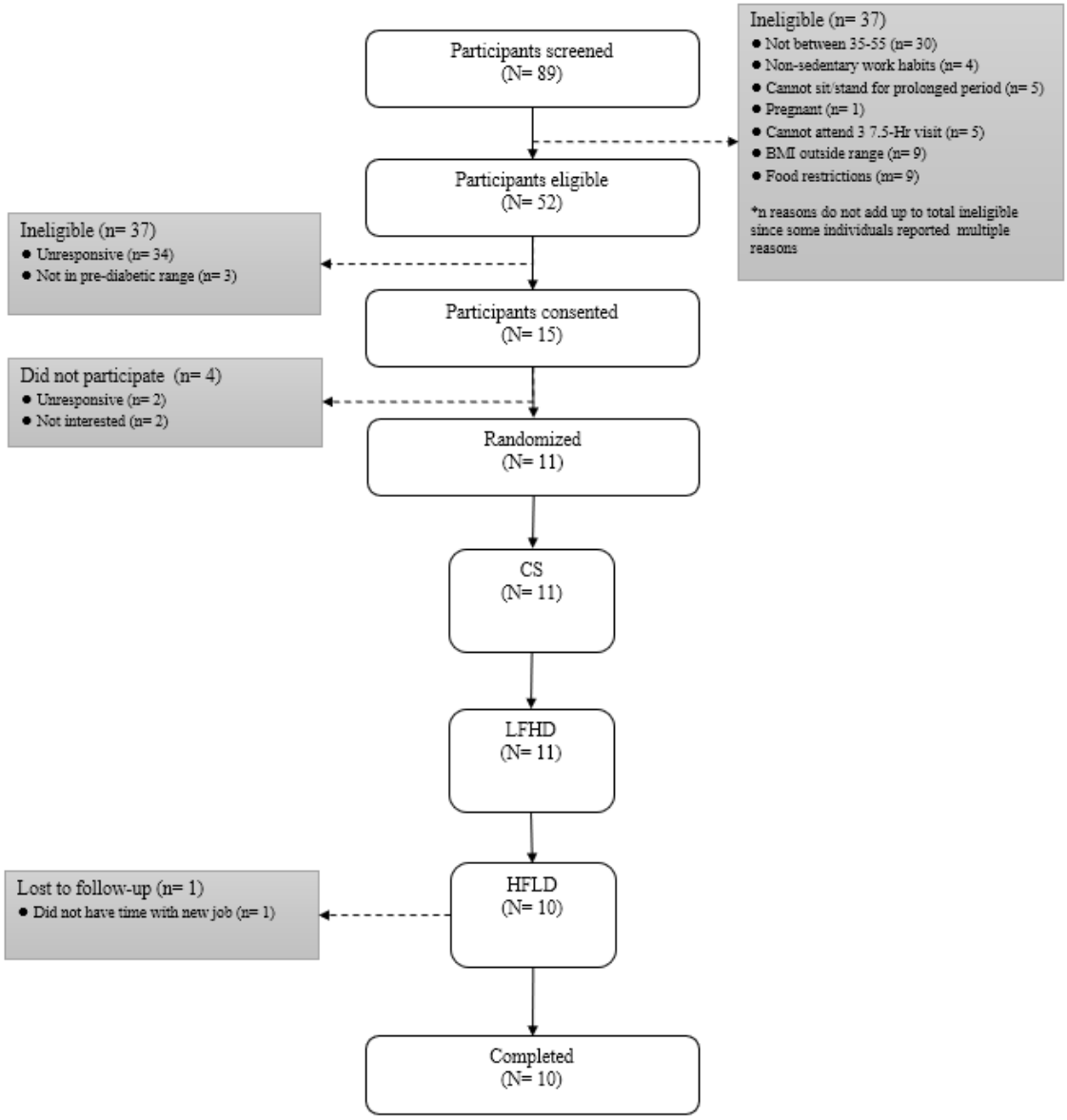

Figure 6. Consort diagram. CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition. This is a shortened version of the consort diagram to clearly illustrate completion rate at each study condition. A full version of the consort can be viewed at Appendix G.

B. Physical Activity

To evaluate how the study sit-stand protocol affects the participant's usual activity level, the weekly objectively measured behavior data are summarized in Table 4. Overall, the participants physical activity behavior did not significantly vary across the three-week period. In addition, their physical activity level during the visit days were similar to their usual physical activity pattern. The participants' objectively measured physical activity data during each visit are summarized in Table 3. Overall, participants complied with the study protocol for all conditions. In the continuous sitting (CS) condition, participants accumulated their total sitting time in a total of 2.7±1.1 bouts of sitting with very minimal standing. As expected, participants performed about 60 minutes of standing and 360 minutes of sitting in both HFLD and LFHD conditions. The only difference between the two groups was on the manner in which they accumulated sitting time. In the HFLD condition, participants performed 26.0±1.4 short bouts of sitting (less than 60 minutes per bout). In contrast, most sedentary bouts in the LFHD condition were accrued through longer bouts of sitting (averaging a total of 5.7±2.4 bouts). These results suggest excellent compliance to the study's protocol.

Table 3. Objectively-measured sedentary and standing bout duration during visits.

	CS	HFLD	LFHD
Total sitting time (min)	416.5±55.7	356.6±93.9	351.1±90.3
Total sitting bouts	3.9±1.6	26.6±1.7	7.9±1.6
Total standing time (min)	3.6±6.7	62.9±4.5	68.9±2.3
Prolonged sitting (≥60 mins) bouts	2.7±1.1	0.4±0.6	5.7±2.4
Short sitting (<60 mins) bouts	1.1±1.4	26.0±1.4	2.5±1.1

CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition.

Table 4. Objectively-Measured Sedentary and Physical Activity Behavior.

	CS			HFLD			LFHD		
	Week	Visit	Mdiff	Week	Visit	Mdiff	Week	Visit	Mdiff
Total sitting time (min)	642.69±144.47	720.54±131.97	7.1±139.03	625.37±155.51	588.22±216.99	-67.18±200.01	694.7±111.12	704.2±146.8	-11.56±123.27
Total sitting bouts	43.39±17.06	35.67±17.47	-3.05±22.78	48.19±16.82	43.71±21.08	4.93±23.2	42.82±16.94	42.9±31.11	-5.92±30.19
Total standing time (min)	236.02±115.65	181.66±94.94	-8.81±97.05	231.63±113.36	282.34±224.29	49.33±182.37	188.57±87.56	197.07±129.54	5.16±99.11
Prolonged sitting (≥60 mins) bouts	5.67±2.76	5.67±2.67	0.85±3.27	5.67±2.98	4.93±3.6	-0.06±5.75	6.75±2.38	6.6±3.6	-1.25±3.28
Short sitting (<60 mins) bouts	37.56±17.94	29.83±17.32	-3.8±22.91	42.37±17.44	38.86±21.24	4.91±24.23	35.86±17.29	36.1±31.81	-4.37±32.13
Total LPA time (min)	61.8±31.67	41.92±39.3	3.76±58.01	78.21±45.04	69.21±48.92	6.68±58.58	58.86±27.98	48.05±24.45	2.23±31.93
Total MVPA time (min)	19.49±11.55	15.87±11.47	-2.06±12.31	24.79±15.89	20.23±17.94	11.16±18.56	17.87±11.44	10.68±8.34	4.17±11.49

CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition. Values are standardized to a 16-hour wakeday except for the total number of sitting, short sitting and prolonged sitting bouts. Weekly column is the daily average of all days prior to the visit, excluding the data from the actual visit day. Visit column is the total physical activity pattern for the specific visit day including the time spent in the lab. Mdiff is the mean of the differences between the weekly averages of the physical activity pattern and the daily activity pattern of the visit day (i.e., Weekly-Visit).

Table 5. Macronutrient content of standardized meals.

	Breakfast	Lunch	Total
Total energy (Kcal)	857.82±97.94	859.09±86.33	1716.91±0.69
Carbohydrates (%)	52.91±0.67	54.55±1.08	53.73±0.69
Protein (%)	14.00±0.74	13.64±0.88	13.82±0.32
Fat (%)	33.09±0.9	31.82±0.57	32.45±0.58

CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition.
Standardized meals were calculated to provide 33% of an individual's total daily caloric needs using Schofield's equation set at 1.5 activity factor.

C. Standardized Meals

The macronutrient content of the standardized meals is summarized in Table 5. In terms of compliance to the standardized meals, fasting states were confirmed before the start of each visit. All participants consumed their standardized meals within 15-20 minutes. Eighty percent of the participants (8/10) were able to consume 100% of the provided meals. For the two participants that were not able to consume the entirety of their meals during the first visit, the meals on their succeeding visits were adjusted to match what they were able to consume in the first visit.

D. Interrupting Sedentary Time via Standing Breaks

Table 6 and figure 7 summarizes the primary results of this study. Compared to the CS condition, conditions where sedentary time was interrupted with a standing break consistently had lower, although non-significant, post-prandial glucose iAUC during breakfast, lunch and total visit time with mean iAUC differences (95% CI) of -597.15 (-2878.33, 1694.03) mg/dL·3.5h, -210.86 (-3118.9, 2697.18) mg/dL·3.5h and -829.00 (-6001.52, 4343.60) mg/dL·7h, respectively. The mean post-prandial glucose were also consistently lower on both conditions where sedentary time were interrupted with standing breaks with mean glucose differences of -3.47 (-8.53, 1.59) mg/dL, -2.61 (-6.32, 1.10) mg/dL and -2.7 (-5.90, 0.47) mg/dL for breakfast, lunch and total time. Similarly, MAGE was also lower for the interrupted sitting conditions. Overall, Cohen's d effect sizes between the CS condition and the interrupted sitting conditions ranges from 0.05 to 0.16.

Comparing the mean post-prandial glucose responses for each condition reveals similar responses for the CS and LFHD condition (see Table 6 and Figure 8), which

ultimately dampen the results of the prior analyses. For example, the mean post-prandial glucose iAUC for total time were similar for both the CS and LFHD conditions with iAUC (mean±SD) of 10638.4±7443.07 mg/dL·7h and 10436.46±7208.7 mg/dL·7h, respectively. To further examine this relationship, a mixed-model with a post-hoc test where each interrupted sitting condition was compared to the CS condition (i.e., HFLD vs CS and LFHD vs CS) was performed using Bonferroni adjustment (Table 7). The results indicated that the mean post-prandial glucose of the HFLD condition were significantly lower ($p < .01$) than the CS condition with a mean difference of -7.70 (-11.98, -3.42) mg/dL and -5.76 (-9.50, -2.03) mg/dL for lunch and total time, respectively.

E. Frequency and Duration of Standing Breaks

Figure 8 illustrate the comparison of the post-prandial glucose responses of two different strategies to interrupt sitting time. The comparison of the two conditions (i.e., HFLD vs. LFHD) revealed small to medium effect size (Cohen's d ranged from 0.02 to 0.42) with the largest effect size occurring during lunch period. The result revealed similar post-prandial iAUC during breakfast with a mean difference of 232.51 (-3400.90, 3865.92) mg/dL·3.5h. However, iAUC during the lunch and total time were consistently lower during in the HFLD condition with mean difference of -1838.05 (-5922.86, 2246.77) mg/dL·3.5h and -1419.42 (-8703.33, 5864.49) mg/dL·7h, respectively. Analysis of the mean post-prandial glucose revealed similar post-prandial glucose levels during breakfast. Mean post-prandial glucose during lunch and total time were significantly lower in the HFLD condition compared to the LFHD condition with mean difference of -9.94 (-14.13, -5.74) mg/dL·3.5h and -6.23 (-9.93, -2.52) mg/dL·7h, respectively. Glucose variability did not differ between the two conditions.

Table 6. CGM-derived post-prandial glucose outcomes during each experimental condition.

	CS	IS[a]	HFLD	LFHD	CS-IS[b]	Cohen's d[b]	HFLD-LFHD[c]	Cohen's d[c]
iAUC								
Breakfast	5414.17±4119.94	4833.96±3496.17	4913.75±3995.09	4754.17±2913.02	-597.15 (-2878.33, 1684.03)	0.16	232.51 (-3400.90, 3865.92)	0.05
Lunch	5097.5±3698.25	4871.95±3633.79	4113.28±2646.19	5630.63±4405.3	-210.86 (-3118.9, 2697.18)	0.07	-1838.05 (-5922.86, 2246.77)	0.42
Total	10638.4±7443.07	9806.82±6559.51	9177.19±5838.59	10436.46±7208.7	-829.00 (-6001.52, 4343.60)	0.11	-1419.42 (-8703.33, 5864.49)	0.19
Mean glucose								
Breakfast	115.87±43.04	112.62±36.77	111.18±40.93	114.06±32.08	-3.47 (-8.53, 1.59)	0.08	-1.86 (-7.85, 4.13)	0.08
Lunch	111.29±34.67	109.54±26.53	105.03±25.16	114.06±27.85	-2.61 (-6.32, 1.10)	0.05	**-9.94 (-14.13, -5.74)**	0.34
Total	113.47±38.88	111±31.79	107.94±33.64	114.06±29.84	-2.7 (-5.90, 0.47)	0.06	**-6.23 (-9.93, -2.52)**	0.19
Variability								
MAGE	57.36±31.06	53.62±27.6	53.86±34.16	53.39±18.9	-3.82 (-19.03, 11.39)	0.16	2.83 (-23.93, 29.60)	0.02

CS: Continuous sitting condition. IS: interrupted sitting conditions. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition. iAUC: Incremental area under the curve. MAGE: Mean amplitude glucose excursions.

Mean glucose and iAUC were over 3.5-hour period for breakfast and lunch and 7-hour period for total time. MAGE was calculated over the entire 7-hour study visit.

CS, IS, HFLD and LFHD presented as means±SD.

Bolded results are significant at $p<.05$.

[a]IS is the mean of both HFLD and LFHD conditions.

[b]HFLD and LFHD conditons vs. continuous sitting condition as mean(95% CI).

[c]HFLD condition vs. LFHD condition as mean(95% CI).

Table 7. Post-prandial glucose comparison between continuous sitting and interrupted sitting conditions.

	CS-HFLD[a]	Cohen's d[a]	CS-LFHD[b]	Cohen's d[b]
iAUC				
Breakfast	-531.53 (-3349.52, 2286.46)	0.12	-648.65 (-3361.47, 2064.17)	0.18
Lunch	-1187.05 (-4582.82, 2208.71)	0.31	624.00 (-2652.86, 3901.65)	0.13
Total	-1663.38 (-7951.06, 4624.31)	0.22	-95.131 (-6163.28, 5973.03)	0.03
Mean glucose				
Breakfast	-4.18 (-10.13, 1.77)	0.11	-2.79 (-8.69, 3.11)	0.05
Lunch	**-7.70 (-11.98, -3.42)**	0.21	2.16 (-2.05, 6.36)	0.09
Total	**-5.76 (-9.50, -2.03)**	0.15	0.19 (-3.49, 3.87)	0.02
Variability				
MAGE	-3.50 (-22.30, 15.30)	0.11	-4.07 (-22.15, 14.00)	0.15

CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition. iAUC: Incremental area under the curve. MAGE: Mean amplitude glucose excursions.

Mean glucose and iAUC were over 3.5-hour period for breakfast and lunch and 7-hour period for total time. MAGE was calculated over the entire 7-hour study visit.

CS, IS, HFLD and LFHD presented as means±SD.

Bolded results are significant at $p < .001$.

[a]CS conditon - HFLD condition presented as mean difference (95% CI).

[b]CS condition - LFHD condition presented as mean difference (95% CI).

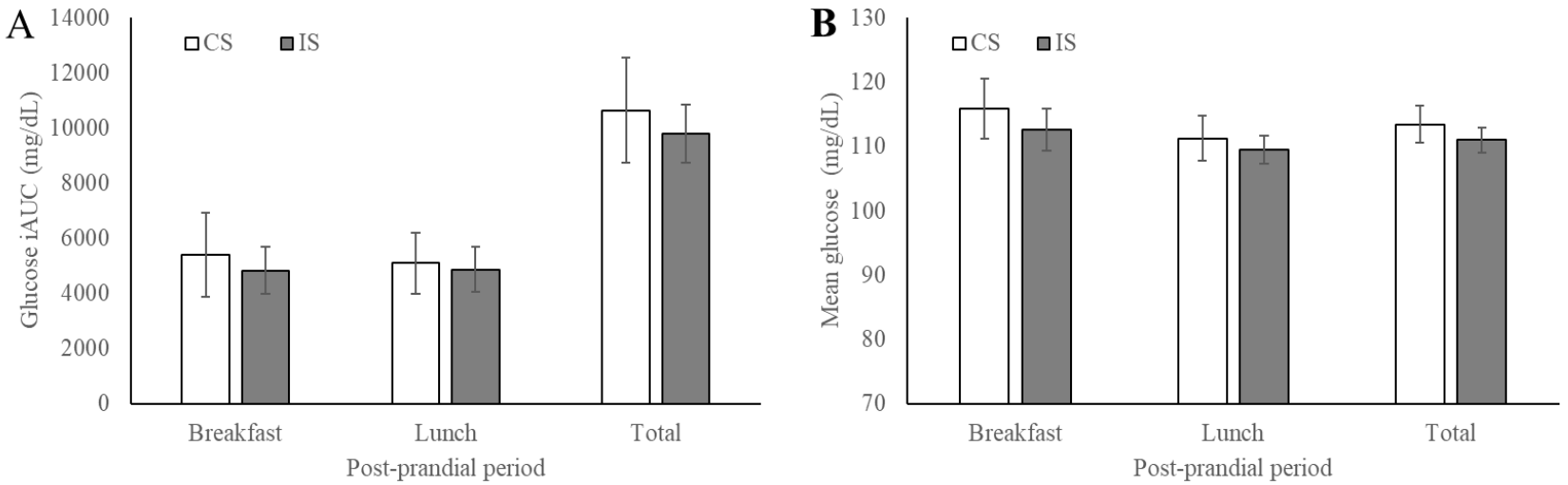

Figure 7. Post-prandial glucose response of continuous sitting vs. interrupted sitting conditions. Figure 7A for glucose incremental area under the curve (iAUC) and 7B for mean glucose. SIT: continuous sitting condition. IS: interrupted sitting conditions. Glucose iAUC were over 3.5-hour period for breakfast and lunch and 7-hour period for total time.

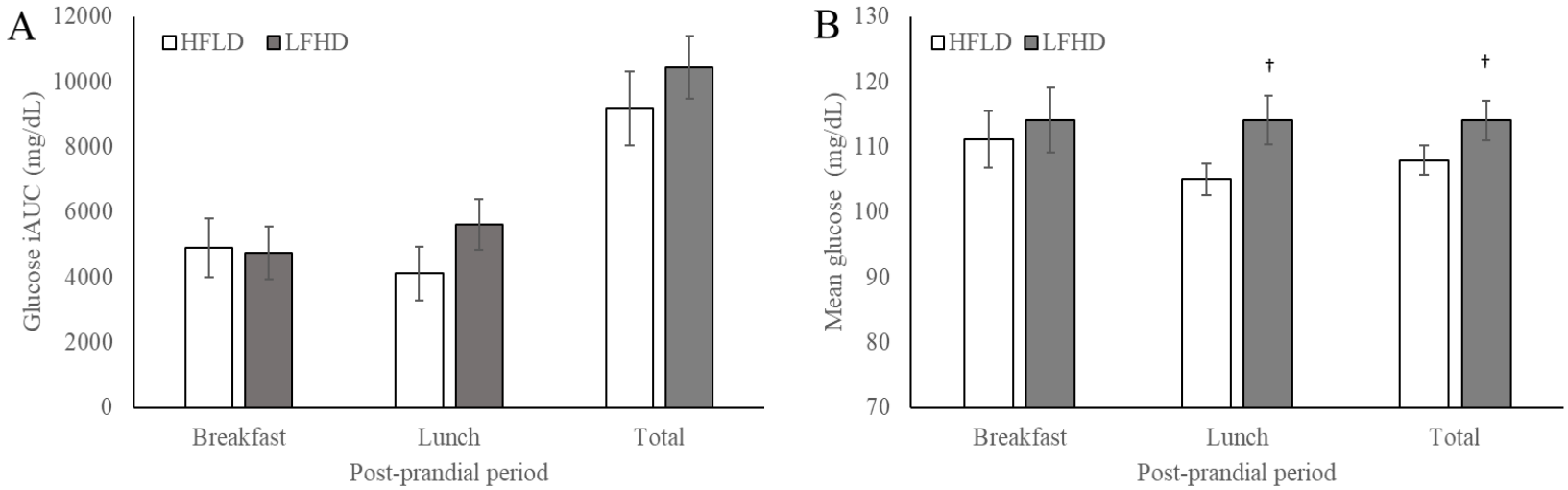

Figure 8. Post-prandial glucose response of continuous sitting vs. interrupted sitting conditions. Figure 8A for glucose incremental area under the curve (iAUC) and 8B for mean glucose. SIT: continuous sitting condition. IS: interrupted sitting conditions. Glucose iAUC were over 3.5-hour period for breakfast and lunch and 7-hour period for total time.

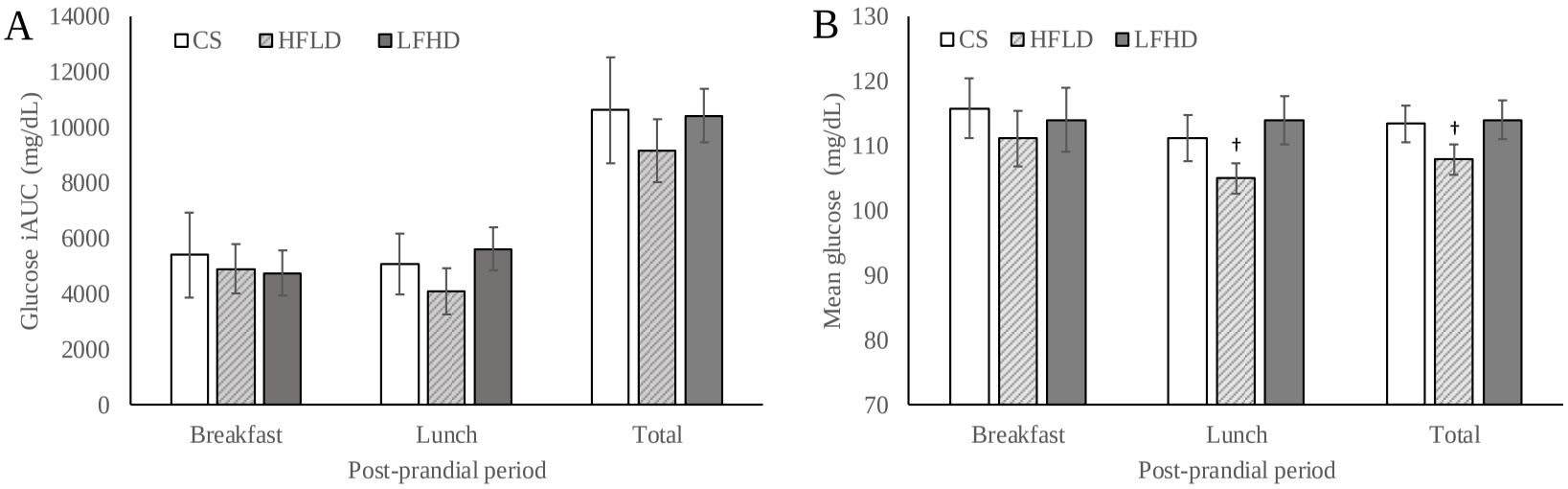

Figure 9. Post-prandial glucose response of all study conditions. Figure 9A for glucose incremental area under the curve (iAUC) and 9B for mean glucose. Values are marginal means with 95% confidence interval. CS: continuous sitting. HFLD: High frequency, low duration condition. LFHD: Low duration, high frequency condtion. Glucose iAUC were over 3.5-hour period for breakfast and lunch and 7-hour period for total time. †Significant against the CS condition at $p < .05$.

CHAPTER 5

SUMMARY AND DISCUSSION

The primary aim of this study was to evaluate the effectiveness of different strategies to interrupt sitting time through standing. High-frequency, low duration and low-frequency, high duration bouts of standing breaks were tested against continuous sitting in their potential to improve the acute post-prandial glucose response of a sample of office employees with impaired fasting glucose. Total sitting and standing time were equal for both interrupted sitting conditions and differed only in the frequency and duration of each sitting bouts. By doing this, any benefits of engaging in physical activity on post-prandial glucose were minimized to focus solely on the benefits of interrupting sitting time with standing. The results indicated that interrupting prolonged sitting time with 2.5-minute bouts of standing every 15 minutes of sitting can improve post-prandial glucose response. However, this improvement did not occur when sitting time was accumulated in 60-minute bouts with 10-minute standing breaks in between even though total sitting and standing time were equal for both conditions.

Currently, there have been multiple studies that demonstrate the detrimental effects of sedentary behavior to health. A number of epidemiological studies have documented that exposure to higher levels of sedentary time is associated with increased risk for cardiometabolic diseases and mortality, even after controlling for the amount of physical activity[37,51,86,97]. These studies were also augmented by a growing number of highly controlled experimental studies that interrupted prolonged sitting through various modalities. A review that evaluated the benefits of interrupting sitting time reported

strong evidence on the benefits of reducing sitting time through light physical activity and moderate-vigorous physical activity but had equivocal results when evaluating the effectiveness of standing as a modality for interrupting sedentary time[22]. Indeed, some (i.e., Crespo et. al., 2016[28], Henson et. al., 2016[59], and Thorp et. al., 2014[119]) but not all studies (i.e., Bailey et. al., 2015[7], Hawari et. al., 2016[48], and Pulsford et. al., 2016[106]) have shown that interrupting sedentary time with standing could have significant (5-30%) improvement in post-prandial glucose response compared to continuous sitting. Unfortunately, most of these studies have focused on understanding the effects of using different modalities (e.g., standing, light physical activity, moderate-vigorous physical activity, and squats) in breaking up sedentary time but very little on the potential effect of using activity breaks of varying frequency and bout length. Although understanding which modality can result in greater benefits in terms of improving glycemic control, it is equally valuable to understand how frequency and duration of breaks can impact this specific outcome. Gaining a full understanding on how different patterns of breaking up sedentary time can shed light to potential mechanisms on how sedentary behavior can be detrimental to health and contribute to the development of efficient interventions to reduce sitting time especially in settings where individuals are limited to the type of activity in which they can engage (e.g., office employees).

This study demonstrated that different patterns of interrupting sitting time can differentially impact post-prandial glucose. Specifically, the results indicated that using frequent, although shorter bouts of standing to interrupt prolonged sitting resulted in better post-prandial glycemic response (5-8% lower mean glucose) compared to engaging in higher duration but less frequent bouts of standing breaks or continuous sitting. These

results are in accordance with previous meta-analysis on interrupting sitting time where authors found that frequent interruptions of sedentary time through light physical activity or moderate-vigorous physical activity was effective in reducing post-prandial glucose[22]. In addition, a similar study in a sample of prediabetic women where they evaluated the effect of interrupting prolonged sitting through frequent standing breaks (5-minute standing breaks every 30 minutes of sitting) also showed improvements in post-prandial glucose[59]. It should be noted that their total sitting and standing time were comparable to this study (i.e., 420 and 60 minutes, respectively) and that their standing breaks protocol was midway between the HFLD (2.5-minute standing breaks every 15 minutes of sitting) and LFHD (10-minute standing breaks every 60 minutes of sitting) conditions used in this study. This suggests that accumulating sedentary time through bouts that are more than 30 minutes, a common threshold for prolonged sitting, can have a significant negative impact on the post-prandial glucose response of dysglycemic individuals. However, two other studies that showed improvement in post-prandial glucose response using standing breaks utilized a protocol allowing for accumulation of sedentary time through bouts longer than 60 minutes[28,119]. This may be due to the fact that their study protocol elicited significantly longer duration standing breaks (up to 30 minutes every hour of sitting) resulting in longer total standing time (150-240 minutes of standing time). This suggest that accumulating a certain amount of standing or a reduction of total sitting time to a certain level could also lead to significant improvement in this outcome. Unfortunately, this study was not designed to determine this threshold nor was it designed to determine any dose-response relationship between the number and duration

of sedentary breaks and post-prandial glucose response. Thus, these questions should be investigated in future studies.

To date, the underlying physiological mechanisms driving the benefits of reducing sedentary time is not well understood. The current prevailing theory that explains how sedentary behavior negatively impact health comes from the inactivity physiology theory[44–46]. The inactivity theory hypothesized that engaging in prolonged sedentary behaviors can lead to unique metabolic effects that are deleterious to the body's biochemical processes (e.g., reduction in plasma high density liproprotein and local liproprotein lipase activity). However, the concept of "breaking up" sedentary time itself is complex and this theory does not completely capture dynamic interactions between varying type, frequency and duration of activity breaks and their combinations[22]. Previous studies indicated that frequent interruptions in sitting time can lead to upregulation of the contraction-mediated glucose uptake pathway[14,75]. However, these studies mainly interrupted sitting time through higher intensity activity breaks. Thus, it is difficult to determine whether these benefits were due to the benefits of engaging in physical activity or the act of interrupting prolonged sitting. No studies have been conducted to explore whether these same mechanisms exist when continuous sitting is interrupted with standing breaks. Another potential explanation for the results observed in this study is the increased in total energy expenditure and total carbohydrate substrate utilization associated with frequent intermittent standing. A recent study found that standing for 1.5 minutes every 2 minutes of sitting increased the 8-hour total energy expenditure by 20% (617±76 kJ) and 9% (296±78 kJ) compared to prolonged continuous sitting and longer standing breaks (15 minutes of standing every 30 minutes sitting),

respectively[48]. In addition, their results also hinted on a higher carbohydrate substrate utilization during the frequent intermittent standing condition compared to the longer standing break condition (mean±SD of 86.1±5.5 g vs. 78.4±5.6 g) although the difference did not reach the significance threshold. It was estimated that a single sit-stand transition consumes roughly ~2 kJ of energy (0.5 kcal)[48]. This is further supported by studies looking at the differences in energy expenditure associated with sitting, standing and sit-stand transitions. These studies demonstrated that in a sample of 50 participants, the energy expenditure associated with performing one sit-stand transition per minute for 10 minutes was significantly higher (1.49 ± 0.25 and 1.16 ± 0.16 kcal/min for men and women, respectively) compared to the energy expenditure of continuous standing (1.23 ± 0.19 and 0.92 ± 0.13 kcal/min) or sitting (1.14 ± 0.18 and 0.88 ± 0.11 kcal/min)[65]. Collectively, these suggest that the difference in post-prandial glucose response of the two strategies in interrupting sitting time may be partially accounted for by the discrepancy in total energy requirement between the two conditions.

To the best of our knowledge, this is the first study to experimentally test the effect of different combinations of frequency and bouts of standing breaks in interrupting sitting time. The results particularly provide evidence that informs the development of future JITAI that aim to reduced sitting time in sedentary office employees. Such interventions could utilize techniques that elicit frequent but short interruptions in sitting time. Previous studies have shown that it is possible to reduce total sedentary time using technology-assisted prompts (i.e., computer-based prompts, text messaging, and email-based prompts)[39,74]. Furthermore, we have also reported that it is possible to invoke an immediate (within 5 minutes of receiving a prompt) break in sitting time [OR(95% CI)=

1.42 (1.10, 1.80)] by sending email prompts to 19 sedentary office employees with sit-stand workstations[122]. Thus, it is possible to develop smart and adaptive interventions that utilize frequent prompts to produce breaks in sitting time. However, finding the proper balance between frequency and timing of these prompts to maximize the effectiveness but still keeping the potentially undesirable impact of such interventions (i.e., loss of productivity and fatigue) at minimum should be explored in future studies.

The study has several strengths. Providing participants with standardized mixed meals allowed for the evaluation of an individual's post-prandial glucose response to a more ecologically valid type of meal. Continuous glucose monitor was used to measure the main outcome of the study. This enabled us to obtain large amount of glucose measurement during the study period without adding significant burden to the participant. The use of activPAL as an objective measure of sedentary and standing time allowed for accurate measurements of bouts of sitting and standing and facilitated higher compliance to the study protocol. This study also focused on standing as a mode for interrupting sitting time. This enabled us to isolate the benefits of interrupting sitting time without confounding it with the benefits from engaging in other higher intensity physical activity behaviors such as light walking or squatting. This study also randomized participants to six blocks that represent different combinations of the three study conditions. This designed resulted in a balance and uniform crossover study and allowed us to full account for potential period and sequence effects that are commonly associated with this type of study design. Lastly, this is the first study that experimentally tested different combinations of frequency and bouts of sedentary breaks provided some novel insights on how to efficiently break up sitting time.

Despite all of these, the study also has several limitations. As with other studies of this nature, we had a very limited sample size with data from only 11 participants included in this analysis. Using the observed effect size of 0.20 for mean glucose during the entire study visit, the sample size of 11 participants and an alpha error probability of 0.05, we calculated our power to be 96% for mean glucose and 22% for the glucose iAUC. This is primarily due to the significant differences in number of observations used during the analysis of the two outcomes (i.e., 308 for mean glucose vs. 28 for iAUC). This highlights the benefits of using continuous glucose monitoring as a measurement tool for this study. Diets were not controlled outside of the laboratory visits, so the results presented in this study were limited to the data collected during the laboratory visits. Several studies have presented evidence on how these types of interventions can potentially impact glycemic profile up to a day after the visits[28,59]. Gaining information on their glycemic profiles outside of the lab visits could lead to insights on the temporality of the observed benefits that resulted from the intervention. However, this approach was outside the scope of this study and should be explored in the future. Another limitation of the study is the lack of control to the menstrual cycle of female participants. It has been previously shown that glucose level can fluctuate depending on the stage of the menstrual cycle. To minimize bias from this, we randomly allocated participants to different blocks to determine the order that they receive the intervention. In addition, only two out of seven female participants were below the age of 45 years and excluding these participants does not significantly deviate the outcome of this study.

To conclude, despite its limitations this study adds to the increasing evidence on the benefits of reducing sedentary time and specifically, favoring the use of frequent

interruptions in sitting time to improve post-prandial glycemic control in individuals with impaired fasting glucose. Previous studies suggest that these results may have been driven by the increased in carbohydrate oxidation during frequent sit-stand transitions although the optimal the number of bouts and the potential for interaction between bout duration and frequency still needs to be investigated in future studies.

CHAPTER 6

CONCLUSION

In summary, this study provides some evidence favoring the use of frequent interruptions in sitting time to improve glycemic control of individuals with impaired glycemic profile. In contrast, less frequent, although longer bouts of standing breaks resulted in similar post-prandial glucose profile to that of the continuous sitting condition despite total standing time being equal to the less frequent, but longer duration bout condition. Previous studies suggest that these results may have been driven by the increase in carbohydrate oxidation during frequent sit-stand transitions which ultimately lead to increased total energy expenditure and improved post-prandial glycemic profile. Overall, our results suggest that frequency and bout duration of sedentary breaks can significantly influence post-prandial glucose response of individuals with impaired fasting glucose. Future studies should explore potential dose response relationship between the number of bouts and the potential for interaction between bout duration and frequency.

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APPENDIX A

STUDY RECRUITEMENT FLYER

TranSITion 2 Study

WHAT: Help us understand how sitting too much can negatively impact your health and test strategies to reduce sitting behavior.

WHO: Full-time office workers who spend majority of their work day being seated and are prediabetic.

HOW: Interested? See if you qualify using the link below.

YOU GET:

- Compensation of up to $60 for completing the study
- Feedback on your glucose level
- Free breakfast and lunch meals on every lab visit

The study will require 3 7.5 hour lab visits that are one week apart.

You will be asked to wear a device to continuously measure glucose level.

Participation is voluntary.

For questions, contact us at: mltoledo@asu.edu

bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2	bit.ly/TranSITion2

APPENDIX B

SCREENING QUESTIONNAIRE

Introduction

We are inviting you to participate in a study to help researchers at Arizona State University understand sedentary behavior and explore strategies to reduce sedentary behavior in the workplace. If you want to learn more about the study, please see our info sheet here.

Participation in this questionnaire is completely voluntary and you may exit the questionnaire at any time. Your responses to this questionnaire will not be published in any way and will only be used for screening purposes.

Please feel free to contact Meynard Toledo at (480)270-0514 or mltoledo@asu.edu for any questions about the study. Thank you!

To proceed, please click next.

Eligibility

Am I eligible?

To participate you MUST (all must apply to be eligible):
Please check the box next to each criteria you meet.

- [ ] Be between 35-55 years old
- [ ] Sedentary work habits
- [ ] Be in a seated position for a majority of your working day (computer, desk-based tasks)
- [ ] Be in the office at least 4 days per week
- [ ] Be at your desk at least 50% of the time when at work
- [ ] NOT be advised by a health professional to avoid long periods of standing
- [ ] NOT be currently pregnant
- [ ] NO known food allergies/restrictions
- [ ] Willing to engage in a 7.5 hour lab visit

BMI between 25-45 kg/m2

Thank you for completing this survey. Based on your responses, you are eligible to participate in this study.

APPENDIX C

CGM INSERTION INSTRUCTIONS

Important Safety Information

Indications for Use

The FreeStyle Libre Pro Flash Glucose Monitoring System is a professional continuous glucose monitoring (CGM) device indicated for detecting trends and tracking patterns in persons (age 18 and older) with diabetes. The System is intended for use by health care professionals and requires a prescription. Readings from the FreeStyle Libre Pro Sensor are only made available to patients through consultation with a health care professional. The System does not require user calibration with blood glucose values.

The FreeStyle Libre Pro System aids in the detection of glucose level excursions above or below the desired range, facilitating therapy adjustments. Interpretation of the FreeStyle Libre Pro Flash Glucose Monitoring System readings should be based on the trends and patterns analyzed through time using the reports available.

IMPORTANT: The device may inaccurately indicate hypoglycemia. The results of the clinical study conducted for this device showed that 40% of the time when the device indicated that user sensor glucose values were at or below 60 mg/dL, user glucose values were actually in the range of 81-160 mg/dL. Therefore, interpretation of the FreeStyle Libre Pro Flash Glucose Monitoring System readings should only be based on the trends and patterns analyzed through time using the reports available per the intended use.

Contraindications

The FreeStyle Libre Pro Flash Glucose Monitoring System must be removed prior to Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, or high-frequency electrical heat (diathermy) treatment. The effect of MRI, CT scans, or diathermy on the performance of the System has not been evaluated. The exposure may damage the Sensor and may impact proper function of the device to detect trends and track patterns in the user's glucose values during the wear period.

WARNING: The FreeStyle Libre Pro Flash Glucose Monitoring System contains small parts that may be dangerous if swallowed.

CAUTION:

- Performance of the System when used with other implanted medical devices, such as pacemakers, has not been evaluated.
- Some individuals may be sensitive to the adhesive that keeps the Sensor attached to the skin. If your patient notices significant skin irritation around or under their Sensor, they should remove the Sensor and stop using the FreeStyle Libre Pro System. Follow your facility's procedures for handling skin reactions.

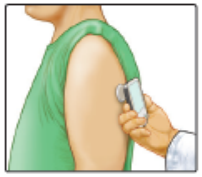
Warnings/Limitations

- Review all product information before use.
- Physiologic differences between the interstitial fluid and capillary blood may result in differences in glucose readings. Differences in glucose readings between interstitial fluid and capillary blood may be observed during times of rapid change in blood glucose, such as after eating, dosing insulin, or exercising.
- Severe dehydration and excessive water loss may cause inaccurate results.
- Do not reuse Sensors. The Sensor and Sensor Applicator are designed for single use. Reuse may result in no glucose readings and infection. Not suitable for re-sterilization. Further exposure to irradiation may cause inaccurate results.
- Interfering Substances: Taking ascorbic acid (vitamin C) while wearing the Sensor may falsely raise Sensor glucose readings. Taking salicylic acid (used in some pain relievers such as aspirin and some skin care products) may slightly lower Sensor glucose readings. The level of inaccuracy depends on the amount of the interfering substance active in the body. Test results did not indicate interference for methyldopa (used in some drugs to treat high blood pressure) or tolbutamide (infrequently used in some drugs to treat diabetes in the US) at maximum circulating levels. However, concentrations of potential interferents in interstitial fluid are unknown compared to circulating blood. Taking medications with acetaminophen (such as Tylenol and some cold medicines) while wearing the Sensor may falsely raise Sensor glucose readings. The level of inaccuracy depends on the amount of acetaminophen active in the body and may be different for each person.
- Take standard precautions for transmission of blood borne pathogens to avoid contamination.
- The Reader should be cleaned between patients.
- If a Sensor breaks inside a patient, remove with tweezers, treat any medical complications and call Customer Service.
- Use of the System is not recommended in the critically ill population since performance is unknown due to different conditions and medications.
- Sensor placement is not approved for sites other than the back of the arm. If placed in other areas, the Sensor may not function properly.
- If the Sensor Kit package or contents or the Reader appear to be damaged, do not use as there may be a risk of electric shock, no results, and/or infection.
- Store the Sensor Kit between 39°F-77°F. While you don't need to keep the Sensor Kit in a refrigerator, you can as long as the refrigerator is between 39°F-77°F.
- Store the Sensor Kit between 10-90% non-condensing humidity.
- The System does not provide real-time results. Patients need to rely on blood glucose readings for monitoring glucose during System use.
- Clean hands prior to Sensor handling/insertion to help prevent infection.

- Clean the application site and ensure that it is dry prior to Sensor insertion. This helps the Sensor stay attached to the body.
- Change the application site for the next Sensor application to prevent discomfort or skin irritation.
- Select an appropriate Sensor site to help the Sensor stay attached to the body and prevent discomfort or skin irritation. Avoid areas with scars, moles, stretch marks, or lumps. Select an area of skin that generally stays flat during normal daily activities (no bending or folding). Choose a site that is at least 1 inch away from an insulin injection site.
- The Sensor should not be worn more than 14 days. Readings are not obtained after 14 days.
- The Sensor should be removed prior to exposing it to an X-ray machine. The effect of X-rays on the performance of the system has not been evaluated. The exposure may damage the Sensor and may impact proper function of the device to detect trends and track patterns in the user's glucose values during the wear period.
- The FreeStyle Libre Pro Flash Glucose Monitoring System has not been evaluated for use in pregnant women, persons on dialysis, or people less than 18 years of age.

Getting to Know the System

The FreeStyle Libre Pro Flash Glucose Monitoring System has three main parts: a handheld Reader, a disposable Sensor, and FreeStyle Libre Pro software. A single FreeStyle Libre Pro Reader can be used to gather data from FreeStyle Libre Pro Sensors on multiple patients.



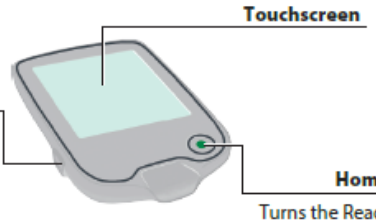
IMPORTANT: Safety information about the System is in this Operator's Manual. Read all of the information in the Operator's Manual before using the System.

When opening the **Reader Kit** and **Sensor Kit**, check that the contents are undamaged and that you have all parts listed. If any parts are missing or damaged, contact Customer Service.

Reader Kit

The Reader Kit includes:

- FreeStyle Libre Pro Reader
- USB Cable
- Power Adapter
- Operator's Manual
- Quick Start Guide



Touchscreen

USB Port
Used to charge the Reader and connect it to a computer.

Home Button
Turns the Reader on/off and takes you to the Home Screen from any other screen.

The Reader is used to start the Sensor on a patient and gather their glucose readings. Multiple patients can have their Sensor started by the same Reader.

Sensor Kit

The Sensor Kit includes:

- Sensor Pack
- Sensor Applicator
- Alcohol wipe
- Product insert

Sensor Pack
Used with the Sensor Applicator to prepare the Sensor for use.

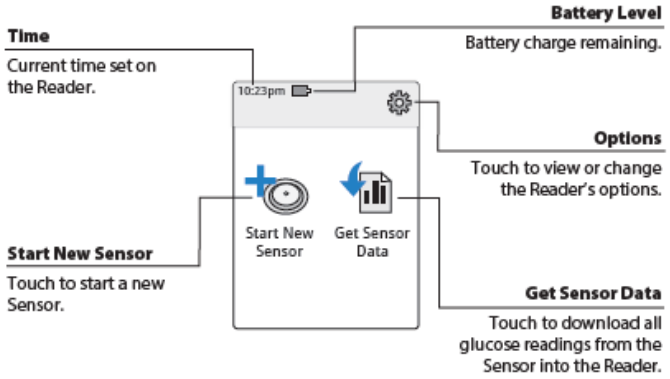
Sensor Applicator
Applies the Sensor to the patient's body.

The Sensor measures and stores glucose readings when worn on the body. It initially comes in two parts: one part is in the Sensor Pack and the other part is in the Sensor Applicator. By following the instructions, prepare and apply the Sensor on the back of the patient's upper arm. The Sensor has a small, flexible tip that is inserted just under the skin. The Sensor can be worn for up to 14 days.

Sensor
Measures glucose while on body (only visible after applied).

The Reader Home Screen provides access to starting a new Sensor, getting Sensor data, and information about the System.

Home Screen



FreeStyle Libre Pro Software

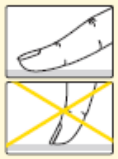
FreeStyle Libre Pro software can be used to create reports based on glucose readings from the most recently downloaded Sensor. The software is compatible with most Windows and Mac operating systems. Go to www.FreeStyleLibrePro.com and follow onscreen instructions to download and install the software.

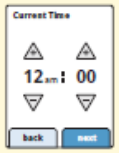
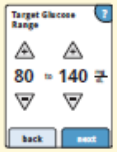
INTENDED USE

The FreeStyle Libre Pro software is intended for use by health care professionals to aid in the review, analysis and evaluation of a patient's glucose readings uploaded from the FreeStyle Libre Pro Flash Glucose Monitoring System in support of an effective diabetes health management program.

Setting up the Reader for the First Time

Before using the System for the first time, the Reader must be set up.

Step	Action
1	 Press the Home Button to turn on the Reader.
2	 If prompted, use the touchscreen to select your preferred language for the Reader. Touch **OK** to continue. **Note:** Use the pad of your finger. Do NOT use your fingernail or any other object on the screen.
3	 Set the **Current Date** using the arrows on the touchscreen. Touch **next** to continue.

Step	Action
4	 Set the **Current Time**. Touch **next** to continue. **CAUTION:** It is very important to set the time and date correctly for correct interpretation of Sensor data.
5	 Set the **Target Glucose Range**. Touch **next** to continue. **Note:** The Target Glucose Range is displayed on the Daily Graph on the Reader once Sensor data has been downloaded. While the glucose data are gathered in the System range of 40-500 mg/dL, the Daily Graph display range is 0-350 mg/dL for ease of review on screen.
6	 The Reader now indicates how to return to the Home Screen from any other screen. Touch **done** to go to the Home Screen.

Note: Charge the Reader if the battery level is low. Only use the USB cable and power adapter included with the System.

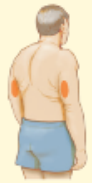
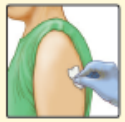
Using the Sensor

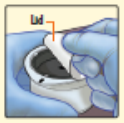
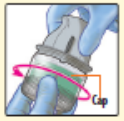
After you assemble and apply the Sensor to your patient's body, start the Sensor with the Reader and confirm it is working. The Sensor stores glucose readings every 15 minutes for up to 14 days. The first reading is stored 1 hour after the Sensor is successfully started.

CAUTION:

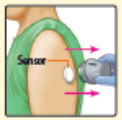
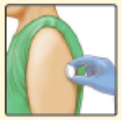
The Sensor Pack and Sensor Applicator are packaged as a set (separately from the Reader) and have the same Sensor code. Check that the Sensor codes match before using the Sensor Pack and Sensor Applicator. Sensor Packs and Sensor Applicators with the same Sensor code should be used together or Sensor glucose readings may be incorrect.

Applying the Sensor

Step	Action
1	 Apply Sensors only on the back of your patient's upper arm. Avoid areas with scars, moles, stretch marks, or lumps. Select an area of skin that generally stays flat during normal daily activities (no bending or folding). Choose a site that is at least 1 inch (2.5 cm) away from an insulin injection site. To prevent discomfort or skin irritation, you should select a different site other than the one most recently used.
2	 Clean application site with an alcohol wipe and allow site to dry before proceeding. This helps the Sensor stay attached to the body. **Note:** The area **MUST** be clean and dry, or the Sensor may not stick to the site.

Step	Action
3	  Open the Sensor Pack by peeling the lid off completely. Unscrew cap from the Sensor Applicator and set the cap aside. **CAUTION:** Do NOT use if the Sensor Pack or the Sensor Applicator seem to be damaged or already opened. Do NOT use if past expiration date.
4	 Line up the dark mark on the Sensor Applicator with the dark mark on the Sensor Pack. Press firmly down on the Sensor Applicator until it comes to a stop.
5	 Lift the Sensor Applicator out of the Sensor Pack.

Step	Action
6	 The Sensor Applicator is prepared and ready to apply the Sensor. **CAUTION:** The Sensor Applicator now contains a needle. Do NOT touch inside the Sensor Applicator or put it back into the Sensor Pack.
7	 Place the Sensor Applicator over the prepared site and push down firmly to apply the Sensor to the body. **CAUTION:** Do NOT push down on the Sensor Applicator until placed over prepared site to prevent unintended results or injury.

Step	Action
8	 Gently pull the Sensor Applicator away from the body. The Sensor should now be attached to the skin. **Note:** Applying the Sensor may cause bruising or bleeding. If there is bleeding that does not stop, remove the Sensor, and apply a new one at a different site.
9	 Make sure the Sensor is secure after application. Put the cap back on the Sensor Applicator. Discard the used Sensor Pack and Sensor Applicator according to your facility's procedures.

Starting the Sensor

Step	Action
1	 Press the Home Button to turn on the Reader.
2	 Touch **Start New Sensor**.
3	  Hold the Reader within 1.5 inches (4 cm) of the Sensor to start it. If sounds are turned on, the Reader beeps when the Sensor has been started. You can check the Sensor has successfully started in 2 minutes. **Note:** If communication is not established within 15 seconds, the Reader displays a prompt to try again. Touch **OK** to return to the Home Screen and touch **Start New Sensor** to start the Sensor.

Step	Action
4	 When prompted, touch **yes** to check the Sensor status. Hold the Reader within 1.5 inches (4 cm) of the Sensor to verify Sensor is working.
5	 Touch **OK** to go to the Home Screen.

Patient Wear

The Sensor stores your patient's glucose readings every 15 minutes for up to 14 days. The first reading is stored 1 hour after the Sensor is successfully started.

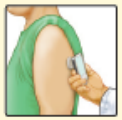
IMPORTANT:

- The Sensor should not be worn for more than 14 days.
- Data can be downloaded at anytime from Sensors that are on or off the body.
- Before your patient goes home, review and give them the "Living with Your FreeStyle Libre Pro Sensor" section of the insert in the Sensor Kit.

CAUTION: Intense exercise may cause the Sensor to loosen due to sweat or movement of the Sensor. If the Sensor becomes loose, the Sensor readings may be unavailable or unreliable. Your patient should return to your facility for application of a new Sensor.

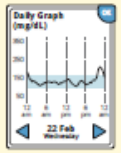
Getting Sensor Data

Data can be downloaded at anytime from Sensors that are on or off the body.

Step	Action
1	 Press the Home Button to turn on the Reader.
2	 Touch **Get Sensor Data**.
3	 Hold the Reader within 1.5 inches (4 cm) of the Sensor. If sounds are turned on, the Reader will beep when all the data has been successfully downloaded from the Sensor. This may take up to 5 seconds. **Note:** If communication is not established within 15 seconds, the Reader displays a prompt to try again. Touch **OK** to return to the Home Screen and touch **Get Sensor Data** again.

Step	Action
4	 The Reader will indicate how many days of Sensor wear are left, if any. Touch **view** to view the daily graph. Touch **next**. For more information about the daily graph, see *Daily Graph* section.
5	 To create reports, connect the Reader to a computer. See *Creating Reports* section in the FreeStyle Libre Pro software User's Manual. The User's Manual can be found in the Help Menu of the software. Touch **done** to return to the Home Screen. **Note:** The Home Screen will show this symbol near the top of the screen when there is new Sensor data in the Reader that has not been transferred to a computer. A report should be generated from this data before the next Sensor is downloaded.

Daily Graph



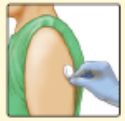
The Daily Graph shows the Sensor glucose readings by day and the Target Glucose Range that is set on the Reader. You can change the target glucose range by touching the Options symbol on the Home Screen and selecting **Target Range**.

Notes:

- If you want the graph to show the current patient's target range, set their target range before downloading their data.
- The graph displays glucose readings up to 350 mg/dL. Glucose readings above 350 mg/dL are displayed at 350 mg/dL. For sequential readings above 350 mg/dL, a line is displayed at 350 mg/dL.

Removing the Sensor

The Sensor automatically stops working and should be removed 14 days after being started. You should also replace the Sensor if there is any irritation or discomfort at the application site or if the Reader reports a problem with the Sensor currently in use.

Step	Action
1	 Pull up the edge of the adhesive that keeps the Sensor attached to the skin. Slowly peel away from the skin in one motion. **Note:** Any remaining adhesive residue on the skin can be removed with warm soapy water or isopropyl alcohol.
2	Discard the used Sensor according to your facility's procedures. See *Maintenance and Disposal* section.

APPENDIX D

PARTICIPANT'S TAKE HOME INSTRUCTIONS

TranSITion 2.0 Libre Pro Sensor

What the FreeStyle Libre Pro Sensor Does

The FreeStyle Libre Pro sensor continually measures and stores your sugar levels for up to 14 days.

After you've worn the sensor for up to 14 days, your doctor will download data from it that will give information about how your sugar levels are trending throughout the day and night. This will help your doctor personalize your treatment plan.

DON'T STOP TESTING!

The FreeStyle Libre Pro sensor is not a replacement for self-monitoring of blood glucose.

Continue your normal testing routine while wearing the sensor.

Indications and Important Safety Information

The FreeStyle Libre Pro Flash Glucose Monitoring System is a professional continuous glucose monitoring (CGM) device indicated for detecting trends and tracking patterns and glucose level excursions above or below the desired range, facilitating therapy adjustments in persons (age 18 and older) with diabetes. The system is intended for use by health care professionals and requires a prescription.

IMPORTANT: The device may inaccurately indicate hypoglycemia. The results of the clinical study conducted for this device showed that 40% of the time when the device indicated that user sensor glucose values were at or below 60 mg/dL, user glucose values were actually in the range of 81-160 mg/dL. Therefore, interpretation of the FreeStyle Libre Pro Flash Glucose Monitoring System readings should only be based on the trends and patterns analyzed through time using the reports available per the intended use.

CONTRAINDICATIONS: Remove the Sensor before MRI, CT scan, X-ray, or diathermy treatment. WARNINGS/LIMITATIONS: The FreeStyle Libre Pro System does not provide real-time results and patients should adhere to their blood glucose monitoring routine while using the system. If a sensor breaks, contact physician and call Customer Service. Patients with high levels of ascorbic acid (Vitamin C) or salicylic acid (used in Aspirin) or severe dehydration or excessive water loss or medications containing acetaminophen (Tylenol) may experience inaccurate results with this system. The FreeStyle Libre Pro System is not approved for pregnant women, persons on dialysis, or recommended for critically ill population. Sensor placement is not approved for sites other than the back of the arm and standard precautions for transmission of blood borne pathogens should be taken.

For question about the study, please contact us at: mltoledo@asu.edu

What You Need To Do

- Wear the sensor on the back of your upper arm for up to 14 days
- Continue regular blood glucose self-testing per your doctor's recommendation
- Maintain a daily log of your blood glucose readings, diet, exercise, and insulin

There's no need for you to interact with the sensor. Just go about your daily routine.

A Little Extra Care

The sensor is comfortable enough to forget it's there. Use care to avoid accidentally loosening or removing it:

Showering, Bathing, and Swimming

Your sensor is water resistant and can be worn while bathing, showering, or swimming as long as you do not:

- take it deeper than 3 feet (1 meter)
- keep it underwater for longer than 30 minutes at a time

Getting dressed

Use care to avoid catching the sensor on clothing while getting dressed

Exercising

Intense exercise may cause the sensor to loosen due to sweat or movement of the sensor

FreeStyle Libre Pro
FLASH GLUCOSE MONITORING SYSTEM

Contact the research team if:

- Your sensor becomes loose or is removed
- You have irritation or discomfort at the sensor site
- You have any questions about your sensor

Special Activities

Medical procedures

If you have an MRI, a CT scan, or a diathermy treatment, you must remove your sensor prior to the procedure. Notify your healthcare provider.

Security checkpoints

Notify security at airport checkpoints

Removing the sensor

If necessary, pull up the edge of the adhesive that keeps your sensor attached to your skin. Slowly peel away from your skin in one motion.

Note: Any remaining adhesive residue on the skin can be removed with warm soapy water or isopropyl alcohol.

APPENDIX E

STANDARDIZED BREAKFAST AND LUNCH MEALS

Meal	Energy(Kcal)	Food	Amount	Macronutrient composition (%)
M1	569	Croissant,plain	35g	P: 15%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 31%
		Ham,leg,non-canned,lean	21g	C: 54%
		Orange juice	280 mL	
		Kelloggs bran flakes	0.8 cup	
		Milk,cow,fluid,regular fat (~3.5%)	0.5 cup	
M2	642	Croissant,plain	50g	P: 16%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 29%
		Ham,leg,non-canned,lean	21g	C: 54%
		Orange juice	300 mL	
		Kelloggs bran flakes	1 cup	
		Skimmed milk	0.75 cup	
M3	659	Croissant,plain	50g	P: 14%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 32%
		Ham,leg,non-canned,lean	21g	C: 53%
		Orange juice	320 mL	
		Kelloggs bran flakes	1 cup	
		Milk,cow,fluid,regular fat (~3.5%)	0.6 cup	
M4	683	Croissant,plain	50g	P: 14%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 31%
		Ham,leg,non-canned,lean	21g	C: 52%
		Orange juice	320 mL	
		Kelloggs bran flakes	1 cup	
		Milk,cow,fluid,regular fat (~3.5%)	0.6 cup	
M5	697	Croissant,plain	50g	P: 14%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 31%
		Ham,leg,non-canned,lean	21g	C: 53%
		Orange juice	350 mL	
		Kelloggs bran flakes	1 cup	
		Milk,cow,fluid,regular fat (~3.5%)	0.6 cup	
M6	711	Croissant,plain	50g	P: 14%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 30%
		Ham,leg,non-canned,lean	21g	C: 54%
		Orange juice	380 mL	
		Kelloggs bran flakes	1 cup	
		Milk,cow,fluid,regular fat (~3.5%)	0.6 cup	
M7	733	Croissant,plain	75g	P: 14%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 31%
		Orange juice	300 mL	C: 53%
		Kelloggs bran flakes	1 cup	
		REV LOW FAT FRESH	0.75 cup	
M8	756	Croissant,plain	75g	P:13%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 30%
		Orange juice	350 mL	C: 54%
		Kelloggs bran flakes	1 cup	
		REV LOW FAT FRESH	0.75 cup	
M9	778	Croissant,plain	75g	P: 15%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 30%
		Ham,leg,non-canned,lean	21g	C: 53%
		Orange juice	350 mL	
		Kelloggs bran flakes	1 cup	
		REV LOW FAT FRESH	0.75 cup	
M10	799	Croissant,plain	75g	P: 15%
		Cheese,cheddar (mild,tasty & vintage styles)	21g	F: 30%
		Ham,leg,non-canned,lean	30g	C: 53%
		Orange juice	375 mL	
		Kelloggs bran flakes	1 cup	
		REV LOW FAT FRESH	0.75 cup	

MEAL	Energy (Kcal)	Food	Amount	Macronutrient content (%)
M1	659.2	Bread,foccacia/turkish style bread,plain	130g	P:15%
		Tuna,canned in brine,drained	50g	F:29%
		Lettuce,iceberg,raw	15g	C:54%
		Sweetcorn,canned in brine,drained	20g	
		GOLDEN CIRCLE DRINK ORANGE MAI	200 mL	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	15g	
M2	698.4	Bread,foccacia/turkish style bread,plain	130g	P:14%
		Tuna,canned in vegetable oil,drained	50g	F:30%
		Lettuce,iceberg,raw	15g	C:54%
		Sweetcorn,canned in brine,drained	20g	
		GOLDEN CIRCLE DRINK PINE MANGO	250 mL	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M3	721.3	Bread,foccacia/turkish style bread,plain	130g	P:14%
		Tuna,canned in vegetable oil,drained	50g	F:29%
		Lettuce,iceberg,raw	15g	C:55%
		Sweetcorn,canned in brine,drained	20g	
		GOLDEN CIRCLE DRINK PINE MANGO	300 mL	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M4	744.7	Bread,foccacia/turkish style bread,plain	130g	P:14%
		Ham,leg,non-canned,lean	30g	F:30%
		Lettuce,iceberg,raw	15g	C:55%
		Cheese,cheddar (mild,tasty & vintage styles)	25g	
		GOLDEN CIRCLE DRINK PINE MANGO	250 mL	
		Mayonnaise,full fat,commercial	20g	
		NABISCO OREO COOKIES	2 biscuits	
M5	765.5	Bread,foccacia/turkish style bread,plain	165g	P:13%
		Ham,leg,non-canned,lean	20g	F:30%
		Lettuce,iceberg,raw	15g	C:57%
		Cheese,cheddar (mild,tasty & vintage styles)	20g	
		GOLDEN CIRCLE DRINK PINE ORANGE	250 mL	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M6	767.4	Bread,foccacia/turkish style bread,plain	165g	P:13%
		Ham,leg,non-canned,lean	20g	F:30%
		Lettuce,iceberg,raw	15g	C:56%
		Cheese,cheddar (mild,tasty & vintage styles)	20g	
		GOLDEN CIRCLE DRINK ORANGE MAI	250 mL	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M7	785.4	Bread,foccacia/turkish style bread,plain	165g	P:12%
		Ham,leg,non-canned,lean	20g	F:29%
		Lettuce,iceberg,raw	15g	C:57%
		Cheese,cheddar (mild,tasty & vintage styles)	20g	
		Soft drink,lemonade or fanta	1 can(375ml)	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M8	805.9	Bread,foccacia/turkish style bread,plain	165g	P:13%
		Ham,leg,non-canned,lean	20g	F:30%
		Lettuce,iceberg,raw	15g	C:55%
		Cheese,cheddar (mild,tasty & vintage styles)	25g	
		Soft drink,lemonade or fanta	1 can(375ml)	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M9	807.1	Bread,foccacia/turkish style bread,plain	165g	P:14%
		Ham,leg,non-canned,lean	40g	F:29%
		Lettuce,iceberg,raw	15g	C:55%
		Cheese,cheddar (mild,tasty & vintage styles)	20g	
		Soft drink,lemonade or fanta	1 can(375ml)	
		Mayonnaise,full fat,commercial	20g	
		Margarine spread,monounsaturated,nfs	10g	
M10	813.3	Bread,foccacia/turkish style bread,plain	165g	P:13%
		Ham,leg,non-canned,lean	30g	F:30%
		Lettuce,iceberg,raw	15g	C:55%
		Cheese,cheddar (mild,tasty & vintage styles)	20g	
		Soft drink,lemonade or fanta	1 can(375ml)	
		Mayonnaise,low fat,commercial	1 tb	
		Margarine spread,monounsaturated,nfs	15g	

APPENDIX F

CHARACTERISTICS OF STUDIES INVESTIGATING THE EFFECT OF BREAKING UP SITTING ON POSTPRANDIAL GLUCOSE LEVELS

Source	Poulation	N (F)†	Age‡ (years)	Protocol duration (hrs)	Intervention			Control Condition	Main results
					Break Activity	Frequency and Duration	Total break time (min)		
Intermittent Standing vs Uninterrupted Sitting									
Bailey et al, 2015*	Healthy adults	10 (3)	24±3	5	Standing	2 min/20 min sitting	30 min	Uninterrupted Sitting	No difference in 5h glucose AUC between uninterrupted sitting and standing breaks with AUC= 22.0 (20.5, 23.5) and 22.2 (20.7, 23.75) mmol/L, respectively.
Crespo et al, 2016	Overweight/obese adults	9 (7)	30±15	8	Standing	Progressive Pattern§	150 min	Uninterrupted Sitting	Mean 8h glucose of standing condition (5.6±0.1.0 mmol/L) was significantly lower than uninterrupted sitting condition (5.3±0.9 mmol/L). No significant diference in AUC between the two conditions.
Hawari et al, 2016	Overweight/obese adults	10(0)	33±13	8	Prolonged vs. interrupted standing	1.5 min/2 min sitting or 15 min/30 min sitting	240 min	Uninterrupted Sitting	No significant differnece in 8h post-prandial glucose AUC between conditions.
Henson et al, 2016*	Prediabetic women	22 (22)	67±5	7.5	Standing	5 min/30 min sitting	60 min	Uninterrupted Sitting	The 7.5h glucose iAUC of standing condtion (3.5±0.8 mmol/L) was significantly lower than that of the uninterrupted sitting condition (5.3±0.8 mmol/L).
Pulsford et al, 2016	Sedentary, healthy men	25 (0)	40±12	7	Standing	2 min/20 min sitting	35 min	Uninterrupted Sitting	No significant difference in Matsuda index between the standing condition vs. uninterrupted sitting condition. Significant mean difference of -1.6 (-3.1, -0.1) mmol/L for 7h glucose AUC.
Thorp et al, 2014	Sedentary, overweight adults	23 (6)	48±8	4	Standing	30 min/hr sitting	240 min	Uninterrupted Sitting	The 4h glucose iAUC of the standing group was 11% lower than that of the uninterrupted sitting group, AUC= 6.4 (5.0, 7.7) mM/h vs. 7.2 (5.8, 8.5) mM/h.

Source	Poulation	N (F)†	Age‡ (years)	Protocol duration (hrs)	Intervention			Control Condition	Main results
					Break Activity	Frequency and Duration	Total break time (min)		
Intermittent LPA vs Uninterrupted Sitting									
Bailey et al, 2015*	Healthy adults	10 (3)	24±3	5	Walking at 3.2 km/h	2 min/20 min sitting	30 min	Uninterrupted Sitting	Light-intensity activity breaks elicited a 15.9% decrease in 5h glucose AUC compared to uninterrupted sitting condition, AUC= 18.5 (17.0, 20.0) and 22.0 (20.5, 23.5) mmol/L, respectively.
Bailey et al, 2016*	Sedentary, healthy adults	13 (7)	27±9	6	Walking at 3.2 km/h	2 min/20 min sitting	30 min	Uninterrupted Sitting	No significant difference in 6h glucose AUC between the uninterrupted sitting condition, iAUC= 2.9±1.7 mmol/L, and light-intensity activity break condition, iAUC=3.5±2.2 mmol/L.
Crespo et al, 2016	Overweight/obese adults	9 (7)	30±15	8	Walking at 1.6 km/h and cycle at 25-30 rpm	Progressive Pattern§	150 min	Uninterrupted Sitting	Mean 8h glucose of walking and cycling condition (5.2±0.9 and 5.1±1.0 mmol/L, respectively) was significantly lower than uninterrupted sitting condition (5.3±0.9 mmol/L). No significant diference in AUC between the three groups
Dempsey et al, 2016	Adults with T2DM	24 (14)	62±6	7	Walking at 3.2 km/h	3 min/30 min sitting	36 min	Uninterrupted Sitting	The 7h glucose iAUC was significanlty lower in the light-intensity activity condition compard to uninterrupted sitting condition, iAUC= 14.8 (11.0, 18.6) mmol/L vs. 24.2 (20.4, 28.0) mmol/L.
Dunstan et al, 2012*	Overweight/obese adults	19 (8)	54±5	5	Walking at 3.2 km/h	2 min/20 min sitting	28 min	Uninterrupted Sitting	The 5h glucose iAUC was significanlty lower in the light-intensity activity condition compard to uninterrupted sitting condition, iAUC= 5.2 (4.1, 6.6) mmol/L vs. 6.9 (5.5, 8.7) mmol/L.
Hansen et al, 2016	Active, healthy adults	14 (8)	22±1	2.5	Walking at 4.1 km/h	2 min/20 min sitting	14 min	Uninterrupted Sitting	The 2.5h glucose iAUCs were not significantly different between the two conditions, iAUC=252 (163, 340) mmol/L for uninterrupted sitting and 214 (146, 282) for light-intensity activity breaks.
Henson et al, 2016	Prediabetic women	22 (22)	67±5	7.5	Walking at 3.0 km/h	5 min/30 min sitting	60 min	Uninterrupted Sitting	The 7.5h glucose iAUC of walking condtion (3.8±0.7 mmol/L) was significantly lower than that of the uninterrupted sitting condition (5.3±0.8 mmol/L).
Larsen et al, 2015	Sedentary, overweight/obese adults	19 (8)	57±7	4	Walking at 3.2 km/h	2 min/20 min sitting	34 min	Uninterrupted Sitting	The hourly glucose iAUC of the light-intensity activity condition were lower at day 1 and 3 compared to the uninterrupted sitting condition, with mean difference of 1.3±0.5 and 1.5±0.5 mmol/L, respectively.
McMarthy et al, 2017	Non-obese adults	34 (18)	40±9	7.5	Walking at 3.0 km/h	5 min/30 min sitting	60 min	Uninterrupted Sitting	Breaking sedentary time with light walking reduced 6.5h glucose iAUC by 35% with mean glucose iAUC= 3.89±0.7 mmol/L for the uninterrupted sitting condition and 2.51±0.7 mmol/L for the light-walking condition.
Miyashita et al, 2016	Sedentary, healthy women	15 (15)	69±3	8	Walking at 3.7 km/h	1.5 min/ 15 min sitting or one 30-min bout at start of visit	30 min	Uninterrupted Sitting	The 8h glucose AUC and iAUC were not significantly different from the uninterrupted sitting condition.
Pulsford et al, 2016*	Sedentary, healthy men	25 (0)	40±12	7	Walking at 3.2 km/h	2 min/20 min sitting	35 min	Uninterrupted Sitting	Walking group have significanlty higher Matsuda index, mean differnce= 1.2 (0.1, 2.2), and lower 7h glucose AUC, mean difference= -2.5 (-3.7, -1.3) mmol/L, compared to the uninterrupted siting condition.
Van Dijk, et al, 2013	Adults with T2DM	20 (0)	64±4	10.5	Slow-paced strolling at 3 METs	Three 15-minute bouts	45 min	Uninterrupted Sitting	The 10.5h glucose iAUC of the slow-paced strolling consition was significantly lower compared to the uninterrupted sitting condtion, iAUC= 365±51 mmol/L vs. 448±54 mmol/L, respectively.

Source	Poulation	N (F)†	Age‡ (years)	Protocol duration (hrs)	Intervention			Control Condition	Main results
					Break Activity	Frequency and Duration	Total break time (min)		
Intermittent MVPA vs Uninterrupted Sitting									
Bailey et al, 2016*	Sedentary, healthy adults	13 (7)	27±9	6	Walking at 5.8-7.9 km/h	2 min/20 min sitting	30 min	Uninterrupted Sitting	Moderate-intensity activity breaks resulted in a significanlty lower 5h glucose iAUC compared to uninterrupted sitting condition, iAUC= 1.5±1.5 mmol/L vs. 2.9±1.7 mmol/L.
Baynard et al, 2005*	Adults with T2DM	9 (9)	53±6	4	Walking at 60-65% VO2max	Three 10-minute bouts	30 min	Uninterrupted Sitting	No significant differneces in 2h post-prandial mean glucose or AUC between the intermittent walking condition and control condition.
Baynard et al, 2005*	Non-obese, healthy adults	6 (6)	49±4	4	Walking at 60-65% VO2max	Three 10-minute bouts	30 min	Uninterrupted Sitting	No significant differneces in 2h post-prandial mean glucose or AUC between the intermittent walking condition and control condition.
Bhammar et al, 2017	Sedentary, overweight/obese adults	10 (5)	32±5	8	Walking at 3.7-4.0 METs	2 min/20 min sitting	42 min	Uninterrupted Sitting	Mean 18.7h glucose was significantly lower during the moderate-intensity breaks condition (5.2±1.1 mmol/L) compared to the uninterrupted sitting condtion (5.6±1.1 mmol/L).
Dunstan et al, 2012*	Overweight/obese adults	19 (8)	54±5	5	Walking at 5.8-6.4 km/h	2 min/20 min sitting	28 min	Uninterrupted Sitting	The 5h glucose iAUC was significanlty lower in the moderate-intensity activity group compard to uninterrupted sitting condition, iAUC= 4.9 (3.8, 6.1) mmol/L vs. 6.9 (5.5, 8.7) mmol/L.
Holmstrup et al, 2014	Obese young-adults with IFG	11 (3)	25(NR)	12	Walking at 60-65% VO2max	5 min/hour	60 min	Uninterrupted Sitting	The 12h glucose iAUC of the intermittent exercise condtion was significantly lower (5457.0±238.8 mmol/L) compared to a single bout of exercise (6249.6±286.3 mmol/L).
Peddie et al, 2013	Sedentary, healthy adults	70(42)	26±5	9	Walking at 45.6% VO2max	100 sec/30 min	30 min	Uninterrupted Sitting	The regular activity break condition lowered the 9h glucose iAUC by 18.9 (8.4, 26.3) mmol/L when compared to the uninterrupted sitting condition.

***** Multiple comparisons were extracted from this study.

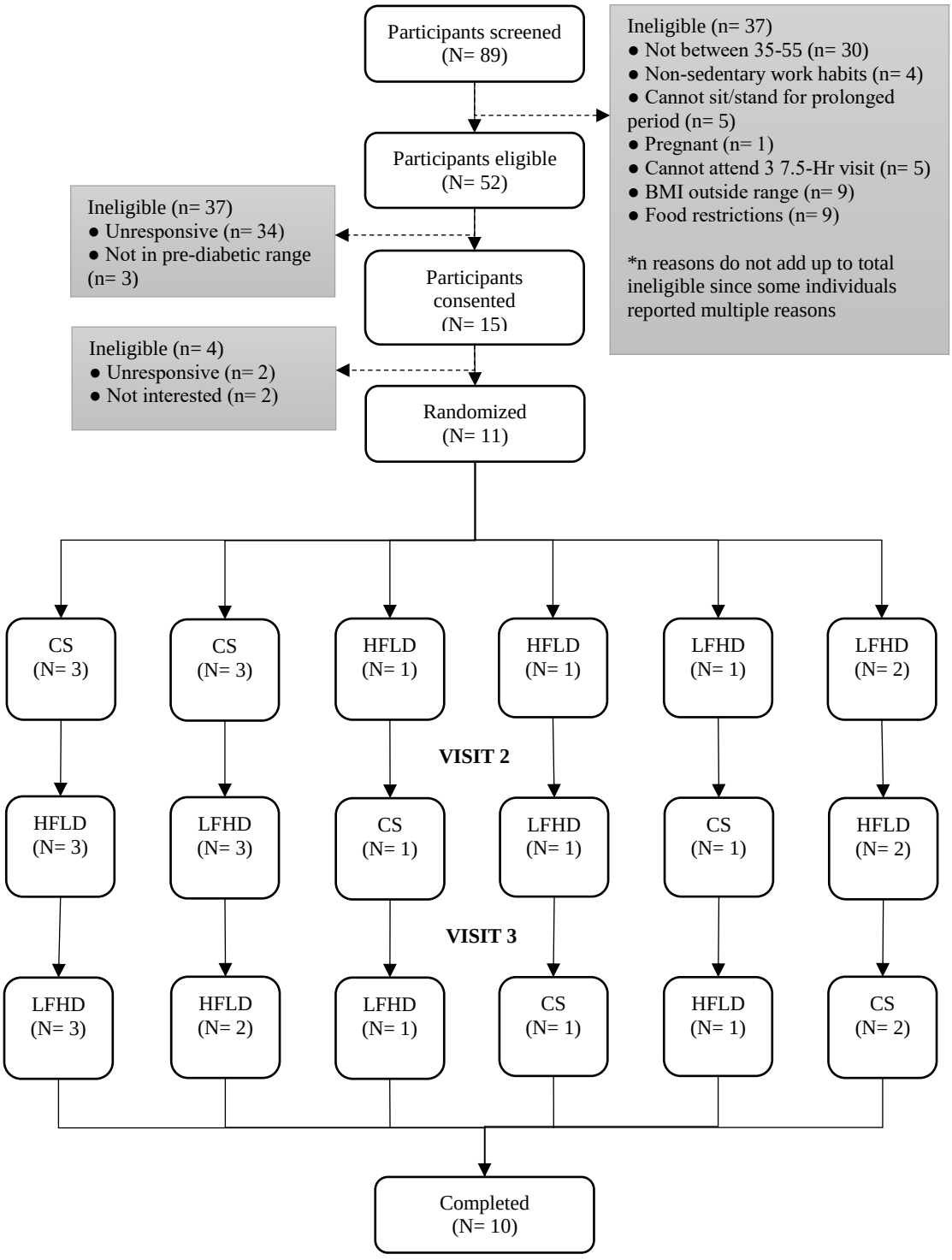
† Total number of subjects (number of female).

‡ Means ± Standard Deviation.

§ Standing time: 10 min, from 0850 to 0950 h; 15 min, from 1045 to 1145 h; 20 min, from 1240 to 1320 h; and 30 min, from 1400 to 1530 h.

APPENDIX G

CONSORT DIAGRAM



Appendix G. Full Consort diagram. CS: Continuous sitting condition. HFLD: High-frequency, low duration condition. LFHD: Low-frequency, high duration standing condition.