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Sedentary behavior and risk for cardiovascular disease and diabetes

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Public Health (Epidemiology)

by

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Publications

1. Michael J. LaMonte, M.J., Lewis, C.E., Buchner, D.M., Evenson, K.R., Rillamas-Sun, E., Di, C., Lee, I., **Bellettiere, J.,** Stefanick, M.L., Eaton, C.B., Howard, B.V., Bird, C., LaCroix A.Z. (2017) Both Light Intensity and Moderate-to-Vigorous Physical Activity Measured by Accelerometry are Favorably Associated with Cardiometabolic Risk Factors in Older Women: The Objective Physical Activity and Cardiovascular Health (OPACH) Study. *The Journal of the American Heart Association*. (*in press*).

2. **Bellettiere, J.,** Winkler, E.A.H., Chastin, S.F.M., Kerr, J., Owen, N., Dunstan, D.W., Healy, G.N. (2017). Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults. *PLoS ONE 12(6): e0180119.*

3. Klepeis, N. E., **Bellettiere, J.**, Hughes, S. C., Liles, S., Blumberg, E., Berardi, V., Obayashi, S., Allen, T. T., Hovell, M. F. (2017). Fine

particles in homes of predominantly low-income families with children and smokers: Key physical and behavioral determinants to inform indoor-air-quality interventions. *PLoS ONE* 12(5): e0177718.

4. **Bellettiere, J.**, Liles, S., Benporat, Y., Bliss, N., Hughes, S., Bishop, B., Robusto, K., Hovell, M.F. (2017). And she's buying a stairway to health: signs and participant factors influencing stair ascent at a public airport. *Journal of Primary Prevention.* (*in press*).

5. **Bellettiere, J.**, Chuang, E., Quintanilla, I., Hofstetter, R., Hughes, S.C., Hovell M.F. (2017). Parental barriers to accessing a usual source of care and their children's receipt of preventive services. *Public Health Reports.* 132(3), 1-10.

6. Hartman, S.J., Marinac, C.R., **Bellettiere, J.**, Godbole. S., Natarajan, L., Patterson. R.E., Kerr, J. (2017). Objectively measured sedentary behavior and quality of life among survivors of early stage breast cancer. *Supportive Care in Cancer.* 25, 2495-2503.

7. LaCroix, A.Z., Rillamas-Sun, E., Buchner, D., Evenson, K.R., Di, C., Lee, I., Marshall, S., LaMonte, M.J., Hunt, J., Tinker, L.F., Stefanick M., Lewis, C.E., **Bellettiere, J.**, Herring, A.H. (2017). The Objective Physical Activity and Cardiovascular Disease Health in Older Women (OPACH) study: design and baseline characteristics. *BMC Public Health.*17(1), 192.

8. Baron-Epel, O., Berardi, V., **Bellettiere, J.,** Shalata, W. (2016). The relation between discrimination, sense of coherence and health varies

according to ethnicity; a study among three distinct populations in Israel. *Journal of Immigrant and Minority Health*. (Available online).

9. **Bellettiere, J.**, Kritz-Sliverstein, D., Laughlin, G.A., LaCroix, A.Z., Barrett-Connor, E. (2016). Relation of depressive symptoms with coronary artery calcium determined by electron-beam computed tomography (From the Rancho Bernardo Study). *The American Journal of Cardiology.* 117(3), 325-332.

10. Berardi, V., **Bellettiere, J.,** Nativ, O., Hovell, M.F., Baron-Epel, O. (2016). Fatalism, diabetes management outcomes, and the role of religiosity. *Journal of Religion and Health.* 55(2), 602-617.

11. **Bellettiere, J.**, Carlson, J.A., Rosenberg, D., Singhania, A., Natarajan, L., Berardi, V., LaCroix, A.Z., Sears, D.D., Moran, K., Crist, K., Kerr, J. (2015). Gender and age differences in hourly and daily patterns of sedentary time in older adults living in retirement communities. *PLoS ONE.* 10(8): e0136161*.*

12. Berardi, V., Carretero, R., Klepeis, N., Palacios, A., **Bellettiere, J.**, Hughes, S., Obayashi, S., Hovell, M.F. (2015). Proper orthogonal decomposition methods for air particle time-series in residences: exploring peak clustering by occupant behavior patterns. *Journal of Computational Science.* 11, 102-111.

13. Rosenberg, D.E., **Bellettiere, J.,** Gardiner, P.A., Villarreal, V.N., Crist, K., Kerr, J. (2015). Independent associations between sedentary behaviors and mental, cognitive, physical and functional health among

older, retirement community-dwelling adults. *Journal of Gerontology Series A: Biological Sciences and Medical Sciences,* 71(1), 78-83.

14. Macera, C.A., Cavanaugh, A., **Bellettiere, J**. (2015). State of the art review: physical activity and older adults. *American Journal of Lifestyle Medicine,* 11(1), 42-57.

15. **Bellettiere, J.**, Hughes, S. C., Liles, S., Boman-Davis, M., Klepeis, N. E., Blumberg, E., Mills, J., Berardi, V., Obayashi, S., Allen, T. T., Hovell, M. F. (2014). Developing and selecting auditory warnings for a real-time behavioral intervention. *American Journal of Public Health Research*, 2(6), 232-238.

16. Klepeis, N. E., Hughes, S. C., Edwards, R. D., Allen, T., Johnson, M., Chowdhury, Z., Smith K. R., Boman-Davis, M., **Bellettiere, J.**, Hovell, M. F. (2013). Promoting smoke-free homes: a novel behavioral intervention using Real-time audio-visual feedback on airborne particle levels. *PLoS ONE, 8*(8), e73251.

17. Chuang, E., Wells, R., **Bellettiere, J.**, Cross, T. P. (2013). Identifying the substance abuse treatment needs of caregivers involved with child welfare. *Journal of Substance Abuse Treatment.* 45(1), 118-25.

Abstract of Dissertation

Sedentary behavior and risk for cardiovascular disease and diabetes

by

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Doctor of Philosophy in Public Health (Epidemiology)

University of California, San Diego, 2017

San Diego State University, 2017

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Background: Sedentary behavior has been associated with increased risk of
diabetes and cardiovascular disease (CVD), but most studies rely on self-reported
sedentary behavior measures, which are subject to reporting bias. Furthermore, there
is little evidence on these associations in adults over 80 years. The way sedentary
time is accumulated (i.e., sedentary accumulation patterns) has shown acute effects
on glucose and lipid metabolism in laboratory studies, but little evidence exists on
how accumulation patterns relate to diabetes risk factors among adults and there are

no previous studies on how accumulation patterns are related to diabetes risk in older adults.

Methods: This dissertation is composed of four separate studies. Data for Chapters 2 through 4 were from the Objective Physical Activity and Cardiovascular Health Study (OPACH), a cohort of older women (n=6489; average age = 79±7) that wore ActiGraph GT3X+ accelerometers around their hip for up to 7 days between 2012-2014 and were followed for incident CVD and diabetes through September 30, 2016. Data for Chapter 5 were from the 2011-2012 wave of the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab), a national, population-based cohort (n=739; average age 58±10 years) established to study the distribution and determinants of diabetes.

Results: Chapter 2 results suggest that women in the highest (≥ ~11 hr/day) quartile of sedentary time had higher risk for CVD events (hazard ratio (HR)=1.44; 95% confidence intervals (CI)=1.05-1.98) and coronary heart disease (CHD) events (HR=2.19; 95% CI=1.09-4.40) than women in the lowest quartile of sedentary time (≤ ~9 hr/day) with a linear dose-response relation (P-linear<.05; P-nonlinear>.05 | all). Results from Chapters 3 and 4 reveal that women in the highest quartile of sedentary time had higher odds of prevalent diabetes (odds ratio (OR) = 1.96; CI, 1.59-2.42) than women in the lowest quartile, after adjustment for covariates. Those that accumulated sedentary time with the most prolonged accumulation patterns (i.e., many long bouts of sedentary time with few short bouts and few interruptions) had higher odds of prevalent diabetes than women with the most interrupted patterns, though the ORs were weaker than for total sedentary time. Due to non-proportional hazards by family history of diabetes (FH+/-), models of diabetes incidence were

stratified by FH. FH+ women in the highest quartile of sedentary time had higher risk for diabetes (HR=2.38; 95% CI=1.05-1.98) than women in the lowest quartile. Women with the most prolonged sedentary accumulation patterns had higher risk for new-onset diabetes (HR=2.32; 95% CI=1.15-4.71) than women with the most interrupted patterns. No significant associations were observed for FH- women. Results from Chapter 5 indicate that accumulation patterns of frequently interrupted sitting (compared to patterns with relatively more prolonged sitting) were significantly beneficially associated with BMI, waist circumference, HDL cholesterol, triglycerides, 2-hour post-load plasma glucose levels (PLG), and fasting plasma glucose levels. Significant interactions (p<0.05) showed that associations of sitting time with HDL, triglycerides and PLG became more deleterious with longer usual bout durations indicating a joint relationship between sedentary behavior and sedentary accumulation patterns.

Conclusion: High levels of sedentary time were associated with increased risk for CVD and diabetes in older women. Furthermore, prolonged sedentary accumulation patterns were associated with increased diabetes risk in older FH+ women and were deleteriously associated with several cardio-metabolic biomarkers in Australian adults. Accumulation patterns interacting with total sitting time in relations with key diabetes-related biomarkers (PLG, HDL, and triglycerides) provides further evidence that the way in which sedentary time is accumulated may be a relevant factor in diabetes etiology, in addition to the total amount of time sedentary.

Chapter 1: Introduction

Introduction

Sedentary behavior is a class of behaviors that result in a low energy expenditure (<1.5 metabolic equivalent of task units (METs)) while awake and sitting or reclining.[1] Sedentary behavior was first identified as a potential cardiovascular disease (CVD) risk factor when British epidemiologists found higher rates of CVD among office workers and bus drivers (jobs that required more sitting) when compared to postal workers and bus conductors (active jobs).[2] Nine years later, American physicians published similar findings showing clerks (whose jobs included more sedentary behavior) had higher death rates than did railroad switchmen and section men, jobs that require higher levels of physical activity (PA).[3] Two laboratory studies followed that showed how low levels of physical activity were associated with changes in total cholesterol[4] and insulin sensitivity.[4,5] At the time, the interpretation of these studies focused on inadequate physical activity. We are now reconsidering such studies in light of the sitting time involved.

In studying the mechanisms through which sedentary behavior affects cardio-metabolic health, evidence emerged that the way in which sedentary behavior was accumulated (i.e., sedentary accumulation patterns) had important consequences for cardio-metabolic health. Experimental studies showed that sitting for long "unbroken" periods compared to regularly interrupting sitting bouts with standing,[6,7] walking,[7–9] or simple resistance exercises,[10] were related to short-term detrimental effects on postprandial glucose metabolism, insulin, C-peptide, triglyceride responses[10], and resting blood pressure;[11] with the short-term effects on postprandial glycaemia being consistently replicated.[12] Epidemiologic studies have examined sedentary

accumulation patterns using primarily two measures, (1) the number of "breaks" in sedentary time per day and (2) the amount of time spent in "long" sedentary bouts. Figure 1.2 displays the human movement spectrum over time; red regions are bouts of sedentary behavior, green regions are bouts of light intensity physical activity (LIPA), and blue regions are bouts of moderate to vigorous physical activity (MVPA). Transitions from sedentary behavior to either LIPA or MVPA are known as breaks in sedentary time, which can be conceptualized as a measure of the frequency with which sedentary behavior occurs.[13]

More breaks per day have been beneficially associated with measures of obesity[12] and measures of glucose and lipid metablism.[14] Time spent in "long" sedentary bouts (≥10 minutes[15], ≥20 minutes[16], and ≥30 minutes[15,17,18]) have shown detrimental cross-sectional associations with HDL-cholesterol and triglycerides and consistent relations with BMI and waist circumference. By contrast, time spent in "short" bouts (≤ 5 minutes[15] and ≤ 30 minutes[18]) have shown beneficial cross-sectional associations with adults' BMI, waist circumference, and HDL-cholesterol levels. Since long and short sedentary bouts are differentially associated with similar cardiometabolic health outcomes (i.e., waist circumference, BMI, HDL cholesterol), focusing only on time spent in longer bouts does not take into account the entire sedentary accumulation pattern.

Two metrics (alpha and usual bout duration) have been developed to take into account time spent in both long and short sedentary bouts, thus measuring the full spectrum of sedentary accumulation patterns.[19] Both metrics are consistent with the underlying power-law distribution[19] of sedentary bout durations (see Figure 1.2). The first metric, alpha, characterizes the shape of the frequency distribution thereby taking

into account both the number of sedentary bouts and their respective durations. The second metric, usual bout duration, indicates the bout duration above which half (50%) of sedentary time is accumulated. Both metrics have been used to examine changes in behavioral interventions,[13,20] but have not yet been tested in associations with adults' cardiometabolic health.

Alpha (α) is a unitless parameter that characterizes the frequency distribution of sedentary bouts (see Figure 1.3). A high alpha is thought to be favorable (compared to a low alpha) because it indicates that sedentary behavior is accumulated in more short bouts and fewer long bouts. Because alpha indicates the slope of the frequency distribution of sedentary bouts, it describes the relative frequency of long vs. short bouts for a given person. Alpha is computed using maximum likelihood estimation by fitting data (i.e., bout lengths for all valid days for each person) to the following equation

$$\alpha = 1 + n \left[\sum_{i=1}^{n} ln \frac{\varphi_i}{\varphi_{min}} \right]^{-1},$$

where φ is the duration of each bout and φ_{min} is the smallest observed bout duration for each person.

The usual bout duration is the median value of the cumulative sedentary bout duration distribution. It provides information about the bout duration in which half of all sedentary time is accumulated. Non-linear regression (the Levenberg-Marquardt algorithm) models are used to fit data (i.e., bout lengths for all valid days for each person) to the following sigmoid:

$$y(\varphi) = \frac{\varphi^n}{\varphi^n + X_{50}^n},$$

where φ is each bout duration, y is the cumulative proportion of sitting time in bouts $\leq$ φ, X_{50} is the usual bout duration, and n is a free parameter.

The four chapters that follow will add to existing literature by examining objectively measured sedentary behavior and incidence rates of two of the world's most debilitating chronic diseases, cardiovascular disease (CVD) and diabetes. Furthermore, sedentary accumulation patterns and diabetes incidence will be investigated for the first time. The studies that follow employ published metrics of sedentary accumulation patterns,[13,19–24] that have never been tested in association with cardio-metabolic health. The first three chapters focus on a cohort (n=6,489) of older women (average age 79±7) that wore ActiGraph GT3X+ accelerometers for up to 7 days and were followed for new diabetes and CVD events.[25] The fifth chapter investigates sedentary accumulation patterns in relation to cardio-metabolic risk biomarkers in Australian adults (average age 58±10) who wore activPAL accelerometers for up to 7 days and underwent an oral glucose tolerance test which included a fasting blood draw.
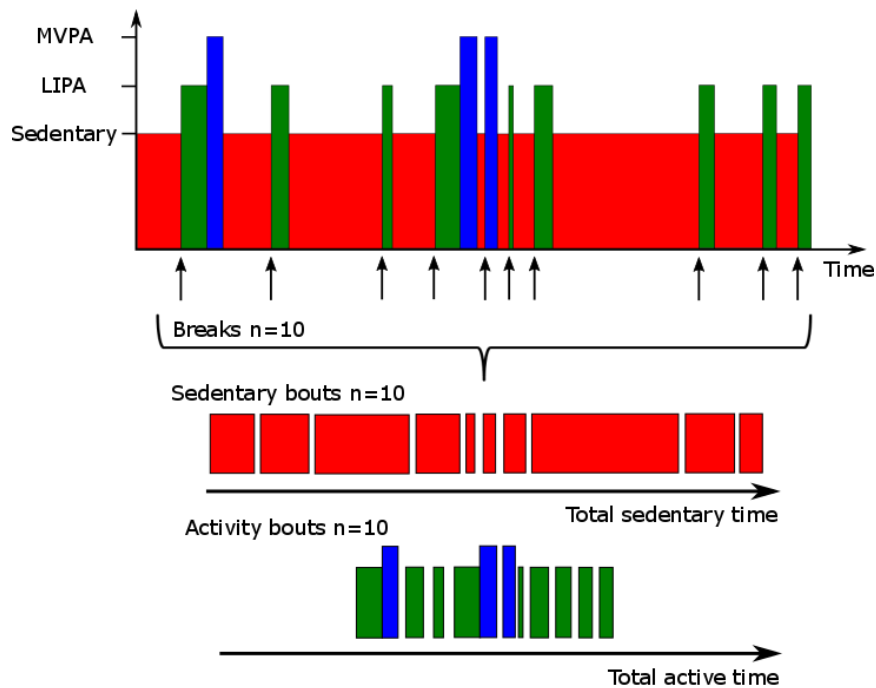
Figure 1.1 Human movement spectrum over time. *Note: Arrows indicate "breaks" in SB. This figure was adapted from work by Chastin et al. (2015).*[13]
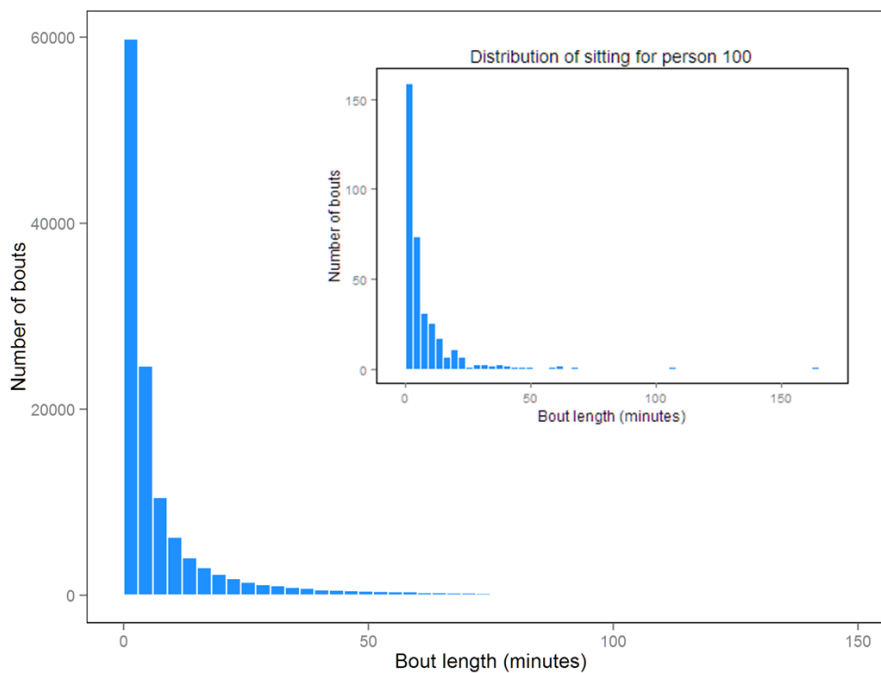
Figure 1.2 Population-level and individual-level (for person #100) frequency distribution of SB bouts, measured using hip-worn ActiGraph GT3X+ accelerometers in 307 older men and women (mean age 84) who participated in Jacqueline Kerr's MIPARC intervention to increase physical activity in retirement community-dwelling adults.[26]
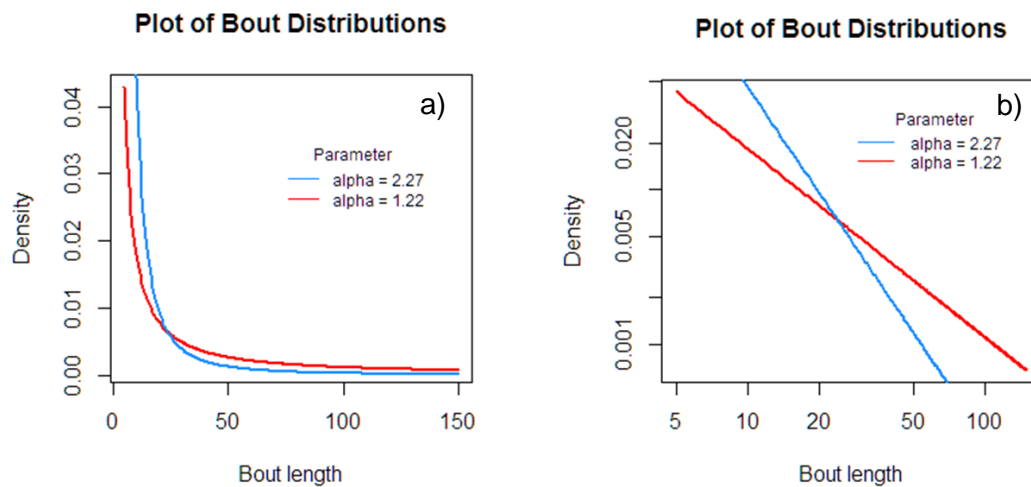
Figure 1.3 Hypothetical frequency distributions of SB bout durations for persons with high and low alpha. Panel a plots distributions on a standard xy plane while panel b plots the same distributions in log-log space.

Chapter 2: Sedentary time and incidence of cardiovascular disease in older women: The Objective Physical Activity and Cardiovascular Health (OPACH) Study

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Background: Prior evidence examining sedentary time and incident cardiovascular disease (CVD) relies mainly on self-reported measures which are known to be inaccurate, especially in older adults. Few studies have investigated accelerometer-measured sedentary behavior and CVD incidence in older adults.

Methods: Women aged 63-97 (n=5638, mean age=79±7) with no history of myocardial infarction or stroke wore accelerometers for 4-7 days and were followed for 2-4 years for incident CVD events. Hazard ratios (HR) for CVD and coronary heart disease (CHD) events comparing the upper three quartiles of accelerometer-measured sedentary time to the lowest quartile were estimated using Cox proportional hazard models first adjusting for 9 confounders, then adding possible mediators body mass index (BMI), diabetes, hypertension, and separately, CVD-risk biomarkers (systolic blood pressure, fasting-glucose, HDL-cholesterol, and triglycerides). Two self-report and 2 accelerometer measures of moderate-to-vigorous physical activity (MVPA) were added separately to cofounder-adjusted models. Accelerometer-measured MVPA, age, ethnicity, BMI, and physical functioning were tested as effect modifiers. Restricted cubic splines tested for nonlinear dose-response relations.

Results: There were 447 and 119 incident CVD and CHD cases, respectively. Women in the highest (≥ ~11 hr/day) vs. the lowest (≤ ~9 hr/day) quartile of sedentary time had higher risk for CVD events (HR=1.44; 95% CI=1.05-1.98) and CHD events (HR=2.19; 95% CI=1.09-4.40). The dose-response relation between sedentary time and both outcomes was linear (P-linear<.05; P-nonlinear>.05 | all). Adjustment for CVD-risk biomarkers attenuated HRs but a significant linear association persisted for

CHD (p= .02). Accelerometer-measured MVPA, highly correlated with sedentary time (r=-.72), attenuated HRs leading to loss of statistical significance after 2 of 4 adjustments (p-values: CVD=.05 & .04, CHD=.04 & .13). HRs were independent of self-reported MVPA (p<.01 | all). No effect modification was observed by MVPA or any other variable tested.

Conclusions: Higher sedentary time increased the risk for CHD in older women in a linear dose-response manner independent of several known CVD-risk factors.

INTRODUCTION

Throughout the 20th century, cardiovascular disease (CVD) killed more Americans than any other disease, currently causing one in three deaths annually.[27] Incidence rates of CVD increase with age and are highest for adults ≥85 years.[28] Incidence rates of coronary heart disease (CHD), responsible for 1 in 7 deaths annually, also increases with age, with the highest rates among adults in the highest age groups.[27]

Significant evidence has amassed that physical inactivity,[29] often defined as failure to meet physical activity guidelines, is a major risk factor for CVD.[30] Despite the known health benefits of moderate to vigorous physical activity (MVPA), guideline recommendations are achieved by few adults, and even fewer older adults.[27,31] In recent years, high levels of sedentary behaviors – which are low energy expenditure activities done while seated or reclining and awake[1] – have been shown to increase CVD risk, often independent of MVPA levels.[32,33] A recent meta-analysis, based only on self-reported measures of sedentary behavior, found an 8% increased risk of CVD associated with each additional hour spent sedentary.[34] Authors of the meta-analysis also reported that the dose response relationship between sedentary behavior and CVD risk was nonlinear with elevated risk only among adults with >10 hours/day of sitting time.[34] Considering correlations between self-report and accelerometer-measured sedentary behavior are low[35] and some self-reports only capture certain behaviors like TV watching, studies with better measurement are needed. Especially for estimates of dose-response relations since they estimate the effect of an *exposure* over increasing levels of the *exposure*, thereby relying on the exposure measure twice.

While several mostly cross-sectional studies have shown that accelerometer-measured sedentary time is associated with adverse levels of CVD risk biomarkers,[14,36,37] no evidence of relations with incident CVD events has been reported. Obtaining evidence using accelerometer-measured sedentary behavior measures is a primary research objective of the American Heart Association[38] and the National Institutes of Health (NIH)[39]. Furthermore, nearly all of the existing evidence was established among predominantly white cohorts of young-to-middle aged adults, making the inclusion of ethnically diverse and America's oldest adults an important advancement to characterize the population-level burden of CVD morbidity and mortality in relation to sedentary behavior.[39]

The objective of this study is to examine associations of accelerometer-measured sedentary time with incident CHD and CVD in an ethnically diverse sample of older adults with no prior history of myocardial infarction (MI) or stroke.

METHODS

Study Participants

Between 2012 and 2014, 7058 ambulatory community-dwelling women aged 63 and older were enrolled in the Objectively Measured Physical Activity and Cardiovascular Health Study (OPACH). All participants were initially recruited from 40 clinical sites throughout the US beginning in 1993 as part of the Women's Health Initiative (WHI). More details on the OPACH study population are published elsewhere.[40]

Upon enrollment, participants were distributed ActiGraph GT3X+ accelerometers to wear 24 hours per day on an elastic band over their right hip for a

requested 7 days, removing devices when they were likely to get wet (e.g., showering or swimming). Participants self-reported in-bed and out-of-bed times using sleep logs on days when the accelerometer was worn.

Of the 6489 women who wore accelerometers, 6133 met the recommended data processing criteria for estimating average daily sedentary time among older adults (i.e., ≥ 10 waking hours on ≥ 4 days per week).[41] After excluding data from 495 women who had an MI or stroke before OPACH baseline, data from 5638 women were available for the present study. The protocol for this study was approved by the Fred Hutchinson Cancer Research Center IRB and all women provided informed consent either in writing or by telephone.

Coronary heart disease and total cardiovascular events

Outcomes for this study included incident CHD events (myocardial infarction or coronary death) and incident CVD events (CHD, revascularization, angina, congestive heart failure, stroke, or death from other cardiovascular disease) which are described in detail elsewhere.[40] At WHI baseline and each subsequent year, standardized medical updates were collected from participants. All reported CVD-related events were ascertained and adjudicated by WHI physicians through a review of medical records.[42] Among WHI adjudicators, inter-rater agreement on a range of CVD-related outcomes was excellent to almost perfect with Kappas ranging from .67 to .94.[43] Women with CHD and CVD events that occurred after OPACH baseline through September 30, 2016 were considered incident cases.

Sedentary time

ActiGraph GT3X+ accelerometers measured acceleration 30 times per second. The resulting data were converted to 1-minute epochs using the low-frequency filter supplied with Actilife version 6.[44] Periods of accelerometer non-wear were removed from data by the commonly-used Choi algorithm applied to the vector magnitude acceleration counts.[45] Then self-reported in-bed and out-of-bed times were used to remove times during which the participant was asleep. Missing bed times were imputed using person-specific averages, when available, or the OPACH population average otherwise (i.e., in-bed =10:45 pm; out-of-bed = 7:22 am).

Sedentary behavior was detected from accelerometer data based on whether each minute had sufficiently low levels of movement (counts per minute of 99 or lower) as measured by the vertical axis on the accelerometer, the most commonly used[41] measure of sedentary time. [46,47] Total sedentary time was computed for each participant by summing the number sedentary minutes for each adherent day (adherent day = waking wear time >10 hours) and dividing by the number of adherent days.

Covariates

At WHI baseline, age, race/ethnicity, education, and family history of MI were measured by questionnaire. At or near the OPACH baseline, self-reported health (measured on a likert scale from 1=excellent to 5=poor), physical functioning (using 10 items from the Rand-36), alcohol consumption, and current smoking were measured by questionnaire nearest to the OPACH baseline. The number of chronic health conditions (cancer, cardiovascular disease; cerebrovascular disease; cognitive impairment; constructive obstructive pulmonary disease; depression; and osteoarthritis) reported at or before OPACH baseline was categorized as 0, 1, or ≥2

to account for multimorbidity.[48] Prevalent diabetes[49] and hypertension at OPACH baseline were measured using self-reports of physician diagnoses over the entire WHI follow-up period. A subset of participants (n=4458) received in-home visits at or near the OPACH baseline as part of the WHI Long Life Study.[40] At those visits, height and weight were measured using a tape measure and calibrated bathroom scale, respectively. BMI was computed from these measures as weight in kg divided by height in meters squared. Fasting serum levels of glucose, insulin, creatinine, C-reactive protein, high- and low-density lipoprotein cholesterol, triglyceride, and total cholesterol were quantified at the University of Minnesota from fasting blood samples collected at the visit.

Moderate to vigorous physical activity (MVPA) was measured using four validated methods: $MVPA_{MATTHEWS}$ = mean minutes per day with vertical-axis accelerometer counts per minute ≥760 over all adherent days;[50] $MVPA_{OPACH}$ = mean minutes per day with ≥519 vector-magnitude accelerometer counts per 15-second (a measure calibrated for OPACH women);[51] $MVPA_{WHI}$ = metabolic equivalent of task (MET) minutes per week from moderate to strenuous activities (including walking) measured by self-report using the WHI physical activity questionnaire;[52] and $MVPA_{CHAMPS}$ = MET-minutes per week spent in moderate intensity exercise activities as measured by self-report using the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.[53]

Statistical Analysis

To account for differences in time spent wearing accelerometers while awake, total sedentary time was adjusted for waking wear time using the residuals method (i.e., regress awake wear time on total sedentary time to get the residuals and

predicted total sedentary time values, then add the residual value from each participant to the population average of predicted values), then stratified into quartiles.[54]

Socio-demographic and health-related characteristics were described using means and standard deviations (continuous variables) or column percentages (categorical variables) across quartiles of total sedentary time. Differences across quartiles were tested using F-tests and Pearson's chi-square tests for continuous and categorical variables, respectively.

Hazard ratios (HR) for CVD and CHD events comparing the upper three quartiles of sedentary time to the lowest quartile were estimated using multivariable Cox proportional hazard models. Time to event was calculated as the number of days from OPACH baseline to either the first event (CHD or CVD, depending on the outcome), death unrelated to the outcome (censored outcome), or the last available medical update (censored outcome). For each outcome, four progressively adjusted Cox models were examined: Model 1 was adjusted for age and ethnicity while Model 2 was additionally adjusted for potential confounders (education level, family history of MI, self-reported health status, physical functioning, alcohol consumption, and smoking status). CVD risk factors thought to be in the causal pathway between sedentary time and incident cardiovascular disease were investigated as possible mediators in models 3a and 3b. Model 3a added history of hypertension, history of diabetes, and BMI to Model 2 while Model 3b added measures of baseline serum fasting glucose, triglycerides, HDL-cholesterol, and systolic blood pressure to Model 2. We report adjusted hazard ratios (aHRs), 95% confidence intervals, and p-values from tests of linear trend, which were computed by re-running Cox models while

treating total sedentary time as continuous. Proportional hazards assumptions were assessed using tests based on Schoenfeld residuals[55] that were confirmed using plots of the scaled Schoenfeld residuals over time. No variables violated the assumption.

There is no consensus about whether MVPA should be treated as a confounder, a causal intermediary, or an effect modifier of the association between sedentary behavior and cardio-metabolic health.[56,57] Furthermore, accelerometer-measures of MVPA were highly correlated with total sedentary time among OPACH participants making results from models employing mutual adjustment difficult to interpret. Thus, to be comprehensive, we examined four measures of MVPA ($MVPA_{MATTHEWS}$, $MVPA_{OPACH}$, $MVPA_{WHI}$, and $MVPA_{CHAMPS}$) as potential confounders in separate proportional hazards models. We also tested for effect modification of associations of CVD and CHD with total sedentary time by $MVPA_{MATTHEWS}$ and $MVPA_{OPACH}$, as well as with age, race/ethnicity, physical functioning, and BMI by including interaction terms (effect modifier*total sedentary time) in confounder-adjusted proportional hazards models.

The dose-response relations between the continuous variable total sedentary time and incident CVD and CHD events were examined using 2 steps. First, we tested the dose-response trajectory for nonlinearity by running confounder-adjusted Cox regression models using restricted cubic spline functions of total sedentary time (included in continuous form) implemented using the Regression Modeling Strategies (rms) package[58] in r. To test whether the shapes of dose-response trajectories were sensitive to the number of number of knots used, we ran models separately with 3, 4, and 5 knots placed at the default locations. Plots of the dose-response trajectories

were reviewed for each outcome after each run and chi-squared tests for nonlinearity performed. After determining the most appropriate functional form of the dose-response trajectories, we plotted them for each outcome specifying the 10th percentile of the sedentary time distribution as the referent category to enhance interpretability of the plots.[59]

To explore the possibility of reverse-causation, all CVD and CHD cases that occurred within 6 months after OPACH baseline were removed and confounder-adjusted proportional hazards models were rerun.

All statistical tests were two-tailed with p ≤ .05 considered significant. All analyses were conducted within the R environment (R Foundation for Statistical Computing; Vienna, Austria).

RESULTS

Over 3.1±0.8 years of follow-up, 447 women reported an incident CVD event and over 3.3±0.7 years, 119 incident CHD events were reported. Nearly all socio-economic and health-related characteristics were associated with total sedentary time (except smoking status and parental history of MI), with women in quartile 4 being the oldest, disproportionately White, having the highest BMI, and often having the most unfavorable cardio-metabolic biomarkers (i.e., systolic blood pressure, fasting plasma glucose, HDL-cholesterol, and triglycerides; Table 2.1).

Incident CVD events were associated with total sedentary time with crude incidence rates per 100 person-years of 14.4, 21.2, 29.4, and 39.0 in quartiles 1, 2, 3, and 4, respectively (Table 2.2). Controlling for potential confounders, women in the quartile 4 had 44% higher risk for incident CVD events than women in quartile 1

(aHR=1.44; 95% CI=1.05-1.98; p-trend =.007). Hazard ratios were attenuated with adjustment for potential mediators, and the linear trend was no longer significant after simultaneously adjusting for fasting plasma glucose, HDL-cholesterol, triglycerides, and systolic blood pressure (p=0.141).

Incident CHD events also increased across increasing quartiles of total sedentary time with crude incidence rates of 2.6, 5.4, 6.5, and 12.6 per 100 person-years, respectively. Risk for incident CHD events was more than two times higher for women in quartile 4 than women in quartile 1 (aHR=2.19; 95% CI=1.09-4.40; p-trend =.003) after adjustment for potential confounders. Adding potential mediators attenuated aHRs slightly, though significant linear trends persisted (p-value =.02).

MVPA$_{MATTHEWS}$ and MVPA$_{OPACH}$ were correlated with total sedentary time with Pearson's r=-.79 and -.72, respectively (Supplemental Table 2.1). Adding measures of MVPA to confounder-adjusted models of incident CVD events, aHRs comparing women in quartile 4 to those in quartile 1 ranged from 1.37 (95% CI=0.90-2.07) to 1.53 (95% CI=1.11-2.12) and were generally higher when MVPA was measured by self-report and lower when measured by accelerometer (Table 2.3). Statistically significant linear trends were observed for all associations except when using MVPA$_{MATTHEWS}$ (p=.05). Inclusion of MVPA to confounder-adjusted models of incident CHD events generally lowered aHRs, with significant linear trends persisting for all measure of MVPA except when using MVPA$_{OPACH}$ (p=.13). After adding MVPA, quartile 4 (compared to quartile 1) aHRs for incident CHD events ranged from 1.39 (95% CI=0.63-3.08) to 2.21 (95% CI=1.09-4.48). Furthermore, the increased risks of incident CVD and CHD associated with higher levels of sedentary time did not differ between women with high and low MVPA$_{MATTHEWS}$ levels (interaction p-values:

CVD=.46 and CHD=.92) and MVPA$_{OPACH}$ levels (interaction p-values: CVD=.53 and CHD=.95; Figure 2.1).

No statistically significant interactions were observed by age, BMI, physical functioning, or race/ethnicity (interaction p-values ≥ .08; Figure 2.1). However, increased risk for CVD events associated with higher sedentary time tended toward being lower for Hispanic women (than Black and White women; interaction p-value=.79) and, similarly for CHD events, tended toward being lower for women <80 years (compared to women ≥80 years; interaction p-value=.11).

Dose-response trajectories were not meaningfully different between models with 3, 4, or 5 knots so chi-squared tests were performed for restricted cubic spline models with 3 knots to maximize statistical power. Chi-square tests indicated the most appropriate functional form of the dose-response trajectories for incident CVD events (P-linear=.02; P-nonlinear=.56) and incident CHD events (P-linear=.01; P-nonlinear=.85) were linear. Trajectories were therefore plotted using multivariable Cox *linear* regression models (Figure 2.2). Each 1 hour increase in sedentary time was associated with a 1.11 times (95% CI; 1.03-1.19) increase in risk for CVD events and a 1.26 times (95% CI; 1.08-1.46) increase in risk for CHD events (Figure 2.1). As shown by the 95% confidence intervals which did not include 1, compared to women with 8 hours/day of sedentary time, there is significantly higher risk of CVD and CHD events for sedentary times above 8 hours/day and significantly lower risk for sedentary times below 8 hours/day.

Sensitivity analyses conducted after removing events that occurred within the first six months of follow-up did not appreciably change the results.

DISCUSSION

In this ethnically diverse cohort study of older community-dwelling women, nearly half of whom were over age 80, we found a linear dose-response relation in that each additional hour of total sedentary time, on average, was associated with an 11% increase in risk for incident CVD events and a 26% increase in the risk for incident CHD events. The associations did not differ for women with high and low MVPA or any other variable that was examined for effect modification. The magnitude of the increased risk for CVD and CHD events was attenuated, though the association for CHD events remained significant, following adjustment for CVD-risk biomarkers, supporting their potential role in the causal pathway and suggesting prospective studies of behavior-biomarker relations may help identify mechanisms through which sedentary behavior impacts cardiovascular health.[36,37,60] Our results were sensitive to statistical adjustment for MVPA (though they remained statistically significant in 6 out of 8 tests), highlighting an interrelation between the activity-related behaviors in relation to CVD and CHD, but suggesting that associations of sedentary time with CVD and CHD are not fully explained or usurped by MVPA.

High sedentary time was associated with a higher risk for CHD events than for CVD events, similar to results from the larger WHI Observational and Extension Study that found women that reported sitting >11 hours per day (compared to women with ≤4 hours per day of sitting time) had higher risk for CHD mortality (HR=1.27) then for CVD mortality (HR=1.13).[61] The aHRs observed in our study were higher than those reported by Seguin et al. (2014) and higher than results from a recent meta-analysis that included data from 10 studies, all of which used self-reported sitting time.[34] Lower effects reported in most previous studies could reflect an attenuation of effect estimates resulting from the measurement error inherent in self-

reported total sitting time.[35,62–69] Differences could also indicate that there is higher

risk of CHD and CVD events attributed to sedentary time among older adults (none of

the studies in the meta-analysis included a large proportion of adults over the age of

80); a hypothesis supported by the higher CHD risk observed in our study for women

≥ 80 but requiring replication, especially since similar results were not observed for

CVD risk.

Higher levels of accelerometer-measured sedentary behavior was related to

CVD mortality among 3809 US adults over 40 (average age 53 years) and among

2918 US men aged 79±5 with similar magnitudes as those observed in our study for

CVD events, however the associations reported in both studies were not statistically

significant.[70,71] CVD mortality reflects cause of death and does not capture all incident

events that occur prior to death. The present study is novel for investigating the

important clinical outcome of incident CVD, which could account for the different

findings. Additionally, both studies of CVD mortality had notably smaller sample sizes

potentially limiting statistical power, and the larger study[70] included sedentary time,

low intensity physical activity, and MVPA in their fully adjusted models, potentially

leading to over adjustment. A separate cross-sectional study among 1477 adults

(aged 62±11 years) from England used an isotemporal substitution approach to

statistically model how the association of CVD is affected by replacing sedentary time

with MVPA and light intensity physical activity.[72] Replacing one hour of sedentary

time with 1 hour of MVPA or low intensity physical activity, respectively was

associated with 50% and 17% lower odds of CVD. These results suggest that

reductions in sedentary time may protect against CVD and the analytic approach

highlights a temporal interdependence between MVPA and sedentary behavior in

relation to CVD.[72] In the present study, sedentary time and MVPA were highly correlated when both were measured by accelerometry, likely explaining the attenuated aHRs observed when mutually adjusting for sedentary behavior and accelerometer-measured MVPA. While the attenuation led to loss of statistical significance on two occasions, the direction and magnitude of aHRs always indicated higher risk associated with higher levels of sedentary time. This higher risk for CVD and CHD events was entirely independent of self-reported MVPA. Furthermore, tests for effect modification showed that women with high and low accelerometer-measured MVPA had similar aHRs for incident CVD and CHD events. The totality of this evidence suggests that in our study of older women without a history of MI and stroke, sedentary time increased the risk for CVD and CHD in ways that are separate from the beneficial effects of MVPA, suggesting the two behavior classes may represent separate, but related, risk factors.

We observed linear dose-response relations of sedentary time with incident CVD and CHD events whereby women across the full spectrum of the sedentary time distribution were at higher risk for CVD and CHD with higher levels of sedentary time. In contrast, results from a recent meta-analysis indicated a nonlinear dose-response relation whereby higher CVD risk in relation to higher sitting times was not observed among adults with <10 hours per day of sitting.[34] These differing results could be attributed age as the average age of women in OPACH was more than 15 years older than mean ages in all studies used in the meta-analysis. Differences could also be attributed to the measurement error inherent in self-reported sitting time measures that were used in all studies included in the meta-analysis.[35,62–69]

One of the proposed mechanisms through which high levels of sedentary behavior increases risk for CVD events is through traditional CVD risk factors such as BMI, lipid metabolism, and glucose metabolism.[14,73] Results from our study, i.e., the attenuated aHRs observed when adjusting for several traditional CVD risk factors, support that hypothesis. It has also been shown that prolonged sedentary behavior directly and indirectly impairs vascular structure and vascular functioning, and is associated with inflammation and oxidative stress; which, taken together suggest additional mechanisms through which high levels of sedentary behavior can increase risk for atherosclerotic CVD.[74]

This was the first prospective study of sedentary behavior in relation to incident CVD events among older adults using objective measures of sedentary time. Other noteworthy strengths include the race/ethnic diversity of women who had a wide range of physical and functional health characteristics. Nearly 50% of our population were over the age of 80, which is a growing segment of the American population who are at highest risk for CVD events and for sedentary behavior.[27,47] Since all women in our study were participants of the initial WHI studies, we were able to use up to 24 years of physician-adjudicated medical histories to help ensure our sample were without a prior history of MI or stroke and to control for multimorbidity. Our large sample size and well characterized cohort enabled us to consider sixteen variables as potential confounders or mediators including physical function which has not typically been examined in past studies. We also conducted a thorough investigation of MVPA using four different measures that included both adjusted and stratified analyses.

Some limitations are worth noting. While accelerometers were used to objectively measure sedentary behavior, devices were worn over the right hip and the resulting data were processed using commonly used techniques, precluding the accurate detection of posture[75] – a key component of the sedentary behavior definition.[1] As a result, standing still could be misclassified as sedentary time. Sedentary behavior was measured during a seven-day period, which has been shown to be a reliable measure 2-to-3 year behavior patterns, but may not fully capture usual sedentary time in all women.[76] Future studies should consider longer measurement periods, if feasible. Finally, this study was conducted among a cohort of women and it is unknown whether these findings can be generalized to older men. Replication in prospective studies of older men is needed, as are studies investigating gender differences.

In conclusion, our study shows that sedentary time is linearly related in a dose-response manner to CVD in older women, with those who accumulate the most sedentary time having a two-fold increased risk of CHD events compared to women with the lowest sedentary time, independent of traditional CVD risk factors. Sedentary behavior guidelines in several industrialized countries call for an overall reduction in sedentary time.[77,78] The results of this study support further consideration by public health entities in the United States of guidelines to reduce sedentary time. Evidence is needed from intervention trials to inform specific guidelines and to inform ecological interventions in an effort to reduce the public health burden of CVD in our growing population of older women and men.

Chapter 2, in full, is currently being prepared for submission for publication of the material. Bellettiere, John; LaMonte, Michael J.; Di, Chongzhi ; Kerr, Jacqueline;

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author of this material.

Table 2.1 Baseline Socio-demographic and health-related characteristics, by quartile of daily sedentary time (n=5638); OPACH (2012-2014).

Characteristics	Total	Total Sedentary Time Quartiles[a]				p
		1 (low)	2	3	4 (high)	
Age, mean (sd)	78.5 (6.7)	75.9 (6.2)	77.3 (6.5)	79.3 (6.4)	81.7 (6.2)	**<.001**
Age category, n (%)						**<.001**
60-<70 years	602 (10.7)	254 (18.0)	183 (13.0)	115 (8.2)	50 (3.5)	
70-<80 years	2,285 (40.5)	704 (49.9)	636 (45.1)	549 (39.0)	396 (28.1)	
80-<90 years	2,523 (44.7)	430 (30.5)	559 (39.7)	686 (48.7)	848 (60.1)	
≥ 90 years	228 (4.0)	22 (1.6)	31 (2.2)	59 (4.2)	116 (8.2)	
Race/ethnicity, n (%)						**<.001**
White	2,773 (49.2)	539 (38.2)	620 (44.0)	724 (51.4)	890 (63.1)	
Black	1,880 (33.3)	504 (35.7)	512 (36.3)	472 (33.5)	392 (27.8)	
Hispanic	985 (17.5)	367 (26.0)	277 (19.7)	213 (15.1)	128 (9.1)	
Highest education level, n (%)						**<.001**
High school/GED or less	1,131 (20.2)	317 (22.5)	285 (20.4)	271 (19.4)	258 (18.4)	
Some college	2,156 (38.5)	530 (37.6)	517 (37.0)	532 (38.1)	577 (41.2)	
College graduate or more	2,314 (41.3)	562 (39.9)	595 (42.6)	592 (42.4)	565 (40.4)	
Smoke now (yes), n (%)	137 (2.4)	23 (1.6)	35 (2.5)	37 (2.6)	42 (3.0)	.12
BMI; kg/m², mean(sd)	28.1 (5.7)	27.1 (5.2)	28.1 (5.6)	28.1 (5.7)	29.0 (6.1)	**<.001**
Obese, n (%)	1,641 (31.1)	325 (24.3)	413 (31.1)	423 (31.8)	480 (37.2)	**<.001**
Self-rated health, n (%)						**<.001**
Excellent or very good	2,926 (52.1)	872 (62.2)	761 (54.2)	729 (51.8)	564 (40.1)	
Good	2,191 (39.0)	450 (32.1)	528 (37.6)	572 (40.7)	641 (45.6)	
Poor or very poor	501 (8.9)	81 (5.8)	115 (8.2)	105 (7.5)	200 (14.2)	
Physical functioning, mean (sd)	69.9 (25.5)	80.2 (20.3)	74.0 (23.3)	69.3 (24.6)	56.2 (27.1)	**<.001**
Has parental history of MI, n (%)	512 (9.2)	117 (8.4)	116 (8.3)	139 (10.0)	140 (10.1)	.19
Has history of diabetes, n (%)	1,096 (19.4)	197 (14.0)	298 (21.1)	248 (17.6)	353 (25.0)	**<.001**
Has history of hypertension, n (%)	3,978 (70.6)	874 (62.0)	977 (69.3)	1,039 (73.7)	1,088 (77.2)	**<.001**
Number of chronic conditions[a], n (%)						**<.001**
Zero	1,823 (32.3)	515 (36.5)	486 (34.5)	434 (30.8)	388 (27.5)	
One	2,571 (45.6)	656 (46.5)	637 (45.2)	664 (47.1)	614 (43.5)	
Two or more	1,244 (22.1)	239 (17.0)	286 (20.3)	311 (22.1)	408 (28.9)	

Table 2.1 Baseline Socio-demographic and health-related characteristics, by quartile of daily sedentary time (n=5638); OPACH (2012-2014), Continued.

Characteristics	Total	Total Sedentary Time Quartiles[a]				p
		1 (low)	2	3	4 (high)	
Systolic blood pressure; mmHg, mean (sd)	125.6 (14.1)	123.8 (13.2)	125.4 (13.9)	125.8 (14.2)	127.6 (14.9)	**<.001**
Fasting glucose; mg/dL, mean (sd)	97.8 (26.6)	94.6 (21.5)	97.9 (26.8)	97.6 (26.6)	101.4 (30.5)	**<.001**
HDL cholesterol; mg/dL, mean (sd)	60.7 (14.9)	64.0 (15.5)	60.6 (14.4)	60.5 (14.6)	57.6 (14.3)	**<.001**
LDL cholesterol; mg/dL, mean (sd)	116.7 (34.2)	120.4 (33.0)	116.9 (34.8)	116.3 (33.9)	112.8 (34.7)	**<.001**
Triglycerides; mg/dL, mean (sd)	108.5 (55.8)	99.6 (51.9)	107.4 (53.5)	108.5 (52.3)	119.4 (63.5)	**<.001**
MVPA$_{MATTHEWS}$[c,d]; min/day, mean (sd)	64.3 (44.1)	111.6 (46.1)	70.9 (29.3)	48.1 (22.8)	26.7 (17.8)	**<.001**
MVPA$_{OPACH}$[c,e]; min/day, mean (sd)	51.6 (33.8)	84.8 (36.5)	56.7 (24.5)	40.6 (19.7)	24.3 (15.6)	**<.001**
MVPA$_{WHI}$[f]; min/day, mean (sd)	11.0 (13.3)	14.9 (15.8)	12.3 (13.8)	10.4 (11.8)	6.4 (9.7)	**<.001**
MVPA$_{CHAMPS}$[g]; min/week, mean (sd)	879 (1,172)	1,245 (1,395)	1,044 (1,324)	770 (983)	451 (686)	**<.001**
Total sedentary time[c]; min/day, mean (sd)	595.6 (89.3)	479.6 (48.4)	569.1 (18.2)	627.8 (16.9)	706.0 (39.3)	**<.001**

Abbreviations: MI = myocardial infarction; MVPA = moderate to vigorous physical activity;

[a] Quartile 1 = 201-536 min, Quartile 2 = 537-599 min, Quartile 3 = 600-657 min, Quartile 4 = 658-875 min

[b] Cancer, cardiovascular disease (not including MI or stroke), cerebrovascular disease (not including stroke), cognitive impairment, chronic obstructive pulmonary disease, depression, and osteoarthritis

[c] Adjusted for awake wear time using the residuals method.

[d] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[e] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[f] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the WHI physical activity questionnaire.

[g] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Table 2.2 Associations of incident cardiovascular disease (CVD) and coronary heart disease (CHD) events with total sedentary time; OPACH (2012-2016)

	Total Sedentary Time[a,b]				P-Trend[c]
	1 (low)	2	3	4 (high)	
Cardiovascular disease (CVD)					
Incident CVD events	65	93	127	162	
Crude CVD incidence rate/1000 PY	14.4	21.2	29.4	39.0	
Model 1, aHR (95% CI), n=5638	1 (ref)	1.34 (0.97-1.84)	1.65 (1.22-2.23)	1.88 (1.40-2.54)	<.001
Model 2, aHR (95% CI), n=5471	1 (ref)	1.27 (0.92-1.75)	1.47 (1.08-2.00)	1.44 (1.05-1.98)	.007
Model 3, aHR (95% CI), n=5132	1 (ref)	1.20 (0.87-1.67)	1.42 (1.04-1.95)	1.30 (0.94-1.80)	.04
Model 4, aHR (95% CI), n=4339	1 (ref)	1.19 (0.84-1.67)	1.30 (0.93-1.82)	1.19 (0.84-1.70)	.14
Coronary heart disease (CHD)					
Incident CHD events	12	24	29	54	
Crude CHD incidence rate/1000 PY	2.6	5.4	6.5	12.6	
Model 1, aHR (95% CI), n=5638	1 (ref)	1.82 (0.91-3.65)	1.89 (0.96-3.73)	3.02 (1.58-5.77)	<.001
Model 2, aHR (95% CI), n=5471	1 (ref)	1.71 (0.83-3.52)	1.67 (0.82-3.41)	2.19 (1.09-4.40)	.003
Model 3, aHR (95% CI), n=5132	1 (ref)	1.57 (0.76-3.26)	1.65 (0.81-3.36)	2.06 (1.02-4.16)	.007
Model 4, aHR (95% CI), n=4339	1 (ref)	1.45 (0.67-3.15)	1.17 (0.53-2.56)	1.78 (0.84-3.79)	.02

[a] Quartile cutpoints (min): Q1=201-536, Q2=537-599, Q3=600-657, Q4=658-875.

[b] Adjusted for awake wear time using the residuals method.

[c] P-values from Cox multivariable linear regression models including total sedentary time in models in continuous form. (Model 1) age and race/ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI + prevalent diabetes + prevalent hypertension, (Model 4) Model 2 + glucose + HDL-cholesterol + log(triglycerides) + systolic blood pressure. Potential confounders include education, self-reported health, family history of MI, number of chronic conditions, physical functioning (SF-36), alcohol consumption, and current smoking status.

Table 2.3 Associations of incident cardiovascular disease (CVD) and coronary heart disease (CHD) events with total sedentary time in models including moderate to vigorous physical activity (MVPA) measured objectively and by self-report; OPACH (2012-2016)

	Total Sedentary Time Quartiles[a,b]				P-Trend[c]
	1 (low)	2	3	4 (high)	
Cardiovascular disease (CVD)					
Model 1[d]	1 (ref)	1.27 (0.92-1.75)	1.47 (1.08-2.00)	1.44 (1.05-1.98)	**.007**
Model 1 + MVPA$_{MATTHEWS}$[b,e]	1 (ref)	1.24 (0.88-1.75)	1.41 (0.98-2.04)	1.37 (0.90-2.07)	.05
Model 1 + MVPA$_{OPACH}$[b,f]	1 (ref)	1.24 (0.89-1.74)	1.42 (1.00-2.02)	1.38 (0.94-2.02)	**.04**
Model 1 + MVPA$_{WHI}$[g]	1 (ref)	1.29 (0.93-1.79)	1.45 (1.05-2.00)	1.53 (1.11-2.12)	**.003**
Model 1 + MVPA$_{CHAMPS}$[h]	1 (ref)	1.28 (0.92-1.77)	1.42 (1.03-1.95)	1.49 (1.07-2.06)	**.007**
Coronary heart disease (CHD)					
Model 1[d]	1 (ref)	1.71 (0.83-3.52)	1.67 (0.82-3.41)	2.19 (1.09-4.40)	**.003**
Model 1 + MVPA$_{MATTHEWS}$[b,e]	1 (ref)	1.51 (0.71-3.24)	1.37 (0.61-3.09)	1.68 (0.70-4.03)	**.04**
Model 1 + MVPA$_{OPACH}$[b,f]	1 (ref)	1.40 (0.67-2.94)	1.20 (0.56-2.57)	1.39 (0.63-3.08)	.13
Model 1 + MVPA$_{WHI}$[g]	1 (ref)	1.63 (0.79-3.38)	1.49 (0.72-3.08)	2.14 (1.06-4.32)	**.004**
Model 1 + MVPA$_{CHAMPS}$[h]	1 (ref)	1.65 (0.80-3.42)	1.51 (0.73-3.13)	2.21 (1.09-4.48)	**.003**

[a] Quartile cutpoints (min): Q1=201-536, Q2=537-599, Q3=600-657, Q4=658-875.

[b] Adjusted for awake wear time using the residuals method.

[c] P-values from Cox multivariable linear regression models including exposure variables in models in their continuous form.

[d] Model 1 is adjusted for age, ethnicity, education, self-reported health; family history of MI, number of chronic conditions; physical functioning; alcohol consumption; and current smoking status.

[e] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[f] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[g] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the WHI physical activity questionnaire.

[h] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Figure 2.1 Adjusted hazard ratios (aHRs) and 95% confidence intervals (CI) for associations of incident cardiovascular disease events and coronary heart disease events with a 1 hour change in total sedentary time, by selected participant characteristics; OPACH (2012-2016). *Associations are adjusted for age, ethnicity, education, self-reported health, family history of MI, number of chronic conditions, physical functioning (SF-36), alcohol consumption, and current smoking status (where appropriate). Physical functioning and moderate to vigorous physical activity (MVPA_{MATTHEWS}) were split at the median value.*

(a) Incident cardiovascular disease events

(b) Incident coronary heart disease events

(c) Distribution of sedentary time
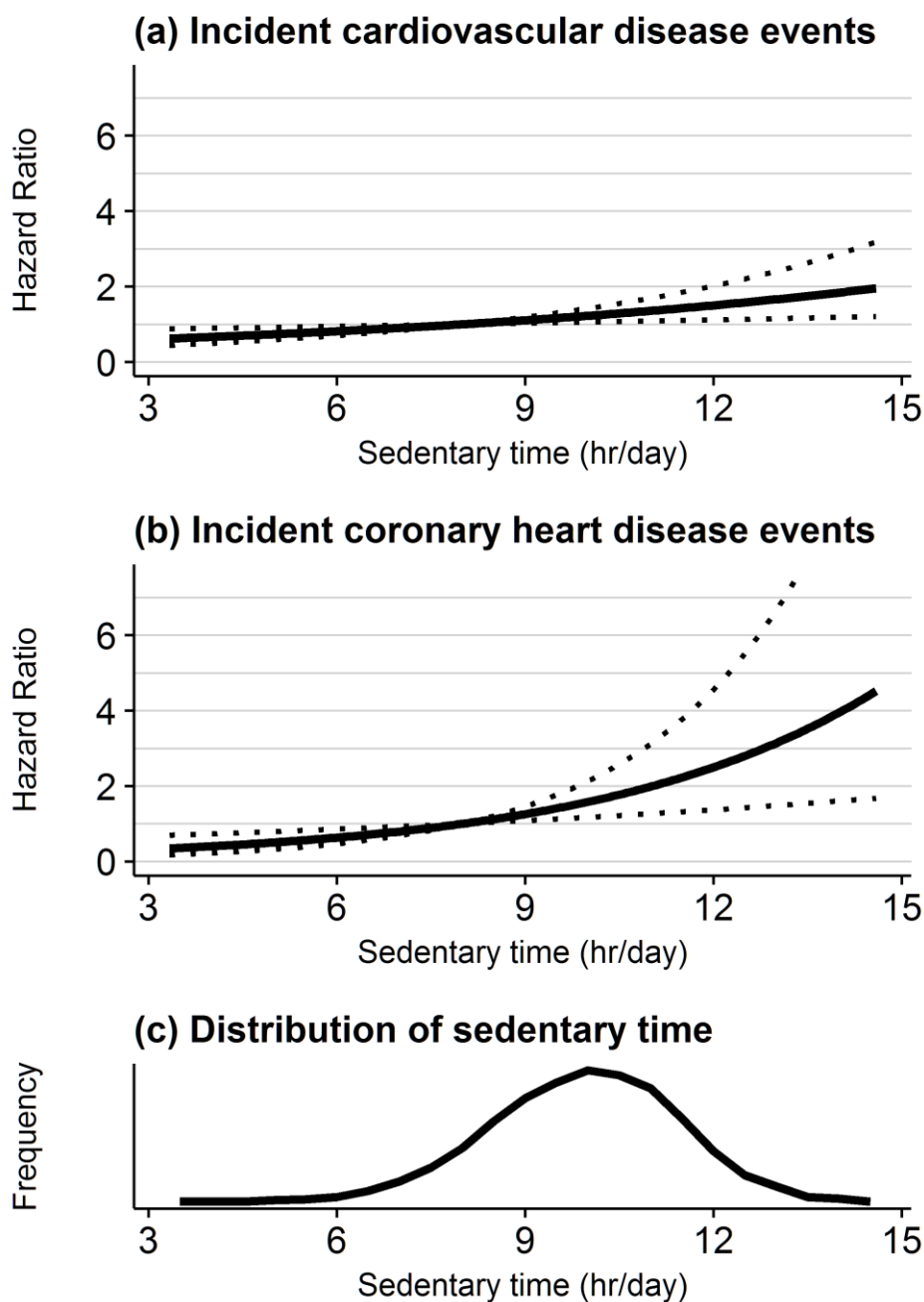
Figure 2.2 Continuous dose-response relation of sedentary time with incident CVD (panel a) and CHD (panel b) events estimated using multivariable linear Cox regression models adjusted for age, race/ethnicity, education, self-reported health, family history of myocardial infarction, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status. *To improve interpretability, the referent was set to the 10th percentile sedentary time duration (i.e., 8 hours per day). Hazard ratios (solid lines) and 95% confidence intervals (dotted lines) are plotted. Panel c shows the frequency distribution of total sedentary time.*

Supplemental Table 2.1 Pearson's correlation coefficients for linear associations of sedentary time and moderate to vigorous physical activity (MVPA)

	(1)	(2)	(3)	(4)	(5)
(1) Total sedentary time[a]	1.00				
(2) MVPA$_{MATTHEWS}$[a,b]	-.79	1.00			
(3) MVPA$_{OPACH}$[a,c]	-.72	.85	1.00		
(4) MVPA$_{WHI}$[d]	-.24	.30	.33	1.00	
(5) MVPA$_{CHAMPS}$[e]	-.26	.30	.30	.53	1.00

[a] Adjusted for awake wear time using the residuals method.

[b] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[c] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[d] Metabolic equivalent of task minutes per day in moderate to strenuous activities (including walking) per day as measured by the WHI physical activity questionnaire.

[e] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Chapter 3: Sedentary time and sedentary accumulation patterns in relation to diabetes in older women: The Objective Physical Activity and Cardiovascular Health Study (OPACH)

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Aims: To examine cross-sectional associations of sedentary time and sedentary behavior patterns with prevalent diabetes in an ethnically diverse cohort of older women, and test whether associations between sedentary time and diabetes are modified by age, race/ethnicity, body mass index (BMI), moderate-to-vigorous physical activity (MVPA), physical functioning, and family history of diabetes.

Methods: Community-dwelling older women (n=6,116; mean age 79±7) wore ActiGraph GT3X+ accelerometers on their hip 24 hours/day for up to 7 days. Prevalent diabetes was measured as self-reported physician diagnosed diabetes requiring oral hypoglycemic medication and/or insulin. Total sedentary time and three measures of sedentary accumulation patterns were derived from accelerometry. Associations between prevalent diabetes and sedentary behavior-related exposures (categorized into quartiles) were assessed using progressively adjusted logistic regression models, with BMI and MVPA added separately to main logistic regression models that contained 9 covariates. Associations between sedentary time and prevalent diabetes were estimated for subcategories of potential effect modifiers using the main regression models to test whether associations varied across subcategories.

Results: Twenty-one percent (n = 1282) of women reported diabetes at or before accelerometry. Women in the highest quartile of sedentary time (≥661 mins/day) had higher odds of prevalent diabetes (odds ratio (OR) = 1.96; 95% confidence intervals (CI), 1.59-2.42) than women in the lowest quartile (≤538 mins/day), after adjustment for main-model covariates. Associations were attenuated, but remained statistically significant, after adjustment for BMI and MVPA. No effect modification was observed. Women that accumulated sedentary time with the most prolonged patterns (i.e., many

long bouts of sedentary time with few short bouts and few interruptions) had higher odds of prevalent diabetes than women with the most interrupted patterns. Associations remained significant after adjustment for BMI and, for one sedentary pattern metric, after additional adjustment for MVPA.

Conclusions/interpretations: Higher levels of sedentary time and accumulating it in prolonged patterns were associated with increased odds for prevalent diabetes among an ethnically diverse cohort of older women. Sedentary time and prolonged sedentary behavior patterns are potentially modifiable lifestyles factors that offer promise as intervention targets for reducing the burden of diabetes in an aging population.

INTRODUCTION

Type II diabetes is an often progressive chronic condition characterized by insulin resistance[79] that can lead to heart disease, stroke, blindness, and renal failure.[80] Approximately 37 million US adults (10.2%) had diabetes in 2010 and prevalence is expected to increase to 53 million by 2030.[81] At present, nearly 1 in 5 older adults (≥65 years) have diagnosed diabetes.[82] Older adults with diabetes are at significantly higher risk than younger adults for hypoglycemia, stroke, ischemic heart disease, and congestive heart failure with adults ≥75 years having the highest risk.[83] Little data exist on the contribution lifestyle risk factors to diabetes burden among adults over the age of 80.[84]

New cases of diabetes occur at similar rates for adults aged 46-64 as adults aged 65-79,[85] suggesting there are opportunities for prevention across the adult lifespan. Among older adults, as many as 90% of new-onset diabetes has been attributed to behavioral risk factors such as lack of physical activity, poor diet, smoking, and alcohol use.[86] Sedentary behavior – defined as activities resulting in low energy expenditure (≤1.5 metabolic equivalents) while seated or reclining [1] – is an emerging factor associated with diabetes.[32,87–89] Meta analytic results indicate that adults with the highest self-reported sedentary time have double the risk for diabetes than adults with the lowest levels of sedentary time.[33] However, since self-reported sedentary time correlates poorly with objective measures,[35,90] especially for older adults,[91] and few studies have included adults over 75 years, studies using accelerometer measures in older age groups are needed to advance our understanding of sedentary behavior in relation to diabetes across the adult lifespan.

Sedentary time is not a binary risk factor, as some is needed for rest and relaxation. Conversely, sedentary time accrued in long, uninterrupted bouts is increasingly being recognized for its acute deleterious effects on glucose control and other cardio-metabolic risk factors.[14,92] Behavior patterns that are composed of many long, uninterrupted sedentary bouts (i.e., prolonged sedentary behavior patterns) are thought to increase risk for metabolic disease such as diabetes, but have seldom been studied in that context outside of the laboratory.[93] Consequently there is little known about how sedentary behavior patterns are associated with diabetes burden and there is no existing data on those associations in older people.

The aims of this study were to examine associations of diabetes with accelerometer-measured sedentary time and sedentary behavior patterns in older postmenopausal women. As the extant literature on diabetes and self-reported sitting time indicates differing associations for high and low risk adults,[94–96] our second aim was to test whether associations of sedentary time and diabetes were modified by age, race/ethnicity, body mass index (BMI), moderate to vigorous physical activity, physical functioning, or family history of diabetes.

METHODS

Sample and Design

The Objective Physical Activity and Cardiovascular Health Study (OPACH) was conducted among a subset of participants from the Women's Health Initiative (WHI) Hormone Therapy Trial and Observational Study who were initially enrolled in the Long Life Study (LLS). Details of the OPACH sample and design are published elsewhere.[40] Briefly, 7048 ambulatory women not residing in an institution and able to provide informed consent were enrolled and given ActiGraph GT3X+ accelerometers

along with wear instructions during a home visit. Accelerometers were worn on a belt around the participant's waist for a requested 24 hours per day (removed only when showering or swimming) for up to 7 continuous days. Sleep logs were concurrently collected to obtain data on participants' in-bed and out-of-bed times. Accelerometers were returned by 6721 (95.4%) participants with 6489 (91.2%) containing evidence of human wear.[97] Sociodemographic, behavioral, and health-related data, including self-reported physician-diagnosed or treated diabetes, were obtained by interviews and through self-administered questionnaires.

Dependent Variable

Women who answered "yes" to the following question at WHI baseline, "Did a doctor ever say that you had sugar diabetes or high blood sugar when you were not pregnant?" or who, before OPACH baseline, reported being treated with insulin or oral hypoglycemic medication at any of the annual medical update collected during the up to 24 years of WHI follow-up were considered to have diabetes. In the larger WHI cohort, self-reported diabetes has a high degree of concordance with results from physician-reviews of medical records with positive and negative predictive values of 91.8% and 94.5%, respectivly.[49]

Accelerometer Data Processing

ActiLife software (Version 6) was used to convert the raw accelerometer data (30 hertz) to 1-minute epochs using the low-frequency filter.[44] Accelerometer non-wear was removed using the Choi algorithm applied to the vector magnitude of acceleration counts per minute.[45] Then, sleep time was removed from the data using self-reported in-bed and out-of-bed times from sleep logs. For missing bed times, each person's mean in-bed and out-of-bed time were used, or if all data were

missing, the population mean in-bed (7:22 am) and/or out-of-bed (10:45 pm) time was used. In accordance with recommended data processing protocol for older adults, calendar days with ≥ 10 hours of awake wear time were considered adherent days and only adherent days were analyzed.[41] Furthermore, sedentary time and sedentary behavior pattern metrics were designed to estimate behavior over the typical week and therefore we required at least 4 adherent days to be considered in the analysis, as recommended.[41]

Exposure Variables

Total sedentary time, prolonged sedentary time, and sedentary accumulation patterns were derived from minute-level accelerometer data. Each 1-minute epoch was classified as sedentary if the acceleration counts per minute (cpm) on the vertical axis was < 100, a data processing protocol commonly used for older adults that has been validated and was previously used to study sedentary behavior patterns.[13,41,47]

Total sedentary time was computed as the average number of sedentary minutes per day, calculated over all adherent days. Consecutive sedentary minutes are referred to as sedentary bouts that can range from 1 minute to several hours in duration. Summarizing the frequency and duration of sedentary bouts is one way to describe how sedentary time is accumulated (i.e., sedentary behavior patterns). This paper used three sedentary behavior pattern metrics, each measuring the frequency and/or duration of sedentary bouts; breaks in sedentary time (frequency), usual bout duration (duration), and alpha (hybrid measure of frequency and duration). We also report associations between diabetes and prolonged sedentary time (here defined as the average number of minutes per day spent in sedentary bouts ≥ 30 minutes) so that results can be compared with other studies.[18,98,99] While prolonged sedentary

time is useful in providing an easy-to-interpret measure related to sedentary behavior patterns, metrics that take into account the frequency and/or duration of long *and* short sedentary bouts is thought to provide a more complete picture of how sedentary time is accumulated.

Breaks in sedentary time are transitions from a sedentary to non-sedentary bouts and therefore account for the frequency with which sedentary bouts occur. Higher frequencies indicate more interrupted patterns. Breaks in sedentary time was computed by summing the number of sedentary bouts over all eligible days and dividing by the number of eligible days.

The usual bout duration measures the bout duration above which half of all sedentary time was accumulated. Higher values indicate a tendency to accumulate sedentary time in longer sedentary bouts. The usual bout duration is the midpoint of the cumulative distribution of sedentary bout durations.

Alpha is a single, unitless metric that simultaneously describes the frequency and duration of all sedentary bouts, making it a hybrid measure of sedentary behavior patterns.[13] Accumulating sedentary time with frequent long bouts and relatively few short bouts would result in a low alpha, while accumulating sedentary time in relatively many short bouts with few long bouts would yield a high alpha. Both usual bout duration and alpha were computed according to the methods described by Chastin et al. (2010).[13,19]

Covariates

Data collected by questionnaire at WHI baseline were used to measure age, race/ethnicity (categorized into Black, White, or Hispanic), education (categorized into

high school/GED or less, some college, college graduate or more), and family history of diabetes (yes/no). At or near OPACH baseline, participants completed questionnaires that measured self-reported health status (categorized into excellent/very good, good, fair/poor), physical function score from the Rand 36-Item Health Survey (10 items, range 0-100), frequency of alcohol consumption (categorized into non-drinker, <1 drink/week, ≥ 1 drink/week, unspecified), and current smoking status (smoker, nonsmoker; missing values (n=536) were coded as nonsmokers). A subset of participants received in-home visits as part of the LLS where height was measured to the nearest half-inch by tape measure and weight measured to the nearest pound using a calibrated analog bathroom scale; from those measures, BMI was computed as (weight, lbs.)*703 / (height, in)^2. A measure of multimorbidity, adapted from Rillamas-Sun et al. (2016), was included as the number of chronic health conditions (cardiovascular disease; cancer; cognitive impairment, depression; osteoarthritis; history of falls; chronic obstructive pulmonary disease, hypertension; cerebrovascular disease) reported at or before OPACH baseline.[48]

Four measures of moderate to vigorous physical activity (MVPA) with differing correlations with sedentary time were used. The primary measure was derived from the vertical axis accelerometer counts to be consistent with the method used for measuring sedentary time; specifically, $MVPA_{MATTHEWS}$ was defined as the average minutes per day with accelerometer levels ≥760 counts per minute.[50] $MVPA_{OPACH}$, calibrated for older adults similar in age to participants of OPACH, was measured as the average minutes per day with vector-magnitude accelerometer counts ≥ 519 per 15-second epoch.[51] The WHI physical activity questionnaire was used to measure typical metabolic equivalent of task (MET) minutes per day from moderate to

strenuous activities (including walking; $MVPA_{WHI}$). MET-minutes per week in moderate exercise activities common to older adult lifestyles was assessed by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire ($MVPA_{CHAMPS}$).[66]

Statistical Analysis:

Variations in accelerometer wear time could impact measures of sedentary behavior and MVPA so total and prolonged sedentary time, breaks in sedentary time, $MVPA_{MATTHEWS}$, and $MVPA_{OPACH}$ were adjusted for awake wear time using the residuals method.[54] By design,[19] usual bout duration and alpha were unrelated to wear time and were therefore not adjusted.

Socio-demographic, health-related, and activity-related variables were summarized for the total sample and by quartile of total sedentary time using means and standard deviations for continuous variables and percentages for categorical variables. Differences in these factors across quartiles of total sedentary time were tested using F-tests for continuous variables and chi-square tests for categorical variables.

Associations of diabetes prevalence (yes/no) with total sedentary time, prolonged sedentary time, and sedentary accumulation patterns were assessed using multivariable logistic regression. Women were grouped in quartiles of each sedentary behavior-related exposure variable and quartiles were ranked so that quartile 1 (Q1; the referent) was the lowest total or prolonged sedentary time and most interrupted sedentary accumulation pattern. In this regard, odds ratios (OR) greater than 1.0 would reflect an adverse association with prevalent diabetes for all sedentary behavior exposure variables. The p-value from linear tests for trend using exposure

variables in their continuous form were reported. Four sequentially adjusted models were fit for each exposure: Model 1 was unadjusted; Model 2 was adjusted for age and race/ethnicity, Model 3 (the main model) added covariates (education level, family history of diabetes, self-reported health status, physical functioning, alcohol consumption, and smoking status) to Model 2, and Model 4 added BMI, a potential mediator, to Model 3.

Relations between sedentary time, MVPA, and cardio-metabolic health are unclear, with most studies treating MVPA as a confounder and some as a mediator, though there is evidence against both treatments.[56,100] Still, others view MVPA as an effect modifier, asserting sedentary time has differing relations with cardio-metabolic health for adults with differing levels of MVPA. In the present study, understanding interrelations between MVPA and sedentary time with respect to diabetes was especially difficult when using accelerometer measures because the two activity-related behaviors were highly correlated. Therefore, we evaluated the impact of four previously validated measurement methods of MVPA, two accelerometer-based and two self-reported, on associations of total sedentary time and prevalent diabetes by repeating Model 3 with addition of each MVPA measure ($MVPA_{MATTHEWS}$, $MVPA_{OPACH}$, $MVPA_{WHI}$, $MVPA_{CHAMPS}$), evaluated separately. Additionally, $MVPA_{MATTHEWS}$ (median split: < 54 minutes/day, ≥ 54 minutes/day) was tested as an effect modifier using Model 3 covariates. Less is known about how MVPA is related to sedentary accumulation patterns so for analyses of diabetes with prolonged sedentary time and accumulation patterns, $MVPA_{MATTHEWS}$ was added to Model 3 treating it as a potential confounder (Model 4b). Variance inflation factors (VIF) were inspected for evidence of multicollinearity (i.e., VIF ≥5); no evidence was observed for any model.

We also tested whether associations between total sedentary time and prevalent diabetes differed between women in high and low risk groups by separately examining age (<80 years, ≥80 years), BMI (<30 kg/m^2, ≥30 kg/m^2), physical functioning (median split: <75, ≥75), race/ethnicity (White, Black, Hispanic), and family history of diabetes (yes, no).   Effect modification was assessed by including interaction terms (effect modifier*total sedentary time) in multivariable logistic regression Model 3 and testing the interaction effect with statistical significance set to p<.05.

All statistical analyses were conducted in R (R Foundation for Statistical Computing; Vienna, Austria). Statistical tests were two-tailed with significance set to p<.05.

RESULTS

Of the 6489 women who returned accelerometers, 6133 provided ≥4 adherent days of accelerometer measures and of them, 6116 had complete data on diabetes diagnosis. Among the 6116 (94.3%) women in the final analytic sample (mean±SD age 78.7±6.7 years), 1,282 (21.0%) reported physician-diagnosed diabetes or treated diabetes at or before OPACH baseline (Table 3.1). Demographic and health-related characteristics are described in Table 3.1.

<u>Total and prolonged sedentary time</u>

High sedentary time was associated with increased risk of prevalent diabetes in unadjusted models and in each successively adjusted model (p-trend <0.001 all; Table 3.2). Compared to women in quartile (Q)1, women in Q2, Q3, and Q4 had 1.48 (95% confidence interval (CI)=1.22-1.80), 1.39 (95% CI=1.13-1.70), and 1.96 (95%

CI=1.59-2.42) times higher odds of prevalent diabetes, after controlling for potential

confounders (Model 3). Additional adjustment for BMI did not measurably change the

associations.

Adjustment for the four different MVPA metrics revealed attenuated odds

ratios (ORs) for sedentary behavior only when MVPA was measured by

accelerometry (Table 3.3). ORs for women in Q4 (compared to women in Q1)

reduced from 1.96 (95% CI=1.59-2.42) when not controlling for MVPA to as low as

1.40 (95% CI=1.06-1.85) when including accelerometer-derived MVPA. Despite

attenuated ORs, significant linear trends between sedentary behavior and diabetes

persisted (p-trend ≤ .003 all).

Associations between total sedentary time and prevalent diabetes were not

modified by categories of MVPA or by age, BMI, physical functioning, race/ethnicity,

or family history of diabetes (Figure 3.1).

Higher volumes of prolonged sedentary time were associated with higher odds

of prevalent diabetes (Table 3.4), though ORs were consistently lower than those for

total sedentary time. The odds of prevalent diabetes was 1.57 time higher for women

in Q4 than for women in Q1 (95% CI=1.28-1.93) after adjustment for Model 3

confounders.

Sedentary accumulation patterns

Women with the most prolonged patterns of sedentary behavior had higher

odds of prevalent diabetes than women with the most interrupted patterns (Table

3.4), but adjustment for Model 3 confounders attenuated all associations and

significant linear trends persisted only for usual bout duration and alpha. ORs for

women with the highest usual bout duration (Q4) and an alpha that indicated

accumulation patterns composed of the fewest short bouts and the most long bouts

(Q4) had 1.57 (95% CI=1.28-1.92) and 1.61 (95% CI=1.32-1.97) times higher odds,

respectively, than women with the most interrupted patterns (Model 3). Additional

adjustment for BMI slightly attenuated ORs, but did not measurably affect

associations or their significance. ORs were also attenuated after adjustment for

MVPA, with significant differences between the highest and lowest quartiles

persisting for usual bout duration and alpha and significant linear trends persisting

only for alpha.

For completeness, results for the continuous functional form of total sedentary

time are in Figure 3.1 and for prolonged sedentary time and sedentary behavior

accumulation metrics are in Supplemental Table 3.1.

DISCUSSION

High levels of sedentary time were associated with increased rates of

prevalent diabetes independent of several covariates and after additional adjustment

for BMI and MVPA. Compared to women with the lowest total sedentary time,

women with the highest total sedentary time had increased odds of prevalent

diabetes by 1.40 to 1.96. There was no evidence of effect modification when the

associations were examined across categories of relevant subgroups within the

cohort.

Prolonged sedentary patterns measured by alpha and usual bout duration

were associated with increased odds of prevalent diabetes, though associations

tended to be weaker than the associations observed with total sedentary time. Breaks

in sedentary time, a measure of how often sedentary bouts occur, was not significantly related to diabetes after adjusting for covariates. One explanation for differing results among the accumulation metrics is that the frequency of sedentary bouts (as measured by breaks sedentary time) may be less relevant for diabetes then the duration of sedentary bouts only (as measured by usual bout duration) or the combination of frequency and duration (as measured by alpha). Breaks in sedentary time computed using data from hip-worn accelerometers may also be more susceptible to measurement error than the other accumulation metrics as evidenced by its accuracy compared to breaks measured with posture-based devices.[101] However, despite the low accuracy, the correlations between hip-worn accelerometer sedentary breaks and those measured by direct observation have Pearson's correlation coefficients of between .64 and .86, suggesting adequate reliability.[75] The different pattern metrics could also be differentially susceptible to confounders, as evidenced by the differential changes in ORs when adjusting for $MVPA_{MATTHEWS}$. However, breaks are necessary for interrupting long bouts of sedentary time to reduce usual bout durations and improve alphas, and while the frequency of breaks are not significantly related to diabetes in this sample, their temporal placement (i.e., breaking long bouts into several shorter bouts) is critical for improving sedentary behavior patterns.

A major strength of this study was the use of accelerometer measures of sedentary behavior obtained during wear periods of 24 hours per day. This 24-hour wear protocol likely gave us better estimates of sedentary behavior than in most previous studies in which participants removed devices each night before going to bed. Waking-day only protocols present opportunities for device non-wear before and

after actual sleep time and are thought to differentially miss sedentary time since that is the most common activity intensity category. The sedentary accumulation measures used in this analysis have been previously characterized, and were specifically designed to summarize patterns of sedentary behavior.[13,19] While there has been one cross-sectional study of alpha and usual bout duration in relation to diabetes risk factors,[102] this is the first time the two sedentary pattern metrics have been used to study the clinically-relevant outcome of diabetes. This is also the first study of diabetes and accelerometer-assessed sedentary behavior, to our knowledge, that focuses exclusively on older postmenopausal women including a large number over the age of 75 years. As the aging population expands, both the absolute number and the proportion of older adults living with diabetes will increase.[81] This makes characterizing potentially modifiable factors associated with diabetes burden in this group of great public health importance.

Our study had some limitations. Accelerometers were unable to discriminate posture because they were worn on the hip and the resulting data were processed using the most commonly used methods.[75] As a result, pattern metrics were more prone to error than if they were measured using devices designed to detect posture.[101]

This study was cross-sectional which does not enable us to establish temporality of associations between sedentary time and diabetes. Furthermore, the WHI did not attempt to identify subclinical diabetes through routine monitoring of fasting blood glucose, glucose challenge tests, or other methods. It was also not possible to distinguish between type 1 and type 2 diabetes with the self-reported diabetes question. However, the self-reported measures were evaluated in WHI for

concordance with physician medical-records reviews and demonstrated high levels of accuracy.[49] It is unknown whether these results can be generalized to populations of older men or those too cognitively or physically disabled to participate in a study such as OPACH. Our study was conducted within a relatively large sample that was ethnically diverse and composed of women originally recruited from 40 sites throughout the US. Furthermore, we observed associations among various subgroups within our cohort including race/ethnicity, physical functioning, and age indicating high levels of external validity of associations between sedentary time and prevalent diabetes among older women.

The results of our study are in general agreement with previous studies of diabetes and sedentary behavior defined as self-reported time spent watching television. In a meta-analysis of 7 prospective and 3 cross-sectional studies, adults with the most television time had 2.12 times higher risk for developing diabetes than those with the least television time.[33] Many of the reviewed studies adjusted for self-reported MVPA, which has low concordance with accelerometer-measured MVPA. Incomplete adjustment for MVPA could lead to overestimated relations between sedentary time and diabetes in these previous studies. To address this, our study included MVPA measured by self-report and by accelerometer, showing that associations for sedentary time with prevalent diabetes were largely unchanged when MVPA was measured by self-report and were smaller, though still statistically significant, when measured by accelerometer. The attenuated associations following adjustment for accelerometer-measured MVPA highlights an important interrelation between the sedentary behavior and MVPA suggesting that replacement of sedentary time with moderate to high intensity activity may reduce odds of diabetes through

reduced sedentary time, increased MVPA, or both. Since linear associations between sedentary time and prevalent diabetes persisted after adjustment for MVPA, our results indicated that associations were independent of MVPA. Future studies are needed to help untangle these related behaviors.

Three recent studies examined incident diabetes in relation to sitting time measured by self-report, all studies demonstrating significant associations only among adults with low leisure time MVPA levels and/or adults who were obese.[94,96,103] Results from our study do not support differential associations between high and low risk groups including those based on obesity and MVPA. The lack of interaction of sedentary time with MVPA and BMI in OPACH differ from those studies,[94,96,103] possibly due to measurement error inherent in self-reported sitting measures used by the previous studies,[35] the weaker correlation between BMI and actual body fat at older ages,[104] or by reduced influence that adiposity appears to have on metabolic pathways in older adults.[105] Accelerometer-measured sedentary behavior has not often been used to evaluate associations of sedentary time and diabetes. Using hip-worn accelerometers to study adults aged 45±3, Gibbs et al. (2015) found a 29% increase in the relative odds for diabetes associated with, on average, each 1 hour increase in total sedentary time.[106] A larger study using posture-based accelerometers among adults aged 60±8 reported nearly identical increases in relative odds for diabetes (28%) associated with a 1 hour increase in total sitting time.[107] Similarly, Stamatakis et al. (2012) reported that each additional hour of total sedentary time was associated with a 24% increase in relative odds for diabetes in adults aged 44±6, though the association did not reach statistical significance (p=0.07). All previous estimates were higher than the 19% increased relative odds

observed in our study (see Figure 3.1), but fell within our 95% CI (OR=1.19; 95% CI = 1.13-1.30). Thus, our results are generally consistent with these reports, but this study extends the evidence base to include postmenopausal women, most of whom were over the age of 75.

Studies of sedentary accumulation patterns and diabetes are scarce. Experimental studies have demonstrated that prolonged sedentary behavior patterns (compared to regularly interrupted accumulation patterns) lead to acute detrimental effects on several key diabetes risk factors including postprandial glucose and lipid metabolism.[6–12,92,108] Epidemiologic studies also suggest that accumulation patterns composed of frequently interrupted sedentary time have favorable associations with diabetes risk factors such as measures of obesity, triglycerides, 2-hour post-load glucose, and HDL cholesterol.[12,14] In the only study of sedentary accumulation patterns and diabetes that we found, the authors reported that breaks in sedentary time were not associated with prevalent diabetes, consistent with findings from the present study.[107] Also consistent with our findings, van der Berg et al. reported that prolonged accumulation patterns (measured as the number of sedentary bouts ≥ 30 minutes and the average sedentary bout duration) were positively associated with odds of diabetes in models that did not mutually adjust for accumulation patterns and total sedentary time.[107] Notably, the sedentary accumulation measures employed by our study, different from those employed by van der Berg et al. (2016),[107] were theoretically grounded (they take into account the highly non-normal distribution of sedentary bout durations) and have been well characterized in previous studies,[13,19,22] which enhances confidence in our results. A recent American Heart Association scientific advisory recommended that standardizing sedentary-behavior related

measurement procedures would enhance syntheses of results across studies and should be a research priority and we believe the theoretically-grounded measures used herein are a good place to start.[38]

In conclusion, our study indicates that higher total sedentary time, and prolonged patterns of sedentary accumulation are positively associated with prevalent diabetes among older women. These data support existing guidelines[77,78] and recommendations[93] that call for reducing overall sedentary behavior and regularly interrupting prolonged sitting in order to mitigate metabolic aberrations associated with chronic diseases such as diabetes.

Prospective epidemiologic studies are needed to establish the temporal order of associations of sedentary behavior and patterns of sedentary behavior with diabetes. Intervention trials comparing various methods of reducing sedentary behavior and modifying sedentary accumulation patterns[109] are also needed to inform more specific public health recommendations that may curb the diabetes epidemic through improved sedentary habits.

Chapter 3, in full, is currently being prepared for submission for publication of the material. Bellettiere, John; Healy, Genevieve; LaMonte, Michael J.; Kerr, Jacqueline; Rillamas-Sun, Eileen; Di, Chongzhi; Buchner, David; Hovell, Melbourne F.; Evenson, Kelly R.; LaCroix, Andrea Z. John Bellettiere was the primary investigator and author of this material.

Table 3.1 Baseline Socio-demographic and health-related characteristics of women, by quartile of daily sedentary time; OPACH (2012-2014), n=6,116. Data are mean (SD) or n (%)

Characteristics	Total	Total Sedentary Time Quartiles[a,b]				p
		1 (low)	2	3	4 (high)	
Age, mean (sd)	78.7 (6.7)	76.0 (6.3)	77.5 (6.4)	79.5 (6.5)	81.8 (6.1)	<.001
Age category, n (%)						<.001
60-<70 years	627 (10.3)	269 (17.6)	184 (12.0)	122 (8.0)	52 (3.4)	
70-<80 years	2437 (39.8)	760 (49.7)	686 (44.9)	576 (37.7)	415 (27.1)	
80-<90 years	2795 (45.7)	473 (30.9)	628 (41.1)	760 (49.7)	934 (61.1)	
≥90 years	257 (4.2)	27 (1.8)	31 (2.0)	71 (4.6)	128 (8.4)	
Race/ethnicity, n (%)						<.001
White	3042 (49.7)	588 (38.5)	684 (44.7)	803 (52.5)	967 (63.2)	
Black	2045 (33.4)	552 (36.1)	562 (36.8)	505 (33.0)	426 (27.9)	
Hispanic	1029 (16.8)	389 (25.4)	283 (18.5)	221 (14.5)	136 (8.9)	
Highest education level, n (%)						.038
High school/GED or less	1235 (20.3)	340 (22.3)	314 (20.7)	291 (19.2)	290 (19.1)	
Some college	2346 (38.6)	580 (38.0)	548 (36.2)	591 (39.1)	627 (41.3)	
College graduate or more	2494 (41.1)	608 (39.8)	653 (43.1)	631 (41.7)	602 (39.6)	
Self-rated health, n (%)						<.001
Excellent or very good	3082 (50.6)	929 (61.0)	806 (52.9)	772 (50.6)	575 (37.7)	
Good	2420 (39.7)	502 (33.0)	581 (38.1)	633 (41.5)	704 (46.2)	
Poor or very poor	594 (9.7)	91 (6.0)	137 (9.0)	120 (7.9)	246 (16.1)	
Family history of diabetes (yes), n (%)	2263 (37.2)	532 (34.9)	602 (39.5)	568 (37.3)	561 (36.9)	.076
Number of chronic conditions[c], n (%)						<.001
Zero	612 (10.0)	221 (14.5)	169 (11.1)	130 (8.5)	92 (6.0)	
One	1983 (32.4)	560 (36.6)	521 (34.1)	486 (31.8)	416 (27.2)	
Two	2194 (35.9)	539 (35.3)	532 (34.8)	583 (38.1)	540 (35.3)	
Three or more	1327 (21.7)	209 (13.7)	307 (20.1)	330 (21.6)	481 (31.5)	
BMI, mean (sd)	28.1 (5.7)	27.16 (5.3)	28.11 (5.6)	28.22 (5.7)	28.96 (6.2)	<.001
Obese (≥30 kg/m2), n (%)	1795 (31.3)	358 (24.7)	444 (30.8)	472 (32.7)	521 (37.3)	
Alcohol intake, n (%)						<.001
Non-drinker	2092 (34.2)	461 (30.2)	498 (32.6)	531 (34.7)	602 (39.4)	
<1 per week	1909 (31.2)	453 (29.6)	513 (33.6)	475 (31.1)	468 (30.6)	
≥1 per week	1587 (25.9)	500 (32.7)	390 (25.5)	402 (26.3)	295 (19.3)	
Unspecified	528 (8.6)	115 (7.5)	128 (8.4)	121 (7.9)	164 (10.7)	

Table 3.1 Baseline Socio-demographic and health-related characteristics of women, by quartile of daily sedentary time; OPACH (2012-2014), n=6,116. Data are mean (SD) or n (%), Continued.

Characteristics	Total	Total Sedentary Time Quartiles[a,b]				p
		1 (low)	2	3	4 (high)	
Current smoker (yes), n (%)	159 (2.6)	28 (1.8)	36 (2.4)	42 (2.7)	53 (3.5)	.04
Physical functioning (SF-36), mean (sd)	68.9 (25.9)	79.5 (20.8)	73.2 (23.5)	68.1 (24.9)	54.6 (27.3)	<.001
MVPA$_{MATTHEWS}$[a,d]; min/day, mean (sd)	62.6 (43.6)	109.3 (45.8)	69.3 (29.2)	46.5 (22.4)	25.4 (17.2)	<.001
MVPA$_{OPACH}$[a,e], min/day, mean (sd)	50.3 (33.5)	83.1 (36.3)	55.6 (24.4)	39.5 (19.3)	23.0 (15.1)	<.001
MVPA$_{WHI}$[f]; min/day, mean (sd)	10.8 (13.3)	14.8 (15.8)	12.2 (13.8)	10.1 (11.9)	6.0 (9.1)	<.001
MVPA$_{CHAMPS}$[g]; min/week, mean (sd)	859 (1,171)	1,226 (1,383)	1,029 (1,344)	743 (968)	433 (679)	<.001
Total sedentary time[a]; min/day, mean (sd)	598 (90)	481 (49)	572 (18)	630 (17)	709 (40)	<.001
Prolonged sedentary time[a]; min/day, mean (sd)	218 (114)	108 (49)	173 (51)	234 (60)	356 (100)	<.001
Breaks in sedentary time[a]; n/day, mean (sd)	85.9 (15.9)	96.3 (13.2)	91.3 (12.2)	85.3 (11.8)	70.7 (13.6)	<.001
Usual sedentary bout duration; min, mean (sd)	18.4 (9.7)	10.9 (3.5)	14.9 (4.0)	18.8 (5.1)	28.8 (12.1)	<.001
Alpha, mean (sd)	1.87 (0.15)	2.04 (0.12)	1.90 (0.07)	1.82 (0.06)	1.71 (0.08)	<.001
Diabetes cases (yes), n (%)	1282 (21.0)	227 (14.8)	334 (21.8)	305 (19.9)	416 (27.2)	<.001

a Adjusted for awake wear time using the residuals method.

b Quartile 1 = 199-538 min, Quartile 2 = 539-601 min, Quartile 3 = 602-660 min, Quartile 4 = 661-921 min

c Cardiovascular disease, cancer, cognitive impairment, depression, osteoarthritis, history of falls, COPD, hypertension, cerebrovascular disease

d MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

e MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

f Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the Women's Health Initiative physical activity questionnaire.

g Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Table 3.2 Adjusted odds ratios and 95% confidence intervals for prevalent diabetes by quartile of total sedentary time; OPACH (2012-2014), n=6,116

	Total Sedentary Time[a,b]				p-trend[c]
	1 (low)	2	3	4 (high)	
Model 1[d] (n=6116)	1 (ref)	1.60 (1.33-1.93)	1.43 (1.18-1.73)	2.14 (1.79-2.57)	**<.001**
Model 2[d] (n=6116)	1 (ref)	1.70 (1.41-2.05)	1.63 (1.34-1.98)	2.75 (2.27-3.34)	**<.001**
Model 3[d,e] (n=5979)	1 (ref)	1.48 (1.22-1.80)	1.39 (1.13-1.70)	1.96 (1.59-2.42)	**<.001**
Model 4[d,e] (n=5611)	1 (ref)	1.44 (1.17-1.76)	1.34 (1.09-1.66)	1.90 (1.53-2.36)	**<.001**

[a] Quartile cutpoints for total sedentary time (min), Q1=199-538, Q2=539-601, Q3=602-660, Q4=661-921.

[b] Total sedentary time is adjusted for awake wear time using the residuals method.

[c] P-values from a linear test for trend chi square test executed using logistic regression including total sedentary time in models in continuous form.

[d] (Model 1) unadjusted, (Model 2) age and race/ethnicity adjusted, (Model 3) Model 2 + covariates, and (Model 4) Model 3 + body mass index.

[e] Covariates include education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Table 3.3 Adjusted odds ratios and 95% confidence intervals for associations of prevalent diabetes and total sedentary time adjusted for covariates and, where indicated, adjusted for moderate to vigorous physical activity (MVPA) measured objectively and by self-report; OPACH (2012-2014)

	Total Sedentary Time Quartiles[a,b]				p-trend[c]
	1 (low)	2	3	4 (high)	
Model 1[d]	1 (ref)	1.48 (1.22-1.80)	1.39 (1.13-1.70)	1.96 (1.59-2.42)	<.001
Model 1 + MVPA$_{MATTHEWS}$[e]	1 (ref)	1.26 (1.01-1.56)	1.07 (0.84-1.37)	1.40 (1.06-1.85)	.003
Model 1 + MVPA$_{OPACH}$[f]	1 (ref)	1.27 (1.03-1.56)	1.09 (0.87-1.38)	1.43 (1.11-1.84)	.002
Model 1 + MVPA$_{WHI}$[g]	1 (ref)	1.50 (1.23-1.83)	1.38 (1.12-1.70)	1.95 (1.58-2.42)	<.001
Model 1 + MVPA$_{CHAMPS}$[h]	1 (ref)	1.49 (1.22-1.82)	1.38 (1.12-1.70)	1.92 (1.55-2.38)	<.001

[a] Quartile cutpoints for total sedentary time (min), Q1=199-538, Q2=539-601, Q3=602-660, Q4=661-921.

[b] Total sedentary time is adjusted for awake wear time using the residuals method.

[c] P-values from a linear test for trend chi square test executed using logistic regression including total sedentary time in models in continuous form.

[d] Model 1 adjusts for age, race/ethnicity, education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

[e] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[f] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[g] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the Women's Health Initiative physical activity questionnaire.

[h] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Figure 3.1 Adjusted odds ratios (aORs) and 95% confidence intervals (CI) for associations of prevalent diabetes and total sedentary time (1 hour), by selected participant characteristics; OPACH (2012-2014). *Associations are adjusted for age, ethnicity, education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning (SF-36), alcohol consumption, and current smoking status (where appropriate). Physical functioning and moderate to vigorous physical activity (MVPA) were split at the median value.*

Table 3.3 Adjusted odds ratios and 95% confidence intervals for prevalent diabetes by quartile of prolonged sedentary time, breaks in sedentary time, usual bout duration, and alpha; OPACH (2012-2014), n=6,116

	Prolonged Sedentary Time, and Sedentary Accumulation Pattern Quartiles[a]				p-trend[b]
	1	2	3	4[c]	
Model 1[d] (n=6116)					
Prolonged sedentary time[e]	1 (ref)	1.17 (0.98-1.41)	1.35 (1.13-1.62)	1.51 (1.27-1.81)	**<.001**
Breaks in sedentary time[e]	1 (ref)	0.97 (0.82-1.15)	0.87 (0.73-1.04)	1.02 (0.86-1.21)	.91
Usual bout duration	1 (ref)	1.19 (1.00-1.43)	1.21 (1.02-1.45)	1.38 (1.16-1.65)	**<.001**
Alpha	1 (ref)	1.29 (1.07-1.54)	1.15 (0.95-1.38)	1.59 (1.33-1.89)	**<.001**
Model 2[d] (n=6116)					
Prolonged sedentary time[e]	1 (ref)	1.24 (1.03-1.50)	1.57 (1.30-1.89)	2.03 (1.68-2.45)	**<.001**
Breaks in sedentary time[e]	1 (ref)	1.05 (0.88-1.25)	1.01 (0.85-1.21)	1.35 (1.13-1.62)	**.001**
Usual bout duration	1 (ref)	1.31 (1.09-1.57)	1.44 (1.20-1.73)	1.90 (1.57-2.30)	**<.001**
Alpha	1 (ref)	1.37 (1.14-1.64)	1.33 (1.10-1.60)	2.04 (1.70-2.46)	**<.001**
Model 3[e,f] (n=5979)					
Prolonged sedentary time[e]	1 (ref)	1.14 (0.94-1.38)	1.33 (1.09-1.62)	1.57 (1.28-1.93)	**<.001**
Breaks in sedentary time[e]	1 (ref)	1.07 (0.89-1.29)	0.94 (0.78-1.14)	1.21 (1.00-1.47)	.14
Usual bout duration	1 (ref)	1.26 (1.04-1.53)	1.28 (1.05-1.55)	1.57 (1.28-1.92)	**.001**
Alpha	1 (ref)	1.34 (1.11-1.62)	1.24 (1.02-1.51)	1.61 (1.32-1.97)	**<.001**
Model 4a[e,f] (n=5611)					
Prolonged sedentary time[e]	1 (ref)	1.13 (0.93-1.39)	1.27 (1.04-1.56)	1.47 (1.19-1.82)	**<.001**
Breaks in sedentary time[e]	1 (ref)	1.04 (0.86-1.26)	0.87 (0.72-1.06)	1.12 (0.91-1.37)	.56
Usual bout duration	1 (ref)	1.24 (1.02-1.51)	1.21 (0.99-1.48)	1.46 (1.18-1.81)	**.005**
Alpha	1 (ref)	1.32 (1.09-1.61)	1.18 (0.97-1.45)	1.56 (1.27-1.92)	**<.001**

Table 3.3 Adjusted odds ratios and 95% confidence intervals for prevalent diabetes by quartile of prolonged sedentary time, breaks in sedentary time, usual bout duration, and alpha; OPACH (2012-2014), n=6,116, Continued.

	Prolonged Sedentary Time, and Sedentary Accumulation Pattern Quartiles[a]				p-trend[b]
	1	2	3	4[c]	
Model 4b[e,f] (n=5979)					
Prolonged sedentary time[e]	1 (ref)	1.01 (0.82-1.23)	1.11 (0.90-1.37)	1.20 (0.96-1.51)	.09
Breaks in sedentary time[e]	1 (ref)	1.08 (0.90-1.30)	0.94 (0.78-1.14)	1.15 (0.94-1.39)	.44
Usual bout duration	1 (ref)	1.13 (0.93-1.38)	1.09 (0.89-1.34)	1.25 (1.01-1.56)	.19
Alpha	1 (ref)	1.18 (0.97-1.44)	1.03 (0.84-1.27)	1.27 (1.02-1.58)	**.04**

[a] Quartile cutpoints: Prolonged sedentary time (min), Q1=-24-134, Q2=135-202, Q3=203-280, Q4=282-850; breaks in sedentary time (n), Q1=97-140, Q2=87-96, Q3=76-86, Q4=17-75; usual bout duration (min), Q1=4-12, Q2=13-16, Q3=17-21, Q4=22-171; alpha (unitless), Q1=1.96-2.85, Q2=1.87-1.95, Q3=1.77-1.86, Q4=1.37-1.76.

[b] P-values from a linear test for trend chi square test executed using logistic regression including total sedentary time in models in continuous form.

[c] Participants in quartile 4 have the highest prolonged sedentary time and the most prolonged pattern of sedentary time accumulation.

[d] (Model 1) unadjusted, (Model 2) Model 1 + age and race/ethnicity, (Model 3) Model 2 + covariates, (Model 4a) Model 3 + BMI, (Model 4b) Model 3 + MVPA.

[e] Adjusted for awake wear time using the residuals method.

[f] Covariates include education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Supplemental Table 3.1 Adjusted odds ratios and 95% confidence intervals for each standard deviation in prolonged sedentary time, breaks in sedentary time, usual bout duration, and alpha; OPACH (2012-2014), n=6,116

	Standardized odds ratio[a]	95% confidence interval	p-value
Model 1[b] (n=6116)			
Prolonged sedentary time[c]	1.16	(1.09-1.23)	**<.001**
Breaks in sedentary time[c,d]	1.00	(0.94-1.07)	.92
Usual bout duration	1.11	(1.05-1.18)	**<.001**
Alpha[d]	1.19	(1.11-1.26)	**<.001**
Model 2[b] (n=6116)			
Prolonged sedentary time[c]	1.30	(1.22-1.39)	**<.001**
Breaks in sedentary time[c,d]	1.11	(1.04-1.19)	**.001**
Usual bout duration	1.22	(1.14-1.30)	**<.001**
Alpha[d]	1.31	(1.22-1.40)	**<.001**
Model 3[b,e] (n=5979)			
Prolonged sedentary time[c]	1.18	(1.10-1.27)	**<.001**
Breaks in sedentary time[c,d]	1.05	(0.98-1.13)	.14
Usual bout duration	1.12	(1.05-1.20)	**.001**
Alpha[d]	1.20	(1.11-1.29)	**<.001**
Model 4a[b,e] (n=5611)			
Prolonged sedentary time[c]	1.16	(1.07-1.25)	**<.001**
Breaks in sedentary time[c,d]	1.02	(0.95-1.10)	.56
Usual bout duration	1.11	(1.03-1.19)	**.005**
Alpha[d]	1.18	(1.09-1.27)	**<.001**
Model 4b[b,e] (n=5979)			
Prolonged sedentary time[c]	1.07	(0.99-1.16)	.09
Breaks in sedentary time[c,d]	1.03	(0.96-1.10)	.44
Usual bout duration	1.05	(0.98-1.13)	.19
Alpha[d]	1.09	(1.00-1.18)	**.04**

[a] Units of measures for prolonged sedentary time and each sedentary behavior pattern variable: prolonged sedentary time = 114 minutes/day; breaks in sedentary time = -16 breaks/day; usual bout duration = 10.7 minute; alpha = -0.15 units.

[b] (Model 1) unadjusted, (Model 2) Model 1 + age and race/ethnicity, (Model 3) Model 2 + potential confounders, (Model 4a) Model 3 + BMI, (Model 4b) Model 3 + MVPA.

[c] Adjusted for awake wear time using the residuals method.

[d] Reverse coded so that higher values indicate higher risk.

[e] Potential confounders include education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Chapter 4: Sedentary time and accumulation patterns in relation to diabetes risk among older women: The Objective Physical Activity and Cardiovascular Health Study (OPACH)

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Background: Evidence is lacking on whether sedentary time affects risk for diabetes in America's oldest age groups. Furthermore, few prospective epidemiologic studies of diabetes have used objective sedentary behavior measures and none has evaluated how sedentary time is accumulated in relation to diabetes risk.

Methods: Women without diabetes (n=4834, age=79±7) wore accelerometers for ≥4 days and were followed for 3.1±0.8 years for new-onset diabetes. Total sedentary time and sedentary accumulation patterns were derived from accelerometer data. Hazard ratios (HRs) for new-onset diabetes were estimated across quartiles of sedentary behavior-related exposure variables using Cox proportional hazard models. Due to non-proportional hazards by family history of diabetes (FH+/-), models were stratified by FH.

Results: FH$_+$ women with the highest total sedentary time (≥ ~11 hours/day) had more than two times higher risk for diabetes (HR=2.38; 95% CI=1.05-1.98) than women with the lowest sedentary time (≤ ~9 hours per day). The most prolonged sedentary accumulation patterns increased risk for new-onset diabetes (HR=2.32; 95% CI=1.15-4.71) compared to the most interrupted patterns. Significant associations were not observed for FH- women.

Conclusions: High levels of sedentary time and accumulating it in patterns indicating frequent long sedentary bouts increased risk for diabetes among older FH$_+$ women.

INTRODUCTION

Type 2 diabetes is epidemic in the US, affecting 12.3% of adults over the age of 19 and 1 in 5 adults over age 64.[82] Older adults with diabetes are at the highest risk for complications such as vascular disease, renal impairment, and severe or fatal hypoglycemia.[110–112] Furthermore, diabetes and its complications accelerate the decline of physical functioning,[113] which is known to reduce independence and overall quality of life.

Each year, 1.2% of older adults are diagnosed with diabetes for the first time[114] and as many as 9 out of 10 of those new cases can be attributed to modifiable lifestyle factors such as physical inactivity, poor diet, smoking, alcohol use, and BMI.[86] In addition, several review papers have concluded that high amounts of sedentary behavior (i.e., actions that take place while sitting) is a factor, separate from physical inactivity, that contributes to the development of diabetes.[32,33,88] However, nearly all the studies that were reviewed measured sedentary behavior using reported time spent watching TV, which underestimates total sedentary time and is confounded by other metabolic risk factors such as socio-economic status and snacking.[115,116] More recent studies found that high levels of *time spent sitting* were related to elevated risk for diabetes, but only among adults with low physical activity levels[94,95] and/or high BMI[95,96]. Low correlations between reported sitting time and objective measures of sedentary time (such as those from accelerometers[35]) may contribute to the inconsistent findings.

Several mostly cross-sectional studies have used accelerometers to assess sedentary behavior, but they have primarily focused on risk factors related to diabetes.[14] The few studies that examined associations specifically with diabetes

have reported mixed results and none had a majority focus on adults over 75 years old.[106,107,117] The paucity of high quality evidence relating sedentary behavior to incident diabetes and other hard outcomes has led to consensus that evidence from prospective studies that use objective sedentary behavior measures is a research priority.[39]

Using data from accelerometers not only improves measurement accuracy, it also enables the separation of each minute of the day into sedentary and non-sedentary time, which allows for the study of sedentary accumulation patterns. Not all sedentary time is harmful, some is needed for rest and relaxation. However, sedentary time accrued in prolonged, uninterrupted periods has particularly adverse acute health consequences including the impairment of glucose control.[8,92] The long-term effects of these sedentary accumulation patterns, including how they relate to risk for metabolic diseases such as diabetes, have not yet been investigated.[93]

To address this lack of evidence, 4834 older women without self-reported diabetes wore accelerometers for up to 7 consecutive days and were followed for new-onset diabetes. We tested whether accelerometer-measured sedentary time and sedentary accumulation patterns were associated with risk for new-onset diabetes among a cohort of older postmenopausal women initially free of diabetes. We hypothesized that the longest total sedentary times and most prolonged accumulation patterns (i.e., patterns composed of many long periods of sedentary time with few interruptions) would be associated with the highest risk for new-onset diabetes in older women.

METHODS

Study Participants

As part of the Women's Health Initiative (WHI) Extension Study, 9252 non-institutionalized women that were ≥63 years consented to join the Long Life Study (LLS) of healthy aging and cardiovascular disease risk factors. Of these, 7058 ambulatory women were enrolled in the Objectively Measured Physical Activity and Cardiovascular Health Study (OPACH). Detailed methods have been previously published.[40] Briefly, at OPACH baseline (March 2012 and April 2014), women were asked to wear ActiGraph GT3X+ accelerometers around their waist 24 hours per day for 7 days (except when showering or swimming) and to record in-bed and out-of-bed times using sleep logs while wearing accelerometers. Women were subsequently followed annually for morbidity and mortality.

The analytic sample for the present study consisted of women that wore accelerometers (n=6489) for at least 4 eligible days (≥10 hours of awake wear time; n=6133), had follow-up data available (n=6116), and were without physician-diagnosed diabetes at OPACH baseline (n=4834).

Identification of diabetes

At WHI baseline, participants were asked whether a doctor ever diagnosed them with sugar diabetes or high blood sugar when not pregnant. At regular intervals (semi-annually or annually) during follow-up through the present, standardized medical history updates were mailed to participants that included questions about new physician-diagnosed diabetes requiring insulin or oral hypoglycemic medication. Prevalent diabetes at OPACH baseline was defined as any reported physician diagnosed diabetes at WHI baseline, or new physician-diagnosed diabetes requiring treatment with insulin or oral medication before OPACH baseline.

New-onset diabetes cases were defined as any participant with physician-diagnosed diabetes treated with insulin or oral medication after OPACH baseline through September 30, 2016 among women initially free of diabetes at the time of accelerometer measures. A separate study of 715 WHI participants showed that self-reports of new-onset diabetes were concordant with expert medical record review in 82% of women and reports of being without diabetes was concordant in 95% of women.[49] This measure of new-onset diabetes has also demonstrated construct validity by having been used in several studies by WHI investigators (e.g., [96,118,119]).

Sedentary time and sedentary accumulation patterns

Accelerometer data measured at 30 Hz were converted to 1-minute epochs using Actilife version 6, employing the low-frequency filter. The Choi algorithm was used to remove data that were collected while devices were unworn,[45] then periods while participants were in-bed were removed from data using recorded times from sleep diaries. When at least one in-bed or out-of-bed time was missing, each woman's average time was used if available, otherwise the overall mean in-bed (10:45pm) and out-of-bed (07:22am) times were used.

Sedentary behavior was operationalized as any 1-minute epoch with vertical axis accelerometer counts per minute < 100.[47] *Total sedentary time* was then computed as the average number of sedentary minutes per day over all eligible days.

Sedentary time is accrued in long and short uninterrupted periods called sedentary bouts that, when summarized, describe patterns of sedentary-time accumulation. *Prolonged sedentary time*, here operationalized as average minutes per day spent in long (≥30 minutes) sedentary bouts,[98] has been commonly used in epidemiologic studies, though it is not an accumulation pattern itself but a product of

sedentary accumulation patterns (i.e., a person with prolonged accumulation patterns will engage in high amounts of prolonged sitting). This measure was included so results could be compared with those from previous studies.

Sedentary accumulation patterns can be targeted by behavioral interventions,[13,109] though it is not yet known whether different aspects of accumulation patterns (frequency and/or duration) relate to disease risk. The present study explores three accumulation pattern metrics; 1) *breaks in sedentary time* (a frequency measure); 2) *usual bout duration* (a duration measure)*; and 3) *alpha* (a hybrid frequency and duration measure).

Breaks in sedentary time summarizes the frequency of sedentary bouts, providing a measure of the number of times sedentary time is typically interrupted. It was computed by summing the total number of sedentary bouts over all eligible days and dividing by the number of eligible days.

Usual bout duration summarizes the duration of sedentary bouts. It is the midpoint of the cumulative distribution of sedentary bout durations[19] and indicates the duration above which 50% of sedentary time is accumulated. Lower usual bout durations reflect accumulation patterns that were more regularly interrupted (i.e., patterns composed of shorter bouts).

Alpha, a unit-less metric that characterizes the highly skewed distribution of sedentary bout durations, summarizes both the frequency *and* duration of sedentary bouts for a given person,[19] making it a hybrid measure of accumulation patterns.[13] A person accumulating sedentary time with frequent long bouts and relatively few short bouts would have a lower Alpha than someone with a regularly interrupted

accumulation pattern. Usual bout duration and alpha were computed as described by Chastin et al (2010).[19]

Covariates

Age, race/ethnicity, education level, and family history of diabetes were measured by questionnaire at WHI baseline. Family history of diabetes was measured with the following question, "Did your mother or father, or full-blooded sisters, full-blooded brothers, daughters, or sons ever have sugar diabetes or high blood sugar that first appeared as an adult?" Self-reported health status, physical functioning (from the Rand 36-Item Health Survey), alcohol consumption, and current smoking status (smoker, nonsmoker; missing values were classified as non-smokers) were measured by questionnaire at OPACH baseline. Near OPACH baseline, trained research assistants conducted in-person visits as part of the LLS and measured height (to the nearest half-inch) and weight (to the nearest pound) after shoes, excess heavy clothing, and pocket contents were removed. BMI was computed as (weight (lb)/[height (in)]^2 * 703) . Multimorbidity was measured as the number of chronic health conditions (cardiovascular disease; cancer; cognitive impairment; depression; osteoarthritis; history of falls; chronic obstructive pulmonary disease, hypertension; cerebrovascular disease) reported at or before OPACH baseline.[48]

Selected apriori to be consistent with the method used to compute sedentary behavior-related exposures, moderate to vigorous physical activity (MVPA) was derived from vertical axis accelerometer counts and was defined as the average daily number of minutes with accelerometer counts per minute ≥760 (MVPA$_{MATTHEWS}$); a cutpoint commonly used for epidemiologic studies of MVPA.[50,59] For sensitivity analyses, we examined 3 additional measures of MVPA; average minutes per day

with accelerometer counts (vector-magnitude) per 15-second epoch ≥ 519 (MVPA$_{OPACH}$), an age-specific cutpoint determined in the OAPCH calibration study;[51] typical metabolic equivalent of task (MET) minutes per day from moderate to strenuous activities (including walking) measured by the WHI physical activity questionnaire (MVPA$_{WHI}$); and self-reported moderate exercise activities (MET-minutes/week) assessed by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire (MVPA$_{CHAMPS}$) designed specifically to obtain detailed information on various types of physical activity germane to older adult lifestyles.[40]

Statistical Analysis

Total and prolonged sedentary time were adjusted for awake wear time using the residuals method.[54] Breaks in sedentary time was adjusted for total sedentary time using the residuals method[54] so the resulting metric represented how frequently *sedentary time* was interrupted.[75] Usual bout duration and alpha were not related to accelerometer wear time, by design,[19] and therefore were not adjusted.

Sedentary time, sedentary accumulation patterns, and potential covariates were summarized for the total sample and by quartile of total sedentary time using means and standard deviations for continuous variables and column percentages for categorical variables. F-tests and Pearson's chi-square tests tested for differences among continuous and categorical variables, respectively.

Multivariable Cox proportional hazards (PH) models estimated hazard ratios (HRs) of new-onset diabetes in relation to total sedentary time, prolonged sedentary time, and sedentary accumulation patterns. Time to event was computed as the number of days from OPACH baseline to the date that new-onset diabetes was

reported or the date of either death or when the last medical update was received.
HRs were estimated for quartiles of each sedentary behavior-related exposure
variable, which were coded such that women in quartile 1 (the referent category) had
the lowest total or prolonged sedentary time and the most interrupted sedentary
accumulation patterns. Four models were fit for each sedentary behavior-related
exposure variable as follows: Model 1 = age and race/ethnicity adjusted, Model 2 =
Model 1 + potential confounders (education level, family history of diabetes, self-
reported health status, physical functioning, alcohol consumption, and smoking
status), Model 3 = Model 2 + BMI, and Model 4 = Model 2 + MVPA$_{\text{MATTHEWS}}$. Tests for
linear trend in diabetes risk were conducted using Cox regression models by treating
all sedentary behavior-related exposure variables as continuous. Cox PH
assumptions were assessed using tests based on Schoenfeld residuals[55] then
reviewing plots of the scaled Schoenfeld residuals over time to visually confirm any
detected violations. Variables that violated the assumption were included in Cox
regression models as multiplicative interaction terms with all other potential
confounders to test for effect modification, which, if found, would require results to be
presented separately for each level of the effect modifier (i.e., the variable in
violation). If no effect modification was detected, any variable in violation was treated
as a stratification variable within the Cox models using the strata() function in R.[120]

Sensitivity analyses

The choice to mutually adjust for sedentary time and MVPA is controversial,[56]
but common practice. In our sample of older women, the decision was additionally
problematic because sedentary time and accelerometer-measured MVPA were highly
correlated, making it difficult to assess "independent" effects. As a result, we explored

how adding various measures of MVPA with differing correlations with sedentary time affected HRs for total sedentary time and new-onset diabetes by repeating Model 2 while separately including each of the 4 MVPA measures described above (MVPA$_{MATTHEWS}$, MVPA$_{OPACH}$, MVPA$_{WHI}$, and MVPA$_{CHAMPS}$). We also acknowledge that new-onset diabetes and its complications could cause increased total sedentary time, meaning any identified associations could reflect reverse causation. To address this, we removed new-onset diabetes cases that occurred in the first 6 months from OPACH baseline and repeated analyses.

All statistical tests were two-tailed with significance levels set to 0.05. Analyses were conducted using R statistical software (R Foundation for Statistical Computing; Vienna, Austria).

RESULTS

During a mean follow-up time of 3.1±0.8 years, 252 women were diagnosed with new-onset diabetes requiring oral medication or insulin. Health-related characteristics are described in Table 4.1. Generally, women in the fourth quartile of total sedentary time were oldest, in the poorest health as measured by self-rated health and physical functioning, and had the highest rates of alcohol consumption and current smoking. Women in the highest quartile of sedentary time also had the lowest levels of MVPA$_{MATTHEWS}$ (mean±SD=27.0±18.2 minutes per day), the highest volume of prolonged sedentary time (350±99 minutes per day), the fewest sedentary breaks (77.0±15.7 breaks per day), and the most prolonged sedentary accumulation patterns as measured by usual bout duration (28.3±11.3 minutes) and alpha (1.72±0.08). In this sample, total sedentary time was strongly correlated with

MVPA$_{MATTHEWS}$ (r= -.78), prolonged sitting time (r= .85), usual bout duration (r= .74), and alpha (r= -.88; Supplemental Table 4.1).

Age, ethnicity, and family history of diabetes violated the PH assumption (p<0.05). Age (categorized as 63-69, 70-79, 80-89, ≥90) and ethnicity were not effect modifiers and were therefore treated as stratification variables in the survival models. A significant interaction between family history of diabetes and total sedentary time was detected (p=0.02); accordingly HRs were estimated separately for women with (FH$_+$) and women without (FH-) a family history of diabetes.

Total sedentary time

Associations between total sedentary time and new-onset diabetes for all women (presented only for context) and separately for FH$_+$ and FH- women are shown in Table 4.2. Among FH- women, there were no significant associations between total sedentary time and new-onset diabetes. For FH$_+$ women, the crude incidence rate of new-onset diabetes increased in a dose response manner over increasing quartiles of total sedentary time from 11.0/1000 person-years in quartile 1 to 17.1, 20.5, and 30.2 per 1000 person-years in quartiles 2, 3, and 4, respectively. After adjustment for potential confounders, the dose response pattern persisted and women in the quartile 4 had 2.38 times higher risk for new onset diabetes than women in quartile 1 (aHR=2.38; 95% CI=1.20-4.74). The aHRs were not appreciably changed after additional adjustment for BMI or MVPA$_{MATTHEWS}$, though following adjustment for MVPA$_{MATTHEWS}$ the p-value for trend increased to 0.06.

Prolonged sedentary time and patterns of sedentary accumulation

New-onset diabetes was not significantly associated with prolonged sedentary time or sedentary accumulation patterns among FH- women (Table 4.3). Among FH+ women, prolonged sedentary time, usual bout duration, and alpha were significantly associated with incident diabetes in age and race/ethnicity-adjusted models (p's <0.05), with the largest aHRs observed for alpha (Table 4.4). After adjustment for confounders, only alpha remained significantly associated with new-onset diabetes. Crude incidence rates for women with the most *interrupted* patterns as measured by alpha were 9.31/1000 person-years in quartile 1, while rates for women in quartiles 2, 3, and 4 were 21.5, 21.4, and 25.8 per 1000 person-years, respectively. After adjustment for potential confounders, FH+ women with successively more *prolonged* accumulation patterns had higher risk for new-onset diabetes than women with the most interrupted patterns (quartile 1); aHRs were 2.05 for quartile 2 (95% CI=1.03-4.09), 2.18 for quartile 3 (95% CI=1.08-4.39), and 2.32 for quartile 4 (95% CI=1.15-4.71). The linear trend of this association persisted after additional adjustment for BMI (p=0.007) and MVPA$_{MATTHEWS}$ (p=0.04).

Sensitivity analyses

Among FH+ women, the hazard ratio for a 1 standard deviation (90 minutes) increase in total sedentary time was 1.37 (95% CI=1.07-1.74) after adjustment for potential confounders (Supplemental Table 4.2). Additional adjustment for MVPA$_{WHI}$ and MVPA$_{CHAMPS}$ slightly increased standardized aHRs to 1.44 (95% CI=1.12-1.85) and 1.44 (95% CI=1.12-1.84). Adjustment for accelerometer-measured MVPA yielded mixed results. When adjusting for MVPA$_{MATTHEWS}$, the standardized aHR slightly increased to 1.42 (95% CI=0.99-2.02); when including MVPA$_{OPACH}$, the standardized aHR was reduced to 1.29 (95% CI=0.95-1.75). Both adjustments for accelerometer-

measured MVPA resulted in loss of statistical significance. Notably, self-report measures of MVPA were positively related to new-onset diabetes among FH$_+$ women, contrary to expectation, while accelerometer-measured MVPA was not significantly related with new-onset diabetes independent of sedentary time.

To account for potential reverse causation, 36 women (out of 252 total cases; 14.3%) treated for new-onset diabetes within the first 6-months of follow-up were removed and analyses repeated. For total sedentary time, magnitudes of the aHRs for FH$_+$ women were slightly reduced, but similar patterns were observed (See Supplemental Tables 4.3-4.5). For prolonged sitting time, usual bout duration, and alpha, aHRs among FH$_+$ women increased, but the overall associations remained unchanged though following adjustment for MVPA$_{MATTHEWS}$ the p-value for trend between alpha and new-onset diabetes increased to 0.07.

DISCUSSION

FH$_+$ women who were sedentary ≥11 hours per day had more than double the risk for new-onset diabetes compared to women who were sedentary for <8.9 hours per day after adjustment for potential confounders and BMI.

The amount of time spent in sedentary bouts ≥30 minutes (i.e., prolonged sedentary time) was not significantly associated with new-onset diabetes among FH$_+$ or FH- women. However, sedentary accumulation patterns measured using alpha were related to increased risk for new-onset diabetes among FH$_+$ women. Those with the most prolonged accumulation patterns compared to FH$_+$ women with the most interrupted accumulation patterns had more than two times higher risk for new-onset diabetes. The magnitude of increased risk was similar for women in quartiles 2, 3,

and 4 suggesting there may be a "threshold effect" associated with prolonged accumulation patterns in older FH₊ women.

While high breaks in sedentary time indicates frequently interrupted sedentary time, it does not directly account for the presence or prevalence of long sedentary bouts (e.g., the interruptions can all occur during the first 20 minutes of a 120-minute bout, resulting in the accumulation of sedentary time in several short bouts and one long 100-minute bout). On the other hand, interrupted accumulation patterns as measured by usual bout duration and alpha indicate that interruptions are spread throughout long sedentary bouts effectively breaking them into several shorter bouts. As shown in the results for this study and those from our previous work,[102] alpha tends to have stronger and more robust associations with cardio-metabolic health than prolonged sitting and other measures of sedentary accumulation for reasons that are not yet known. It could be that the combined effects of frequency and duration (of sedentary bouts), which is captured only by alpha, are stronger than the cardio-metabolic health effects of each aspect of sedentary accumulation (frequency and duration) individually. Alpha may also be differentially susceptible to confounders as evidenced by the varying correlations among different measurements of MVPA (Supplemental Table 4.1).

We did not observe significant associations of total sedentary time, prolonged sedentary time, or sedentary accumulation patterns with new-onset diabetes among FH- women. The decision to stratify on family history was not hypothesized a-priori, but was made based on model diagnostics for the proportional hazards assumption. This finding may have occurred by chance, or may reflect the importance of genetic influences on risk of incident diabetes in women who have survived to ages 63 and

above without a prior diabetes diagnosis.[121,122] The short duration of follow-up could also influence this finding in that the highest risk women as evidenced by FH+ may have developed diabetes the earliest with high volumes of sedentary time and more prolonged accumulation patterns. Results could also be attributed to ascertainment bias since new-onset diabetes was measured by physician diagnosis. According to American Diabetes Association guidelines, testing in asymptomatic adults should be considered when BMI ≥25 and at least one other risk factor is present (e.g., family history of diabetes) which could lead to higher rates of diabetes detected in FH+ women and more occult diabetes in FH- women. The importance of this interaction should be evaluated in other prospective studies and in this one after longer durations of follow-up.

Our results for FH+ women were similar to those reported for FH+ and FH- adults combined in a comprehensive meta-analysis based on sedentary behavior measured using TV time.[33] In the meta-analysis, adults with the most TV time had 2.12 times higher risk for diabetes compared to adults with the lowest; in the present study, FH+ women with the highest versus lowest sedentary time had 2.38 times higher risk for diabetes. Most of the studies included in the review controlled for family history of diabetes, but none reported on effect modification by family history of diabetes, preventing us from making direct comparisons.

Similarly, three studies of incident diabetes and self-reported sitting time did not test for effect modification by family history of diabetes. However, all three studies reported significant effect modification in other high risk groups, specifically, among adults with low physical activity and/or who were obese.[94,96,103] For example, 88,829 participants of the WHI Observational Study (aged 62±7 years) were followed for an

average 14.4 years and obese women that self-reported sitting ≥16 hours per day

had 1.25 times higher odds of new-onset diabetes than obese women reporting

sitting ≤7 hours per day.[96] Significant associations were not observed for normal

weight or overweight women. Several differences between the studies could account

for the varying results such as durations of follow-up times, differences in age at

baseline,[110,123] or our use of objective measures of sedentary time vs. the self-reports

used in the previous study, which have low correlation with accelerometer

measures.[35]

Accelerometer-based measures of sedentary behavior were used in just one

prospective study of diabetes, to our knowledge. Over a 5-year period, 81 cases of

incident diabetes were reported among 1718 American adults aged 45±3 and the

odds of incident diabetes did not significantly vary by total sedentary time.[106] This

study was limited by a relatively small sample size without time to event data, which

may have led to insufficient statistical power to detect associations. Differences could

also be attributed to underlying effect modification by environmental or genetic factors

that were not tested for by Gibbs et al. or by their sample's relatively young age,

which is particularly relevant considering diabetes pathophysiology differs for younger

vs. older adults.[110,123]

Our study was the first prospective epidemiologic study of sedentary

accumulation patterns and incident diabetes. After thorough investigation, our

findings revealed that accumulation patterns measured by alpha were associated with

incident diabetes in FH$_+$ women only. Experimental studies consistently show that

prolonged compared to interrupted sitting patterns lead to higher postprandial plasma

glucose levels in both high risk and low risk adults.[12,92] Similarly, several cross-

sectional studies have demonstrated that time spent in long sedentary bouts was deleteriously associated with some cardio-metabolic risk factors while time in short bouts was beneficially associated with cardio-metabolic health.[15,18,98] The totality of the evidence to date suggests that prolonged sedentary accumulation patterns may contribute to diabetes risk through frequent prolonged postprandial glucose excursions. If these hypothesized long-term effects of prolonged sedentary accumulation patterns are confirmed, this indicates that interventions to reduce sedentary time may lead to enhanced improvements in diabetes risk if they focus on regularly interrupting long sedentary bouts in a way that shifts alpha to higher, potentially safer, levels.

Associations between high sedentary time and risk for new onset diabetes among FH_+ women were sensitive to adjustment for MVPA with aHRs always indicating higher risk associated with higher levels of total sedentary time, but the magnitude of associations and the statistical significance of the linear trend varied depending on the measurement method used. Objective measures of MVPA were highly correlated with objective measures of sedentary behavior, tended to have lower standardized aHRs than seen for total sedentary time, and were not independently related to new-onset diabetes. Nevertheless, the sensitivity of sedentary time associations with new-onset diabetes in mutually adjusted models highlights an interrelation between sedentary time and MVPA with respect to incident diabetes that should be carefully investigated in future studies of incident diabetes in older women.

This study was limited by having shorter follow-up time than previous studies of sedentary behavior and incident diabetes. Despite the short follow-up, we had

sufficient power to detect associations even after stratification by family history of diabetes, likely due to the relatively large effect size, our use of objectively measured sedentary behavior, and our relatively large sample size. The self-reported diabetes question used to identify diabetes did not distinguish between type 1 and type 2 diabetes, however, the measure has demonstrated high accuracy for any diabetes diagnosis when evaluated for concordance with physician medical-records reviews.[49] The detection of sedentary accumulation patterns in the present study was limited by measurement using hip-worn accelerometers, which are less accurate for identifying postural transitions than devices specifically designed for this purpose (e.g. activPAL[TM]).[75,101] Such measurement error may account for the null findings for breaks in sedentary time and usual bout duration observed in this study. Future studies should employ posture-based devices to evaluate sedentary accumulation patterns in relation to new-onset diabetes. Additionally, accelerometer data were collected over a period of ≤ 7-days, which is adequately reproducible over a two-to-three year period,[76] but may not reflect longer-term patterns of sedentary behavior. The present study was also conducted among older women only and generalizability would be enhanced by testing these associations in cohorts of older women and men.

Notable strengths of our study are the relatively large cohort in which new-onset diabetes was assessed using valid outcome measures and objective measures of sedentary behavior using a 24-hour per day wear protocol. Objective measures are important for characterizing sedentary behavior because sitting is the default position for many older adults, making it an automatic behavior that is difficult to quantify by self-report.[35] Furthermore, the availability of high-resolution objective measures of sedentary behavior permitted us to explore how sedentary accumulation patterns

relate to diabetes, thereby adding insight into how sedentary behavior interventions might approach interrupting sedentary time to optimize health benefits. This was the first study to investigate new-onset diabetes in a racially/ethnically diverse cohort. Furthermore, this is the first diabetes study we are aware of with more than half the participants over the age of 75. The need to better characterize diabetes among this older age group was a major theme of a recent consensus statement by the American Diabetes Association.[124] The prospective design of the study is also a distinct strength in that it helps establish temporal order of the association between sedentary behavior and diabetes.

In conclusion, our study shows that high levels of sedentary time and prolonged sedentary accumulation patterns were related to new-onset diabetes among women with a family history of diabetes. At least one government agency has already issued guidelines targeting improved sedentary accumulation patterns, even in the context of limited evidence to date.[77] The results from this study support conducting intervention trials to inform guidelines that recommend both reductions in sedentary time and regularly interrupting prolonged sedentary time to prevent diabetes and other salient health outcomes. Until such data are available, the totality of the evidence supports recommendations to reduce sedentary time in whatever ways are feasible, safe, and easy to incorporate into daily life and suggests the benefits may be optimized by regularly interrupting long sedentary bouts.

Chapter 4, in full, is currently being prepared for submission for publication of the material. Bellettiere, John; Healy, Genevieve; LaMonte, Michael J.; Kerr, Jacqueline; Rillamas-Sun, Eileen; Di, Chongzhi; Buchner, David; Hovell, Melbourne

F.; Evenson, Kelly R.; LaCroix, Andrea Z. John Bellettiere was the primary

investigator and author of this material.

Table 4.1 Baseline Socio-demographic and health-related characteristics of women, by quartile of daily sedentary time; OPACH (2012-2014), n=4,834. Data are mean (SD) or n (%).

Characteristics	Total	Total Sedentary Time Quartiles[a,b]				p
		1 (low)	2	3	4 (high)	
Age, mean (sd)	78.9 (6.7)	76.0 (6.2)	77.9 (6.6)	79.6 (6.4)	82.2 (6.1)	**<.001**
Age Category, n (%)						**<.001**
60-<70 years	486 (10.1)	212 (17.5)	140 (11.6)	92 (7.6)	42 (3.5)	
70-<80 years	1861 (38.5)	598 (49.5)	525 (43.5)	447 (37.0)	291 (24.1)	
80-<90 years	2267 (46.9)	379 (31.3)	508 (42.1)	617 (51.1)	763 (63.1)	
≥90 years	220 (4.6)	20 (1.7)	35 (2.9)	52 (4.3)	113 (9.3)	
Race/ethnicity, n (%)						**<.001**
White	2567 (53.1)	491 (40.6)	590 (48.8)	670 (55.5)	816 (67.5)	
Black	1467 (30.3)	406 (33.6)	405 (33.5)	368 (30.5)	288 (23.8)	
Hispanic	800 (16.5)	312 (25.8)	213 (17.6)	170 (14.1)	105 (8.7)	
Highest education level, n (%)						.35
High school/GED or less	949 (19.8)	260 (21.5)	238 (19.8)	235 (19.6)	216 (18.0)	
Some college	1816 (37.8)	447 (37.0)	436 (36.4)	456 (38.1)	477 (39.8)	
College graduate or more	2038 (42.4)	501 (41.5)	525 (43.8)	506 (42.3)	506 (42.2)	
Self-rated health, n (%)						**<.001**
Excellent or very good	2632 (54.6)	783 (65.1)	686 (56.9)	639 (53.0)	524 (43.4)	
Good	1816 (37.7)	360 (30.0)	440 (36.5)	486 (40.3)	530 (43.9)	
Poor or very poor	370 (7.7)	59 (4.9)	79 (6.6)	80 (6.6)	152 (12.6)	
Family history of diabetes (yes), n (%)	1597 (33.2)	391 (32.5)	416 (34.6)	416 (34.6)	374 (31.1)	.20
Number of chronic conditions[c], n (%)						**<.001**
Zero	548 (11.3)	193 (16.0)	150 (12.4)	121 (10.0)	84 (6.9)	
One	1655 (34.2)	458 (37.9)	443 (36.7)	394 (32.6)	360 (29.8)	
Two	1720 (35.6)	414 (34.2)	402 (33.3)	464 (38.4)	440 (36.4)	
Three or more	911 (18.8)	144 (11.9)	213 (17.6)	229 (19.0)	325 (26.9)	
BMI, mean (sd)	27.6 (5.4)	26.8 (5.0)	27.6 (5.4)	27.7 (5.5)	28.3 (5.8)	**<.001**
BMI Categories, n (%)						**<.001**
<25 kg/m2	1591 (35.1)	483 (42.1)	390 (34.2)	386 (33.9)	332 (30.2)	
25 - <30 kg/m2	1700 (37.5)	415 (36.2)	445 (39.0)	424 (37.3)	416 (37.8)	
≥30 kg/m2	1237 (27.3)	249 (21.7)	307 (26.9)	328 (28.8)	353 (32.1)	

Table 4.1 Baseline Socio-demographic and health-related characteristics of women, by quartile of daily sedentary time; OPACH (2012-2014), n=4,834. Data are mean (SD) or n (%), Continued.

Characteristics	Total	Total Sedentary Time Quartiles[a,b]				p
		1 (low)	2	3	4 (high)	
Alcohol intake, n (%)						<.001
Non-drinker	1547 (32.0)	354 (29.3)	407 (33.7)	387 (32.0)	399 (33.0)	
<1 per week	1391 (28.8)	429 (35.5)	355 (29.4)	335 (27.7)	272 (22.5)	
≥1 per week	1524 (31.5)	339 (28.0)	362 (30.0)	398 (32.9)	425 (35.2)	
Unspecified	372 (7.7)	87 (7.2)	84 (7.0)	88 (7.3)	113 (9.3)	
Smoke now (yes), n (%)	133 (2.8)	23 (1.9)	29 (2.4)	31 (2.6)	50 (4.1)	.006
Physical functioning, mean (sd)	70.6 (25.1)	80.8 (20.0)	74.6 (22.7)	69.7 (24.4)	57.4 (26.9)	<.001
MVPA$_{MATTHEWS}$[a,d]; min/day, mean (sd)	65.3 (44.7)	113.3 (46.5)	71.8 (30.1)	49.2 (23.1)	27.0 (18.2)	<.001
MVPA$_{OPACH}$[a,e]; min/day, mean (sd)	52.4 (34.5)	86.5 (37.4)	57.6 (24.9)	41.2 (19.9)	24.5 (16.1)	<.001
MVPA$_{WHI}$[a,f]; min/day, mean (sd)	11.4 (13.7)	15.7 (16.3)	12.9 (14.1)	10.6 (11.9)	6.6 (9.7)	<.001
MVPA$_{CHAMPS}$[g]; min/week, mean (sd)	894 (1,198)	1,293 (1,455)	1,052 (1,350)	766 (961)	462 (696)	<.001
Total sedentary time[a]; min/day, mean (sd)	593 (90)	476 (48)	566 (19)	626 (17)	704 (40)	<.001
Prolonged sed. time[a]; min/day,mean (sd)	214 (113)	106 (48)	169 (51)	231 (60)	350 (99)	<.001
Breaks in sedentary time[a]; n/day, mean (sd)	85.9 (16.3)	90.5 (16.0)	89.6 (14.9)	86.5 (14.9)	77.0 (15.7)	<.001
Usual sed. bout duration; min, mean (sd)	18.1 (9.4)	10.8 (3.5)	14.7 (4.0)	18.7 (5.1)	28.3 (11.3)	<.001
Alpha, mean (sd)	1.87 (0.15)	2.05 (0.12)	1.91 (0.07)	1.83 (0.06)	1.72 (0.08)	<.001

Abbreviations: MVPA = moderate to vigorous physical activity;

[a] Total sedentary time and prolonged sedentary time are adjusted for awake wear time using the residuals method, breaks in sedentary time are adjusted for total sedentary time using the residuals method.

[b] Quartile 1 = 199-538 min, Quartile 2 = 539-601 min, Quartile 3 = 602-660 min, Quartile 4 = 661-921 min

[c] Cardiovascular disease, cancer, cognitive impairment, depression, osteoarthritis, history of falls, chronic obstructive pulmonary disease, hypertension, cerebrovascular disease

[d] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[e] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[f] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the Women's Health Initiative physical activity questionnaire.

[g] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Table 4.2 Adjusted hazard ratios for new-onset diabetes across quartiles of total sedentary time; OPACH (2012-2016)

	Total Sedentary Time Quartiles[a,b]				P-Trend[c]
	1 (low)	2	3	4 (high)	
All women (n=4,834[d])					
Diabetes Cases	59	51	69	73	
Crude incidence rate/1000 PY	15.2	13.5	18.5	20.7	
Model 1, aHR (95% CI)	1 (ref)	0.95 (0.65-1.38)	1.33 (0.93-1.89)	1.57 (1.10-2.26)	**.007**
Model 2, aHR (95% CI)	1 (ref)	0.86 (0.59-1.27)	1.17 (0.81-1.69)	1.38 (0.94-2.03)	.05
Model 3, aHR (95% CI)	1 (ref)	0.82 (0.55-1.22)	1.20 (0.82-1.74)	1.33 (0.89-1.97)	.07
Model 4, aHR (95% CI)	1 (ref)	0.78 (0.51-1.19)	1.01 (0.65-1.57)	1.13 (0.68-1.88)	.51
Women without a family history of diabetes (n=3,216)					
Diabetes Cases	45	28	42	40	
Crude incidence rate/1000 PY	17.4	11.3	17.2	16.4	
Model 1, aHR (95% CI)	1 (ref)	0.72 (0.45-1.15)	1.13 (0.74-1.75)	1.15 (0.73-1.80)	.40
Model 2, aHR (95% CI)	1 (ref)	0.69 (0.43-1.13)	1.03 (0.66-1.62)	1.08 (0.67-1.73)	.61
Model 3, aHR (95% CI)	1 (ref)	0.62 (0.38-1.04)	1.04 (0.66-1.64)	1.01 (0.61-1.65)	.74
Model 4, aHR (95% CI)	1 (ref)	0.61 (0.36-1.03)	0.84 (0.49-1.45)	0.83 (0.44-1.54)	.58
Women with a family history of diabetes (n=1,597)					
Diabetes Cases	14	22	26	32	
Crude incidence rate/1000 PY	11.0	17.1	20.5	30.2	
Model 1, aHR (95% CI)	1 (ref)	1.59 (0.81-3.11)	1.93 (1.00-3.72)	3.01 (1.57-5.78)	**.001**
Model 2, aHR (95% CI)	1 (ref)	1.38 (0.69-2.74)	1.66 (0.85-3.25)	2.38 (1.20-4.74)	**.01**
Model 3, aHR (95% CI)	1 (ref)	1.38 (0.68-2.82)	1.75 (0.88-3.49)	2.41 (1.18-4.93)	**.01**
Model 4, aHR (95% CI)	1 (ref)	1.38 (0.64-2.97)	1.67 (0.73-3.81)	2.40 (0.94-6.09)	.06

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] Adjusted for awake wear time using the residuals method.

[b] Quartile 1 = 199-532 min, Quartile 2 = 533-597 min, Quartile 3 = 598-655 min, Quartile 4 = 656-873 min.

[c] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPA_MATTHEWS.

[d] 21 women were missing family history of diabetes data, of whom 3 were diabetes cases.

Table 4.3 Adjusted hazard ratios for new-onset diabetes across quartiles of prolonged sedentary time, breaks in sedentary time, usual bout duration and alpha for women without a family history of diabetes (n=3,216); OPACH (2012-2014)

Total Sedentary Time, Prolonged Sedentary Time, and Sedentary Accumulation Pattern

	Quartiles[a,b]				P-Trend[c]
	1	2	3	4[d]	
Prolonged sedentary time					
Diabetes Cases	39	33	42	41	
Crude incidence rate/1000 PY	15.6	13.1	17.1	16.5	
Model 1[e], aHR (95% CI)	1 (ref)	0.88 (0.55-1.41)	1.26 (0.80-1.97)	1.25 (0.78-2.00)	.11
Model 2[e,f], aHR (95% CI)	1 (ref)	0.88 (0.55-1.41)	1.18 (0.74-1.88)	1.22 (0.75-1.97)	.15
Model 3[e,f], aHR (95% CI)	1 (ref)	0.81 (0.49-1.32)	1.13 (0.71-1.82)	1.13 (0.68-1.86)	.28
Model 4[e,f], aHR (95% CI)	1 (ref)	0.85 (0.53-1.38)	1.12 (0.69-1.83)	1.12 (0.65-1.93)	.28
Breaks in sedentary time					
Diabetes Cases	36	35	52	32	
Crude incidence rate/1000 PY	14.3	14.3	20.5	13.0	
Model 1[e], aHR (95% CI)	1 (ref)	1.07 (0.67-1.72)	1.50 (0.98-2.31)	1.01 (0.62-1.65)	.52
Model 2[e,f], aHR (95% CI)	1 (ref)	0.99 (0.61-1.59)	1.47 (0.95-2.27)	0.87 (0.53-1.45)	.92
Model 3[e,f], aHR (95% CI)	1 (ref)	0.93 (0.57-1.54)	1.45 (0.93-2.27)	0.88 (0.52-1.48)	.90
Model 4[e,f], aHR (95% CI)	1 (ref)	1.00 (0.62-1.61)	1.51 (0.98-2.33)	0.91 (0.54-1.51)	.78
Usual bout duration					
Diabetes Cases	39	38	36	42	
Crude incidence rate/1000 PY	15.7	15.1	14.8	16.7	
Model 1[e], aHR (95% CI)	1 (ref)	1.07 (0.68-1.69)	1.10 (0.69-1.75)	1.29 (0.80-2.05)	.13
Model 2[e,f], aHR (95% CI)	1 (ref)	1.10 (0.69-1.74)	1.09 (0.67-1.76)	1.19 (0.73-1.95)	.19
Model 3[e,f], aHR (95% CI)	1 (ref)	1.01 (0.63-1.63)	1.06 (0.65-1.73)	1.11 (0.66-1.84)	.32
Model 4[e,f], aHR (95% CI)	1 (ref)	1.06 (0.67-1.69)	1.03 (0.62-1.69)	1.09 (0.64-1.85)	.32

Table 4.3 Adjusted hazard ratios for new-onset diabetes across quartiles of prolonged sedentary time, breaks in sedentary time, usual bout duration and alpha for women without a family history of diabetes (n=3,216); OPACH (2012-2014), Continued.

	Total Sedentary Time, Prolonged Sedentary Time, and Sedentary Accumulation Pattern Quartiles[a,b]				P-Trend[c]
	1	2	3	4[d]	
Alpha					
Diabetes Cases	45	31	40	39	
Crude incidence rate/1000 PY	17.6	12.8	15.9	15.9	
Model 1[e], aHR (95% CI)	1 (ref)	0.79 (0.50-1.25)	1.00 (0.65-1.55)	1.03 (0.65-1.61)	.84
Model 2[e,f], aHR (95% CI)	1 (ref)	0.78 (0.49-1.24)	0.92 (0.59-1.44)	0.89 (0.55-1.44)	.70
Model 3[e,f], aHR (95% CI)	1 (ref)	0.87 (0.54-1.40)	0.97 (0.61-1.55)	0.92 (0.56-1.51)	.70
Model 4[e,f], aHR (95% CI)	1 (ref)	0.73 (0.45-1.17)	0.83 (0.52-1.33)	0.77 (0.46-1.29)	.34

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] Prolonged sitting time is adjusted for awake wear time using the residuals method. Breaks in sedentary time is adjusted for total sitting time using the residuals method. Alpha and usual bout duration are unrelated to awake wear time and are therefore not adjusted for wear time.

[b] Variables coded so that quartile 4 contains the highest prolonged sedentary time and the fewest breaks in sedentary time.

[c] Prolonged sedentary quartile ranges: quartile 1 = -11.8-130.6 min, quartile 2 = 130.7-198.2 min, quartile 3 = 198.3-276.7 min, quartile 4 = 276.8-779.5 min; Breaks in sedentary time quartile ranges: quartile 1 = 97.4-145.5, quartile 2 = 85.9-97.3, quartile 3 = 74.8-85.8, quartile 4 = 26.7-74.7; Usual bout duration quartile ranges: quartile 1 = 3.6-11.9 min, quartile 2 = 12-16.1 min, quartile 3 = 16.2-21.7 min, quartile 4 = 21.8-138.1 min; Alpha quartile ranges: quartile 1 = 1.97-2.85, quartile 2 = 1.87-1.96, quartile 3 = 1.78-1.86, quartile 4 = 1.37-1.77.

[d] P-Tend values are from Cox multivariable linear regression models including exposure variables in models in continuous form.

[e] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPA[MATTHEWS].

[f] Potential confounders include education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Table 4.4 Adjusted hazard ratios for new-onset diabetes across quartiles of prolonged sedentary time, breaks in sedentary time, usual bout duration and alpha for women with a family history of diabetes (n=1,597); OPACH (2012-2014)

	Total Sedentary Time, Prolonged Sedentary Time, and Sedentary Accumulation Pattern Quartiles[a,b]				P-Trend[c]
	1	2	3	4[d]	
Prolonged sedentary time					
Diabetes Cases	20	23	27	24	
Crude incidence rate/1000 PY	14.9	18.4	21.6	23.0	
Model 1[e], aHR (95% CI)	1 (ref)	1.23 (0.67-2.25)	1.42 (0.79-2.56)	1.59 (0.85-2.98)	**.02**
Model 2[e,f], aHR (95% CI)	1 (ref)	1.22 (0.66-2.25)	1.30 (0.71-2.38)	1.30 (0.67-2.50)	.13
Model 3[e,f], aHR (95% CI)	1 (ref)	1.28 (0.67-2.43)	1.43 (0.77-2.67)	1.39 (0.70-2.77)	.07
Model 4[e,f], aHR (95% CI)	1 (ref)	1.10 (0.59-2.06)	1.10 (0.58-2.09)	1.01 (0.49-2.08)	.44
Breaks in sedentary time					
Diabetes Cases	24	25	24	21	
Crude incidence rate/1000 PY	18.6	19.5	20.5	18.4	
Model 1[e], aHR (95% CI)	1 (ref)	1.06 (0.60-1.86)	1.09 (0.62-1.94)	1.00 (0.55-1.83)	.60
Model 2[e,f], aHR (95% CI)	1 (ref)	1.01 (0.56-1.80)	1.11 (0.62-1.99)	0.88 (0.47-1.64)	.76
Model 3[e,f], aHR (95% CI)	1 (ref)	1.12 (0.62-2.03)	1.18 (0.65-2.15)	0.90 (0.47-1.74)	.71
Model 4[e,f], aHR (95% CI)	1 (ref)	1.05 (0.59-1.88)	1.18 (0.66-2.11)	0.94 (0.50-1.75)	.60
Usual bout duration					
Diabetes Cases	21	20	29	24	
Crude incidence rate/1000 PY	15.4	16.6	22.5	23.6	
Model 1[e], aHR (95% CI)	1 (ref)	1.09 (0.59-2.02)	1.43 (0.80-2.54)	1.65 (0.89-3.05)	**.04**
Model 2[e,f], aHR (95% CI)	1 (ref)	1.12 (0.60-2.10)	1.34 (0.74-2.42)	1.40 (0.74-2.66)	.24
Model 3[e,f], aHR (95% CI)	1 (ref)	1.05 (0.54-2.02)	1.38 (0.76-2.52)	1.46 (0.75-2.83)	.10
Model 4[e,f], aHR (95% CI)	1 (ref)	1.04 (0.55-1.96)	1.18 (0.64-2.18)	1.17 (0.59-2.32)	.55

Table 4.4 Adjusted hazard ratios for new-onset diabetes across quartiles of prolonged sedentary time, breaks in sedentary time, usual bout duration and alpha for women with a family history of diabetes (n=1,597); OPACH (2012-2014), Continued.

	Total Sedentary Time, Prolonged Sedentary Time, and Sedentary Accumulation Pattern Quartiles[a,b]				P-Trend[c]
	1	2	3	4[d]	
Alpha					
Diabetes Cases	12	28	25	29	
Crude incidence rate/1000 PY	9.3	21.5	21.4	25.8	
Model 1[e], aHR (95% CI)	1 (ref)	2.28 (1.16-4.50)	2.32 (1.16-4.65)	2.83 (1.42-5.66)	.001
Model 2[e,f], aHR (95% CI)	1 (ref)	2.05 (1.03-4.09)	2.18 (1.08-4.39)	2.32 (1.15-4.71)	.01
Model 3[e,f], aHR (95% CI)	1 (ref)	1.91 (0.95-3.86)	2.09 (1.03-4.24)	2.27 (1.11-4.65)	.007
Model 4[e,f], aHR (95% CI)	1 (ref)	1.94 (0.96-3.91)	1.99 (0.96-4.15)	2.08 (0.98-4.42)	.04

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] Prolonged sitting time is adjusted for awake wear time using the residuals method. Breaks in sedentary time is adjusted for total sitting time using the residuals method. Alpha and usual bout duration are unrelated to awake wear time and are therefore not adjusted for wear time.

[b] Variables coded so that quartile 4 contains the highest prolonged sedentary time and the fewest breaks in sedentary time.

[c] Prolonged sedentary quartile ranges: quartile 1 = -11.8-130.6 min, quartile 2 = 130.7-198.2 min, quartile 3 = 198.3-276.7 min, quartile 4 = 276.8-779.5 min; Breaks in sedentary time quartile ranges: quartile 1 = 97.4-145.5, quartile 2 = 85.9-97.3, quartile 3 = 74.8-85.8, quartile 4 = 26.7-74.7; Usual bout duration quartile ranges: quartile 1 = 3.6-11.9 min, quartile 2 = 12-16.1 min, quartile 3 = 16.2-21.7 min, quartile 4 = 21.8-138.1 min; Alpha quartile ranges: quartile 1 = 1.97-2.85, quartile 2 = 1.87-1.96, quartile 3 = 1.78-1.86, quartile 4 = 1.37-1.77.

[d] P-Tend values are from Cox multivariable linear regression models including exposure variables in models in continuous form.

[e] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPA[MATTHEWS].

[f] Potential confounders include education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Supplemental Table 4.1 Pearson's correlation coefficients describing linear relations between sedentary behavior and physical activity measures

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Total sedentary time[a]	1.00									
(2) Prolonged sedentary time[a]	.85	1.00								
(3) Breaks in sedentary time[b]	-.29	-.57	1.00							
(4) Usual bout duration[c]	.75	.95	-.61	1.00						
(5) Alpha[c]	-.88	-.78	.49	-.72	1.00					
(6) MVPA$_{MATTHEWS}$[a,d]	-.78	-.56	-.05	-.44	.52	1.00				
(7) MVPA$_{OPACH}$[a,e]	-.72	-.53	-.02	-.42	.50	.86	1.00			
(9) MVPA$_{WHI}$[f]	-.24	-.19	.05	-.16	.17	.31	.34	1.00		
(8) MVPA$_{CHAMPS}$[g]	-.26	-.20	.05	-.16	.19	.31	.30	.52	1.00	
(10) Awake wear time	.01	.01	.55	.02	-.02	.01	-.02	.06	.08	1.00

Abbreviations: MVPA = moderate to vigorous physical activity

[a] Total sedentary time and prolonged sedentary time are adjusted for accelerometer wear time using the residuals method.

[b] Breaks in sedentary time is adjusted for total sedentary time using the residuals method.

[c] Usual bout duration and alpha are not adjusted for wear time or total sedentary time.

[d] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[e] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

[f] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the WHI physical activity questionnaire.

[g] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Supplemental Table 4.2 Adjusted hazard ratios for associations of incident diabetes with sedentary time (1 standard deviation) and moderate to vigorous physical activity (MVPA; 1 standard deviation) measured objectively and by self-report, stratified by family history of diabetes; OPACH (2012-2014)

	Total sedentary time[a,b]			MVPA[a]		
	aHR	95% CI	p-value	aHR	95% CI	p-value
All women						
Model 1[c]	1.14	(0.99-1.32)	0.069	-	-	-
Model 1 + MVPA$_{MATTHEWS}$[a,d]	1.08	(0.87-1.33)	0.492	0.92	(0.73-1.15)	.46
Model 1 + MVPA$_{OPACH}$[a,e]	1.04	(0.86-1.25)	0.676	0.86	(0.70-1.05)	.13
Model 1 + MVPA$_{WHI}$[f]	1.17	(1.01-1.36)	**0.032**	1.10	(0.97-1.25)	.15
Model 1 + MVPA$_{CHAMPS}$[g]	1.19	(1.02-1.37)	**0.023**	1.17	(1.06-1.29)	**.003**
Women without family history of diabetes						
Model 1[c]	1.03	(0.86-1.24)	0.740	-	-	-
Model 1 + MVPA$_{MATTHEWS}$[a,d]	0.92	(0.70-1.20)	0.521	0.85	(0.64-1.12)	.25
Model 1 + MVPA$_{OPACH}$[a,e]	0.92	(0.72-1.16)	0.477	0.83	(0.65-1.07)	.15
Model 1 + MVPA$_{WHI}$[f]	1.04	(0.87-1.26)	0.646	1.00	(0.85-1.19)	.96
Model 1 + MVPA$_{CHAMPS}$[g]	1.06	(0.88-1.28)	0.513	1.15	(1.02-1.29)	**.03**
Women with family history of diabetes						
Model 1[c]	1.37	(1.07-1.74)	**0.011**	-	-	-
Model 1 + MVPA$_{MATTHEWS}$[a,d]	1.42	(0.99-2.02)	0.056	1.05	(0.72-1.55)	.79
Model 1 + MVPA$_{OPACH}$[a,e]	1.29	(0.95-1.75)	0.104	0.90	(0.64-1.27)	.55
Model 1 + MVPA$_{WHI}$[f]	1.44	(1.12-1.85)	**0.005**	1.25	(1.02-1.53)	**.03**
Model 1 + MVPA$_{CHAMPS}$[g]	1.44	(1.12-1.84)	**0.004**	1.25	(1.02-1.54)	**.04**

Abbreviations: aHR = adjusted hazard ratio; CI=Confidence interval; MVPA=moderate to vigorous physical activity

[a] Adjusted for awake wear time using the residuals method.

[b] One standard deviation of total sedentary time = 89.8 minutes.

[c] Model 2 adjusts for age, ethnicity, education, self-reported health, family history of diabetes, number of chronic conditions, physical functioning (SF-36), alcohol consumption, and current smoking status.

[d] MVPA measured using 760 count per minute cutpoint with data from the accelerometer (vertical axis only).

[e] MVPA measured using 519 count per 15-second epoch cutpoint with data from the accelerometer (vector magnitude).

Supplemental Table 4.2 Adjusted hazard ratios for associations of incident diabetes with sedentary time (1 standard deviation) and moderate to vigorous physical activity (MVPA; 1 standard deviation) measured objectively and by self-report, stratified by family history of diabetes; OPACH (2012-2014), Continued.

[f] Metabolic equivalent of task minutes per day from self-reported number of minutes spent in moderate to strenuous activities (including walking) per week as measured by the WHI physical activity questionnaire.

[g] Metabolic equivalent of task minutes per week spent in moderate intensity exercises as measured by the Community Health Activities Model Program for Seniors (CHAMPS) questionnaire.

Supplemental Table 4.3 Associations of incident diabetes with total and prolonged sedentary time after removing incident cases in first 6 months of follow-up.; OPACH (2012-2014)

		Total Sedentary Time Quartiles[a]			
	1	2	3	4[c,d]	P-Trend[b]
Women without a family history of diabetes					
Diabetes Cases	42	25	39	38	
Crude incidence rate/1000 PY	16.2	10.1	16	15.5	
Model 1[e], aHR (95% CI)	1 (ref)	0.69 (0.42-1.14)	1.14 (0.73-1.79)	1.19 (0.75-1.90)	.37
Model 2[e,f], aHR (95% CI)	1 (ref)	0.69 (0.42-1.15)	1.03 (0.65-1.64)	1.10 (0.67-1.80)	.64
Model 3[e,f], aHR (95% CI)	1 (ref)	0.62 (0.37-1.06)	1.03 (0.64-1.66)	1.06 (0.64-1.75)	.66
Model 4[e,f], aHR (95% CI)	1 (ref)	0.60 (0.35-1.02)	0.81 (0.46-1.42)	0.80 (0.42-1.52)	.41
Women with a family history of diabetes					
Diabetes Cases	14	13	23	22	
Crude incidence rate/1000 PY	11.0	10.1	18.2	20.8	
Model 1[e], aHR (95% CI)	1 (ref)	0.96 (0.45-2.06)	1.77 (0.90-3.48)	2.23 (1.11-4.48)	**.004**
Model 2[e,f], aHR (95% CI)	1 (ref)	0.81 (0.37-1.77)	1.50 (0.75-3.00)	1.70 (0.81-3.60)	**.04**
Model 3[e,f], aHR (95% CI)	1 (ref)	0.77 (0.34-1.75)	1.54 (0.76-3.15)	1.77 (0.82-3.81)	**.03**
Model 4[e,f], aHR (95% CI)	1 (ref)	0.70 (0.30-1.66)	1.23 (0.52-2.90)	1.31 (0.48-3.57)	.20

		Prolonged Sedentary Time Quartiles[a]			
	1	2	3	4[c,d]	P-Trend[b]
Women without a family history of diabetes					
Diabetes Cases	38	30	39	37	
Crude incidence rate/1000 PY	15.2	11.9	15.9	14.9	
Model 1[e], aHR (95% CI)	1 (ref)	0.84 (0.52-1.37)	1.22 (0.77-1.93)	1.2 (0.74-1.94)	.20
Model 2[e,f], aHR (95% CI)	1 (ref)	0.84 (0.51-1.36)	1.13 (0.7-1.82)	1.18 (0.72-1.93)	.26
Model 3[e,f], aHR (95% CI)	1 (ref)	0.76 (0.46-1.27)	1.08 (0.66-1.75)	1.12 (0.68-1.86)	.32
Model 4[e,f], aHR (95% CI)	1 (ref)	0.79 (0.48-1.3)	1.03 (0.63-1.7)	1.03 (0.59-1.78)	.59

Supplemental Table 4.3 Associations of incident diabetes with total and prolonged sedentary time after removing incident cases in first 6 months of follow-up.; OPACH (2012-2014), Continued.

	Prolonged Sedentary Time Quartiles[a]				P-Trend[b]
	1	2	3	4[c,d]	
Women with a family history of diabetes					
Diabetes Cases	19	16	19	18	
Crude incidence rate/1000 PY	14.2	12.8	15.2	17.3	
Model 1[e], aHR (95% CI)	1 (ref)	0.92 (0.47-1.8)	1.1 (0.58-2.1)	1.34 (0.68-2.63)	.06
Model 2[e,f], aHR (95% CI)	1 (ref)	0.93 (0.47-1.84)	0.99 (0.51-1.93)	1.09 (0.53-2.22)	.25
Model 3[e,f], aHR (95% CI)	1 (ref)	1.02 (0.5-2.05)	1.07 (0.53-2.13)	1.18 (0.56-2.46)	.18
Model 4[e,f], aHR (95% CI)	1 (ref)	0.81 (0.4-1.62)	0.8 (0.39-1.62)	0.79 (0.36-1.73)	.69

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] Adjusted for awake wear time using the residuals method.

[b] P-Trend values are from Cox multivariable linear regression models including exposure variables in models in continuous form.

[c] Variables coded so that quartile 4 contains the highest total and prolonged sedentary time.

[d] Total sedentary time quartile ranges: quartile 1 = 199-532 min, quartile 2 = 533-597 min, quartile 3 = 598-655 min, quartile 4 = 656-873 min. Prolonged sedentary quartile ranges: quartile 1 = -11.8-130.6 min, quartile 2 = 130.7-198.2 min, quartile 3 = 198.3-276.7 min, quartile 4 = 276.8-779.5 min.

[e] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPAMATTHEWS.

[f] Potential confounders include education, self-reported health, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Supplemental Table 4.4 Associations of incident diabetes with breaks in sedentary time and usual bout duration after removing incident cases in first 6 months of follow-up.; OPACH (2012-2014)

	Breaks in Sedentary Time Quartiles[a]				P-Trend[b]
	1	2	3	4[c,d]	
Women without a family history of diabetes					
Diabetes Cases	34	33	50	27	
Crude incidence rate/1000 PY	13.6	13.5	19.7	11.0	
Model 1[e], aHR (95% CI)	1 [ref]	1.06 (0.65-1.71)	1.52 (0.98-2.35)	0.9 (0.54-1.51)	.73
Model 2[e,f], aHR (95% CI)	1 [ref]	0.96 (0.59-1.57)	1.48 (0.95-2.30)	0.81 (0.48-1.37)	.99
Model 3[e,f], aHR (95% CI)	1 [ref]	0.88 (0.53-1.47)	1.42 (0.90-2.22)	0.78 (0.45-1.34)	.91
Model 4[e,f], aHR (95% CI)	1 [ref]	0.98 (0.60-1.60)	1.54 (0.99-2.40)	0.85 (0.50-1.44)	.79
Women with a family history of diabetes					
Diabetes Cases	19	20	17	16	
Crude incidence rate/1000 PY	14.7	15.6	14.5	14.1	
Model 1[e], aHR (95% CI)	1 [ref]	1.08 (0.58-2.03)	1.01 (0.52-1.96)	1.00 (0.51-1.96)	.65
Model 2[e,f], aHR (95% CI)	1 [ref]	1.08 (0.56-2.07)	1.08 (0.55-2.12)	0.91 (0.45-1.83)	.67
Model 3[e,f], aHR (95% CI)	1 [ref]	1.21 (0.62-2.36)	1.22 (0.61-2.43)	0.99 (0.48-2.04)	.56
Model 4[e,f], aHR (95% CI)	1 [ref]	1.13 (0.59-2.16)	1.14 (0.58-2.25)	0.99 (0.49-2.01)	.50

	Usual Bout Duration Quartiles				P-Trend[b]
	1	2	3	4[c,d]	
Women without a family history of diabetes					
Diabetes Cases	37	35	35	37	
Crude incidence rate/1000 PY	14.9	13.9	14.4	14.7	
Model 1[e], aHR (95% CI)	1 [ref]	1.05 (0.66-1.68)	1.14 (0.7-1.83)	1.23 (0.76-1.99)	.23
Model 2[e,f], aHR (95% CI)	1 [ref]	1.08 (0.67-1.73)	1.13 (0.69-1.84)	1.15 (0.70-1.91)	.30
Model 3[e,f], aHR (95% CI)	1 [ref]	0.98 (0.60-1.61)	1.11 (0.67-1.82)	1.10 (0.66-1.84)	.36
Model 4[e,f], aHR (95% CI)	1 [ref]	1.02 (0.63-1.65)	1.04 (0.63-1.72)	1.02 (0.60-1.74)	.57

Supplemental Table 4.4 Associations of incident diabetes with breaks in sedentary time and usual bout duration after removing incident cases in first 6 months of follow-up.; OPACH (2012-2014), Continued.

	Usual Bout Duration Quartiles				P-Trend[b]
	1	2	3	4[c,d]	
Women with a family history of diabetes					
Diabetes Cases	19	15	19	19	
Crude incidence rate/1000 PY	13.9	12.4	14.8	18.7	
Model 1[e], aHR (95% CI)	1 (ref)	0.93 (0.47-1.83)	1.11 (0.58-2.13)	1.49 (0.77-2.88)	.07
Model 2[e,f], aHR (95% CI)	1 (ref)	0.97 (0.49-1.95)	1.04 (0.53-2.04)	1.28 (0.64-2.56)	.28
Model 3[e,f], aHR (95% CI)	1 (ref)	0.94 (0.46-1.92)	1.07 (0.54-2.12)	1.30 (0.64-2.65)	.20
Model 4[e,f], aHR (95% CI)	1 (ref)	0.87 (0.43-1.77)	0.88 (0.44-1.77)	1.01 (0.48-2.14)	.62

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] Adjusted for total sitting time using the residuals method.

[b] P-Trend values are from Cox multivariable linear regression models including exposure variables in models in continuous form.

[c] Variables coded so that quartile 4 contains the most prolonged sedentary accumulation patterns.

[d] Breaks in sedentary time quartile ranges: quartile 1 = 97.4-145.5, quartile 2 = 85.9-97.3, quartile 3 = 74.8-85.8, quartile 4 = 26.7-74.7. Usual bout duration quartile ranges: quartile 1 = 3.6-11.9 min, quartile 2 = 12-16.1 min, quartile 3 = 16.2-21.7 min, quartile 4 = 21.8-138.1 min.

[e] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPA_{MATTHEWS}.

[f] Potential confounders include education, self-reported health, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Supplemental Table 4.5 Associations of incident diabetes with alpha after removing incident cases in first 6 months of follow-up.; OPACH (2012-2014)

	Alpha Quartiles				P-Trend[a]
	1	2	3	4[b,c]	
Women without a family history of diabetes					
Diabetes Cases	40	31	37	36	
Crude incidence rate/1000 PY	15.7	12.8	14.7	14.7	
Model 1[d], aHR (95% CI)	1 (ref)	0.91 (0.57-1.47)	1.07 (0.68-1.69)	1.12 (0.7-1.78)	.68
Model 2[d,e], aHR (95% CI)	1 (ref)	0.90 (0.56-1.45)	0.98 (0.62-1.57)	0.99 (0.61-1.61)	.91
Model 3[d,e], aHR (95% CI)	1 (ref)	1.02 (0.63-1.67)	1.05 (0.64-1.71)	1.06 (0.64-1.77)	.97
Model 4[d,e], aHR (95% CI)	1 (ref)	0.83 (0.51-1.35)	0.87 (0.54-1.43)	0.84 (0.49-1.42)	.41
Women with a family history of diabetes					
Diabetes Cases	10	22	17	23	
Crude incidence rate/1000 PY	7.8	16.9	14.6	20.5	
Model 1[d], aHR (95% CI)	1 (ref)	2.22 (1.05-4.69)	2.01 (0.91-4.41)	2.94 (1.38-6.26)	**.004**
Model 2[d,e], aHR (95% CI)	1 (ref)	1.96 (0.92-4.21)	1.91 (0.86-4.23)	2.46 (1.13-5.35)	**.02**
Model 3[d,e], aHR (95% CI)	1 (ref)	1.77 (0.81-3.85)	1.87 (0.84-4.14)	2.39 (1.09-5.24)	**.02**
Model 4[d,e], aHR (95% CI)	1 (ref)	1.83 (0.84-4.00)	1.72 (0.75-3.95)	2.16 (0.94-4.98)	.07

Abbreviations: PY=person years; aHR = adjusted hazard ratio, CI=Confidence interval

[a] P-Trend values are from Cox multivariable linear regression models including exposure variables in models in continuous form.

[b] Variables coded so that quartile 4 contains the most prolonged sedentary accumulation patterns.

[c] Alpha quartile ranges: quartile 1 = 1.97-2.85, quartile 2 = 1.87-1.96, quartile 3 = 1.78-1.86, quartile 4 = 1.37-1.77.

[d] (Model 1) age and ethnicity adjusted, (Model 2) Model 1 + potential confounders, (Model 3) Model 2 + BMI, and (Model 4) Model 2 + moderate to vigorous physical activity MVPA_MATTHEWS.

[e] Potential confounders include education, self-reported health, number of chronic conditions, physical functioning, alcohol consumption, and current smoking status.

Chapter 5: Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults

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Background: High amounts of time spent sitting can increase cardiovascular disease risk and are deleteriously associated cardio-metabolic risk biomarkers. Though evidence suggests that accruing sitting time in prolonged periods may convey additional risk, verification using high-quality measures is needed. We examined this issue in adults from the Australian Diabetes, Obesity and Lifestyle Study, using accurate measures of sitting accumulation.

Methods: In 2011/12, 739 adults aged 36 to 89 years (mean±SD 58±10 years) wore activPAL3™ monitors (which provide accurate objective measures of sitting); 678 provided ≥4 valid days of monitor data and complete cardio-metabolic biomarker and confounder data. Multivariable linear regression models examined associations of sitting time, sitting time accrued in ≥30 minute bouts (prolonged sitting time), and three measures of sitting accumulation patterns with cardio-metabolic risk markers: body mass index (BMI), waist circumference, blood pressure, high- and low- density lipoprotein (HDL and LDL) cholesterol, triglycerides, glycated haemoglobin (HbA1c), fasting plasma glucose (FPG) and 2-hour post-load glucose (PLG). Interactions tests examined whether associations of sitting time with biomarkers varied by usual sitting bout duration.

Results: Adjusted for potential confounders, greater amounts of sitting time and prolonged sitting time were significantly (p<0.05) deleteriously associated with BMI, waist circumference, HDL cholesterol, and triglycerides. Total sitting time was also significantly associated with higher PLG. Sitting accumulation patterns of frequently interrupted sitting (compared to patterns with relatively more prolonged sitting) were significantly beneficially associated with BMI, waist circumference, HDL cholesterol, triglycerides,PLG, and with FPG. Effect sizes were typically larger for accumulation

patterns than for sitting time. Significant interactions ($p<0.05$) showed that associations of sitting time with HDL, triglycerides and PLG became more deleterious the longer at a time sitting was usually accumulated.

Conclusions: Adding to previous evidence reliant on low-quality measures, our study showed that accumulating sitting in patterns where sitting was most frequently interrupted had significant beneficial associations with several cardio-metabolic biomarkers and that sitting for prolonged periods at a time may exacerbate some of the effects of sitting time. The findings support sedentary behavior guidelines that promote reducing and regularly interrupting sitting.

INTRODUCTION

Diabetes mellitus and cardiovascular disease (CVD), on a global scale, account for more than one in four deaths annually.[125] In addition to lack of physical activity, sedentary behaviors — defined as time spent sitting or reclining while awake with low energy expenditure[1] — have emerged as a new risk factor.[32,33,88] Moreover, time spent sedentary has also been shown to be detrimentally associated with key biomarkers pertinent to both type 2 diabetes mellitus and CVD, notably excess adiposity and disordered lipid and glucose metabolism.[14,37]

Australian and UK sedentary behavior guidelines[77,78] incorporate messages specifically targeting the reduction of prolonged sitting — that is, sitting for prolonged periods at a time. Reducing prolonged sitting time may yield benefits by reducing the total time spent sedentary and increasing activity, and may convey further benefits that are specific to reducing this type of sedentary behavior. Experimental studies have shown that by comparison with sitting that has been interrupted with small amounts of activity, sitting continuously for prolonged periods has acute detrimental effects on blood pressure and lipid metabolism[11,92,126] and on postprandial glucose control,[8,9] with some effects persisting for up to 24 hours.[7,12,92,108,127] The observed beneficial effects could be attributed to breaking up sitting into shorter periods and/or to the small amounts of additional activity. Cross-sectional studies have observed statistically significant, detrimental associations of prolonged sitting time (variously defined) with waist circumference,[15,18,98] BMI,[15,18,98] HDL-cholesterol,[15,18] triglycerides,[15] and diastolic blood pressure.[16] Likewise, this may reflect benefits of sitting less and/or specifically avoiding sitting for long periods at a time. Contrary to results from experimental studies, cross-sectional studies have typically not observed

significant associations between prolonged sitting and biomarkers of glucose control.[16,18,98]

More rigorous evaluation of the effects of sedentary accumulation patterns is needed to better inform whether sedentary behavior guidelines[38] should be placing emphasis on prolonged sitting time and regularly interrupting sitting. Sedentary accumulation patterns refer to the degree to which sedentary time is accumulated in long, uninterrupted periods versus shorter, interrupted periods. Variously defined, sedentary accumulation patterns have shown cross-sectional associations with cardio-metabolic risk biomarkers, including BMI, waist circumference, insulin sensitivity, and triglycerides.[12,14] Many associations have persisted after statistical adjustment for the amount of time spent sedentary,[15,16,37,128–130] suggesting that not all of the effects of prolonged sedentary accumulation patterns are produced by a greater amount of sedentary time they likely entail. When examined separately as time spent in long and short sedentary bouts, the effects of sedentary time have typically appeared larger for time spent in long bouts.[18,98] However, verification with valid measures is needed, as nearly all of the evidence regarding sedentary accumulation patterns has been derived using low-validity measures.[75,101]

Using data from an activity monitor with good validity for measuring both sedentary behavior and sedentary accumulation patterns,[46,75,131] we examined sedentary accumulation patterns in relation to cardio-metabolic biomarkers in a population-based study of Australian adults aged 35 years and over (n=678). Specifically, we tested associations of sitting time, prolonged sitting time and sitting accumulation patterns with cardio-metabolic biomarkers. We also tested whether

sitting time has associations with cardio-metabolic biomarkers that vary depending on how long at a time the sitting time was usually accumulated.

METHODS

Sample and design

The Australian Diabetes, Obesity, and Lifestyle study (AusDiab) is a national, population-based cohort study established to understand the distribution and determinants of diabetes and other cardiovascular risk factors. Details of the original sampling methods and response rates are presented elsewhere.[132] Briefly, in 1999–2000, 11,247 adults aged ≥25 years completed questionnaires and underwent biomedical assessments. Participants were followed up in 2004-2005[133] and again in 2011-2012,[134] with 4,562 adults (all now aged >35 years) attending one of the 46 testing centers across Australia in the 2011-2012 follow-up. Participants were ineligible for the 2011-2012 follow up if they requested not to be contacted, were deceased, moved overseas, or if they were severely/terminally ill and/or moved into a nursing facility classified for high care. A sub-sample of 1,014 participants attending the 2011-2012 onsite assessment were invited to join an ancillary study described in detail elsewhere[36] where participants were asked to wear activity monitors, including the activPAL3[TM], for seven consecutive days (beginning the next day). A total of 782 adults (77%) agreed to participate. Pregnant and/or non-ambulatory participants were not eligible for the ancillary study. All participants provided informed written consent. Protocols for the study were approved by the Alfred Health Human Ethics Committee (project no. 39/11).

Measures

Cardio-metabolic outcomes. Upon arrival to the testing center, a fasting blood sample was drawn from each participant by venipuncture. Serum triglycerides, high-density lipoprotein (HDL) and total cholesterol were assayed by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was estimated using the Freidewald equation.[135] Glycated hemoglobin (HbA1c) was measured by a high-performance liquid chromatography method (Bio-Rad Variance Hemoglobin Testing System; Bio-Rad, Hercules, CA, USA). Participants underwent a 75 g oral glucose tolerance test unless it was contraindicated (e.g., pregnancy, taking medication for diabetes). Fasting plasma glucose (FPG) and 2-hour post-load glucose (PLG) were determined by the hexokinase method using a Seimens Advia 2400 (Siemens AG, Munich, Germany). All blood specimens were analyzed at a central laboratory operated by Healhscope Pathology in Clayton, Victoria. Systolic and diastolic blood pressure were each calculated as the mean of the first two (of three) readings from an automated sphygmomanometer (Dinamap DP 101-NIBP; GE Medical Systems, Freiburg, Germany) after ≥ 5 minutes rest. Body mass index (BMI; kg/m^2) was calculated from height and weight, measured to the nearest 0.5 cm and 0.1 kg (respectively) with participants removing shoes and excess clothing. Waist circumference was measured to the nearest 0.5 cm by tape measure between the lowest point on the ribs and the iliac crest on a horizontal plane, using the mean of two measures (or three measures, when the first two differed by ≥ 2 cm).

Minimum differences of interest (MDI) for the cardio-metabolic biomarkers, selected in discussion with a clinician to reflect clinically meaningful differences, were: 5% BMI (i.e., 1.36 kg/m^2); 2 cm waist circumference; 5% HDL- and LDL-cholesterol (i.e., 0.08 and 0.15 mmol/L, respectively); 10% triglycerides (i.e., 0.11 mmol/L); 5

mmHg systolic blood pressure; 3 mmHg diastolic blood pressure; and 10% FPG, PLG
and HbA1c (i.e., 0.54, 0.56, and 0.57 mmol/mol, respectively) [36].

 Potential confounders. Socio-demographic, behavioral, and health-related
characteristics measured by interviewer-administered questionnaire are described
elsewhere[132] and listed in Supplemental Table 1. Overall energy intake (MJ/day), fiber
intake (g/day), alcohol consumption (g/day), sodium intake (mg/day), and percentage
of energy intake derived from fat and saturated fat were measured using the 80-item
Dietary Questionnaire for Epidemiological Studies v2.[136]

Sedentary time and sedentary accumulation patterns

 Being sedentary a certain number of times (bout frequency), each for a certain
period (bout duration), adds up to the total volume of sedentary time[13] and,
collectively, bout frequency and bout duration constitute sedentary accumulation
patterns. There is no universally accepted indicator of sedentary accumulation. Most
studies have examined "breaks" in sedentary time, which is a measure of how often
people sit (when not accounting for sitting time) or of how often a certain amount of
sedentary time is interrupted with activity (when accounting for the amount of sitting
time). We examined three indicators of sedentary accumulation: transitioning from a
seated to upright posture (sit-stand transitions, a similar concept to "breaks"), usual
bout duration (also known as w50 or x50), and alpha.[19] Usual bout duration and alpha
are theoretically sound measures of sedentary accumulation based on the distribution
of sedentary bout duration[13,19] that each have slightly different measurement
properties [13]. Usual bout duration is the midpoint of the cumulative distribution of
sedentary bout durations (Supplemental Figure 5.1).[13,19] Half of all sedentary time is
accumulated in bouts longer than the usual bout duration. Alpha is a unitless

measure that characterizes the frequency distribution of sedentary bout durations (Supplemental Figure 5.1).[19] Lower values of alpha indicate sedentary time has been accumulated in relatively more long bouts and relatively fewer short bouts.

All of the activity measures were collected using the thigh-worn activPAL3[TM] monitor, which has high accuracy for measuring time spent sitting, standing, stepping and sitting accumulation patterns.[46,75,137] Rather than using the term sedentary, as this monitor specifically measures sitting, we refer to our results in terms of sitting time, prolonged sitting time (here, time spent sitting continuously for ≥30 minutes) and sitting accumulation patterns. The protocols and data processing procedures are described previously.[36] Briefly, participants were asked to wear the monitor 24 hours per day, and record sleep and monitor removals in a diary. Data were downloaded and initially processed using activPAL software version 6.4.1 (PAL Technologies Limited, Glasgow, UK) using default settings. Time spent sleeping, monitor non-wear, and invalid days (wear for <80% of waking hours and waking wear time <10 hours when diary data on sleep were missing) were removed using the diary and monitor data. Totals each day, averaged across valid days, were obtained for the number of sit-stand transitions and time spent: sitting; sitting in ≥30 min bouts; standing; stepping; and, stepping at ≥ 3 METs (i.e., moderate to vigorous physical activity; MVPA). The residuals method[54,60] was used to correct sitting time, prolonged sitting time, and MVPA for waking wear time, and to correct sit-stand transitions for sitting time. The accumulation measures were calculated as outlined elsewhere[19,138] (and detailed in Supplemental Figure 5.1).

Statistical analyses

Out of the initial monitor subsample (n=782), only participants who wore the monitor (n=741) for at least four valid days (n=720) who were not pregnant (n=718) and provided data on covariates and outcomes (n=678, and n=639 for PLG) were included. All statistical analyses were performed using Stata 14.0 (StataCorp LP, College Station, TX, USA) using linearized variance estimation to account for the survey design of AusDiab3. Significance was set at p<0.05 (two-tailed).

Multivariable linear regression was used to model the associations with each cardio-metabolic outcome of the prolonged sitting time and sitting accumulation patterns, adjusting for age, gender, and potential confounders. Results for sitting time have been reported previously[36] and are included here to place the effect sizes observed for prolonged sitting time and sitting accumulation patterns in context. Log transformation was used to improve the normality of BMI, triglycerides, HbA1c, glucose, and PLG. The sitting-related exposures were all examined as quintiles, with the first quintile (Q1; the referent category) always denoting the most time spent sitting or most prolonged (i.e., least interrupted) sitting accumulation pattern (see Supplemental Table 5.2). From the linear regression models, we report on pairwise comparisons of marginal means with all covariates set to their mean values, overall p-values, and p-for-trend. Potential confounders (Supplemental Table 5.1) were determined for each outcome using backwards elimination (p<0.20 for retention). Detrimental effects on biomarkers may occur through sitting displacing MVPA and via increases in body weight. Though MVPA and BMI could be confounders and/or causal intermediates, they were treated as the latter and therefore not adjusted as potential confounders in the main analyses.[56] MVPA-adjusted results are in Supplemental Tables 5.5 and 5.6 to allow comparison to results of prior research and

assess how sensitive conclusions were to the choice to adjust or not adjust. Age and gender interactions were explored in all models with a strict level of significance of p<0.001 because of the large number of tests performed.

Models do not adjust for sitting time as a confounder[139] because increasing the volume of sitting is one of the ways in which sitting for long periods may impact biomarkers. Instead, we tested whether the associations with the biomarker outcomes of sitting time (h/day, mean-centered) varied by usual bout duration (minutes, mean-centered) using interaction terms. Interactions meeting a generous threshold of p<0.1 were reported. To better describe the magnitude of any interaction detected, we report what the effects each hour per day of sitting time were when accumulated in "very long" and "very short" bouts. The mean value of Q1 and Q5 were used to represent "very long" and "very short" bouts.

RESULTS

The analytic sample included 678 adults (n=639 for analyses of PLG) with a mean age (± SD) of 57.8±9.8 years, after excluding participants who were pregnant or had any missing data (Table 5.1). Additional participant characteristics are described in Table 5.1 and Supplemental Table 5.3.

Figure 5.1 depicts participants' sitting accumulation patterns (bout frequency and bout duration) in relation to total sitting volume. The longer at a time that participants usually accumulated their sitting, typically the fewer the number of sitting bouts. An increase in either bout frequency or bout duration appeared to be non-linearly related to accruing a greater amount of sitting time. Sitting times of 6 to <10 h/day were seen over a diverse range and combination of sitting bout frequencies and durations. Notably, sitting times of 10 h/day or more almost exclusively occurred

with above-average usual bout duration. Sitting times of < 6 h/day almost exclusively occurred when participants had both fewer and shorter bouts than average.

Associations with cardio-metabolic biomarkers

Table 5.2 shows the associations of daily sitting time, prolonged sitting time, and sitting accumulation patterns with measures of adiposity and lipid measures. BMI and waist circumference decreased significantly across increasing quintiles of each of the measures. Mean differences (95% CI) between the top and bottom quintiles (Q5 versus Q1) were often of a sizeable magnitude (i.e., equivalent to the MDI or greater), ranging from -1.34 (-2.55, -0.13) to -3.54 (-4.90, -2.18) kg/m^2 for BMI and -3.48 (-6.69, -0.27) to -10.54 (-13.93, -7.16) cm for waist circumference. The observed differences were largest by alpha and smallest by sitting time. Only sitting time, prolonged sitting time, and alpha showed significant associations with HDL-cholesterol and triglycerides. These observed differences were also sizeable, ranging 0.14–0.15 mmol/L (HDL-cholesterol) and 0.20–0.29 mmol/L triglycerides. Associations of the other sitting accumulation measures with HDL-cholesterol and triglycerides were also beneficial in direction, but weaker and non-significant. No significant associations were observed with LDL cholesterol.

Table 5.3 shows the results for blood pressure and glucose. All associations with blood pressure and HbA1c were small (i.e., less than the MDI) and not statistically significant. Only alpha showed a statistically significant association with FPG; a small difference favoring patterns with more interrupted sitting (-0.20, 95% CI: -0.36, -0.04 mmol/L for Q5 versus Q1) was observed. Significantly lower PLG was observed with less sitting time (-0.50, 95% CI: -0.85, -0.14 mmol/L for Q5 versus Q1) and higher alpha (-0.64, 95% CI: -1.00, -0.29 mmol/L for Q5 versus Q1).

None of the associations reported in Tables 5.2 and 5.3 differed by age or gender at p<0.001 (Supplemental Table 5.4). Mostly, the associations reported in Tables 5.2 and 5.3 were only partially attenuated by statistical adjustment for MVPA (Supplemental Tables 5.5 and 5.6). Complete loss of significance was observed only for associations of usual bout duration with adiposity, and of sitting time with PLG.

Effect modification by usual bout duration

Usual sitting bout duration significantly modified associations of sitting time with HDL-cholesterol (p=0.005), triglycerides (p=0.03), and PLG (p=0.04) (Supplemental Table 5.7). In all instances, sitting time showed associations with biomarkers that were more strongly detrimental the longer at a time that sitting time was usually accumulated (Figure 5.2). For example, at an average usual bout duration (26.2 minutes), each hour per day spent sitting was associated with 0.04 (95% CI: 0.03, 0.06) mmol/L lower HDL cholesterol (Figure 5.2). By contrast, this lowering of HDL cholesterol with each hour per day spent sitting was 0.03 (95% CI: 0.01, 0.04) mmol/L with sitting time usually accumulated in very short bouts and 0.07 (95% CI: 0.04, 0.09) mmol/L with sitting time usually accumulated in very long bouts (Figure 5.2).

DISCUSSION

This study evaluated sedentary accumulation in relation to cardio-metabolic biomarkers in a large, general population sample of adults. To our knowledge, this study is among the first to examine this topic with accurate measures of sitting accumulation.[13,19] Many of the previous findings concerning sedentary accumulation that had been established with low-validity measures were corroborated. Although sitting many times per day and accumulating sitting time in long bouts were both

relevant in terms of how much sitting time adults ultimately accrued, additional time spent sitting did not appear to be the only relevant correlate of prolonged accumulation patterns.

The only other study of which the authors are aware that has tested associations of patterns with adult cardiometabolic biomarker outcomes using high-quality measures of sitting patterns was the Maastricht Study.[107] Adjusting only for confounders, not competing time uses, the authors found that sitting patterns (measured as breaks, number of ≥30 min sitting bouts and average sitting bout duration) had statistically significant associations with both metabolic syndrome and glucose metabolism status (normal, impaired fasting glucose/impaired glucose tolerance, type 2 diabetes mellitus). Effects typically indicated the healthiest participants had the most interrupted sedentary patterns though associations were not significant with all of the pattern measures. A meta-analysis of associations between adiposity and sedentary accumulation patterns concluded that there is "some certainty" that more interrupted patterns (specifically, more breaks in sedentary time) are significantly associated with lower BMI and, with less certainty, smaller waist circumference.[12] These same associations were present using our three indicators of sitting accumulation patterns. Previously, a review had concluded there was "some evidence" that sedentary accumulation patterns are associated with triglycerides and there was "inconclusive evidence" of an association with HDL-cholesterol.[14] Our findings were in favor of an association with both cardio-metabolic biomarkers. Consistent with prior studies,[16,18] we did not observe significant associations of sitting accumulation patterns with LDL cholesterol. The null results for HbA1c were consistent with the typically null results in the extant literature[18,98] and

our null results for blood pressure did not conflict with prior results, which are mixed.[16,98,128,140] Despite the potential biases in previous findings, our findings with high-quality measures did not contradict any of the previous conclusions regarding these biomarkers.

The greatest dissimilarity between our findings and the extant literature based on observational studies was for glucose metabolism. In most — but not all [16,128] — previous cross-sectional research, significant associations of sedentary accumulation patterns with FPG and PLG have not been observed.[14] Notably, our findings were dependent on the accumulation measure employed; both these associations were detected only with alpha. Similarly, in the Maastricht study, associations with glucose metabolism were not significant for "breaks" as a measure but were significant for average bout duration and number of prolonged bouts.[107] It is possible that different indicators of accumulation patterns may have different capabilities to detect true effects, and different susceptibilities to unmeasured confounders. Alternatively, our findings could be an aberration or the result of multiple hypothesis testing.

Though the adjustment or non-adjustment for MVPA is a contentious issue on both epidemiologic and statistical grounds,[56,141] it did not appear to strongly affect what conclusions were drawn in our study concerning sedentary accumulation patterns. Adjustment for MVPA (not a procedure we advocate in general) led to only partial attenuation of results — seldom to complete loss of statistical significance. The limited degree of attenuation also suggests that the beneficial associations with cardio-metabolic biomarkers that we observed for our sitting-related exposures are likely to involve mechanisms other than those induced by, or operating through, additional MVPA. By contrast, causation more generally, particularly as concerns

adiposity, remains unresolved and is important to establish in further research with longitudinal and/or experimental designs. Many of our findings could be explained by heavier bodyweight inducing individuals to transition between postures less frequently.

The present study provides some evidence to support prolonged sitting as a specific target of sedentary reduction messages. Waist circumference, BMI, HDL cholesterol and triglycerides were significantly associated with sitting time and prolonged sitting time. PLG was further significantly associated with total sitting time. Although effect sizes for these outcomes were similar when examining sitting and prolonged sitting, rather than suggesting all types of sitting are the same, this likely reflects the problems in examining only one subtype of sitting in isolation. Comparing participants according to their sitting accumulation patterns consistently showed greater differences between the top and bottom quintiles than either sitting or prolonged sitting time, especially by the alpha measure. Crucially, the longer at a time participants accumulated their sitting, the stronger the effects of each hour per day of sitting time on several biomarkers (HDL cholesterol, triglycerides and PLG). This is consistent with the previous research that has aimed to examine or compare short and long sedentary bouts,[18,98] but has been limited by the reliance on low-quality measures and other analytic issues, including the somewhat arbitrary divisions between short and long bouts. Though generally supportive that being sedentary for longer periods at a time may magnify the health risks of sedentary time, more research is needed to develop specific recommendations, such as how long is too long to be sitting without taking a break.

Measurement quality of the exposure variables was a key study strength. The exposure variables were measured over a requested 7-days, which is sufficient to produce reliable measures of total sedentary time over a 3-year period.[76] That said, to date, no studies have assessed the degree to which measures from a 7-day sedentary accumulation pattern measurement protocol reflect longer-term patterns of behavior and our results should be interpreted in consideration of this potential limitation. Future studies should consider longer measurement protocols. Key limitations were the cross-sectional design, which makes results subject to reverse-causality bias (e.g., cardiovascular disease, diabetes, and/or BMI could cause prolonged patterns of sitting accumulation), and the sample size, which was not chosen a priori and sometimes provided insufficient precision as indicated by the 95% confidence intervals of some associations that were not statistically significant containing effects of a magnitude ≥ MDIs. The sample, though covering a broad cross-section of Australian adults, was not population representative, with loss to follow-up prior to this third wave of data collection, and some biases in the subsampling and subsample participation,[36] with potential consequences both to internal and external validity. Residual confounding may exist from unmeasured variables and variables measured with error (e.g., educational status was not current as at 2011/12). Many hypotheses were tested, and some results could be spurious. Of all the findings, the most caution is warranted concerning glucose — the literature has mixed findings and our results varied depending on the measure and statistical adjustment choices. In general, the internal consistency within this study in the direction of the associations and the similarity between our findings and those of other studies suggest that most of our findings are sound.

This study adds important, robust evidence to a growing body of research supporting that in addition to high volumes of time spent sitting, the manner in which sitting time is accumulated has relevance for key areas of cardiovascular and metabolic health — lipid metabolism, glucose metabolism, and adiposity. Evidence concerning causation for long-term effects, such as from longitudinal and/or long-term intervention studies, is needed.

Chapter 5, in full, has been published in PLOS ONE. Bellettiere, John; Winkler, Elisabeth A.H.; Chastin, Sebastien F.M.; Kerr, Jacqueline; Owen, Neville; Dunstan, David W.; Healy, Genevieve N. (2017) *Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults*. PLoS ONE 12(6): e0180119. John Bellettiere was the primary investigator and author of this material.

Table 5.1 Selected sociodemographic, medical, cardio-metabolic, and sitting-related characteristics of the final analytic sample, (AusDiab 2011-12; n=678).

Age, years	57.8 (9.8)
Men, n(%)	297 (45)
Ethnicity, n(%)	
Australia/New Zealand (Non-Indigenous)	550 (81)
Australia/New Zealand (Aboriginal/Torres Strait Islander)	4 (1)
Other English speaking	75 (11)
South Europe	8 (1)
Other Europe	19 (3)
Asia	18 (3)
Other	4 (1)
Family history of diabetes, n(%)	191 (28)
Prior cardiovascular disease diagnosis[a], n(%)	41 (6)
Body Mass Index, kg/m2	27.4 (4.9)
Waist circumference, cm	93 (13.7)
Systolic blood pressure, mmHg	126.3 (17.3)
Diastolic blood pressure, mmHg	72.7 (10.5)
Fasting blood glucose, mmol/L	5.3 (0.73)
HbA1c (IFCC), mmol/L	5.6 (0.35)
High-density lipoprotein cholesterol, mmol/L	1.6 (0.41)
Low-density lipoprotein cholesterol, mmol/L	3.0 (0.82)
Triglycerides, mmol/L	1.3 (0.66)
2-hour postload plasma glucose, mmol/L[b]	5.6 (2.02)
Daily sitting time[c,f], h/day	8.8 (1.7)
Time in sedentary bouts ≥30 minutes [c,f], h/day	4.0 (1.6)
Sit-stand transitions[d], n/day	54.1 (14.5)
Usual bout duration (min)	26.2 (8.9)
Alpha[g]	1.3 (0.039)
Moderate to Vigorous Physical Activity[e,f], h/day	1.2 (0.4)

Abbreviations: IFCC, International Federation of Clinical Chemistry and Laboratory Medicine

Table reports mean (standard deviation) or n(%); means, standard deviations, and % are corrected for the complex sampling design using linearized variance estimation. Results are for the analytic sample that was obtained using complete case analysis.

[a] Heart attack, stroke or angina.

[b] 2-hr postload plasma glucose data were missing from 39 participants.

[c] Variables standardized to device wear time using the residuals method.

[d] Variable standardized to daily sitting time using the residuals method.

[e] Measured via activPAL as "stepping" equivalent to ≥ 3 METs.

[f] Estimates are similar to those previously reported in Healy et al. Eur Heart J. 2015, differing slightly due to small differences in inclusion criteria.

[g] Alpha is a unitless measure of sitting accumulation ranging from 1.22 to 1.51. Higher values indicate accumulation patterns with relatively more interrupted sitting than prolonged sitting.
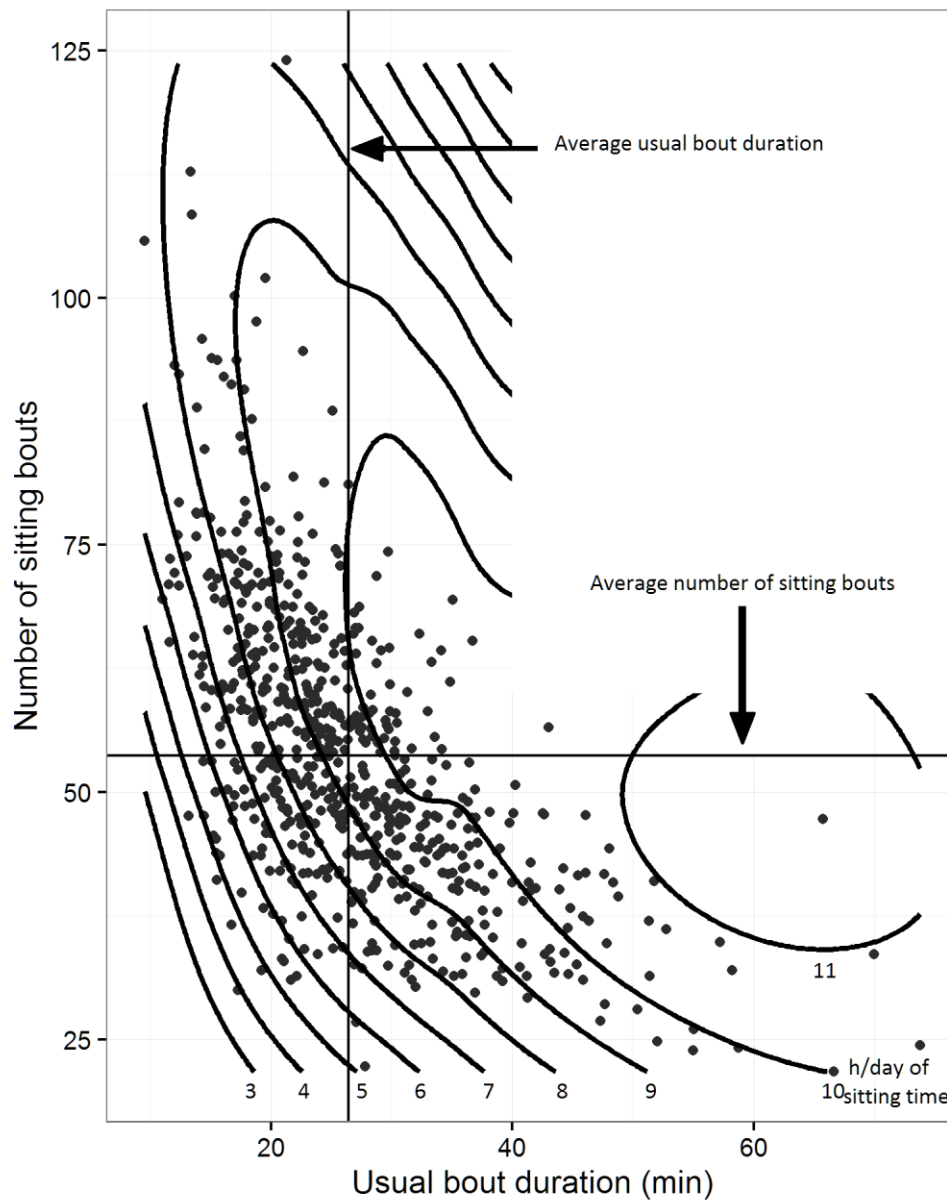
Figure 5.1 Sitting accumulation patterns. *Number of bouts (y axis), usual bout duration (x axis) and the amount of sitting time accumulated (contour lines). Each point represents one participant.*

Table 5.2 Associations and all potential covariates, of sitting and prolonged sitting time, and sitting accumulation with measures of adiposity and lipid metabolism in Australian adults aged 36 to 80 (AusDiab 2011-12; n=678).

	Quintile 1[a]	Quintile 2 Mean diff.[b]	Quintile 2 95% CI	Quintile 3 Mean diff.[b]	Quintile 3 95% CI	Quintile 4 Mean diff.[b]	Quintile 4 95% CI	Quintile 5 Mean diff.[b]	Quintile 5 95% CI	P-for-trend	P-overall
Body Mass index (kg/m2)											
Total sitting time[c,d]	referent	0.28	(-0.91, 1.48)	-0.70	(-1.55, 0.25)	-1.57	(-2.45, -0.69)	-1.34	(-2.55, -0.13)	**0.001**	**<.001**
Prolonged sitting time[c]	referent	0.29	(-0.93, 1.51)	-0.90	(-2.00, 0.25)	-1.40	(-2.47, -0.33)	-1.64	(-2.82, -0.46)	**0.001**	**0.01**
Sit-stand transitions[e]	referent	-0.57	(-1.53, 0.39)	-1.50	(-2.56, -0.48)	-1.26	(-2.44, -0.08)	-2.76	(-3.82, -1.70)	**<.001**	**<.001**
Usual bout duration	referent	-1.26	(-2.32, -0.21)	-1.20	(-2.27, -0.11)	-1.32	(-2.54, -0.10)	-2.04	(-3.34, -0.74)	**0.007**	0.06
Alpha	referent	-0.30	(-1.53, 0.92)	-1.70	(-3.11, -0.20)	-2.33	(-3.65, -1.02)	-3.54	(-4.90, -2.18)	**<.001**	**<.001**
Waist circumference (cm)											
Total sitting time[c,d]	referent	-0.49	(-3.47, 2.49)	-2.10	(-4.76, 0.48)	-5.46	(-7.74, -3.17)	-3.48	(-6.69, -0.27)	**0.001**	**<.001**
Prolonged sitting time[c]	referent	0.16	(-3.15, 3.46)	-3.00	(-6.11, 0.01)	-4.12	(-6.73, -1.51)	-4.22	(-7.25, -1.20)	**0.001**	**0.01**
Sit-stand transitions[e]	referent	-2.39	(-4.80, 0.03)	-4.80	(-7.52, -2.06)	-3.70	(-6.43, -0.97)	-7.46	(-10.32, -4.59)	**<.001**	**<.001**
Usual bout duration	referent	-2.90	(-6.01, 0.22)	-3.20	(-6.14, -0.18)	-3.90	(-6.82, -0.98)	-4.97	(-8.11, -1.83)	**0.004**	0.06
Alpha	referent	-2.30	(-5.26, 0.67)	-5.60	(-9.1, -2.04)	-7.29	(-10.55, -4.02)	-10.54	(-13.93, -7.16)	**<.001**	**<.001**

Table 5.2 Associations and all potential covariates, of sitting and prolonged sitting time, and sitting accumulation with measures of adiposity and lipid metabolism in Australian adults aged 36 to 80 (AusDiab 2011-12; n=678), Continued.

	Quintile 1[a]	Quintile 2		Quintile 3		Quintile 4		Quintile 5		P-for-trend	P-overall
	Mean diff.[b]	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI		
HDL Cholesterol (mmol/L)											
Total sitting time[c,d]	referent	0.01	(-0.06, 0.09)	0.10	(-0.02, 0.14)	0.16	(0.08, 0.25)	0.14	(0.07, 0.22)	**<.001**	**<.001**
Prolonged sitting time[c]	referent	0.09	(0.00, 0.17)	0.10	(-0.02, 0.17)	0.12	(0.06, 0.18)	0.14	(0.06, 0.22)	**<.001**	**0.001**
Sit-stand transitions[e]	referent	0.03	(-0.07, 0.13)	0.00	(-0.07, 0.10)	0.03	(-0.06, 0.12)	0.06	(-0.04, 0.15)	0.26	0.67
Usual bout duration	referent	0.05	(-0.02, 0.11)	0.00	(-0.05, 0.08)	0.02	(-0.04, 0.08)	0.06	(0.00, 0.13)	0.13	0.36
Alpha	referent	0.03	(-0.05, 0.12)	0.10	(0.02, 0.20)	0.09	(0.02, 0.16)	0.15	(0.07, 0.24)	**<.001**	**0.002**
LDL Cholesterol (mmol/L)											
Total sitting time[c,d]	referent	0.07	(-0.06, 0.21)	0.00	(-0.19, 0.13)	-0.02	(-0.17, 0.14)	0.00	(-0.17, 0.18)	0.68	0.81
Prolonged sitting time[c]	referent	0.08	(-0.08, 0.24)	0.00	(-0.14, 0.23)	0.06	(-0.14, 0.26)	0.05	(-0.12, 0.23)	0.67	0.91
Sit-stand transitions[e]	referent	0.05	(-0.16, 0.27)	0.00	(-0.22, 0.16)	0.00	(-0.22, 0.22)	0.10	(-0.09, 0.29)	0.44	0.77
Usual bout duration	referent	-0.03	(-0.24, 0.18)	0.10	(-0.09, 0.23)	-0.03	(-0.24, 0.17)	0.12	(-0.06, 0.29)	0.25	0.37
Alpha	referent	0.03	(-0.15, 0.21)	0.00	(-0.19, 0.19)	0.00	(-0.17, 0.16)	-0.01	(-0.16, 0.14)	0.76	0.99

Table 5.2 Associations and all potential covariates, of sitting and prolonged sitting time, and sitting accumulation with measures of adiposity and lipid metabolism in Australian adults aged 36 to 80 (AusDiab 2011-12; n=678), Continued.

	Quintile 1[a]	Quintile 2		Quintile 3		Quintile 4		Quintile 5		P-for-trend	P-overall
		Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI		
Triglycerides (mmol/L)											
Total sitting time[c,d]	referent	-0.04	(-0.17, 0.08)	-0.10	(-0.25, -0.03)	-0.32	(-0.41, -0.22)	-0.23	(-0.35, -0.11)	**<.001**	**<.001**
Prolonged sitting time[c]	referent	-0.13	(-0.26, 0.00)	-0.20	(-0.34, -0.07)	-0.18	(-0.29, -0.07)	-0.20	(-0.32, -0.08)	**0.003**	**0.007**
Sit-stand transitions[e]	referent	0.02	(-0.10, 0.15)	0.00	(-0.17, 0.12)	-0.07	(-0.20, 0.06)	-0.09	(-0.24, 0.05)	0.09	0.41
Usual bout duration	referent	-0.09	(-0.23, 0.05)	-0.10	(-0.22, 0.02)	-0.05	(-0.18, 0.07)	-0.11	(-0.24, 0.03)	0.27	0.21
Alpha	referent	-0.13	(-0.25, 0.00)	-0.10	(-0.25, 0.03)	-0.16	(-0.29, -0.03)	-0.29	(-0.41, -0.18)	**<.001**	**<.001**

[a] Participants in quintile 1 have the highest total sitting time / prolonged sitting time / the most prolonged pattern of sitting time accumulation. Quintile cutpoints are in S2 Table.
[b] Difference in adjusted mean in contrast to quintile 1, adjusted for age, gender and potential confounders (Supplementary Table 1), from linear regression model with linearized variance estimation accounting for state/testing center strata/clusters.
[c] Variables adjusted for device wear time using the residuals method.
[d] Associations are similar to those previously reported in Healy et al. Eur Heart J. 2015, differing slightly due to small differences in inclusion criteria and differences in the functional form of total sitting time.
[e] Variable adjusted for total sitting time using the residuals method.
Bolded p-values indicate statistically significant relations at p < 0.05.

Table 5.3 Associations of sitting and prolonged sitting time, and sitting accumulation with measures of blood pressure and glucose control in Australian adults aged 36 to 80 (AusDiab 2011-12; n=678[a])

	Quintile 1[b]	Quintile 2		Quintile 3		Quintile 4		Quintile 5		P-for-trend	P-overall
		Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI		
Systolic BP (mmHg)											
Total sitting time[d,e]	referent	0.69	(-2.44, 3.82)	-1.60	(-5.10, 1.84)	1.43	(-2.46, 5.31)	1.05	(-1.83, 3.93)	0.44	0.57
Prolonged sitting time[d]	referent	2.68	(-2.02, 7.37)	4.70	(-0.18, 9.49)	2.17	(-2.08, 6.41)	2.84	(-1.27, 6.95)	0.24	0.38
Sit-stand transitions[f]	referent	-2.14	(-5.66, 1.38)	-1.80	(-5.78, 2.14)	-1.40	(-5.86, 3.05)	-0.50	(-5.05, 4.06)	0.98	0.72
Usual bout duration	referent	0.12	(-4.20, 4.43)	3.30	(-0.67, 7.33)	0.88	(-3.87, 5.63)	1.84	(-2.06, 5.73)	0.37	0.56
Alpha	referent	1.36	(-2.51, 5.23)	0.40	(-4.34, 5.10)	-0.07	(-5.47, 5.33)	0.52	(-4.00, 5.05)	0.94	0.94
Diastolic BP (mmHg)											
Total sitting time[d,e]	referent	1.94	(-0.17, 4.04)	-1.40	(-3.38, 0.67)	0.01	(-2.25, 2.27)	-0.98	(-3.12, 1.17)	0.16	0.06
Prolonged sitting time[d]	referent	2.52	(0.37, 4.66)	1.90	(-0.89, 4.75)	0.81	(-1.65, 3.28)	1.20	(-1.39, 3.80)	0.85	0.29
Sit-stand transitions[f]	referent	0.59	(-1.66, 2.78)	-0.60	(-3.03, 1.81)	-0.67	(-3.21, 1.87)	-0.18	(-2.92, 2.57)	0.61	0.81
Usual bout duration	referent	0.44	(-1.66, 2.53)	0.90	(-1.64, 3.34)	0.45	(-2.13, 3.04)	1.05	(-1.52, 3.62)	0.49	0.95
Alpha	referent	-0.47	(-2.56, 1.62)	-1.60	(-4.14, 1.00)	-1.96	(-4.71, 0.80)	-2.05	(-4.54, 0.44)	0.08	0.47

Table 5.3 Associations of sitting and prolonged sitting time, and sitting accumulation with measures of blood pressure and glucose control in Australian adults aged 36 to 80 (AusDiab 2011-12; n=678[a]), Continued.

	Quintile 1[b]	Quintile 2		Quintile 3		Quintile 4		Quintile 5		P-for-trend	P-overall
		Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI		
2-hour post-load glucose (mmol/L)											
Total sitting time[d,e]	referent	-0.29	(-0.77, 0.20)	-0.40	(-0.82, 0.09)	-0.36	(-0.78, 0.07)	-0.50	(-0.85, -0.14)	**0.009**	0.12
Prolonged sitting time[d]	referent	-0.21	(-0.52, 0.11)	-0.40	(-0.74, 0.04)	-0.33	(-0.71, 0.04)	-0.18	(-0.60, 0.24)	0.33	0.44
Sit-stand transitions[f]	referent	-0.16	(-0.57, 0.25)	-0.10	(-0.38, 0.25)	-0.34	(-0.77, 0.08)	-0.12	(-0.57, 0.32)	0.42	0.50
Usual bout duration	referent	-0.16	(-0.42, 0.11)	-0.20	(-0.67, 0.18)	-0.16	(-0.51, 0.19)	0.02	(-0.37, 0.40)	0.93	0.47
Alpha	referent	-0.40	(-0.80, 0.00)	-0.20	(-0.55, 0.10)	-0.71	(-1.20, -0.21)	-0.64	(-1.00, -0.29)	**0.002**	**0.009**

[a] Models of 2-hour post-load glucose had n=639.

[b] Participants in quintile 1 have the highest total sitting time / prolonged sitting time / the most prolonged pattern of sitting time accumulation. Quintile cutpoints are in S2 Table.

[c] Difference in adjusted mean in contrast to quintile 1, adjusted for age, gender and potential confounders (Supplementary Table 1), from linear regression models with linearized variance estimation accounting for state/testing center strata/clusters.

[d] Variables adjusted for device wear time using the residuals method.

[e] Associations are similar to those previously reported in Healy et al. Eur Heart J. 2015, differing slightly due to small differences in inclusion criteria and differences in the functional form of total sitting time.

[f] Variable adjusted for total sitting time using the residuals method.

Bolded p-values indicate statistically significant relations at p < 0.05.
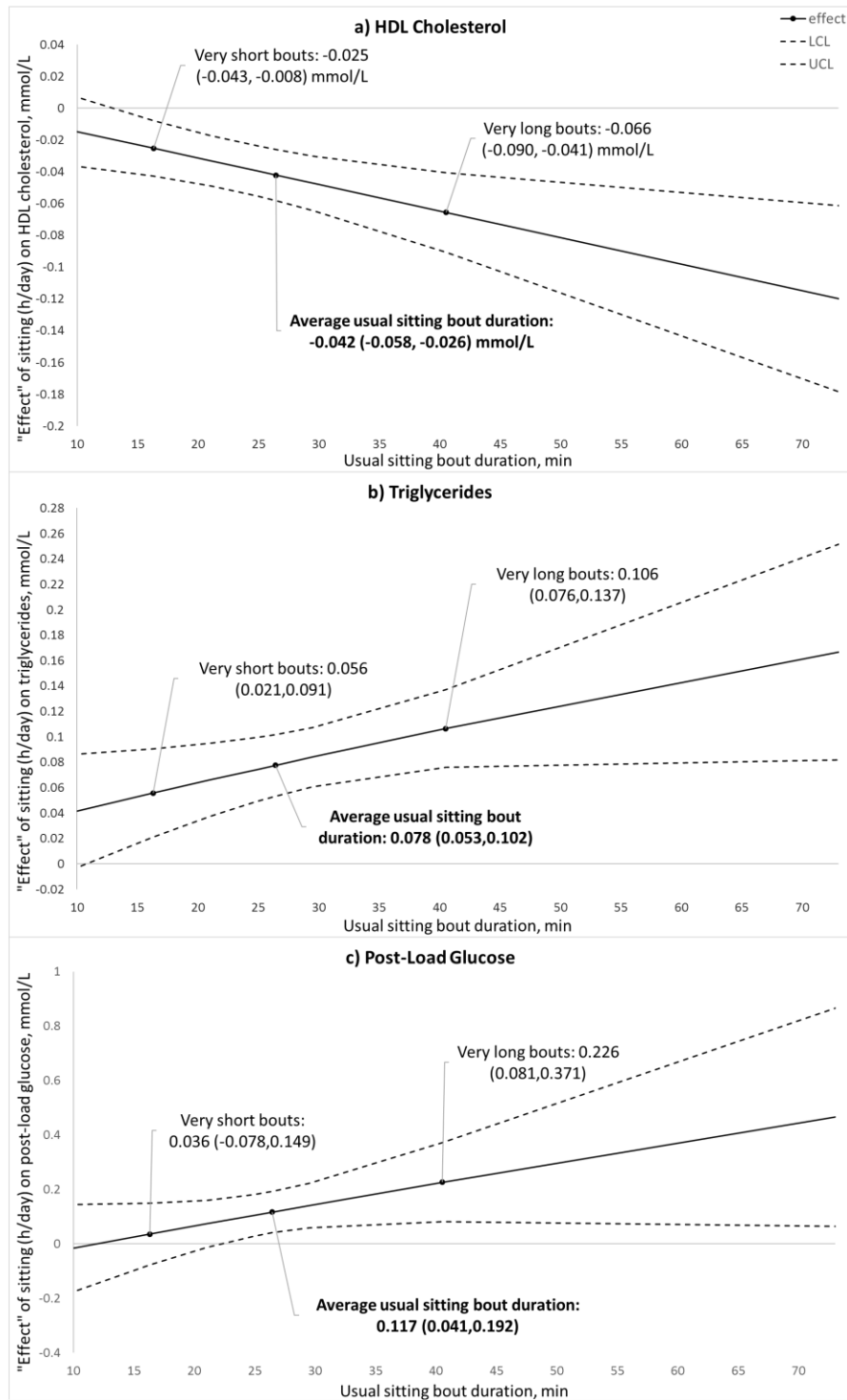
Figure 5.2 Cross-sectional associations of each hour of daily sitting time with (a) HDL-cholesterol, (b) triglycerides, and (c) PLG glucose by how long at a time the sitting was usually accumulated. *Solid lines indicate the estimated association between total sitting time and each biomarker with the dashed lines indicating 95% confidence intervals.*

Supplemental Table 5.1 List of all variables considered or adjusted for as potential confounders in multivariable models.

Model	Potential covariates considered or adjusted in final analyses
Considered for all outcomes	*Age* (as continuous [years] or categorical [35-44 years; 45-54 years; 55-64 years; 65-74 years; ≥75 years]) *Gender* (male/female) *Menopausal status* (post-menopausal/going through menopause/pre-menopausal/not applicable [male]) *Contraceptive pill use* (yes/no/not applicable [male]) *Blood pressure tablets* (yes/no) *Cholesterol tablets* (yes/no) *Diabetes medication* (yes/no) *Ethnicity* (Australian or New Zealand/Other English speaking/Other) *Present occupation or previous if not working* (managers or professionals/technical & trade or community & personal service/clerical & administrative or sales/machinery operator & driver or laborer/never worked or unknown) *Annual household income before tax* (< $30k/$30 to < $60k/$60k to < 100k/≥ $100k/refused or don't know or missing) *Employment status* (full time/part time/retired/other not working/missing) *Fiber intake* (g/day) *Fat, %E* *Saturated fat, %E* *Alcohol intake* (g/day) *Sodium intake* (mg/day) *Potassium intake* (mg/day) *Fruit and vegetable serves* (serves/day)
Body mass index (kg/m^2)	age category, gender, blood pressure tablets, cholesterol tablets, depression score category, diabetes medications, fat intake, energy-adjusted fiber intake
Waist circumference (cm)	age category, gender, blood pressure tablets, cholesterol tablets, depression score category, diabetes medication, height, employment status, saturated fat intake, energy intake, alcohol intake, sodium intake
Systolic blood pressure (mmHg)	age, gender/menopausal status, blood pressure tablets, main lifetime occupation, diabetes medication
Diastolic blood pressure (mmHg)	age category, gender, blood pressure tablets, ethnicity, main lifetime occupation, family history of diabetes, ownership of current residence, height, saturated fat intake, energy intake, alcohol intake, sodium intake
Fasting glucose (mmol/L)	age, gender/menopausal status, cholesterol tablets, main lifetime occupation, diabetes medication, housing type, sodium intake, calcium intake
HbA$_{1c}$ (%)	age, gender/ menopausal status, blood pressure tablets, cholesterol tablets, depression score category, main lifetime occupation, diabetes medication, housing type, height, employment status, alcohol intake, fruit and vegetable intake
Total cholesterol (mmol/L)	age category, gender/menopausal status, cholesterol tablets, ethnicity, diabetes medication, saturated fat intake, alcohol intake
HDL cholesterol (mmol/L)	age category, gender/menopausal status, cholesterol tablets, smoking, housing type, main lifetime occupation, history of CVD, alcohol intake
LDL cholesterol (mmol/L)	age category, gender/menopausal status, cholesterol tablets, ethnicity, saturated fat, energy-adjusted fiber intake
Triglycerides (mmol/L)	age category, gender/menopausal status, cholesterol tablets, smoking, depression score category, ownership of current residence, income category, fruit and vegetable intake
2-hour post load glucose (mmol/L)	age, gender, ethnicity, smoking, married or defacto, family history of diabetes, housing type, height, calcium intake

Supplemental Table 5.2 Range of values within each quintile of sitting, sitting in 30+ min bouts, sit-stand transitions, usual bout duration, and alpha.

	Quintile 1			Quintile 2			Quintile 3			Quintile 4			Quintile 5		
	n	Mean (SD)	Min, Max	n	Mean (SD)	Min, Max	n	Mean (SD)	Min, Max	n	Mean (SD)	Min, Max	n	Mean (SD)	Min, Max
Total sitting time[a], h/day	135	11.1 (0.6)	10.3, 13.2	136	9.8 (0.3)	9.3, 10.2	135	8.9 (0.2)	8.4, 9.2	136	7.9 (0.3)	7.3, 8.3	136	6.3 (0.9)	3.1, 7.2
Prolonged sitting time[a], h/day	135	6.5 (1.0)	5.3, 9.7	136	4.8 (0.3)	4.3, 5.2	135	3.9 (0.2)	3.5, 4.2	136	3.1 (0.2)	2.6, 3.4	136	2.0 (0.5)	0.5, 2.5
Sit-stand transitions[b], n/day	136	35.6 (4.5)	19.8, 41.8	136	45.3 (1.8)	41.9, 48.2	135	52.1 (2.3)	48.3, 55.8	136	60.2 (2.7)	55.9, 64.8	135	75.5 (10.6)	64.9, 120.6
Usual bout duration, min	135	40.5 (7.9)	32.8, 73.8	136	29.5 (1.6)	27.0, 32.7	135	24.9 (1.2)	22.9, 26.9	136	21.1 (1.1)	19.2, 22.8	136	16.3 (2.1)	9.6, 19.1
Alpha	136	1.29 (0.01)	1.225, 1.304	136	1.32 (0.01)	1.305, 1.324	135	1.33 (0.01)	1.325, 1.342	136	1.36 (0.01)	1.343, 1.367	135	1.40 (0.03)	1.368, 1.516

Table reports mean (standard deviation).
[a] Variables adjusted for device wear time using the residuals method.
[b] Variable adjusted for daily sitting time using the residuals method.

Supplemental Table 5.3 Sociodemographic, behavioral, medical, cardiometabolic, and sitting-related characteristics of the final analytic sample, Australia, 2011/12.

	Final analytic sample (n=678)
Socio-demographic	
Age, *years*	57.8 (9.8)
Age category, *n(%)*	
35-44 years	68 (10)
45-54 years	191 (28)
55-64 years	238 (35)
65-74 years	142 (21)
≥75 years	39 (6)
Men, *n(%)*	297 (45)
Height, *cm*	169.4 (8.9)
Ethnicity, *n(%)*	
Australia/New Zealand	554 (82)
Other English speaking	75 (11)
Other non-English speaking	49 (7)
Married/defacto, *n(%)*	524 (78)
Employment status, *n(%)*	
Full time	254 (37)
Part time	148 (22)
Retired	200 (30)
Other/not working/missing	76 (11)
Occupation (current or previous if retired or not currently working) *n(%)*	
Managers/Professionals	282 (42)
Technical & Trade/Community & Personal service	85 (13)
Clerical & Administrative/Sales	159 (23)
Machinery operator & driver/Laborer	45 (6)
Never worked/Unknown	107 (16)
Housing (house), *n(%)*	619 (92)
Owns residence (yes), *n(%)*	602 (89)
Annual household income before taxes, *n(%)*	
< $30k	92 (14)
$30k to <$60k	161 (24)
$60k to <100k	145 (22)
>= $100k	241 (36)
refused/don't know/missing	39 (5)
Behavioral	
Dietary intake	
Fat, *%E*	34.8 (5.2)
Saturated fat, *%E*	13.8 (2.8)
Energy intake, *MJ/day*	7621.9 (2937.4)
Fiber intake, *g/day*	21.4 (8.8)
Total alcohol, *g/day*	13.5 (17.2)
Sodium, *g/day*	2296.7 (377.4)
Potassium, *g/day*	2859.2 (926.2)
Calcium, *mg/day*	904.7 (326.2)
Fruit and vegetables, *serves/day*	3.6 (1.5)

Supplemental Table 5.3 Sociodemographic, behavioral, medical, cardiometabolic, and sitting-related characteristics of the final analytic sample, Australia, 2011/12, Continued.

	Final analytic sample (n=678)
Smoking status, *n(%)*[c]	
Never smoker	383 (56)
Ex-smoker	247 (37)
Current smoker	48 (7)
Medical	
Family history of diabetes, *n(%)*	191 (28)
Diabetes medications, *n(%)*	32 (5)
Blood pressure tablets, *n(%)*	69 (10)
Cholesterol tablets, *n(%)*	131 (20)
Oral contraceptives, *n(%)*	
Not applicable (male)	297 (45)
No	143 (22)
Yes	221 (33)
Prior CVD diagnosis [d], *n(%)*	41 (6)
Menopause, *n(%)*	
Post-menopausal	219 (32)
Going through menopause	58 (8)
Pre-menopausal	104 (15)
Not applicable (male)	297 (45)
Cardio-metabolic biomarkers	
Body Mass Index, *kg/m^2*	27.4 (4.9)
Waist circumference, *cm*	93 (13.7)
Systolic blood pressure, *mmHg*	126.3 (17.3)
Diastolic blood pressure, *mmHg*	72.7 (10.5)
Fasting blood glucose, *mmol/L*	5.3 (0.73)
HbA$_{1c}$ (IFCC), *mmol/L*	5.6 (0.35)
HDL cholesterol, *mmol/L*	1.6 (0.41)
LDL cholesterol, *mmol/L*	3 (0.82)
Triglycerides, *mmol/L*	1.3 (0.66)
2-hr postload plasma glucose, *mmol/*L	5.6 (2.02)
Sitting time and sitting accumulation	
Total sitting time[a], *h/day*	8.8 (1.7)
Prolonged sitting time [a], *h/day*	4 (1.6)
Sit-stand transitions[b], *n/day*	54.1 (14.5)
Usual bout duration, *min*	26.2 (8.9)
Alpha	1.3 (0.039)
Moderate to vigorous physical activity[c], h/day	1.2 (0.4)

Table reports mean (standard deviation) or n(%) for categorical variables where means, standard deviations, and % are corrected for the complex sampling design using linearized variance estimation.

[a] Variables adjusted for device wear time using the residuals method.

[b] Variable adjusted for daily sitting time using the residuals method.

[c] Measured via activPAL as "stepping" equivalent to ≥ 3 METs.

Supplemental Table 5.4 Statistical significance of interactions by age categories and gender in associations of sitting and prolonged sitting time, and sitting accumulation with cardio-metabolic biomarkers, adjusted for potential confounders.

	Age interactions (p-value)					Gender interactions (p-value)				
	Total sitting time, h/day	Prolonged sitting time, h/day	Sit-stand transitions, n/day	Usual bout duration, min	Alpha	Total sitting time, h/day	Prolonged sitting time, h/day	Sit-stand transitions, n/day	Usual bout duration, min	Alpha
Body Mass index (kg/m2), β	0.93	0.78	0.46	0.41	0.28	**0.04**	**0.02**	**0.02**	0.05	**0.002**
Waist circumference (cm), β	0.71	0.85	0.52	0.48	0.69	**0.02**	**0.008**	0.15	**0.04**	**0.01**
HDL Cholesterol (mmol/L), β	0.52	0.91	0.92	0.16	0.66	0.73	0.96	0.36	0.56	0.92
LDL Cholesterol (mmol/L), β	0.06	0.62	0.95	0.87	**0.05**	0.21	0.23	0.76	0.51	0.8
Triglycerides (mmol/L), RR	0.53	0.94	0.71	0.87	0.70	0.41	0.27	0.99	0.23	0.73
Systolic BP (mmHg), β	0.06	0.16	0.75	0.82	0.18	0.08	**0.04**	0.26	0.05	0.23
Diastolic BP (mmHg), β	0.34	0.54	0.71	0.96	0.27	0.15	0.10	0.83	0.28	0.28
HbA1c (mmol/mol), RR	0.96	0.90	0.62	0.88	0.27	0.68	0.80	0.11	0.35	0.87
Glucose (mmol/L), RR	0.06	0.18	0.58	0.47	0.16	0.28	0.51	0.08	0.79	0.62
2-hour post-load glucose (mmol/L), RR	0.23	0.72	0.47	0.16	0.85	0.19	0.27	0.94	0.62	0.43

Supplemental Table 5.5 Mean differences between Quartile 1 and Quartiles 2 - 5 for measures of adiposity and lipids by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678.

	Quantile 1[a]	Quantile 2 Mean diff.[b]	Quantile 2 95% CI	Quantile 3 Mean diff.[b]	Quantile 3 95% CI	Quantile 4 Mean diff.[b]	Quantile 4 95% CI	Quantile 5 Mean diff.[b]	Quantile 5 95% CI	P-for-trend	P-overall
Body Mass index (kg/m2)											
Total sitting time[c,d]	referent	0.82	(-0.37, 2.01)	0.09	(-0.92, 1.10)	-0.85	(-1.88, 0.18)	-0.29	(-1.67, 1.08)	0.10	**0.002**
Prolonged sitting time[c]	referent	0.60	(-0.68, 1.88)	-0.41	(-1.67, 0.86)	-0.91	(-2.12, 0.29)	-0.80	(-2.21, 0.61)	**0.04**	0.11
Sit-stand transitions[e]	referent	-0.51	(-1.47, 0.45)	-1.29	(-2.41, -0.18)	-1.02	(-2.26, 0.23)	-2.44	(-3.59, -1.29)	**0.001**	**0.001**
Usual bout duration	referent	-1.02	(-2.10, 0.07)	-0.81	(-1.99, 0.36)	-0.86	(-2.18, 0.46)	-1.40	(-2.77, -0.03)	0.10	0.3
Alpha	referent	-0.22	(-1.49, 1.05)	-1.44	(-3.01, 0.12)	-2.01	(-3.59, -0.42)	-3.16	(-4.79, -1.53)	**<.001**	**<.001**
Waist circumference (cm)											
Total sitting time[c,d]	referent	1.17	(-1.86, 4.20)	0.15	(-2.40, 2.71)	-3.22	(-5.63, -0.82)	-0.21	(-3.45, 3.02)	0.13	**<.001**
Prolonged sitting time[c]	referent	1.15	(-2.26, 4.57)	-1.58	(-5.06, 1.90)	-2.58	(-5.44, 0.28)	-1.51	(-4.97, 1.96)	0.07	0.11
Sit-stand transitions[e]	referent	-2.20	(-4.62, 0.21)	-4.10	(-7.07, -1.12)	-2.95	(-5.87, -0.03)	-6.45	(-9.52, -3.38)	**0.002**	**0.003**
Usual bout duration	referent	-2.17	(-5.12, 0.96)	-2.00	(-5.13, 1.13)	-2.48	(-5.58, 0.63)	-2.97	(-6.10, 0.16)	0.10	0.48
Alpha	referent	-2.05	(-5.12, 1.02)	-4.91	(-8.70, -1.12)	-6.23	(-10.05, -2.42)	-9.26	(-13.22, -5.30)	**<.001**	**0.001**

Supplemental Table 5.5 Mean differences between Quartile 1 and Quartiles 2 - 5 for measures of adiposity and lipids by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678, Continued.

	Quantile 1[a]	Quantile 2 Mean diff.[b]	95% CI	Quantile 3 Mean diff.[b]	95% CI	Quantile 4 Mean diff.[b]	95% CI	Quantile 5 Mean diff.[b]	95% CI	P-for-trend	P-ovarall
HDL Cholesterol (mmol/L)											
Total sitting time[c,d]	referent	-0.01	(-0.09, 0.08)	0.03	(-0.05, 0.10)	0.13	(0.05, 0.22)	0.10	(0.01, 0.19)	**<.001**	**0.001**
Prolonged sitting time[c]	referent	0.07	(-0.02, 0.15)	0.05	(-0.05, 0.15)	0.09	(0.03, 0.16)	0.09	(0.00, 0.18)	**0.04**	0.09
Sit-stand transitions[e]	referent	0.02	(-0.04, 0.13)	0.00	(-0.08, 0.09)	0.01	(-0.08, 0.10)	0.03	(-0.06, 0.13)	0.62	0.80
Usual bout duration	referent	0.03	(-0.06, 0.10)	-0.01	(-0.06, 0.05)	-0.01	(-0.08, 0.05)	0.02	(-0.05, 0.08)	0.90	0.77
Alpha	referent	0.03	(-0.06, 0.11)	0.09	(0.00, 0.18)	0.06	(-0.01, 0.14)	0.12	(0.03, 0.21)	**0.01**	0.06
LDL Cholesterol (mmol/L)											
Total sitting time[c,d]	referent	0.04	(-0.11, 0.19)	-0.08	(-0.26, 0.11)	-0.06	(-0.24, 0.12)	-0.06	(-0.27, 0.15)	0.33	0.744
Prolonged sitting time[c]	referent	0.06	(-0.17, 0.23)	0.02	(-0.23, 0.23)	0.03	(-0.18, 0.24)	0.01	(-0.21, 0.22)	0.92	0.94
Sit-stand transitions[e]	referent	0.05	(-0.26, 0.26)	-0.04	(-0.12, 0.15)	-0.01	(-0.28, 0.21)	0.09	(-0.11, 0.28)	0.60	0.83
Usual bout duration	referent	-0.04	(-0.16, 0.17)	0.05	(-0.22, 0.23)	-0.06	(-0.22, 0.17)	0.09	(-0.11, 0.28)	0.45	0.48
Alpha	referent	0.02	(-0.16, 0.20)	-0.02	(-0.21, 0.17)	-0.04	(-0.22, 0.14)	-0.06	(-0.22, 0.11)	0.40	0.94

Supplemental Table 5.5 Mean differences between Quartile 1 and Quartiles 2 - 5 for measures of adiposity and lipids by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678, Continued.

	Quantile 1[a]	Quantile 2		Quantile 3		Quantile 4		Quantile 5		P-for-trend	P-overall
		Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI	Mean diff.[b]	95% CI		
Triglycerides (mmol/L)											
Total sitting time[c,d]	referent	-0.02	(-0.15, 0.10)	-0.11	(-0.22, 0.00)	-0.29	(-0.38, -0.20)	-0.19	(-0.32, -0.06)	**<.001**	**<.001**
Prolonged sitting time[c]	referent	-0.11	(-0.23, 0.02)	-0.17	(-0.31, -0.03)	-0.14	(-0.26, -0.03)	-0.14	(-0.28, 0.00)	0.06	0.08
Sit-stand transitions[e]	referent	0.03	(-0.20, 0.15)	-0.01	(-0.18, 0.14)	-0.05	(-0.13, 0.08)	-0.06	(-0.20, 0.08)	0.21	0.62
Usual bout duration	referent	-0.07	(-0.25, 0.06)	-0.07	(-0.24, 0.05)	-0.01	(-0.27, 0.12)	-0.05	(-0.39, 0.10)	0.86	0.41
Alpha	referent	-0.12	0.01	-0.09	0.06	-0.13	0.01	-0.26	-0.13	**0.001**	**0.01**

[a] Participants in quintile 1 have the highest total sitting time / prolonged sitting time / the most prolonged pattern of sitting time accumulation. Quintile cutpoints are in S2 Table.

[b] Difference in adjusted mean in contrast to quintile 1, adjusted for age and gender, covariates (see S1 Table), and MVPA measured using from linear regression model with linearized variance estimation accounting for state/testing centre strata/clusters.

[c] Variables adjusted for device wear time using the residuals method.

[d] Associations are similar to those previously reported in Healy et al. Eur Heart J. 2015, differing slightly due to small differences in inclusion criteria and differences in the functional form of total sitting time.

[e] Variable adjusted for total sitting time using the residuals method.

Supplemental Table 5.6 Mean differences between Quartile 1 and Quartiles 2 - 5 for blood pressure and measures of glucose control by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678.

	Quantile 1[b]	Quantile 2		Quantile 3		Quantile 4		Quantile 5		P-for-trend	P-overall
		Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI		
Systolic BP (mmHg)											
Total sitting time[d,e]	referent	0.68	(-2.54, 3.90)	-1.64	(-5.34, 2.06)	1.42	(-2.38, 5.21)	1.03	(-1.86, 3.92)	0.39	0.56
Prolonged sitting time[d]	referent	2.83	(-2.01, 7.67)	4.89	(0.23, 9.55)	2.42	(-1.84, 6.69)	3.27	(-1.25, 7.79)	0.21	0.29
Sit-stand transitions[f]	referent	-2.16	(-5.71, 1.39)	-1.87	(-5.91, 2.18)	-1.46	(-6.03, 3.12)	-0.57	(-5.26, 4.12)	0.96	0.73
Usual bout duration	referent	0.17	(-4.32, 4.66)	3.42	(-0.81, 7.64)	0.99	(-4.00, 5.97)	1.99	(-2.30, 6.27)	0.36	0.57
Alpha	referent	1.32	(-2.56, 5.20)	0.29	(-4.52, 5.09)	-0.22	(-5.77, 5.32)	0.34	(-4.43, 5.11)	0.88	0.94
Diastolic BP (mmHg)											
Total sitting time[d,e]	referent	2.21	(-0.06, 4.49)	-0.97	(-3.50, 1.56)	0.40	(-2.17, 2.98)	-0.42	(-3.00, 2.16)	0.38	0.08
Prolonged sitting time[d]	referent	2.86	(0.70, 5.02)	2.45	(-0.30, 5.20)	1.36	(-1.29, 4.01)	2.14	(-0.62, 4.90)	0.50	0.18
Sit-stand transitions[f]	referent	0.65	(-1.56, 2.87)	-0.45	(-2.95, 2.05)	-0.48	(-3.10, 2.13)	0.07	(-2.76, 2.90)	0.76	0.84
Usual bout duration	referent	0.69	(-1.38, 2.75)	1.25	(-1.23, 3.73)	0.91	(-1.75, 3.58)	1.71	(-0.93, 4.35)	0.27	0.78
Alpha	referent	-0.43	(-2.50, 1.65)	-1.45	(-4.11, 1.20)	-1.76	(-4.81, 1.29)	-1.81	(-4.53, 0.90)	0.17	0.71

Supplemental Table 5.6 Mean differences between Quartile 1 and Quartiles 2 - 5 for blood pressure and measures of glucose control by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678, Continued.

	Quantile 1[b]	Quantile 2 Mean diff.[c]	95% CI	Quantile 3 Mean diff.[c]	95% CI	Quantile 4 Mean diff.[c]	95% CI	Quantile 5 Mean diff.[c]	95% CI	P-for-trend	P-ovarall
HbA1c (mmol/mol)											
Total sitting time[d,e]	referent	0.05	(-0.06, 0.16)	0.00	(-0.09, 0.08)	-0.05	(-0.14, 0.05)	0.00	(-0.10, 0.09)	0.28	0.47
Prolonged sitting time[d]	referent	0.04	(-0.07, 0.15)	0.01	(-0.10, 0.13)	0.01	(-0.07, 0.09)	0.01	(-0.08, 0.10)	0.87	0.92
Sit-stand transitions[f]	referent	-0.03	(-0.12, 0.06)	0.04	(-0.09, 0.17)	0.03	(-0.05, 0.12)	0.01	(-0.09, 0.12)	0.40	0.56
Usual bout duration	referent	0.07	(-0.02, 0.16)	0.08	(-0.03, 0.19)	0.06	(-0.03, 0.16)	0.05	(-0.02, 0.12)	0.23	0.51
Alpha	referent	0.01	(-0.09, 0.12)	-0.03	(-0.15, 0.08)	-0.03	(-0.13, 0.08)	-0.04	(-0.14, 0.06)	0.30	0.83
Fasting glucose (mmol/L)											
Total sitting time[d,e]	referent	0.13	(-0.06, 0.32)	0.00	(-0.17, 0.17)	-0.09	(-0.27, 0.10)	-0.10	(-0.32, 0.11)	0.07	0.15
Prolonged sitting time[d]	referent	0.06	(-0.12, 0.23)	0.11	(-0.10, 0.33)	-0.01	(-0.16, 0.14)	-0.05	(-0.29, 0.20)	0.50	0.49
Sit-stand transitions[f]	referent	-0.07	(-0.26, 0.12)	0.03	(-0.19, 0.26)	0.07	(-0.15, 0.29)	0.05	(-0.13, 0.22)	0.25	0.43
Usual bout duration	referent	0.06	(-0.27, 0.24)	0.11	(-0.11, 0.32)	0.14	(-0.06, 0.34)	-0.01	(-0.20, 0.19)	0.78	0.48
Alpha	referent	-0.05	(-0.27, 0.18)	-0.28	(-0.44, -0.13)	-0.14	(-0.29, 0.02)	-0.19	(-0.34, -0.03)	**0.002**	**<.001**

Supplemental Table 5.6 Mean differences between Quartile 1 and Quartiles 2 - 5 for blood pressure and measures of glucose control by quintiles of sitting, prolonged sitting, sit-stand transitions, usual bout duration, and alpha after additional adjustment for MVPA; AusDiab (2011-12), n=678, Continued.

	Quantile 1[b]	Quantile 2		Quantile 3		Quantile 4		Quantile 5		P-for-trend	P-ovarall
		Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI	Mean diff.[c]	95% CI		
2-hour post-load glucose (mmol/L)											
Total sitting time[d,e]	referent	-0.15	(-0.66, 0.35)	-0.19	(-0.70, 0.32)	-0.17	(-0.63, 0.28)	-0.24	(-0.60, 0.12)	0.19	0.74
Prolonged sitting time[d]	referent	-0.10	(-0.42, 0.21)	-0.19	(-0.59, 0.20)	-0.16	(-0.53, 0.21)	0.12	(-0.30, 0.55)	0.72	0.46
Sit-stand transitions[f]	referent	-0.14	(-0.54, 0.26)	0.01	(-0.30, 0.32)	-0.26	(-0.69, 0.18)	0.00	(-0.43, 0.43)	0.81	0.58
Usual bout duration	referent	-0.09	(-0.35, 0.18)	-0.11	(-0.52, 0.29)	0.01	(-0.33, 0.36)	0.26	(-0.12, 0.64)	0.17	0.30
Alpha	referent	-0.36	(-0.77, 0.05)	-0.14	(-0.45, 0.18)	-0.57	(-1.08, -0.07)	-0.48	(-0.83, -0.12)	**0.02**	0.06

[a] Models of 2-hour post-load glucose had n=639.

[b] Participants in quintile 1 have the highest total sitting time / prolonged sitting time / the most prolonged pattern of sitting time accumulation. Quintile cutpoints are in S2 Table.

[c] Difference in adjusted mean in contrast to quintile 1, adjusted for age and gender, covariates (see S1 Table), and MVPA measured using from linear regression model with linearized variance estimation accounting for state/testing centre strata/clusters.

[d] Variables adjusted for device wear time using the residuals method.

[e] Associations are similar to those previously reported in Healy et al. Eur Heart J. 2015, differing slightly due to small differences in inclusion criteria and differences in the functional form of total sitting time.

[f] Variable adjusted for total sitting time using the residuals method.

Bolded p-values indicate statistically significant relations at p< 0.05.

Supplemental Table 5.7 Results from multivariable linear regression models testing effect modification of usual bout duration on associations of daily sitting time and cardiometabolic risk biomarkers, AusDiab (2011-12), n=678.

	Daily Sitting Time[c]			Usual Bout Duration[c]			Daily Sitting Time[c] by Usual Bout Duration[c]		
	β or RR	95% CI	p-value	β or RR	95% CI	p-value	β or RR	95% CI	p-value
Body Mass index (kg/m2), RR	1.01	(1.00,1.02)	0.07	1.02	(1.00,1.04)	0.08	1.00	(0.99,1.01)	0.58
Waist circumference (cm), β	0.66	(-0.03,1.36)	0.06	1.16	(-0.08,2.40)	0.07	-0.16	(-0.83,0.51)	0.64
HDL Cholesterol (mmol/L), β	-0.04	(-0.06,-0.03)	**<0.001**	0.02	(-0.01,0.06)	0.18	-0.02	(-0.03,-0.01)	**0.005**
LDL Cholesterol (mmol/L), β	0.03	(-0.01,0.07)	0.21	-0.05	(-0.13,0.03)	0.23	0.00	(-0.03,0.03)	0.88
Triglycerides (mmol/L), RR	1.07	(1.05,1.09)	**<0.001**	0.97	(0.93,1.02)	0.23	1.02	(1.00,1.04)	**0.03**
Systolic BP (mmHg), β	-0.12	(-0.85,0.62)	0.75	-0.30	(-2.13,1.53)	0.74	-0.21	(-0.75,0.34)	0.45
Diastolic BP (mmHg), β	0.56	(0.12,1.01)	**0.02**	-0.78	(-1.76,0.19)	0.11	-0.04	(-0.39,0.31)	0.82
HbA1c (mmol/mol), RR	1.00	(1.00,1.01)	0.22	1.00	(0.99,1.00)	0.42	1.00	(1.00,1.00)	0.80
Glucose (mmol/L), RR	1.01	(1.00,1.02)	**0.01**	0.99	(0.98,1.01)	0.33	1.00	(0.99,1.01)	0.51
2-hour post-load glucose (mmol/L), RR	1.02	(1.01,1.04)	**<0.001**	0.97	(0.94,1.01)	0.12	1.02	(1.00,1.03)	**0.04**

β = unstandardized regression coefficient; RR = relative rate.

RRs were computed by exponentiating beta coefficients from models where outcome variables were log-transformed.

[a] Models controlled for age, gender, and other potential covariates listed in S1 Table.

[b] Models of 2-hour post-load glucose had n=639.

[c] Variables were first mean centered.

Bolded p-values indicate statistically significant relations p < 0.05.
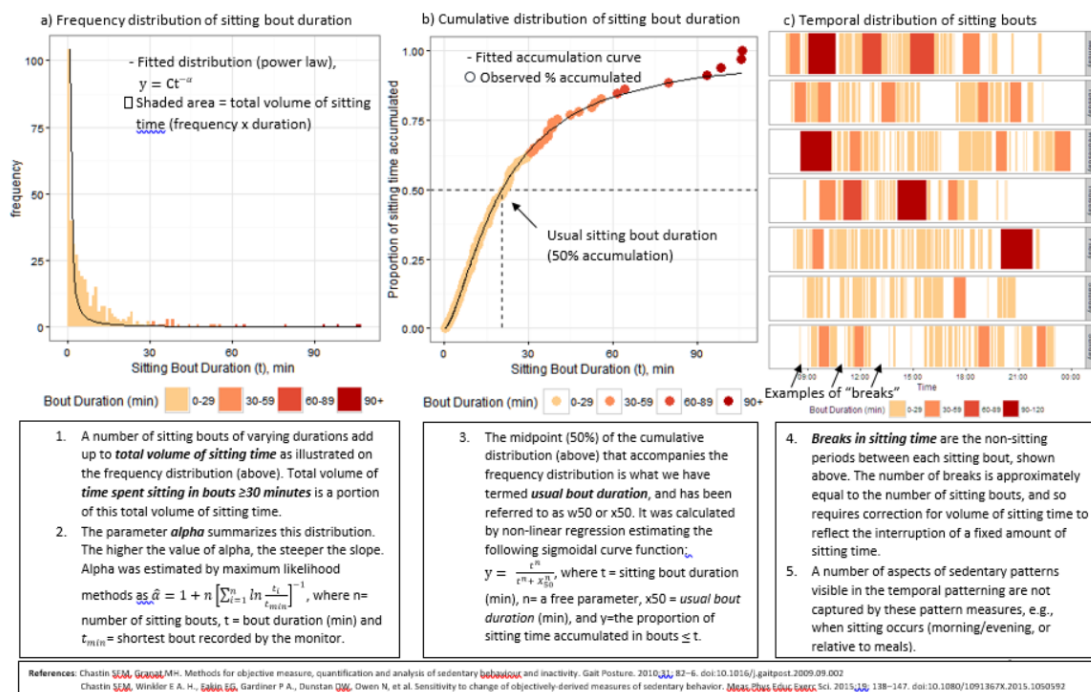
Supplemental Figure 5.1 Distributions of sitting bouts shown for one participant over a seven-day measurement period.

Chapter 6: Conclusion

While evidence is mounting that sedentary behavior is associated with cardiovascular disease (CVD) and diabetes, few studies have tested associations among older adults, the segment of the US population that is most sedentary and has the highest risk for CVD and diabetes.[27,47,82] Furthermore, much of the existing evidence stems from self-reported measures of sedentary time, which often capture only one sedentary behavior (television watching) or are based on recall of hours spent sitting in a usual day. Measures based on recall of time spent sitting have low correlations with measures derived from accelerometer data, potentially leading to biased estimates. Chapters 2 through 4 examine associations of sedentary time with CVD and diabetes among adults with an average age of 80 years using measures derived from accelerometer data, which are not subject to the same errors as measures derived from self-report.

Total sedentary time, cardiovascular disease, and diabetes

The first study (Chapter 2) showed that, after adjustment for several covariates, increased CVD risk associated with higher levels of sedentary time (an association previously observed in younger adults[34]) was present among OPACH women who range in age from 63 to 99 years. The associations followed a linear dose-response pattern, with higher CVD risk associated with higher sedentary time across the sedentary time distribution. For CVD events, linear associations persisted after adjustment for body mass index (BMI), were slightly attenuated after adjustment for moderate to vigorous physical activity (MVPA) leading to a marginally-significant p-for-trend (p=.05), and were more strongly attenuated following adjustment for CVD risk biomarkers suggesting at least partial mediation by these factors (i.e., serum

fasting glucose, triglycerides, HDL-cholesterol, and systolic blood pressure; Table 6.1). For coronary heart disease (CHD) events, linear associations persisted after adjustment for BMI, MVPA, and CVD-risk biomarkers. Tests for effect modification revealed that associations of sedentary time with CVD *and* CHD events did not differ between women with high and low levels of MVPA (high and low values were determined using the median split of daily MVPA estimates). These results combined with persistent linear associations after adjustment for MVPA suggest that higher levels of sedentary time are related to incident CVD and CHD through mechanisms that are not fully explained by MVPA.

It is noteworthy that associations between sedentary time and CHD were attenuated by adjustment for CVD-risk biomarkers, but remained strong and statistically significant, suggesting that higher sedentary time may increase risk for CHD through evoking changes in traditional risk factors, but that other mechanisms are also at play. Some research suggests that higher CVD risk may result from damaged vascular structure that occurs both directly from high levels of sedentary time and through reduced blood flow and reduced shear rate that are associated with high sedentary time.[74,142,143] Other proposed mechanisms include increased inflammation and oxidative stress.[74] Further investigation of these potential mechanisms using intervention and epidemiologic studies are needed as there is little data available from prospective studies conducted "in the wild" and the intermediaries could serve as intervention targets.

Chapter 3 extends previous findings (that high levels of sedentary time are related to prevalent diabetes[33,87,88]) to older adults. The study showed that the increased relative odds associated with 1 hour of sedentary time were similar in our

cohort of women aged 79±7 (odds ratio (OR) =1.19; 95% confidence interval (CI) = 1.13-1.30) than for men and women of younger ages.[106,107,144] The magnitude and statistical significance of higher odds of diabetes associated with higher sedentary time were similar in women with high and low MVPA (using a median split), in women <80 years and ≥80 years, in obese and non-obese women, among those with high and low physical functioning (using a median split), across racial/ethnic groups (White, Black, and Hispanic), and among women with and without a family history of diabetes, suggesting a high degree of generalizability.

Analyses of incident diabetes (Chapter 4) revealed that family history of diabetes was a significant effect modifier of associations between sedentary time and now-onset diabetes and results were therefore presented stratified by this characteristic. Among those with a family history, the 25% of women with the highest sedentary time had 2.4 times higher risk for diabetes than the 25% of women with the lowest sedentary time. The 2.4 times higher diabetes risk associated with higher sedentary time was not attenuated by adjustment for BMI or MVPA, though the linear trend was only marginally statistically significant after adjustment for MVPA (p=0.06). Taken together, however, the results suggest that for woman *with* a family history of diabetes, risk for diabetes was higher with higher levels of sedentary behavior independent of BMI and MVPA. Contrary to expectations, significant associations between sedentary time and incident diabetes among women *without* a family history of diabetes were not observed.

Family history of diabetes is a crude indicator of genetic predisposition for the disease. Accordingly, our results could reflect a gene-behavior interaction where sedentary time is associated with incident diabetes only among women with high

gene-related risk for the disease. Consistent evidence is available that adults with a

family history of diabetes have a 2-fold higher risk for diabetes and the increased risk

is up to 5-fold when both parents have the condition.[145] However, evidence of gene-

lifestyle interactions with respect to diabetes is mixed, perhaps because the genetic

component of type 2 diabetes is still poorly understood. In a post-hoc analysis of the

Diabetes Prevention Program, 10 single nucleotide polymorphisms (SNPs) were

examined as effect modifiers of the lifestyle intervention that definitively improved

insulin functioning, on average, in adults at high risk for diabetes. None of the SNPs

were associated with insulin resistance or secretion, and just one SNP, in the

CDKN2A/B loci, had a trend toward modifying the lifestyle intervention.[122] Similar

findings were observed in a cohort study among 8,600 Swedish adults with the same

genotype.[146] These findings motivate that gene-behavior interactions could play an

important role in diabetes etiology, but no diabetes studies specifically exploring

gene-sedentary behavior interactions have been conducted yet.

 Family history of diabetes is also a marker for shared environmental

exposures, which play an important role in the diabetes epidemic as evidenced by the

rapid rise in new-onset diabetes coincident in time with increasing over-nutrition,

increasing sedentary behavior, and decreasing physical activity. As one example, it

is possible that increased risk of diabetes associated with sedentary behavior could

vary by shared familial dietary patterns that differ between FH+ and FH- women.

Exploration of environment explanations for the observed sedentary time-family

history interaction are needed. Type 1 error could also explain the observed

interaction, as it was discovered during routine data analysis and was not

hypothesized a priory. Accordingly, interpretation of these results should be made

with caution, especially considering (1) that among OPACH women, sedentary behavior increased the odds of prevalent diabetes irrespective of family history and (2) no previous study has reported similar effect modification. Given that follow-up time was relatively short (2-4 years) in this study, another possible explanation is that women with family history of diabetes experienced increased rates of incident diabetes rapidly due to their genetic risk, but that longer term follow-up could reveal associations among women without family history. Replication of the differential risk associated with high levels of sedentary time among women with and without a family history of diabetes is needed, including in prospective studies with longer follow-up.

Table 6.1 summarizes the results related to total sedentary time from Chapters 2 through 4. Overall, sedentary behavior had strong relations with CVD and diabetes with women in the highest quartile of sedentary time having a nearly 2 times the risk for CHD and (among women with a family history of diabetes) diabetes compared to women in the lowest quartile of sedentary time. The difference between those in the highest and lowest sedentary time was as little as 2 hours per day, which is a reasonable duration of time to target in interventions. The observed associations were independent of several covariates commonly controlled for, in addition to MVPA and physical function, meaning that sedentary time may be an important modifiable risk factor associated with two of the most burdensome chronic diseases on Earth.

Sedentary accumulation patterns, cardiovascular disease, and diabetes

Evidence has been emerging that the way in which sedentary time is accumulated (sedentary accumulation patterns) has acute effects on postprandial glucose control and lipid metabolism, but these findings have not yet been tested in relation to clinical outcomes such as diabetes. Chapters 3 through 5 showed that

prolonged sedentary accumulation patterns (vs. interrupted patterns) were

significantly associated with higher rates of prevalent and incident diabetes and were

deleteriously associated with biomarkers of obesity, glucose metabolism, and lipid

metabolism. Of the three accumulation pattern metrics used in this study, alpha

tended to have the largest effect sizes and was consistently more robust to

multivariable adjustment, especially adjustment for accelerometer-measured MVPA.

As was found in the only other study of accumulation patterns and diabetes,

our results indicated that the number of breaks in sedentary time per day was not

significantly related to diabetes. We also observed among Australian adults that more

breaks in sedentary time were associated with lower BMI and lower waist

circumference, but not significantly associated with any other cardio-metabolic

biomarkers. These observations are largely in agreement with the extant

epidemiologic literature which shows consistent associations between breaks and

obesity and mixed results for associations with other biomarkers.[12,14] The overall

evidence, including results from this dissertation, suggests that while the frequency of

breaks may not be relevant for diabetes risk, the strategic disbursement of breaks

throughout long sedentary bouts would improve alpha and usual bout durations, and

therefore may reduce diabetes risk. Intervention studies are needed to test this

hypothesis.

It is important to note that among the older women (aged 79±7 years) studied

in Chapters 2 through 4, sedentary time and sedentary accumulation patterns were

highly correlated and therefore could reflect similar behavior patterns. The high

correlations prohibited formal testing for joint effects, which would better characterize

how the two exposures together associate with cardio-metabolic health. Among the

Australian adults (aged 58±10 years) studied in Chapter 5, correlations between sedentary time and sedentary accumulation patterns were notably weaker, possibly due to the different ages of participants, or more likely due to differences in measurement devices used (ActiGraph vs. activPAL) and/or the wear location of the devices (hip vs. thigh). The lower correlations enabled analyses of joint associations which showed that both sedentary time and usual bout duration were jointly related to key diabetes-related biomarkers (2-hr post-load glucose, triglycerides, and HDL cholesterol) in that high levels of sedentary time were more strongly (detrimentally) associated with biomarkers when accumulated in longer sedentary bouts. This joint relation is the first epidemiologic evidence that suggests both total sedentary time *and* the way in which it is accumulated are relevant to diabetes.

Future directions

Most of the studies in this dissertation relied on data from hip-worn accelerometers that, once processed using commonly used techniques,[41] provided objective measures of sedentary time *and* the way in which it was accumulated. There is evidence that the resulting measures of breaks in sedentary time have low accuracy,[75,101] potentially leading measurement error in all accumulation pattern metrics used in Chapters 2 through 4. Future studies are needed to characterize the measurement properties of accumulation pattern metrics that are computed using hip-worn and thigh-worn accelerometers.

The results in this study were similar to those reported in studies relying on self-reported TV time, but were less similar to results from studies relying on self-reported total sitting time.[32,33] There is mounting evidence that self-reported sedentary behavior poorly estimates accelerometer measures with most correlations

ranging between 0.12 and 0.33; furthermore, in all reported cases, the bias and/or variability of estimates was systematically related to sedentary time.[35,62–69] These systematically inaccurate measures of self-reported sedentary behavior indicate that previous associations of self-reported sedentary time with CVD and diabetes, especially estimates of dose-response relationships,[34] may be differentially biased. Summarizing and quantifying the measurement error in self-reported sitting time is an important first step toward understanding the potential bias in previous estimates. Concurrently, analyses of sedentary behavior in relation to CVD and diabetes risk should be replicated using accelerometer measured sedentary time, as was done in this dissertation.

High correlations between sedentary time and sedentary accumulation pattern metrics in studies using OPACH data prevented investigations of joint relations between the two sedentary behavior-related exposures, which may jointly increase risk of diabetes as shown in Chapter 5. Improved measures of accumulation patterns metrics, such as those computed using data from activPAL accelerometers, may reduce the linear relation between exposures and enable studies of joint effects. It is also possible that adults in the highest age groups with the most prolonged accumulation patterns, even when accurately measured, also have the highest sedentary times. Further work is needed to characterize the joint relation between sedentary time and accumulation patterns and to examine how the two exposures are related to diabetes.

As technology advances, improved measures of sedentary behavior will be available that will help increase the accuracy of point estimates of associations of sedentary time and sedentary accumulation patterns with diabetes and CVD. Using

improved measures, randomized trials are needed to confirm the results of this dissertation that CVD and diabetes risk is related to sedentary time and sedentary accumulation patterns. Furthermore, long-term trials that test whether reducing sedentary time and/or improving accumulation patterns can reduce incident diabetes and cardiovascular disease are needed.

Table 6.1 Summary of associations of sedentary time with cardiovascular disease and diabetes; OPACH (2012-2016)

Outcome	Quartile 1 vs. quartile 4 ratio and 95% CI	Linear associations persist after adjustment for:[a]		
		BMI, diabetes, hypertension	CVD risk bio-markers	MVPA
CVD events, HR	1.44 (1.05-1.98)	yes	no	no[b]
CHD events, HR	2.19 (1.09-4.40)	yes	yes	yes
Prevalent diabetes, OR	1.96 (1.59-2.42)	yes	n/a	yes
Incident diabetes, HR				
Without family history	1.08 (0.67-1.73)	no	n/a	no
With family history	2.38 (1.20-4.74)	yes	n/a	no[c]

Abbreviations: CI = confidence interval; BMI = body mass index; CVD = cardiovascular disease; CHD = coronary heart disease; MVPA = moderate to vigorous physical activity; HR = hazard ratio; OR = odds ratio; n/a = not applicable

[a] Linear trend was assessed using covariate-adjusted models by including sedentary time as a continuous variable. Linear associations were said to persist after adjustment for additional covariates if the p-value for sedentary time was < 0.05 after including additional covariates to the confounder-adjusted model.

[b] The p-value was .054 after adjustment for MVPA

[c] The p-value was .056 after adjustment for MVPA

References

1. Sedentary Behavior Research Network. Letter to the Editor: Standardized use of the terms "sedentary" and "sedentary behaviours." *Appl Physiol Nutr Metab*. 2012;37:540-542. doi:10.1016/j.mhpa.2012.06.001.

2. Morris J, Heady J, Raffle P, Roberts C, Parks J. Coronary heart-disease and physical activity of work. *Lancet*. 1953;265(6796):1111-1120. http://www.ncbi.nlm.nih.gov/pubmed/13110075. Accessed October 30, 2014.

3. Taylor L, Keys A, Blackburn H. Death rates among physically active and sedentary employees of the railroad industry. *Am J Public Heal Nations Heal*. 1962;52:1697-1707.

4. Jennings G, Nelson L, Nestel P, et al. The effects of changes in physical activity on major cardiovascular risk factors, hemodynamics, sympathetic function, and glucose utilization in man: a controlled study of four levels of activity. *Circulation*. 1986;73(1):30-40. doi:10.1161/01.CIR.73.1.30.

5. Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq FR, Schnure JJ. Glucose Intolerance During Decreased Physical Activity in Man. *Diabetes*. 1972;21(2):101-107.

6. Thorp A a., Kingwell B a., Sethi P, Hammond L, Owen N, Dunstan DW. Alternating bouts of sitting and standing attenuates postprandial glucose responses. *Med Sci Sports Exerc*. 2014;(5 d):2053-2061. doi:10.1249/MSS.0000000000000337.

7. Henson J, Davies MJ, Bodicoat DH, et al. Breaking up prolonged sitting with standing or walking attenuates the postprandial metabolic response in post-menopausal women: A randomised acute study. *Diabetes Care*. 2015:In Press. doi:10.2337/dc15-1240.

8. Dunstan DW, Kingwell B a, Larsen R, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care*. 2012;35(5):976-983. doi:10.2337/dc11-1931.

9. Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *Am J Clin Nutr.* 2013;98(2):358-366. doi:10.3945/ajcn.112.051763.

10. Dempsey PC, Larsen RN, Sethi P, et al. Benefits for Type 2 Diabetes of Interrupting Prolonged Sitting With Brief Bouts of Light Walking or Simple Resistance Activities. *Diabetes Care.* 2016:dc152336. doi:10.2337/dc15-2336.

11. Larsen RN, Kingwell B a., Sethi P, Cerin E, Owen N, Dunstan DW. Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults. *Nutr Metab Cardiovasc Dis.* 2014;24(9):976-982. doi:10.1016/j.numecd.2014.04.011.

12. Chastin SFM, Egerton T, Leask C, Stamatakis E. Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. *Obesity.* 2015;23(9):1800-1810. doi:10.1002/oby.21180.

13. Chastin SF, Winkler EA, Eakin EG, et al. Sensitivity to change of objectively-derived measures of sedentary behavior. *Meas Phys Educ Exerc Sci.* 2015;19(3):138-147. doi:10.1080/1091367X.2015.1050592.

14. Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. *Prev Med (Baltim).* 2015;76:92-102. doi:10.1016/j.ypmed.2015.04.013.

15. Kim Y, Welk GJ, Braun SI, Kang M. Extracting Objective Estimates of Sedentary Behavior from Accelerometer Data: Measurement Considerations for Surveillance and Research Applications. *PLoS One.* 2015;10(2):e0118078. doi:10.1371/journal.pone.0118078.

16. Carson V, Wong SL, Winkler E, Healy GN, Colley RC, Tremblay MS. Patterns of sedentary time and cardiometabolic risk among Canadian adults. *Prev Med (Baltim).* 2014;65:23-27. doi:10.1016/j.ypmed.2014.04.005.

17. Healy_2015_European Heart Journal Supplimental Material.

18. Falconer CL, Page AS, Andrews RC, Cooper AR. The Potential Impact of Displacing Sedentary Time in Adults with Type 2 Diabetes. *Med Sci Sport Exerc.* 2015;(February):1. doi:10.1249/MSS.0000000000000651.

19.   Chastin SFM, Granat MH. Methods for objective measure, quantification and analysis of sedentary behaviour and inactivity. *Gait Posture*. 2010;31(1):82-86. doi:10.1016/j.gaitpost.2009.09.002.

20.   Stephens SK, Winkler E a H, Trost SG, et al. Intervening to reduce workplace sitting time: how and when do changes to sitting time occur? *Br J Sports Med*. 2014;2:1037-1042. doi:10.1136/bjsports-2014-093524.

21.   Chastin SFM, Baker K, Jones D, Burn D, Granat MH, Rochester L. The pattern of habitual sedentary behavior is different in advanced Parkinson's disease. *Mov Disord*. 2010;25(13):2114-2120. doi:10.1002/mds.23146.

22.   McVeigh JA, Winkler EAH, Howie EK, et al. Objectively measured patterns of sedentary time and physical activity in young adults of the Raine study cohort. *Int J Behav Nutr Phys Act*. 2016;13(1):41. doi:10.1186/s12966-016-0363-0.

23.   Hallsworth K, Thoma C, Moore S, et al. Non-alcoholic fatty liver disease is associated with higher levels of objectively measured sedentary behaviour and lower levels of physical activity than matched healthy controls. *Frontline Gastroenterol*. 2014;0:1-8. doi:10.1136/flgastro-2014-100432.

24.   Chastin SFM, Mandrichenko O, Helbostadt JL, Skelton DA. Associations between objectively-measured sedentary behaviour and physical activity with bone mineral density in adults and older adults, the NHANES study. *Bone*. 2014;64:254-262. doi:10.1016/j.bone.2014.04.009.

25.   Edwardson CL, Gorely T, Davies MJ, et al. Association of sedentary behaviour with metabolic syndrome: A meta-analysis. *PLoS One*. 2012;7(4):3-7. doi:10.1371/journal.pone.0034916.

26.   Kerr J, Rosenberg DE, Nathan A, et al. Applying the ecological model of behavior change to a physical activity trial in retirement communities: Description of the study protocol. *Contemp Clin Trials*. 2012;33(6):1180-1188. doi:10.1016/j.cct.2012.08.005.

27.   Mozaffarian D, Benjamin EJ, Go AS, et al. *Heart Disease and Stroke Statistics—2016 Update: A Report From the American Heart Association*.; 2015. doi:10.1161/CIR.0000000000000350.

28.   Mozaffarian D, Benjamin EJ, Go AS, et al. *Heart Disease and Stroke Statistics-*

2015 Update : A Report from the American Heart Association.; 2015. doi:10.1161/CIR.0000000000000152.

29. Thompson PD, Buchner D, Piña IL, et al. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical. *Arterioscler Thromb Vasc Biol.* 2003;23(8):42e-49. doi:10.1161/01.ATV.0000089628.63625.D4.

30. Artinian NT, Fletcher GF, Mozaffarian D, et al. Interventions to promote physical activity and dietary lifestyle changes for cardiovascular risk factor reduction in adults: A scientific statement from the american heart association. *Circulation.* 2010;122(4):406-441. doi:10.1161/CIR.0b013e3181e8edf1.

31. Troiano RRP, Berrigan D, Dodd KWK, et al. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc.* 2008;40(1):181-188. doi:10.1249/mss.0b013e31815a51b3.

32. Biswas A, Oh PI, Faulkner GE, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults. *Ann Intern Med.* 2015;162(10):123-132. doi:10.7326/M14-1651.

33. Wilmot EG, Edwardson CL, Achana FA, et al. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia.* 2012;55:2895-2905. doi:10.1007/s00125-012-2677-z.

34. Pandey A, Salahuddin U, Garg S, et al. Continuous dose-response association between sedentary time and risk for cardiovascular disease: a meta-analysis. *JAMA Cardiol.* 2016;75390(5):575-583. doi:10.1001/jamacardio.2016.1567.

35. Jefferis BJ, Sartini C, Ash S, Lennon LT, Wannamethee SG, Whincup PH. Validity of questionnaire-based assessment of sedentary behaviour and physical activity in a population-based cohort of older men; comparisons with objectively measured physical activity data. *Int J Behav Nutr Phys Act.* 2016;13(1):14. doi:10.1186/s12966-016-0338-1.

36. Healy GN, Winkler EA, Owen N, Anuradha S, Dunstan DW. Replacing sitting time with standing or stepping : associations with cardio-metabolic risk biomarkers. *Eur Heart J.* 2015:EHV308. doi:10.1093/eurheartj/ehv308.

37. Healy GN, Matthews CE, Dunstan DW, Winkler EAH, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J*. 2011;32(5):590-597. doi:10.1093/eurheartj/ehq451.

38. Young DR, Hivert M-F, Alhassan S, Camhi SM, Ferguson JF, Katzmarzyk PT, Lewis CE, Owen N, Perry CK, Siddique J YC on behalf of the PAC of the C on L and CHC on CCC on E and PC on FG and TB and S. Sedentary behavior and cardiovascular morbidity and mortality: A science advisory from the American Heart Association. *Circulation*. 2016;134. doi:10.1161/CIR.0000000000000440.

39. Gibbs BB, Hergenroeder AL, Katzmarzyk PT, Lee IM, Jakicic JM. Definition, measurement, and health risks associated with sedentary behavior. *Med Sci Sport Exerc*. 2015;47(6):1295-1300. doi:10.1249/MSS.0000000000000517.

40. LaCroix AZ, Rillamas-Sun E, Buchner D, et al. The Objective Physical Activity and Cardiovascular Disease Health in Older Women (OPACH) Study. *BMC Public Health*. 2017;17(1):192. doi:10.1186/s12889-017-4065-6.

41. Migueles JH, Cadenas-Sanchez C, Ekelund U, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sport Med*. 2017:1-25. doi:10.1007/s40279-017-0716-0.

42. Curb JD, Mctiernan A, Heckbert SR, et al. Outcomes ascertainment and adjudication methods in the women's health initiative. *Ann Epidemiol*. 2003;13(9 SUPPL.). doi:10.1016/S1047-2797(03)00048-6.

43. Heckbert SR, Kooperberg C, Safford MM, et al. Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. *Am J Epidemiol*. 2004;160(12):1152-1158. doi:10.1093/aje/kwh314.

44. ActiGraphcorp.com. *ActiGraph White Paper Low Frequency Extension Filter*.; 2015. http://actigraphcorp.com/wp-content/uploads/2015/06/Low-Frequency-Extension-Filter.pdf.

45. Choi L, Liu Z, Matthews CE, Buchowski MS. Validation of accelerometer wear and nonwear time classification algorithm. *Med Sci Sports Exerc*. 2011;43(2):357-364. doi:10.1249/MSS.0b013e3181ed61a3.Validation.

46. Kozey-Keadle S, Libertine A, Lyden K, Staudenmayer J, Freedson PS. Validation of wearable monitors for assessing sedentary behavior. *Med Sci Sports Exerc.* 2011;43(8):1561-1567. doi:10.1249/MSS.0b013e31820ce174.

47. Matthews CE, Chen KY, Freedson PS, et al. Amount of time spent in sedentary behaviors in the United States, 2003-2004. *Am J Epidemiol.* 2008;167(7):875-881. doi:10.1093/aje/kwm390.

48. Rillamas-Sun E, LaCroix AZ, Bell CL, Ryckman K, Ockene JK, Wallace RB. The impact of multimorbidity and coronary disease comorbidity on physical function in women aged 80 years and older: The Women's Health Initiative. *he Journals Gerontol Ser A Biol Sci Med Sci.* 2016;71(Suppl 1):S54-S61. doi:https://doi.org/10.1093/gerona/glv059.

49. Jackson JM, DeFor TA, Crain AL, et al. Validity of Diabetes Self-Reports in the Women's Health Initiative. *Menopause.* 2014;21(8):861-868. doi:10.1097/GME.0000000000000189.Validity.

50. Matthews C. Calibration for Accelerometer Output for Adults. *Med Sci Sport Exerc.* 2005;S512(Supplement):S512-S522. doi:10.1249/01.mss.0000185659.11982.3d.

51. Evenson KR, Wen F, Herring AH, et al. Calibrating physical activity intensity for hip-worn accelerometry in women age 60 to 91years: The Women's Health Initiative OPACH Calibration Study. *Prev Med Reports.* 2015;2:750-756. doi:10.1016/j.pmedr.2015.08.021.

52. Meyer A-M, Evenson KR, Morimoto L, Siscovick D, White E. Test-retest reliability of the Women's Health Initiative physical activity questionnaire. *Med Sci Sports Exerc.* 2009;41(3):530-538. doi:10.1249/MSS.0b013e31818ace55.

53. Stewart AL, Mills KM, King AC, Haskell WL, Gillis D, Ritter PL. CHAMPS physical activity questionnaire for older adults: outcomes for interventions. *Med Sci Sports Exerc.* 2001;33(7):1126-1141. doi:10.1097/00005768-200107000-00010.

54. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124(1):17-27. doi:10.1017/S1368980013001390.

55. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81(3):515-526.

56. Page A, Peeters G, Merom D. Adjustment for physical activity in studies of sedentary behaviour. *Emerg Themes Epidemiol*. 2015;12(1):10. doi:10.1186/s12982-015-0032-9.

57. Ekelund U, Steene-Johannessen J, Brown WJ, et al. Can physical activity elminate the detrimental association of sitting time with mortality? A harmonized meta-analysis of data from more than one million men and women. *Lancet*. 2016. doi:10.1017/CBO9781107415324.004.

58. Harrell FE. rms: Regression Modeling Strategies. 2017. https://cran.r-project.org/package=rms.

59. Matthews CE, Keadle SK, Troiano RP, et al. Accelerometer-measured dose-response for physical activity , sedentary time , and mortality in US adults. *Am J Clin Nutr*. 2016;104:1424-1432. doi:10.3945/ajcn.116.135129.

60. Qi Q, Strizich G, Merchant G, et al. Objectively Measured Sedentary Time and Cardiometabolic Biomarkers in US Hispanic/Latino Adults: The Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Circulation*. 2015;132(16):1560-1569. doi:10.1161/CIRCULATIONAHA.115.016938.

61. Seguin R, Buchner DM, Liu J, et al. Sedentary behavior and mortality in older women: The Women's Health Initiative. *Am J Prev Med*. 2014;46(2):122-135. doi:10.1016/j.amepre.2013.10.021.

62. Chastin SF, Culhane B, Dall PM. Comparison of self-reported measure of sitting time (IPAQ) with objective measurement (activPAL). *Physiol Meas*. 2014;35(11):2319-2328. doi:10.1088/0967-3334/35/11/2319.

63. Cleland CL, Hunter RF, Kee F, Cupples ME, Sallis JF, Tully MA. Validity of the Global Physical Activity Questionnaire (GPAQ) in assessing levels and change in moderate-vigorous physical activity and sedentary behaviour. *BMC Public Health*. 2014;14:1-11. doi:10.1186/1471-2458-14-1255.

64. Gardiner PA, Clark BK, Healy GN, Eakin EG, Winkler EAH, Owen N. Measuring older adults' sedentary time: Reliability, validity, and responsiveness. *Med Sci Sports Exerc*. 2011;43(11):2127-2133.

doi:10.1249/MSS.0b013e31821b94f7.

65.   Gennuso KP, Matthews CE, Colbert LH. Reliability and Validity of 2 Self-Report Measures to Assess Sedentary Behavior in Older Adults. *J Phys Act Health*. 2015;12(5):727-732. doi:10.1123/jpah.2013-0546.

66.   Hekler EB, Buman MP, Haskell WL, et al. Reliability and validity of CHAMPS self-reported sedentary-to-vigorous intensity physical activity in older adults. *J Phys Act Health*. 2012;9(2):225-236.

67.   Marshall S, Kerr J, Carlson J, et al. Patterns of weekday and weekend sedentary behavior among older adults. *J Aging Phys Act*. 2015;23(4):534-541. doi:10.1123/japa.2013-0208.

68.   Wijndaele K, De Bourdeaudhuij I, Godino JG, et al. Reliability and validity of a domain-specific last 7-d sedentary time questionnaire. *Med Sci Sports Exerc*. 2014;46(6):1248-1260. doi:10.1249/MSS.0000000000000214.

69.   Van Cauwenberg J, Van Holle V, De Bourdeaudhuij I, Owen N, Deforche B. Older adults' reporting of specific sedentary behaviors: validity and reliability. *BMC Public Health*. 2014;14(1):734. doi:10.1186/1471-2458-14-734.

70.   Evenson KR, Wen F, Herring AH. Associations of Accelerometry-Assessed and Self-Reported Physical Activity and Sedentary Behavior with All-Cause and Cardiovascular Mortality among US Adults. *Am J Epidemiol*. 2016;184(9):621-632. doi:10.1093/aje/kww070.

71.   Ensrud KE, Blackwell TL, Cauley JA, et al. Objective measures of activity level and mortality in older men. *J Am Geriatr Soc*. 2014;62(11):2079-2087. doi:10.1111/jgs.13101.

72.   Wellburn S, Ryan CG, Azevedo LB, et al. Displacing sedentary time: Association with cardiovascular disease prevalence. *Med Sci Sports Exerc*. 2016;48(4):641-647. doi:10.1249/MSS.0000000000000816.

73.   Dempsey PC, Owen N, Yates TE, Kingwell BA, Dunstan DW. Sitting less and moving more: Improved glycaemic control for Type 2 Diabetes prevention and management. *Curr Diab Rep*. 2016. doi:10.1007/s11892-016-0797-4.

74. Carter S, Hartman Y, Holder S, Thijssen D, Hopkins N. Sedentary Behavior and Cardiovascular Disease Risk. *Exerc Sport Sci Rev*. 2017;(28):1. doi:10.1249/JES.0000000000000106.

75. Lyden K, Kozey Keadle SL, Staudenmayer JW, Freedson PS. Validity of two wearable monitors to estimate breaks from sedentary time. *Med Sci Sports Exerc*. 2012;44(11):2243-2252. doi:10.1249/MSS.0b013e318260c477.

76. Kozey-Keadle S, Shiroma EJ, Kamada M, Matthews CE, Harris TB, Lee I-M. Reproducibility of Accelerometer-Assessed Physical Activity and Sedentary Time. *Am J Prev Med*. 2017;52(4):541-548. doi:10.1016/j.amepre.2016.11.010.

77. The Australian Government. Australia's Physical Activity and Sedentary Behaviour Guidelines. *Dep Heal website*. 2014.

78. Chief Medical Officers of England, Scotland W and N. *Start Active, Stay Active. A Report on Physical Activity for Health from the Four Home Countries' Chief Medical Officers*. London; 2011.

79. American Diabetes Association. Facts about type 2. *Diabetes Basics*. 2015. http://www.diabetes.org/diabetes-basics/type-2/facts-about-type-2.html. Accessed January 1, 2016.

80. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: From pathophysiology to prevention and management. *Lancet*. 2011;378(9786):169-181. doi:10.1016/S0140-6736(11)60614-4.

81. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*. 2010;87(1):4-14. doi:10.1016/j.diabres.2009.10.007.

82. Centers for Disease Control and Prevention. *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014*. Atlanta, GA; 2014.

83. Halter JB, Musi N, Horne FM, et al. Diabetes and cardiovascular disease in older adults: Current status and future directions. *Diabetes*. 2014;63(8):2578-2589. doi:10.2337/db14-0020.

84.   Kirkman MS, Briscoe VJ, Clark N, et al. Diabetes in older adults. *Diabetes Care*. 2012;35(12):2650-2664. doi:10.2337/dc12-1801.

85.   Centers for Disease Control and Prevention. Incidence of diagnosed diabetes per 1,000 population aged 18–79 years, by sex and age, United States, 1997–2014. *Natl Surveill*. 2015. http://www.cdc.gov/diabetes/statistics/incidence/fig5.htm. Accessed January 1, 2016.

86.   Mozaffarian D, Kamineni A, Carnethon M, Djoussé L, Mukamal KJ, Siscovick D. Lifestyle Risk Factors and New-Onset Diabetes Mellitus in Older Adults. *Arch Intern Med*. 2009;169(8):798-807. doi:10.1001/archinternmed.2009.21.

87.   Proper KI, Singh AS, Van Mechelen W, Chinapaw MJM. Sedentary behaviors and health outcomes among adults: A systematic review of prospective studies. *Am J Prev Med*. 2011;40(2):174-182. doi:10.1016/j.amepre.2010.10.015.

88.   Thorp A a., Owen N, Neuhaus M, Dunstan DW. Sedentary behaviors and subsequent health outcomes in adults: A systematic review of longitudinal studies, 1996-2011. *Am J Prev Med*. 2011;41(2):207-215. doi:10.1016/j.amepre.2011.05.004.

89.   Grontved A, Hu FB. Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. *J Am Med Assoc*. 2011;305(23):2448-2455.

90.   Celis-Morales CA, Perez-Bravo F, Ibañez L, Salas C, Bailey MES, Gill JMR. Objective vs. Self-Reported Physical Activity and Sedentary Time: Effects of Measurement Method on Relationships with Risk Biomarkers. *PLoS One*. 2012;7(5):e36345. doi:10.1371/journal.pone.0036345.

91.   van Uffelen JGZ, Heesch KC, Hill RL, Brown WJ. A qualitative study of older adults' responses to sitting-time questions: do we get the information we want? *BMC Public Health*. 2011;11:458. doi:10.1186/1471-2458-11-458.

92.   Dunstan DW, Howard BJ, Bergouignan A, Kingwell BA, Owen N. Physiological implications of sedentary behavior: emerging human experimental evidence on reducing and breaking up sitting time. In: Zhu W, Owen N, eds. *Sedentary Behavior and Health: Concepts, Assessment & Intervention*. Human Kinetics; 2016.

93.   Colberg SR, Sigal RJ, Yardley JE, et al. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care*. 2016;39(11):2065-2079. doi:10.2337/dc16-1728.

94.   Asvold BO, Midthjell K, Krokstad S, Rangul V, Bauman A. Prolonged sitting may increase diabetes risk in physically inactive individuals: an 11 year follow-up of the HUNT Study, Norway. *Diabetologia*. 2017. doi:10.1007/s00125-016-4193-z.

95.   Petersen CB, Bauman A, Tolstrup JS. Total sitting time and the risk of incident diabetes in Danish adults (the DANHES cohort) over 5 years: a prospective study. *Br J Sport Med*. 2016;23:1-6. doi:10.1136/bjsports-2015-095648.

96.   Manini TM, Lamonte MJ, Seguin RA, et al. Modifying effect of obesity on the association between sitting and incident diabetes in post-menopausal women. *Obesity*. 2014;22(4):1133-1141. doi:10.1002/oby.20620.

97.   Rillamas-Sun E, Buchner DM, Di C, Evenson KR, LaCroix AZ. Development and application of an automated algorithm to identify a window of consecutive days of accelerometer wear for large-scale studies. *BMC Res Notes*. 2015;8:270. doi:10.1186/s13104-015-1229-2.

98.   Healy GN, Winkler E a. H, Brakenridge CL, Reeves MM, Eakin EG. Accelerometer-Derived Sedentary and Physical Activity Time in Overweight/Obese Adults with Type 2 Diabetes: Cross-Sectional Associations with Cardiometabolic Biomarkers. *PLoS One*. 2015;10(3):e0119140. doi:10.1371/journal.pone.0119140.

99.   English C, Healy GN, Coates A, Lewis LK, Olds T, Bernhardt J. Sitting time and physical activity after stroke: physical ability is only part of the story. *Top Stroke Rehabil*. 2015:1945511915Y.000. doi:10.1179/1945511915Y.0000000009.

100.   Prince S, Saunders T. Do Physical activity interventions also decrease sedentary time? *PLOS Blogs*. 2014:1-6. http://blogs.plos.org/obesitypanacea/2014/10/21/do-physical-activity-interventions-also-decrease-sedentary-time/.

101.   Barreira T V., Zderic TW, Schuna JM, Hamilton MT, Tudor-Locke C. Free-living activity counts-derived breaks in sedentary time: Are they real transitions from sitting to standing? *Gait Posture*. 2015;42(1):70-72.

doi:10.1016/j.gaitpost.2015.04.008.

102. Bellettiere J, Winkler EAH, Chastin SFM, et al. Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults. *PLoS One.* 2017;12(6):e0180119.

103. Petersen CB, Bauman A, Tolstrup JS. Total sitting time and the risk of incident diabetes in Danish adults (the DANHES cohort) over 5 years : a prospective study. *Br J Sport Med.* 2016;23:1-6. doi:10.1136/bjsports-2015-095648.

104. Goodman-Gruen D, Barrett-Connor E. Sex differences in measures of body fat and body fat distribution in the elderly. *Am J Epidemiol.* 1996;143(9):898-906. http://www.ncbi.nlm.nih.gov/pubmed/8610703.

105. Taylor H a, Coady S a, Levy D, et al. Relationships of BMI to cardiovascular risk factors differ by ethnicity. *Obesity (Silver Spring).* 2010;18(8):1638-1645. doi:10.1038/oby.2009.407.

106. Gibbs BB, Gabriel KP, Reis JP, Jakicic JM, Carnethon MR, Sternfeld B. Cross-sectional and Longitudinal Associations Between Objectively Measured Sedentary Time and Metabolic Disease: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Diabetes Care.* 2015;38(10):1835-1843. doi:10.2337/dc15-0226.

107. van der Berg JD, Stehouwer CDA, Bosma H, et al. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: The Maastricht Study. *Diabetologia.* 2016;59(4):709-718. doi:10.1007/s00125-015-3861-8.

108. Dempsey PC, Blankenship JM, Larsen RN, et al. Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control. *Diabetologia.* 2016. doi:10.1007/s00125-016-4169-z.

109. Kerr J, Takemoto M, Bolling K, et al. Two-arm randomized pilot intervention trial to decrease sitting time and increase sit-to-stand transitions in working and non-working older adults. *PLoS One.* 2016;11(1):1-12. doi:10.1371/journal.pone.0145427.

110. Meneilly GS, Tessier D. Diabetes in elderly adults. *J Gerontol A Biol Sci Med Sci.* 2001;56(1):M5-M13. doi:10.1093/gerona/56.1.M5.

111. Booth GL, Kapral MK, Fung K, Tu J V. Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. *Lancet*. 2006;368(9529):29-36. doi:10.1016/S0140-6736(06)68967-8.

112. Atkins R. The epidemiology of chronic kidney disease. *Kidney Int*. 2005;67:14-18. doi:10.1111/j.1523-1755.2005.09403.x.

113. Kalyani RR, Corriere M, Ferrucci L. Age-related and disease-related muscle loss: The effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol*. 2014;2(10):819-829. doi:10.1016/S2213-8587(14)70034-8.

114. Centers for Disease Control and Prevention. *Incidence of Diagnosed Diabetes per 1,000 Population Aged 18–79 Years, by Age, United States, 1980–2014*. Atlanta, Georgia; 2015.

115. Gore SA, Foster JA, DiLillo VG, Kirk K, Smith West D. Television viewing and snacking. *Eat Behav*. 2003;4(4):399-405. doi:10.1016/S1471-0153(03)00053-9.

116. Ding D, Sugiyama T, Winkler E, Cerin E, Wijndaele K, Owen N. Correlates of change in adults' television viewing time: A four-year follow-up study. *Med Sci Sports Exerc*. 2012;44(7):1287-1292. doi:10.1249/MSS.0b013e31824ba87e.

117. Stamatakis E, Pulsford RM, Brunner EJ, et al. Sitting behaviour is not associated with incident diabetes over 13 years: the Whitehall II cohort study. *Br J Sports Med*. 2017:bjsports-2016-096723. doi:10.1136/bjsports-2016-096723.

118. Margolis KL, Bonds DE, Rodabough RJ, et al. Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. *Diabetologia*. 2004;47(7):1175-1187. doi:10.1007/s00125-004-1448-x.

119. Culver AL, Ockene IS, Balasubramanian R, et al. Statin Use and Risk of Diabetes Mellitus in Postmenopausal Women in the Women's Health Initiative. *Arch Intern Med*. 2012;172(2):144-152. doi:10.1001/archinternmed.2011.625.

120. Therneau TM. A Package for Survival Analysis in S. 2015. https://cran.r-project.org/package=survival%3E.

121. Barwell ND, Malkova D, Moran CN, et al. Exercise training has greater effects on insulin sensitivity in daughters of patients with type 2 diabetes than in women with no family history of diabetes. *Diabetologia*. 2008;51(10):1912-1919. doi:10.1007/s00125-008-1097-6.

122. Moore AF, Jablonski KA, McAteer JB, et al. Extension of type 2 diabetes genome-wide association scan results in the Diabetes Prevention Program. *Diabetes*. 2008;57:2503-2510. doi:10.2337/db08-0284.

123. Sinclair A, Dunning T, Rodriguez-Mañas L. Diabetes in older people: new insights and remaining challenges. *lancet Diabetes Endocrinol*. 2014;3(4):275-285. doi:10.1016/S2213-8587(14)70176-7.

124. Sue Kirkman M, Briscoe VJ, Clark N, et al. Diabetes in older adults: A consensus report. *J Am Geriatr Soc*. 2012;60(12):2242-2256. doi:10.1111/jgs.12035.

125. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128. doi:10.1016/S0140-6736(12)61728-0.

126. Dempsey PC, Sacrea JW, Larsena RN, et al. Interrupting prolonged sitting with brief bouts of light walking or simple resistance activities reduces resting blood pressure and plasma noradrenaline in type 2 diabetes. *J Hypertens*. 2016;34:2376-2382. doi:10.1097/HJH.0000000000001101.

127. Dempsey PC, Owen N, Yates TE, Kingwell BA, Dunstan DW. Sitting Less and Moving More: Improved Glycaemic Control for Type 2 Diabetes Prevention and Management. *Curr Diab Rep*. 2016;16(11):114. doi:10.1007/s11892-016-0797-4.

128. Healy G, Dunstan DW, Salmon J, et al. Breaks in sedentart time: beneficial associations with metabolic risk. *Diabetes Care*. 2008;31(4):661-666. doi:10.2337/dc07-2046.Abbreviations.

129. Cooper a. R, Sebire S, Montgomery a. a., et al. Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia*. 2012;55(3):589-599. doi:10.1007/s00125-011-2408-x.

130. Henson J, Yates T, Biddle SJH, et al. Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health. *Diabetologia*. 2013;56(5):1012-1020. doi:10.1007/s00125-013-2845-9.

131. Grant PM, Ryan CG, Tigbe WW, Granat MH. The validation of a novel activity monitor in the measurement of posture and motion during everyday activities. *Br J Sports Med*. 2006;40(12):992-997.

132. Dunstan DW, Zimmet PZ, Welborn T a, et al. The Australian Diabetes, Obesity and Lifestyle Study (AusDiab)--methods and response rates. *Diabetes Res Clin Pract*. 2002;57(2):119-129. doi:10.1016/S0168-8227(02)00025-6.

133. Barr E, Magliano D, Zimmet P, et al. *AUSDIAB 2005: The Australian Diabetes, Obesity and Lifestyle Study.*; 2005. https://www.bakeridi.edu.au/Assets/Files/AUSDIAB_Report_2005.pdf.

134. Tanamas SK, Magliano DJ, Lynch B, et al. AUSDIAB 2012: The Australian Diabetes, Obesity and Lifestyle Study. 2013:1-92. http://www.bakeridi.edu.au/AusDiabFindings.

135. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502. doi:10.1177/107424840501000106.

136. Giles G, Ireland P. *Dietary Questionnaire for Epidemiological Studies (Version 2).*; 1996.

137. Grant PM, Ryan CG, Tigbe WW, Granat MH. The validation of a novel activity monitor in the measurement of posture and motion during everyday activities. *Br J Sports Med*. 2006;40(12):992-997. doi:10.1136/bjsm.2006.030262.

138. Chastin SF, Winkler EA, Eakin EG, et al. Supplementary material: Sensitivity to change of objectively-derived measures of sedentary behavior: *Meas Phys Educ Exerc Sci*. 2015. http://www.tandfonline.com/doi/suppl/10.1080/1091367X.2015.1050592/suppl_file/hmpe_a_1050592_sm0678.docx. Accessed January 26, 2016.

139. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology*. 2009;20(4):488-495.

doi:10.1097/EDE.0b013e3181a819a1.

140. Scheers T, Philippaerts R, Lefevre J. SenseWear-determined physical activity and sedentary behavior and metabolic syndrome. *Med Sci Sports Exerc*. 2013;45(3):481-489. doi:10.1249/MSS.0b013e31827563ba.

141. Chastin SFM, Palarea-Albaladejo J, Dontje ML, Skelton DA. Combined effects of time spent in physical activity, sedentary behaviors and sleep on obesity and cardio-metabolic health markers: A novel compositional data analysis approach. *PLoS One*. 2015;10(10). doi:10.1371/journal.pone.0139984.

142. Grace MS, Climie RED, Dunstan DW. Sedentary Behavior and Mechanisms of Cardiovascular Disease-Getting to the Heart of the Matter. *Exerc Sport Sci Rev*. 2017;45(2):55-56. doi:10.1249/JES.0000000000000107.

143. Same R V., Feldman DI, Shah N, et al. Relationship Between Sedentary Behavior and Cardiovascular Risk. *Curr Cardiol Rep*. 2016;18(1):1-7. doi:10.1007/s11886-015-0678-5.

144. Stamatakis E, Hamer M, Tilling K, Lawlor DA. Sedentary time in relation to cardio-metabolic risk factors: differential associations for self-report vs accelerometry in working age adults. *Int J Epidemiol*. 2012;41(5):1328-1337. doi:10.1093/ije/dys077.

145. Scott RA, Langenberg C, Sharp SJ, et al. The link between family history and risk of type 2 diabetes is not explained by anthropometric, lifestyle or genetic risk factors: The EPIC-InterAct study. *Diabetologia*. 2013;56(1):60-69. doi:10.1007/s00125-012-2715-x.

146. Brito EC, Lyssenko V, Renstrom F, et al. Previously associated type 2 diabetes variants may interact with physical activity to modify the risk of impaired glucose regulation and type 2 diabetes. *Diabetes*. 2009;58(June):1411-1418. doi:10.2337/db08-1623.