SHORT-TERM CHANGES IN DAILY MOVEMENT BEHAVIOUR INFLUENCE C-REACTIVE PROTEIN IN HEALTHY, MIDDLE-AGED WOMEN

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B.Sc. Kinesiology (Hons), University of Lethbridge, 2016

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

M.SC. EXERCISE SCIENCE

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Date of Defence: December 16, 2016

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ABSTRACT

Despite growing awareness of the health consequences of sedentary time (ST), the underlying physiological mechanisms are poorly understood. C-reactive protein (CRP), a marker of systemic inflammation, represents a potential link between ST and adverse health. It has also become apparent that prolonged, uninterrupted bouts of ST are particularly harmful to health. The primary purpose of this thesis was to explore the effect of (a) increased ST and (b) increased physical activity (PA) on salivary CRP in healthy, middle-aged women. A secondary objective was to examine how the pattern of activity changes in response to these behavioural interventions.

After completing a 7-day preliminary assessment of daily step count, ST, and PA, 20 healthy, middle-aged women were randomly assigned to one of two 10-day interventions, either sedentary or active. The sedentary group reduced their step count to <5000 steps/day. The active group added 3,000 steps/day to their preliminary average. During both the preliminary assessment and intervention period, participants wore a pedometer to monitor their daily step count and an accelerometer to objectively assess their activity profile. Salivary CRP samples were taken during the preliminary assessment, pre-intervention, and post-intervention.

During the intervention, the sedentary group (n=9; 49.6±5.6 yrs; 28.4±3.5 $kg \bullet m^{-2}$) significantly increased ST by 70 minutes/day, and decreased both light physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). They also increased the number of prolonged sedentary bouts ($p = 0.004$) and decreased the frequency of

interruptions to ST ($p = 0.006$). The active group (n=10; 49.9±5.2 yrs; 26.6±3.7 $kg \bullet m^{-2}$) increased MVPA by 19.4 minutes/day, but there was no change in ST, LPA, or the pattern of ST accumulation. After 10 days of behavior change, CRP increased by 31% in the sedentary group ($p<0.05$) and decreased by 22% in the active group ($p<0.01$).

These results suggest that CRP, and thus inflammation, may represent a physiological link between movement behavior and health in middle-aged women. The decrease in CRP in the active group despite no change in ST challenges current data that shows ST impacts health independent of MVPA. Importantly, interventions focused on increasing MVPA may not effectively reduce ST.

ACKNOWLEDGEMENTS

Dr. Jennifer Copeland

"If it doesn't challenge you, it doesn't change you." - Fred DeVito

- It is impossible to fully acknowledge the role Copeland has played in the completion of this work and in my development as a young researcher. I am beyond grateful for her influence, both inside and outside of the lab. I truly could not have chosen a better mentor as I move forward in life and my career.

My committee members, **Dr. Jon Doan & Dr. Cheryl Currie,** both of whom were there to answer my endless flow of questions and requests.

Clayton, whose support throughout this journey has meant more than he knows. His unrelenting patience never ceases to amaze me.

My dedicated **participants**, without whom this work would not exist.

University of Lethbridge Research Fund, for funding this project and allowing me the opportunity to do research that truly interests me.

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ABBREVIATIONS

BMI, body mass index

CVD, cardiovascular disease

CRP, C-reactive protein

HOMA-IR, homeostatic model assessment - insulin resistance

LPA, light physical activity

MVPA, moderate-to-vigorous physical activity

PA, physical activity

ST, sedentary time

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

As an increasingly technologized society, Canadian men and women spend 68% and 69% of respective waking hours sedentary [1] and similar values have been reported in other developed nations.[2-5] Current evidence suggests that excessive sedentary time is related to cardiovascular disease risk independent of moderate-to-vigorous physical activity (MVPA).[6-12] This means that an individual can accumulate high amounts of sedentary time, which increases their risk of developing cardiometabolic disease, while simultaneously reaching current physical activity guidelines. Although the association between sedentary time and adverse health outcomes has been shown to be independent of MVPA, less is known about the underlying biological mechanisms.

C-reactive protein (CRP) may represent a mechanistic link as it is elevated in sedentary individuals even after controlling for MVPA.[2 13] Using a nationally representative sample of US adults, Healy et al. [2] showed a significant positive relationship between objectively measured total sedentary time and circulating concentration of CRP after adjusting for age, sex, ethnicity, and exercise. Interestingly, the quartile of participants with the most breaks in sedentary time had lower CRP levels than the quartile with the fewest breaks, which suggests the pattern of accumulation of sedentary time may influence CRP. Similarly, Leon-Latre et al. [13] found a significant positive relationship between CRP and self-reported sedentary time in Spanish factory workers.

C-reactive protein (CRP) is a non-specific component of innate immunity that is synthesized during the acute phase immune response. CRP is used clinically as a marker

of endothelial health and function and can be indicative of systemic, low-grade inflammation.[14 15] Even within normal range, epidemiological studies have repeatedly shown a strong predictive relationship between CRP concentration and future cardiovascular (CV) events.[16] Serum CRP concentrations of <1, 1 – 3, and >3 mg/L in apparently healthy persons correspond to low-, moderate-, and high- risk for future CV events.[14] Associations between CRP and various metabolic abnormalities (i.e. obesity, insulin resistance, hypertension, etc.) have also been identified.[17-19]

Although cross-sectional studies have shown an association between CRP and sedentary time, the experimental evidence supporting these data is limited and equivocal.[20 21] After 7 days of reduced stepping, Dixon et al. [21] observed no change in the CRP concentration of highly active, middle-aged men. Conversely, Breen et al. [20] reported a 25% increase in CRP following 14 days of reduced stepping in 10 older adults (50% male). Given the harmful effects of prolonged sedentary time, more experimental research is needed to further explore the relationship between sedentary time and inflammation. Further investigation into how various intervention strategies affect the pattern of sedentary behaviour is also warranted.

SEDENTARY TIME

The specific biological outcomes related to sedentary behaviours and their unique nature makes it important to recognize sedentary physiology as distinct from exercise physiology. Physiological responses to sedentary behaviour and physical activity are not necessarily opposite of one another [22] and can vary between systems (ex. musculoskeletal

vs. cardiovascular). For example, lipoprotein lipase (LPL) kinetics are qualitatively different in response to hind-limb unloading, ambulatory activity and physical exercise in animal models (discussed in more detail later).[23]

Figure 1.1 represents the movement continuum, wherein sedentary behaviour and exercise behaviour lie at opposite ends. Energy expenditure is altered as an individual moves up or down the movement continuum, producing distinct acute and chronic biological adaptations. These responses may (i) occur in a linear fashion, (ii) occur in a non-linear fashion, (iii) emerge once a given threshold of sedentary behaviours or physical activity is reached, or (iv) be non-existent.[24] Thus, physical activity and sedentary behaviours may affect health-related outcomes differently.

There remains an inconsistency in terminology related to sedentary physiology and sedentary behaviours. The term "sedentary" has taken on two operationally opposing definitions within the literature.[25] The first generally characterizes sedentary behaviours by a low energy expenditure of ≤1.5 metabolic equivalents (METs) while in a sitting or reclined position.[26] In this, a person can be defined as living a sedentary lifestyle if they spend a significant amount of time engaged in sedentary behaviour, regardless of time spent in moderate-to-vigorous physical activity (MVPA). An individual can also engage in high levels of MVPA and still be considered sedentary if they are accumulating high levels of sedentary time (at work, while commuting, during leisure time, etc.). Comparatively, sport and exercise specific literature often define sedentary as failing to reach a given threshold of MVPA, regardless of time spent in lifestyle-related ambulation or light physical activity. As a result, many studies proclaiming the deleterious effects of a sedentary

lifestyle did not actually measure sedentary time[27-30]

Figure 1.2 shows two drastically different movement profiles.[26] As represented in Figure 2a, it is possible for an individual to engage in high levels of both sedentary behaviours and MVPA. Moreover, it is equally as feasible to obtain low levels of sedentary time and not reach recommended levels of MVPA (Figure 1.2b). Dunstan et al. [6] introduced the terms "active couch potato" and "active non-couch potato" to describe the movement profiles represented in Figure 1.3. Although both individuals are reaching daily physical activity recommendations and are therefore classified as active, divergence occurs in time spent sedentary throughout the day. Finally, accelerometry can be used to explore patterns specific to sedentary behaviour. Despite the same amount of time spent sedentary, Figure 1.4 portrays different interruption patterns to sedentary time wherein a "prolonger" and "breaker" are shown Dunstan et al. [6] Interestingly, frequent interruptions to sedentary time are inversely associated with abdominal obesity and markers of cardiometabolic health.[31 32]

Practical attempts to reduce sedentary time will also be different than approaches used in motivating people to increase purposeful exercise.[33] Health recommendations focused on decreasing sedentary behaviours are likely to be more accessible to low income individuals, those with limited time and/or those apathetic toward structured physical activity involvement. It is likely that reducing sedentary time is an achievable short- term goal for most individuals, whereas increasing MVPA might be self-limiting in nature (time, equipment, competence, etc.).[24]

As is becoming increasingly accepted, this paper defines sedentary behaviour as any

waking behaviour characterized by a reclined or sitting position and an energy expenditure of ≤1.5 METS. The term "inactive" is reserved for those not engaging in the recommended amount of MVPA, defined here as a minimum of 150 minutes at an intensity ≥3 METs.[25] Light physical activity (LPA) refers to activities eliciting an energy expenditure of 1.6 – 2.9 METs, including slow walking, light cleaning, and other activities of daily living.

Sedentary Time & Health

The global prevalence of numerous lifestyle-related chronic diseases and risk factors has increased over the past 3 decades. The World Health Organization (WHO) [34] reported that worldwide obesity has nearly doubled since 1980, equating to approximately 11% of the adult population. The prevalence of diabetes has followed similar trends in Canada,[35] the United States,[36 37] and the UK.[38] The prevalence of CVD risk factors has also increased within the last 30 years.[39 40] Furthermore, Canadians lose approximately 164,400 years of life due to CVD and another 166,000 from diabetes, and the economic burdens of CVD and diabetes in Canada are estimated to be $24.8 and $4.8 billion per year, respectively.[41] It is clear the individual, economic, and public health impact of chronic disease is extensive. As such, it is crucially important that scientific research pursue a deeper understanding of the physiological underpinnings of chronic disease in hope of mitigating disease incidence.

A growing body of research suggests time spent sedentary may play a role in the development of chronic disease and a substantial portion of this research has focused on cardiometabolic outcomes. Sedentary time has been correlated with measures of metabolic

dysfunction such as impaired glucose tolerance and low-density lipoprotein concentrations.[31] Since metabolic dysfunction is highly predictive of CVD,[42 43] the relationship between sedentary time and health outcomes is of particular importance. Over the past decade, the interplay between sedentary behaviours, metabolic health, and cardiovascular disease has become better understood.

Initial experimental research in humans was conducted on individuals undergoing bouts of bed rest due to injury or by experimental design. Although complete bed rest facilitates the study of sedentary physiology, the practical application of such methodology is limited as it neglects lifestyle-related ambulation and light physical activity.[44] Studies exploring cessation of MVPA and detraining effects in trained individuals have also been used to explore physiological adaptions. This research, although still modest in magnitude, shows reversal of several training adaptations as quickly as a few weeks.[45] Animal models (i.e. hind-limb unloading and wheel-locking) have also been used extensively to expose the underlying mechanisms of sedentary behaviours, and are particularly useful in identifying metabolic changes.[22 46] Hind-limb unloading was introduced by Morey [47] and has since been extensively used to study the physiological consequences of weightlessness and disuse in rats and mice. Briefly, the hind limbs are suspended above the cage floor by a cord attached at the base of the tail; the forelimbs remain on the floor allowing locomotion and access to food and water. The hind-limb unloading method is minimally invasive; animals show immediate stress-related responses that return to baseline within ~7 days.[48] Over the last ~6 years, more experimental studies in humans have emerged and range in duration from 6 hrs to 14 days. These experimental studies are limited in number but provide

important insight into how sedentary time impacts health.

Much of our current knowledge in sedentary physiology has been informed by large population-based studies such as the National Health and Nutrition Examination Survey (NHANES) in the US and the Canadian Health Measures Survey (CHMS). These large studies provide insight into where experimental research is needed and the most recent cross-sectional designs have begun to use more sophisticated statistical analysis approaches to explore sedentary time.[49 50]

Sedentary time and cardiovascular disease. Cardiovascular disease (CVD) is an umbrella term used to describe a range of abnormalities related to the heart and/or the vasculature surrounding or encapsulated within the heart. Cerebrovascular events, or different types of strokes, are often categorized under the 'CVD umbrella' and are reported alongside cardiac-specific outcomes such as coronary heart disease (CHD), coronary revascularization procedures, and myocardial infarction.

Cross-sectional and follow-up studies on London transport and postal workers were some of the first epidemiological models suggesting a relationship between sedentary behaviours, light physical activity and risk of CVD.[51 52] Prevalence of CVD among those with sedentary jobs (e.g. bus drivers and postal receptionists) was twice that of those with more active jobs (e.g. conductors and mail delivery men). Drivers were shown to have a higher mortality rate than conductors after controlling for central adiposity. Moreover, a three-year follow-up of the same cohort reported conductors had half the mortality rate relative to drivers.[52] Weller and Corey [53] found similar associations in women, suggesting a 2.7-fold increase in CVD mortality risk for high-sitters versus low-sitters. Several

population-based longitudinal studies indicate those engaging in high amounts of sedentary behaviours are 1.5 to 2.5 times more likely to develop CVD than those spending less time sedentary. The dose-response relationship found in these studies remained after adjusting for possible cofounders such as age, sex, smoking status, diet, body mass index and physical activity levels.[9 54 55] This research further supports sedentary behaviours as an independent correlate to CVD risk, a relationship that has been reproduced by other researchers in a variety of populations.[56-58]

Several studies have used objectively measured sedentary time in assessing CVD risk and mortality. Stamatakis and colleagues [59] conducted a cross-sectional analysis using subjective and objective measures of sedentary behaviours in 2765 mean and women 60 years and older. Their results show a trend toward a higher percentage of CVD among the more sedentary tertile, however this was not shown to be significant ($p>0.27$). The authors suggested a lack of contextual information regarding sedentary behaviours (i.e. watching TV may be more deleterious to cardiac health compared to reading a book) might have affected statistical outcomes. Matthews et al. [54] further showed those watching TV for > 7 h • day^{-1} were 85% more likely to die of CVD than those watching < 1 h • day^{-1}. However, this trend was not significant for overall sitting time (including work, transportation, and TV time), suggesting the context in which sedentary time is accumulated has an effect on health outcomes. Using accelerometry data from 1906 men and women over age 50, Koster et al. [60] reported a similar trend, supporting a positive relationship between sedentary behaviours and CVD mortality rate. The relationship between sedentary time and CVD has repeatedly been reported and many studies support sedentary time as a CVD

risk factor independent of MVPA.[9 55 61] Longitudinal data further supports sedentary time as a CVD risk factor independent of MVPA.[62]

Given the chronic nature of CVD, it is difficult to study it in relation to sedentary time using an experimental study design. For this reason, intervention studies have relied on various vascular, metabolic, and immune biomarkers that respond acutely to changes in activity to study the effect of sedentary time on health. Metabolic abnormality is highly related to CVD such that the two variables are difficult to separate; that is, one rarely occurs without the other.[43] Thus much of our mechanistic understanding of sedentary physiology has been informed by exploring the effect of short-term exposure to sedentary time on metabolic outcomes.

Sedentary time and metabolic dysfunction. Metabolic syndrome (MetS) has become the commonly used term to describe the clustering of risk factors (i.e. insulin resistance, central obesity, high triglyceride levels, low high-density lipoprotein cholesterol (HDL-C), hypertension, etc.) suggestive of systemic metabolic dysfunction, though the clinical criterion for diagnosis of MetS varies between organizations.[63] A meta-analysis of ten cross-sectional studies exploring the association between MetS and sedentary behaviours suggests there is a 73% increased risk of MetS with more time spent sedentary, independent of age, sex, MetS definition and physical activity ($p<0.0001$).[7] Although the authors failed to identify how much sedentary time was associated with the elevated risk, a recent analysis comparing the prevalence of MetS across tertiles of sedentary time in 930 Swedish adults reported a similar odds ratio of 1.72 (95% CI: 1.08–2.74) in the most sedentary tertile versus the least sedentary.[64] Data from van der Berg et al. [65] is more

specific, suggesting that each additional hour of daily sedentary time increases the risk of developing MetS by 39%. Longitudinal data also supports an adverse influence on cardiometabolic disease risk, independent of MVPA.[66] CVD has been identified as the primary clinical outcome of MetS [43] and in 2011, WHO [34] reported CVD to be the leading cause of death around the world. Thus, insight into what might be contributing to metabolic dysfunction is important for its prevention and treatment.

Insulin resistance. A significant amount of the research into the physiological effects of chronic exposure to sedentary behaviours has focused on insulin resistance and glucose metabolism. This is primarily a product of the global prevalence of insulin resistance and diabetes, in low socioeconomic and high socioeconomic populations alike.[67] Insulin resistance has also been suggested to be the primary driver of several CVD risk factors, such as obesity, hypertension and hyperlipidemia,[68] although this conclusion remains controversial.

Epidemiological studies show sedentary time to be significantly associated with diabetes and insulin resistance. Whether measured subjectively or objectively, several studies have reported a linear relationship between sedentary time and prevalence of diabetes that is independent of MVPA.[3 54 59 60 69] Moreover, other large cross-sectional studies have shown that interrupting sedentary time may positively affect insulin sensitivity.[31 69]

In 2011, Thyfault & Krogh-Madsen proposed that the best model to study the consequences of sedentary time is to transition individuals from high levels of daily ambulation (10,000-12,000 steps/day) to low levels, (<1,500 or <4,000 steps/day) within a free-living environment.[11] A few experimental trials have used this model to study the

effects of sedentary time on insulin sensitivity and glucose metabolism.[21 70 71] Based on preliminary work by Olsen et al. [72] Krogh-Madsen and colleagues [70] took ten healthy males who routinely took >10,000 steps per day and transitioned them to <1,500 steps per day with no recreational physical activity/MVPA. Participants were directed to maintain their normal diet and change no other aspect of daily living (i.e. work, socializing, etc.). Two weeks of reduced stepping and MVPA resulted in significant changes in the metabolic response to an infused glucose load. In addition to a reduced rate of insulin-stimulated glucose disappearance (a marker of peripheral insulin sensitivity), subjects experienced an increased insulin response to both a glucose tolerance test and an oral fat tolerance test.[73] Using a hyperinsulinemic-euglycemic clamp technique, the measured glucose infusion rate was slower post-intervention. A slower glucose infusion rate combined with no change in hepatic glucose turnover suggests reduced peripheral insulin sensitivity and a diminished ability to utilize blood glucose in skeletal muscle. Dixon et al. [21] and Lyden et al. [71] have demonstrated comparable outcomes using similar experimental models. Human bed rest studies [44 74-78] and data from animal models [46 79-81] also support these findings.

Importantly, the lack of tight dietary control in some studies [70 72] makes it difficult to conclude whether changes in post-prandial metabolic outcomes are primarily due to metabolic dysfunction or the result of drastic alterations in energy balance. Stephens et al. [82] addressed this question by examining the effect of one day of sitting (~17 hours) and energy intake on insulin action using a counter balanced cross-over design with three experimental conditions: (1) a limited-sitting condition with light physical activity (LPA) and a neutral energy balance (NO-SIT), (2) prolonged sitting with no PA and an energy

surplus (SIT), and (3) prolonged sitting with no PA and a neutral energy balance (SIT-BAL). Participants were given standardized meals and slept in the laboratory following each experimental condition. Insulin action and the rate of glucose disappearance were assessed the following morning via a continuous isotope-labeled glucose infusion and venous blood draws.[83] Compared to the NO-SIT condition, mean plasma insulin concentrations during the glucose infusion were 41% higher in the SIT condition and 20% higher in the SIT-BAL condition ($p < 0.05$). Between the two sitting conditions, plasma insulin concentrations were 18% higher in the SIT condition compared to SIT-BAL. The rate of glucose disappearance was also significantly lower in the SIT condition compared to NO-SIT and SIT-BAL, but there was no difference between NO-SIT and SIT-BAL. These data suggest that the relationship between sedentary time, and both insulin sensitivity and glucose metabolism can be moderated by an isocaloric energy intake. They also suggest that, compared to sitting, standing and performing activities of daily living can acutely enhance metabolic function. Together these studies provide compelling evidence for a relationship between sedentary time and insulin resistance/glucose metabolism. However, future experimental research needs to explore whether sedentary time affects insulin action independent of MVPA and consider macronutrient composition.

Dyslipidemia. Dyslipidemia is characterized by high serum triglycerides, high low-density lipoprotein cholesterol (LDL-C), and decreased high-density lipoprotein cholesterol (HDL-C) concentrations. Dyslipidemia contributes to atherosclerosis,[84] which is affiliated with CVD incidence.[85] As with insulin resistance, dyslipidemia and compromised fat oxidation are potential outcomes of chronically high sedentary time.[86]

Accelerometer-derived cross-sectional data suggests there is an association between sedentary time and HDL-C levels,[2 59 69] as well as fasting triglycerides [2] at a population-wide level. However, these results are inconclusive.[87]

Current experimental data suggests sedentary time may have an effect on blood lipids but the extent and magnitude of these effects remain largely uncharacterized. Krogh-Madsen et al. [70] found no changes in either serum free-fatty acids or total triglyceride levels following two weeks of < 1,500 • day^{-1}. Lyden et al. [71] also showed no change in lipid concentrations (total cholesterol, LDL, HDL, and triglycerides) following 7 days of reduced ambulation in 10 young, healthy adults (aged 20-25 yrs). However, using slightly older participants (aged 45-65 yrs), Dixon et al. [21] reported a significant increase in fasting triglycerides, but not free-fatty acids after 7 days of reduced ambulation in active, healthy men. This suggests that age may be an important factor in sedentary time related health risk. Moreover, in a study exploring the effects of detraining in overweight and obese men, the control group (with no previous exercise training) experienced detrimental increases in LDL particle number and a decrease in LDL particle size after 15 days compared to an experimental group who ceased their exercise program that consisted of low levels of moderate walking.[88] A positive association between sedentary time and serum lipids is supported by Fung et al. [89] who reported significantly lower of HDL-C concentrations (~ 5 mg/dl) in men who spent 12.9–32.9 h • week^{-1} watching TV compared to those who watched 0.1-5.8 h • week^{-1}. This difference persisted across all levels of physical activity (MET-hrs/week).[89]

Perhaps the most compelling evidence that sedentary behaviours increase plasma lipid concentrations independent of physical activity is provided from the analysis of lipoprotein lipase (LPL) kinetics. Examination of LPL activity has provided insight regarding lipoprotein deregulation [22 23] and triglyceride metabolism [90] during inactivity. LPL is a central enzyme in fat metabolism [91 92] and influences plasma cholesterol metabolism, partioning of triglyceride-derived fatty acids into different tissues, and intracellular lipid availability.[8] In rat models, LPL activity decreased after ~4 hours of hind-limb unloading (analogous to bed rest in humans), and continued to decrease for ~18 hours. Simultaneously, plasma triglyceride uptake by skeletal muscle decreased dramatically (~75%) and HDL-C concentration declined ~20% after just 1 day of unloading, and remained unchanged to day eleven.[23] Oxidative, slow-twitch muscle fibres show profound decreases in LPL activity after unloading compared to glycolytic, fast-twitch fibres, likely due to the pattern of neuromuscular recruitment during ambulatory movement.[22] Similar changes in LPL activity have been observed in humans.[93] After 20 days of bed rest, Yanagibori et al. [94] reported an 18% decline in LPL activity in healthy subjects, concomitant with significant increases in plasma triglycerides and decreased HDL-C. LPL activity has also been shown to decrease in endurance athletes during detraining.[95] Interestingly, inactivity and intense exercise have qualitatively different effects, such that LPL activity will swiftly and dramatically decrease in response to unloading while intense exercise has comparatively little effect on LPL kinetics.[22 96]

Obesity and central adiposity. The relationship between obesity and sedentary behaviours is well supported by population-based data. Prevalence of obesity and BMI

increase linearly with self-reported and objectively-measured sedentary time.[31 54 97 98] A significant association was shown between accelerometer-derived sedentary time and waist circumference, a measure of central adiposity, in US [2] and UK adults.[59] Interestingly, Sugiyama et al. [99] concluded that individuals engaging in low levels of sedentary time but insufficient physical activity are at a similar risk for obesity compared to those with sufficient activity but high levels of sedentary time. Intuitively, individuals with high sedentary time and insufficient physical activity have the highest risk of developing obesity.[99]

Significant alterations in adipose distribution and fat mass have been quantified in animal and human models undergoing periods of decreased physical activity. Given the chance, rats will habitually run between 1 and 20 km/day depending on the phenotype. This pattern of running is suggested to be analogous to regular lifestyle ambulation in humans. Once the rat's wheel is locked, normal cage activity remains but higher amounts of physical activity are not achieved. Thus, the wheel-lock (WL) model represents the transition from an active lifestyle to a sedentary lifestyle in humans.[46] A series of studies comparing 5, 53 and 173 hours of WL produced novel findings related to adiposity.[46 79-81] Compared to the 5 hour WL control, 53 hours of WL resulted in a 30% increase in epididymal (abdominal) fat pad mass and a 48% increase in omental (subcutaneous) fat pad mass, both of which reside in the abdominal cavity. Within the epididymal fat pad, the number and size of adipose cells increased.[46] Laye et al. [81] showed these effects to be independent of energy intake as abdominal fat pad mass increased despite a controlled

energy intake wherein intake was age-matched to sedentary rats that never had access to a running wheel.

Outcomes from similar reductions to daily ambulation in young, healthy males have also been studied. After 14 days of reduced stepping, visceral adiposity increased 7% despite a decrease in body weight and lower body fat-free mass,[70] indicating a positive energy intake did not cause variations in adiposity. Breen et al. [20] reported similar increases in central adiposity and decreases in lower body fat-free mass and skeletal muscle, despite a decrease in total body mass, after 14 days of reduced stepping in 10 older adults (72.3 ± 1.0 yrs). Similar findings have also been reported in middle-aged, overweight men and women.[30]

Current evidence supports sedentary behaviour as a contributing factor to obesity and that MVPA is not completely protective against obesity in adults. Notably, it has not been conclusively established that sedentary behaviours produce obesity and weight gain. It is feasible that heavier people are instinctively more sedentary. Although current research is compelling, more longitudinal and experimental evidence is needed to establish a cause-effect relationship between sedentary time and obesity.

Finally, although observational data correlating sedentary time to poor cardiometabolic health outcomes independent of MVPA are convincing, they are not conclusive. Systematic reviews of both prospective [100] and longitudinal [101] studies demonstrated mixed findings for the relationship between sedentary time and disease outcomes. Moreover, there remains a paucity in the number of experimental trials exploring the physiological

link between sedentary time and health outcomes, and the existing research largely focuses on glucose metabolism and insulin sensitivity.[20 21 70 71]

The metabolic system and immune system are inextricably linked as a specie's survival depends on its ability to both metabolize substrate for energy and mount a defense against pathogens.[102] The chronic nature of metabolic dysfunction, which is often marked by risk factors such as insulin resistance, dyslipidemia, and obesity, results in a chronic, low-grade activation of the immune system that contributes to CVD risk and development.[102 103] Given the known effects of physical activity on inflammation,[104] and cross-sectional evidence of an association between sedentary time and inflammatory markers, such as CRP,[2 13 105] there is increasing interest in how movement behaviours might affect inflammation. This, along with current data linking sedentary time to CRP, has catalyzed speculation into the potential effect of sedentary time on C-reactive protein.

C-REACTIVE PROTEIN

C-reactive protein (CRP) is a non-specific, acute-phase plasma protein with an enduring evolutionary history. Homologs of human CRP have been traced back as far as the horseshoe crab, an ancient species with roots extending more than 70 million years.[106] The preservation of CRP across time and species is a testament to its central role in host defense. CRP was discovered in humans less than 90 years ago by Tillett and Francis [107] After observing the precipitation and subsequent reaction between an unknown substance and C-polysaccharide of the *pneumococcal* cell wall, Tillett and Francis [107] introduced a "C-reactive substance" to the literature. The researchers observed a rapid accumulation of the

substance at the onset of pneumonia, succeeded by a similarly prompt decline following resolution of the illness.[107] In 1941, Avery and Abernethy[108] established the reactive substance to be a protein and renamed it "C-reactive protein". The binding of CRP was further recognized to be calcium (Ca^{2+})-dependent, an important property in elucidating the diverse biological role of the reactive protein.[108]

Structure and Function. Along with serum amyloid P component, CRP comprises the short-arm of the pentraxin family, an evolutionarily preserved [109] group of proteins that play an essential role in the humoral component of innate immunity.[110] It is a cyclical, disk-like plasma protein composed of five identical, non-glycosylated subunits that are arranged symmetrically around a central pore. Each subunit is a single polypeptide strand that is comprised of 206 amino acids with a molecular weight of ~23 kDa. Each subunit is able to bind two calcium ions to facilitate Ca^{2+}-dependent binding of specific ligands.[111]

The diverse physiological functions of CRP are largely explained by its various binding properties and ability to activate the classical pathway of human complement (Figure 1.5).[112] Briefly, human complement refers to a group of plasma and membrane proteins in humans that, when activated, respond in a cascade-like fashion and act as opsonins. Opsonins are chemical attractants for leukocytes and other phagocytotic agents that cause mast cell degranulation and eventual disposal of pathogenic, damaged or dead microorganisms.[113] Phosphocholine, a common component of pathogenic cell walls and damaged cellular membranes, is Ca^{2+}-dependent and popularly considered the prototypical ligand of CRP.[114] The CRP-Ca^{2+}-PCh complex initiates a cascade of molecular signals that results in the activation of classical human complement.[115] [116] Opsonisation and

phagocytosis of PCh-containing microorganisms occurs through the terminal membrane attack complex. This terminal complex uses a pore in the bilayer of the pathogenic cell membrane to destroy its integrity and disrupt the electrochemical gradient, thus terminating the cell.[113]

Similar mechanisms are used in the disposal of necrotic host cells: CRP binds nuclear or other cellular material such as small nuclear ribonucleoproteins, chromatin or histone in a Ca^{2+}-dependent manner to activate classical complement and opsonophagocytosis.[112] Furthermore, CRP can bind to apoptotic cells and work in concert with the classical complement pathway to enhance opsonisation and phagocytosis by macrophages in a manner that protects cells from terminal complement components.[117] In this way, CRP can display both anti- and pro-inflammatory properties.[109 117]

Another important mechanism through which CRP promotes phagocytosis of pathogenic microorganisms or damaged/dead host cells is the binding of CRP-ligand complexes to FcγR receptors of immunoglobulin G (IgG) molecules, specifically FcγRI, FcγRIIa and FcγRIIb (Figure 5).[118] Activation and inhibitory receptors are recognized as the two general classes of FcR receptors. The role of activation FcR receptors in initiating cellular host defense and dictating the efficacy of antibodies is mediated through ligand action on effector cells.[119] Inhibitory FcR receptors regulate peripheral activation and terminate IgG effector stimulus. Thus the absence or dysregulation of inhibitory receptors by genetic or environmental factors can culminate into autoimmunity via a magnified effector response by cytotoxic antibodies or immune complexes.[118] Therefore, binding of

CRP-ligand complexes to FcγR receptors both activate and inhibit phagocytotic activity to promote host defense and defend against autoimmunity.

The roles of CRP in innate immunity are multifaceted and function dynamically in a cohesive fashion with other compounds and systems to protect the host from infection, aid in the disposal of damaged/dead cells and mediate clearance of apoptotic cells.[120] CRP's ability to activate the classical pathway of human complement is central to its anti-pathogenic properties. Moreover, depending on the duration, intensity and nature of the stimulus, CRP will respond in a pro-inflammatory or anti-inflammatory fashion.[109 117]

Plasma concentrations. Synthesis of CRP occurs primarily in the liver and is stimulated by pro-inflammatory cytokines, interleukin (IL)-6 and IL-1, with IL-6 being the predominant inducer.[120] In young, healthy blood donors, the median serum CRP concentration was 0.8 mg/l, with 3.0 mg/l and 10 mg/l representing the 90th and 99th percentile, respectively.[121] Slightly higher median values were reported in 4,494 ostensibly healthy men and women, which might be explained by the existence of more subclinical disease in the general population versus eligible blood donors.[122] CRP is also recognized to increase with age and may be slightly higher in women than men.[122]

Following an acute-phase stimulus, concentrations increase rapidly and can increase from <50 μg/l to >500 mg/l in less than 48 hours (a 10,000-fold increase), with levels reaching about 5 mg/l above baseline after 6 hours and peaking after 48.[123] Vigushin et al. [124] demonstrated the 19-hour half-life of CPR to be unaffected by age or disease, suggesting circulating concentrations of CRP are solely dependent on the rate of synthesis. Thus serum CRP concentration is proportional to the intensity and duration of the pathological

stimuli.[124] Importantly, chronically elevated levels of CRP, even within the range generally considered normal, are indicative of systemic low-grade inflammation and predictive of atherosclerotic vascular diseases (i.e. cerebrovascular and cardiovascular).[14]

CRP & Health

Cardiovascular disease and atherosclerosis. Because of its well-established role in acute-phase immunity, the clinical use of CRP was largely ignored beyond the 1970s. Interest in the protein was revived in the late 1980s to mid-1990s when utilization of higher-sensitivity immunoassays revealed strong predictive associations between serum CRP concentrations and future cardiovascular events. In 1995, a two-year follow-up of 2960 men and women revealed those who experienced a coronary event had an average of 20.2% higher serum CRP concentrations at baseline than those free of such events.[125] Similar outcomes were reported shortly thereafter [126] and numerous large epidemiological studies have since supported a predictive relationship between CRP and adverse cardiovascular outcomes.[18] [127-129] In the early 2000's, seminal work by Ridker et al. [16] showed CRP to be the best univariate predictor of future cardiovascular events compared to 12 other biomarkers. Relative to women in the lowest quartile of CRP levels, women in the highest quartile had a relative risk for future events of 4.4 (95% CI, 2.2-8.9).[16] Evidence supporting CRP as a reliable predictive marker of cardiovascular disease is extensive and, to date, unrefuted.

The lipoprotein-binding [130] and complement initializing properties of CRP, combined with its localization in perturbed vessels [131] catalyzed investigations into its role in atherosclerotic processes. Current epidemiological evidence [126] [132] [133] and clinical

observations [128] [134] support a pathogenic relationship between CRP and atherosclerosis, however this relationship remains largely uncharacterized. It is unclear whether CRP is simply a marker of inflammation and endothelial dysfunction, or if it contributes to atherosclerotic disease progression. It is clear, however, that higher CRP concentrations are associated with higher levels of atherosclerosis in peripheral and coronary arteries,[133] and significantly linked to both unstable [132] and stable angina.[126] Evidence strongly points to CRP levels as representative of endothelial tissue injury and inflammation in dysfunctional vasculature, making CRP a reliable marker of the progression and severity of atherosclerotic processes.[133] Cardiovascular and/or cerebrovascular events (i.e. myocardial infarction, stroke, surgical revascularization, etc.) are often the clinical endpoints of atherosclerosis and such events are commonly used as proxy markers of atherosclerotic disease.

Atherosclerosis is an inflammatory disease characterized by plaque formation on vessel walls. Chronic exposure to CV risk factors such as smoking, hypertension, and hyperglycaemia overwhelms the vascular system, compromising its integrity and often resulting in endothelial dysfunction.[135] Endothelial dysfunction is characterized by an inability of the endothelium to vasodilate appropriately in response to stimuli and plays a central role in atherogenesis. The vessels take on a pro-constrictory state, leading to pathophysiological changes that culminate into pro-inflammatory, proliferative, pro-coagulation and pro-vascular adhesion features.[136] Although the exact role of CRP in atherosclerosis has not been confirmed, a causal role has been speculated.[137] [138] CRP has been found in atherosclerotic plaque, co-localized with the terminal complement complex

in areas of extracellular lipid deposition. Aggregated CRP binds to modified very-low density lipoprotein and LDL, activating human complement, and potentially plays a role in coagulation and early atherogenesis.[137] Notably, CRP's causal role in atherosclerosis is controversial and not widely accepted.[139]

Metabolic syndrome. A commonly used definition of MetS was proposed by the National Heart Lung and Blood Institute [140] and includes five diagnostic criteria (Table 1.1). CRP concentrations sequentially increase with the number of MetS characteristics a person presents.[18] For example, Ridker et al. [18] showed those presenting 0, 1, 2, 3, 4 or 5 MetS markers had baseline CRP levels of 0.68, 1.09, 1.93, 3.01, 3.88 and 5.57 mg/L, respectively. These findings have been supported by several other studies and in different populations.[17 19 141-143] Furthermore, in a 6-year prospective study, Han et al. [144] found women with CRP in the highest tertile were four times more likely to develop MetS than those in the lowest tertile (95% CI, 2.0-7.9), suggesting CRP may be useful in predicting future occurrence of metabolic dysfunction.

There are several feasible mechanisms that might explain the link between CRP and MetS. High circulating low-density lipoprotein (LDL) is a criterion for MetS and plays a role in endothelial dysfunction. Further, CRP binds to non-oxidized LDL molecules and is present in injured atherosclerotic vessels. It follows that CRP concentrations reflect vascular health and will be elevated in persons with hypertension and high LDL concentrations.[145] Second, since pro-inflammatory cytokines such as IL-6 are detectable in atherosclerosis and CRP's expression is regulated by such cytokines,[146] it is reasonable that CRP is simply a marker of cytokine activity, rather than a risk factor for atherosclerosis.

Most explanations for the relationship between CRP and MetS maintain that CRP is indirectly related to atherosclerosis as a result of its role in acute-phase immunity, however this theory is not universally accepted.[147-149] Although the biological mechanism through which each component of MetS is related to CRP are not fully established, endothelial dysfunction and atherosclerotic processes are widely accepted as the central catalyst for CRP production.[143] Because MetS is exhibited as a clustering of simultaneously occurring abnormalities, it may be inappropriate to isolate variables. Instead, emphasis should be focused on the interrelationship between multiple MetS manifestations and CRP as most studies show a linear association does exist.

CRP & Sedentary Time

There is some indication that sedentary time and CRP are positively associated independent of MVPA,[2 13 105] though some studies have found no correlation.[69 87] Using a nationally representative sample, Healy et al. [2] showed a significant positive relationship between accelerometer-derived total sedentary time and CRP after adjusting for age, sex, race and exercise. Interestingly, the quartile with the most breaks in sedentary time had lower CRP levels than the quartile with the fewest breaks ($p < 0.001$), which supports earlier work by Healy et al. [31] Similar outcomes have been published by Leon-Latre et al. [13] in a different population despite the use of subjectively measured sedentary time. In both instances the link between sedentary time and CRP was shown to be independent of MVPA. Conversely, in a representative population of Canadian adults, Carson et al. [87] observed an inverse relationship between MVPA and CRP, but no significant association

between total sedentary time and CRP. However, breaks in sedentary time had a favorable impact on CRP concentration,[87] which is consistent with data published by Healy et al. [2] Henson et al. [69] have also shown that the relationship between sedentary time and CRP is attenuated when MVPA is controlled for. Although inconclusive, these cross-sectional studies have informed experimental research and recent emphasis has been placed on experimental studies to further explore the relationship between sedentary time and CRP.

Experimental studies exploring sedentary time and CRP have relied on bed rest studies and interventions that reduce daily ambulation. Bosutti et al. [150] observed an increase in CRP concentrations after 14 days of eucaloric bed rest in 9 young healthy males, while Hamburg et al. [44] reported no change after 5 days of bed rest in health young adults (n=20; 6 females). Interestingly, the increase in CRP after 14 days of bed rest was prevented with a hypocaloric diet,[150] suggesting energy intake may play a mediating role. Two experimental studies have used a reduction in daily step count while participants remain in a free-living environment to explore the effect of decreased ambulatory activity on CRP concentration, and one study looked at IL-6. Dixon et al. [21] showed no change in CRP after transitioning middle-aged men from ~13,000 steps/day to <4,000 steps/day for 7 days. Similarly, using young healthy males, Krogh-Madsen et al. [70] found no significant change in IL-6 after 14 days of reduced ambulation (>10,000 steps to <1,500 steps/day). However, Breen et al. [20] reported a 25% increase in CRP concentration following 14 days of reduced stepping in 10 older adults (50% male). The divergence in these results might be explained by a few factors. First, the lack of change in the Dixon et al. [21] study might be explained by the short duration and/or the highly active sample. Results from Hamburg et al. [44] and Bosutti et

al. [150] suggest time is a factor in when exploring CRP and it is unlikely that 7 days is long enough to observe significant changes. Additionally, inclusion criteria for the Dixon et al. [21] study mandated that participants be 'highly active.' The, activity status of the participants may have prevented or blunted an immune response as regular physical activity is known to be immunoprotective.[151] The sample of Krogh-Madsen et al. [70] was both young (23.8 +- 1.5 yrs) and active, and ostensibly free of sub-clinical cardiovascular and/or metabolic abnormalities, whereas the sample used by Breen et al. [20] was comparatively much older (72.3 ± 1.0 yrs) and less active. Therefore, it could be that the age and activity status of the participants explains the different outcomes. It is worth noting that both Dixon et al. [21] and Krogh-Madsen et al. [70] focused exclusively on males, while Breen et al. [20] used 5 males and 5 females. Unfortunately, Breen et al. [20] did not present male and female data separately but it is possible that sex had an effect on these results. It is important to emphasize that there remains a profound lack of experimental research in this area that is focused on females, despite numerous biological differences between sexes, including differences in endothelial function [152] and average circulating concentrations of CRP.[122] It is therefore difficult to generalize existing androcentric research to the broader population.

CRP & Physical Activity

Relative to the study of sedentary time, exercise physiology is an established area of study and our understanding of how exercise impacts the immune system and response is further developed. Physical activity is well-known to positively affect physiological health [153] [154] and has been proposed as a viable way to protect against and reduce chronic

inflammation.[104 155 156] CRP's response to a single bout of exercise and to regular exercise training has been explored.

Acute phase response (APR). The APR of CRP to exercise is proportional to the extent of muscle damage, and is largely dependent on the duration and intensity of the exercise bout.[157] Serum CRP has been shown to increase after long duration, low-to-moderate intensity aerobic exercise such as a marathon, [158 159] ultramarathon, [160] and long-distance triathlon.[161] The observed increases in CRP are marked, increasing between 122% to 2,000% 24 hours after exercise.[158 159 161] However, these changes are transient and typically return to baseline within 2-8 days.[157 159]

The APR to shorter duration activity also depends on the intensity of exercise. Some of the earliest research on shorter duration aerobic activity reported "races of 15 and 21 km produced only a minute increase in C reactive protein concentrations." [157] Unfortunately, the authors failed to report p-values and race completion times so exercise intensity cannot be commented on.[157] Drenth et al. [162] also observed a small but significant rise in CRP 24 hours after a 5 km running race. Despite the shorter duration of a 5 km race, participants completed it in 20.5 ± 1.5 mins and maintained a mean heart rate of ~95% of heart rate reserve (i.e. exercise intensity was very high). Similar increases in CRP have been reported following short duration, anaerobic intervals,[163] competitive athletics,[164] and plyometric exercise.[165]

Long and short duration exercise exhibit a similar APR pattern that is proportionate to the intensity and duration of the exercise bout. CRP transiently increases ~24 hours after exercise cessation and returns to baseline within 2-8 days depending on the extent of

muscle damage.[157-159 161 164 165] Additionally, the APR to strenuous exercise seems to be attenuated with regular training.[166] Subsequent to 9 weeks of run training, Liesen et al. [166] observed that CRP rose to 0.65 mg/L following a 2-hr run test. This was approximately 40% lower than the pre-training APR that followed the same 2-hr run test (1.1 mg/L). The tempered rise in CRP occurred despite the fact that participants ran 10-20% further post-training.

Regular exercise training. Observational evidence from population-based studies overwhelmingly support an inverse association between physical activity and CRP,[167-175] and the majority of this evidence shows the association to be independent of BMI.[104] Using 1988-1994 NHANES data (n=13,748), Ford [174] reported an odds ratios of having elevated CRP levels to be 0.98, 0.85, and 0.53 for participants who engaged in light, moderate, and vigorous intensity exercise, respectively, compared to those who engage in no leisure-time physical activity. These results were observed after adjusting for potential cofounding variables such as age, sex, ethnicity, education, work status, smoking status, body mass index, waist-to-hip ratio, and HDL-C concentration.[174] A decade later, Loprinzi et al. [172] reported similar results using 2003-2004 NHANES data: compared to those with low CRP levels, participants with average and high CRP levels were 41% and 54% less likely to meet PA guidelines, respectively. Others have also shown an inverse association between aerobic fitness and CRP concentrations.[169 176 177]

Although the majority of cross-sectional evidence shows a correlation between CRP and physical activity, a couple of studies have reported no association.[178 179] However, both Rawson et al. [178] and Verdaet et al. [179] used a relatively small sample (n = 109 and 892,

respectively) and both used self-reported, rather than objectively-measured physical activity data. While Ford [174] and Mora et al. [175] also relied upon self-reported physical activity data, they both used significantly larger samples (n = 13,748 and 27,158, respectively). The difference in sample size may explain the discrepant results. Taken together, cross-sectional data strongly supports an association between CRP and leisure-time physical activity.

Results from experimental studies are inconclusive but support a relationship between physical activity and CRP. While a large number of trials have reported a decrease in CRP following an aerobic exercise training intervention [156 180-189] several others have shown no change.[155 190-194] Many of the intervention trials that have observed reductions in CRP were 10 months or longer,[180 185 188 189] while many shorter trials showed no change.[191 195 196] However, Kadoglou et al. [184] reported significant reductions after a 6-month trial in diabetic patients and other 12- to 18-month trials have shown no change.[155 156 190] There is no observable difference in frequency and/or duration between trials that observed reductions and those that did not.[104] Few trials have examined the effect of resistance training on CRP and, similar to aerobic interventions, the results are mixed.[197 198] In a sample of Hispanic older adults with type 2 diabetes, there was a significant reduction in CRP following 16 wks of resistance training.[197] In contrast to these findings, Levinger et al. [198] observed no change in CRP concentration after 10 wks of resistance training in middle-aged men and women with either a high or low number of metabolic risk factors.

A 2010 systematic review suggests some reasons for the mixed findings across intervention studies may be (a) underpowered studies, (b) differences in exercise energy expenditure and thus differences in the effect on body fat across studies, (c) differences in

the duration, intensity, and type of exercise, (d) variation in participants' baseline inflammatory status, and/or (e) a lack of studies with appropriate control groups.[104] To date, the role of aerobic exercise training in moderating CRP is not well understood but is likely related to IL-6 production in some way.[199] However, the extent of this relationship is questionable as low intensity exercise can result in dramatic increases in IL-6 without a parallel rise in CRP.[199] Similarly, exercise training can reduce IL-6 concentrations without reducing CRP.[156]

Studies focusing on individuals with existing chronic disease or high baseline CRP levels generally respond to aerobic exercise to a greater extent than individuals who are healthy or have low baseline CRP levels.[182 183 186 200] For instance, following 20 weeks of aerobic training, data from the HERITGAGE Family Study showed a significant decrease in plasma CRP in the high CRP subgroup (> 3.0 mg/L), but not the low (< 1.0 mg/L) or moderate (1.0 – 3.0 mg/L) subgroups. Moreover, the change in CRP was independent of changes in body weight.[186] Similar results have been observed in obese post-menopausal women with type 2 diabetes.[182] CRP decreased by 15% following 14 weeks of aerobic exercise,[182] and Goldhammer et al. [183] observed a significant decrease in CRP after 12 weeks of aerobic activity in 28 coronary heart disease patients. Similar to Lakka et al. [186] the reduction in CRP was not mediated by alterations in body weight.[183] A recent meta-analysis of 15 randomized controlled trials (500 patients) exploring the effects of exercise on CRP in type 2 diabetics reported exercise to be associated with a -0.66 mg/L change in CRP levels.[200] Conversely, an earlier meta-analysis of 5 trials and 323 participants with different health status' found a nonsignificant reduction of 3% following exercise

training.[201] These data suggest that the relationship between physical activity and CRP is stronger in those with chronic disease or an elevated concentration of CRP.

To conclude, the role of physical activity in moderating CRP concentration is not well characterized. However, the relationship between CRP and physical activity is suggested to be independent of BMI. Current evidence indicates aerobic exercise training is most effective for high risk individuals with serum CRP levels > 3.0 mg/L or those with an established chronic disease. CRP concentrations in physically active individuals have overwhelmingly been reported to be lower when compared their inactive counterparts. Future research in this area should consider other cofounding variables that might explain the inconsistent results between observational and experimental data. Moreover, an effort to homogenize exercise protocols is needed to develop our understanding of this relationship.

CONCLUSION

The negative effect of excessive amounts of sedentary time has become increasingly apparent over the past decade. There is a need for more human research focused on the biological mechanisms that underlie the deleterious impact of sedentary time on health. In particular, experimental studies should focus on manipulating total sedentary time to examine the acute consequences.[24] C-reactive protein is a well-known and highly studied biomarker that may provide insight into the biological underpinnings of sedentary time. Much of the literature exploring the health effects of sedentary time suggest it is independent of MVPA. However, no experimental trial to date has explored this issue.

The anti-inflammatory effects of MVPA are well documented [104 172] and thus may provide a novel way to explore the independent nature of sedentary time. Epidemiological and experimental evidence suggest that frequent interruptions to sedentary time are associated with better health outcomes.[2 31 32 69 87 202] As such, attention should be paid to the pattern of behaviour change in response to both physical activity and sedentary behaviour interventions.

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Table 1.1. ATP III criteria for diagnosis of metabolic syndrome. At least 3 markers must be present.[63]

Criteria	Cutpoints
1. Abdominal obesity	Women: **WC >88 cm** Men: **WC >102 cm**
2. Hypertriglyceridemia	**≥150 mg/dL (1.69 mmol • L^{-1})**
3. Low HDL	Women: **<50 mg/dL (1.29 mmol • L^{-1})** Men: **<40 mg/dL (1.04 mmol • L^{-1})**
4. Hypertension	**≥130/85 mm Hg**
5. High fasting glucose	**≥110 mg/dL (6.1 mmol • L^{-1})**

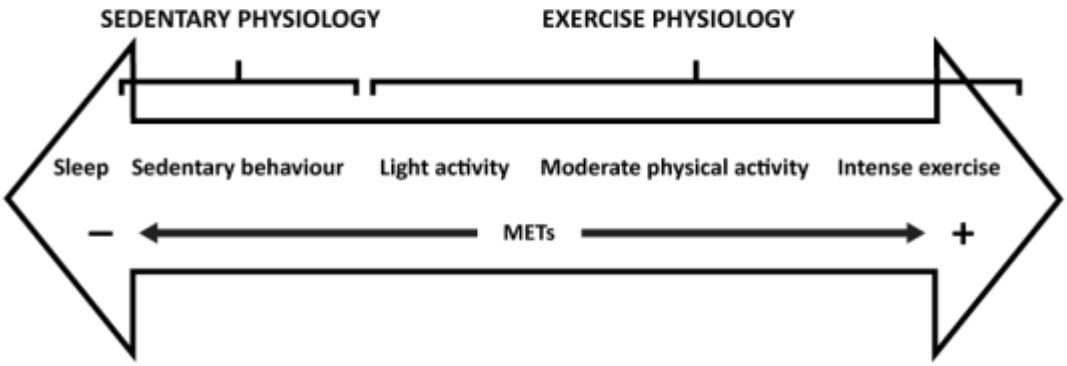
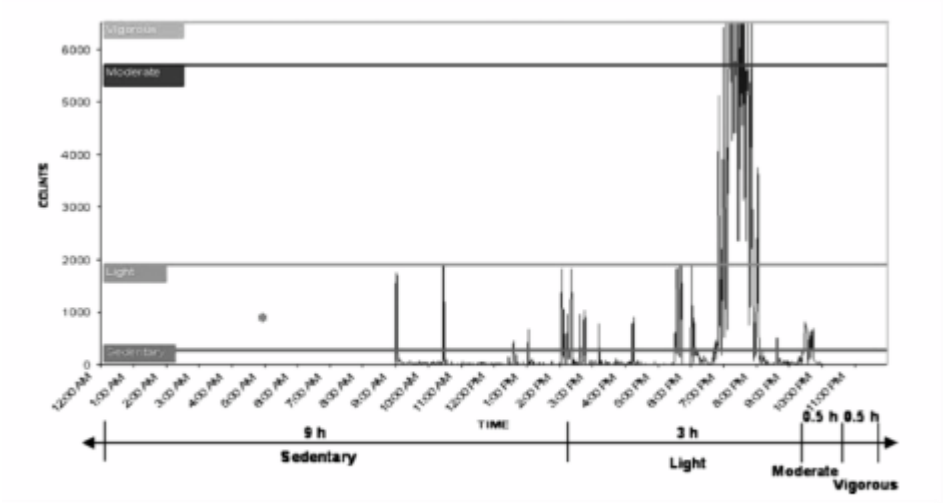


Figure 1.1. The movement continuum. Reproduced from Tremblay et al. [24]

1.2a.



1.2b.

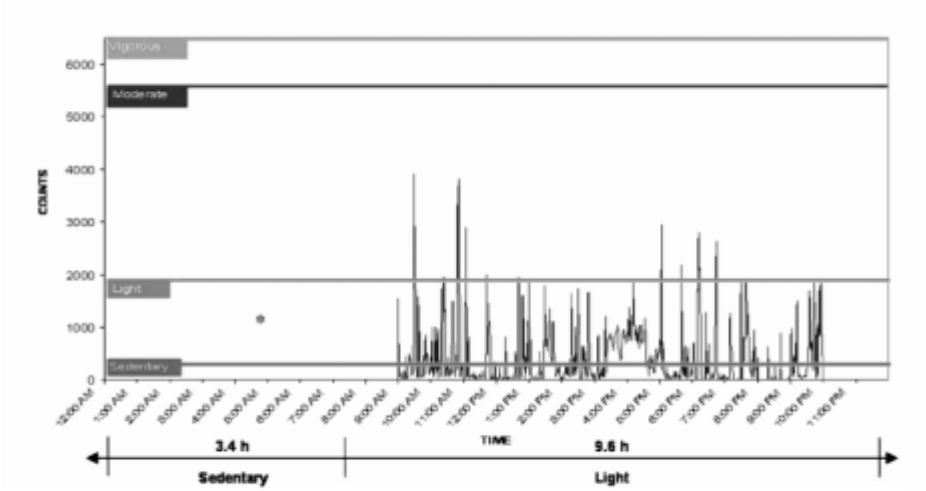


Figure 1.2 a. & b. Two accelerometer-derived movement patterns are represented. 1.2a represents an "active" individual who fulfills the recommended level of MVPA while still exhibiting high levels of sedentary behaviour. 1.2b represents an "inactive" individual who does not fulfill the recommended level of MVPA but is spending less time in sedentary behaviours. Intermittent and regular interruptions to sedentary behaviours are also shown in 1.2b. Reproduced from Pate et al. [26]

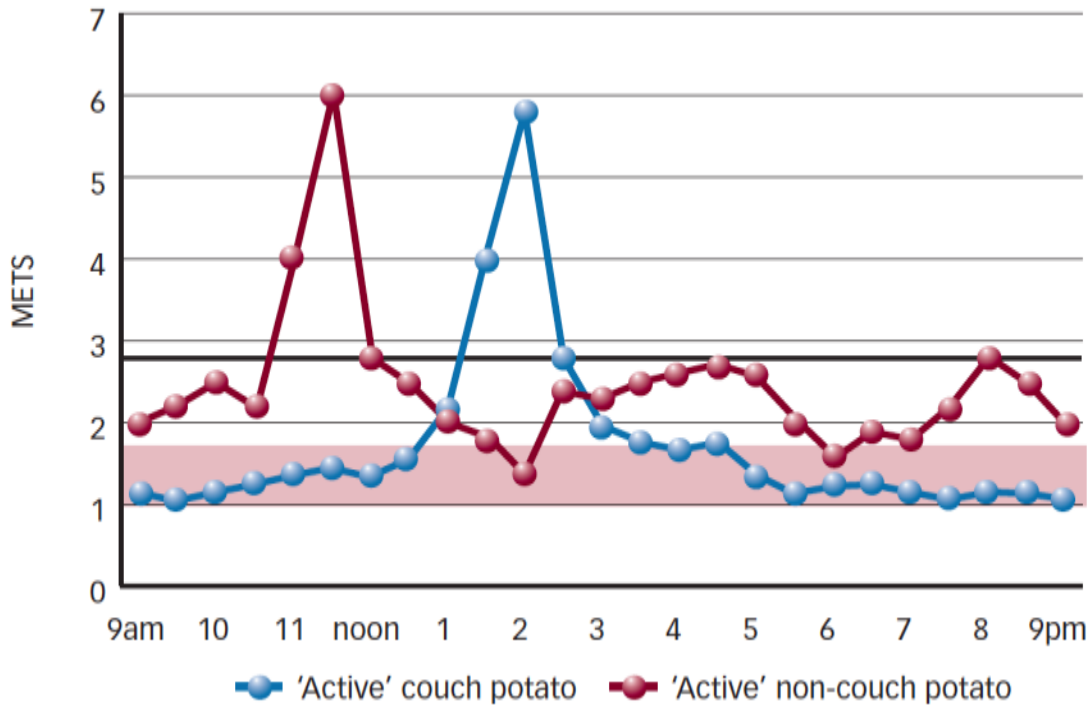


Figure 1.3. Graphical depiction of accelerometer data representing an active couch potato (meets physical activity guidelines with high levels of sedentary behaviours) and an active non-couch potato (meets physical activity guidelines with high levels of light activity/low sedentary behaviours). Reproduced from Dunstan et al. [203]

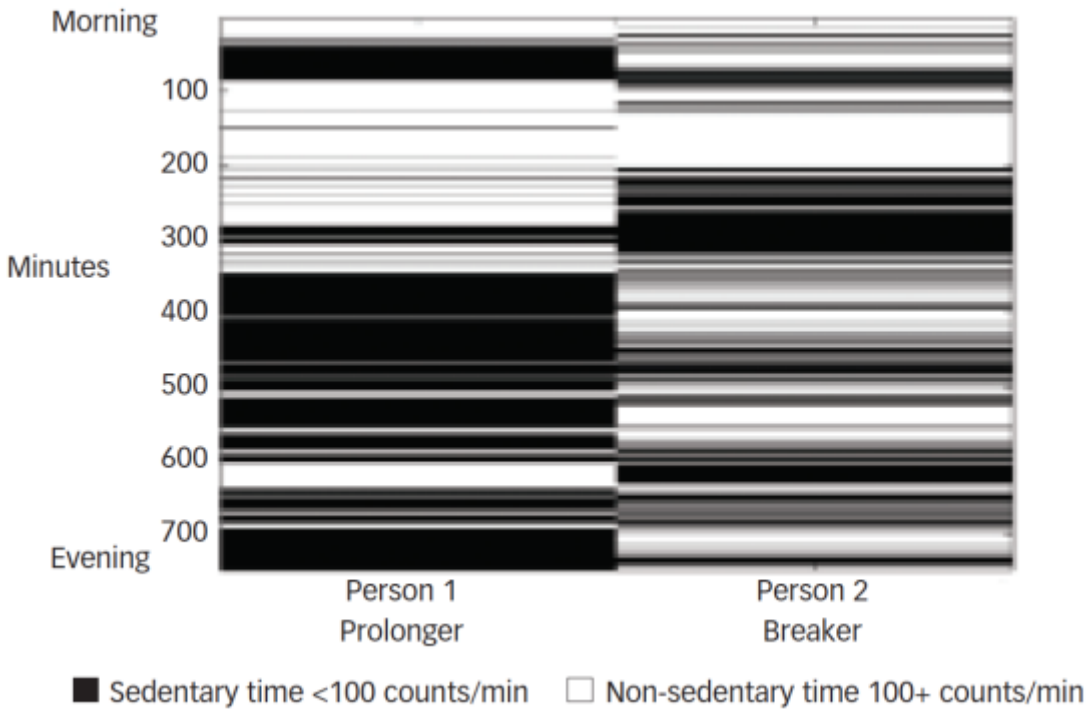


Figure 1.4. Accelerometer-derived data depicting sedentary behaviour patterns of a "prolonger" and "breaker." A "prolonger" will remain sedentary for extended periods of time whereas a "breaker" will interrupt sedentary behaviours with ambulatory activity. Reproduced from Dunstan et al. [203]

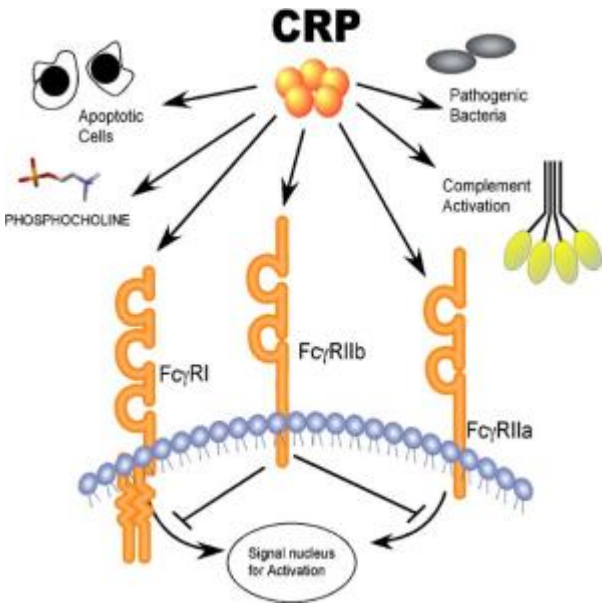


Figure 1.5. Summary of CRP ligands. Reproduced with permission from Marnell et al. [118]

CHAPTER 2: THE EFFECT OF 10 DAYS OF INCREASED SEDENTARY TIME OR INCREASED PHYSICAL ACTIVITY ON C-REACTIVE PROTEIN IN WOMEN.

ABSTRACT

Despite growing awareness of the health consequences of sedentary time, the underlying physiological mechanisms are poorly understood. The purpose of this study was to explore the effect of (a) increasing sedentary time, and (b) increasing physical activity on C-reactive protein (CRP) in women. Nineteen healthy females aged 40-60 years participated in the study. After completing a 7-day preliminary assessment of daily step count, sedentary time, and physical activity, they were randomly assigned to one of two 10-day interventions, either sedentary or active. The sedentary group reduced their step count to <5000 steps/day and the active group added 3,000 steps/day to their preliminary average. During both the preliminary assessment and intervention period, participants wore a pedometer to monitor their daily step count and an ActiGraph GT3X accelerometer to objectively assess sedentary time and physical activity. CRP concentrations were measured via saliva, and samples were taken during the preliminary assessment, pre-intervention and post-intervention. During the intervention, the sedentary group (n=9, age: 49.6 ± 5.6 yr, BMI: 28.4 ± 3.5 $kg \bullet m^{-2}$) significantly increased sedentary time by 69.8 minutes/day, and decreased both light physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). The active group (n=10, age: 49.9 ± 5.2 yr, BMI: 26.6 ± 3.7 $kg \bullet m^{-2}$) increased MVPA by 19.4 minutes/day, but there was no change in sedentary time or LPA. After 10 days of behavior change, CRP concentrations

increased by 31% in the sedentary group (0.38 to 0.49 μg/L; $p<0.05$) and decreased by 22% in the active group (0.41 to 0.26 μg/L; $p<0.01$). These results suggest that CRP, and thus inflammation, may be a physiological link between movement behavior and health in middle-aged women.

INTRODUCTION

Sedentary behaviour is defined as any waking activity with an energy expenditure of ≤1.5 metabolic equivalents while in a seated or reclined position.[1] Accumulating evidence suggests that the ubiquity of sedentary behaviours in modern society is contributing to the increasing prevalence of chronic diseases such as cardiovascular disease (CVD), obesity, and type 2 diabetes.[2 3] Associations between sedentary time and cardiometabolic health may be independent of moderate-to-vigorous physical activity (MVPA) as many of these associations persist after accounting for activity.[2] This distinction is important as it is possible for an individual to meet minimum recommendations for physical activity yet still accumulate substantial sedentary time.[4] Despite growing awareness of the health consequences of sedentary time, the underlying physiological mechanisms remain poorly understood.

C-reactive protein (CRP) is a plasma protein that is a constituent of innate immunity. The roles of CRP are multifaceted and it functions dynamically with other compounds and systems to protect the host from infection, aid in the disposal of damaged/dead cells, and mediate the clearance of apoptotic cells. Synthesis of CRP occurs primarily in the liver and is predominantly stimulated by the pro-inflammatory cytokine interleukin (IL)-6.[5] Following an acute-phase immune stimulus, such as illness or tissue injury, circulating

concentrations of CRP can increase dramatically in less than 48 hours.[6] Importantly, chronically elevated levels of CRP, even within the normal range, are indicative of systemic low-grade inflammation and predictive of both atherosclerotic vascular diseases [6] and metabolic syndrome.[7]

Chronic inflammation has been linked to many diseases such as diabetes and CVD.[8 9] Physical activity has been shown to have anti-inflammatory effects [10 11] and markers of inflammation, such as C-reactive protein (CRP), are lower in physically active individuals.[12] These studies have not accounted for sedentary time in their analyses and more recently, several cross-sectional studies have shown an inverse relationship between CRP and sedentary time [3 13] that was independent of physical activity. Furthermore, it has been suggested that CRP is not simply a marker of inflammation but also a direct cause of atherosclerosis and CVD.[14] Thus, CRP may represent a mechanistic link between prolonged sedentary time and increased risk of cardiometabolic disease. CRP is found in various body fluids and can be measured in serum and saliva. Salivary concentrations for CRP are moderately to strongly correlated with serum concentrations,[15] and salivary CRP has been associated with CVD risk and subclinical atherosclerosis.[16 17]

Although cross-sectional evidence supports an association between sedentary time and cardiometabolic health, few studies have used an experimental study design to further our understanding of this relationship in healthy subjects. Longitudinal data published by Falconer et al. [18] suggests that moderate decreases in sedentary time, even in the absence of increased MVPA, can reduce CRP concentrations in women with newly diagnosed type 2 diabetes. Conversely, Dixon et al.,[19] using a non-randomized controlled trial design,

found no evidence of increased inflammation in active men who reduced their step count from ~13,000 steps/day to <4,000 steps/day for 7 days. To our knowledge no studies have assessed the effect of short-term changes in sedentary time and activity on inflammation in women, despite important biological differences between sexes, including differences in endothelial function [20] and circulating concentrations of CRP.[21] Thus, the purpose of this study was to explore the effect of (a) increasing sedentary time, and (b) increasing physical activity on CRP among healthy women.

METHODS

Participants. Healthy females aged 40-60 years were recruited via list-serves, online postings, and word of mouth. Inclusion criteria mandated that participants be free of any known chronic or inflammatory diseases including diabetes, cardiovascular disease, arthritis, asthma, or any inflammatory gastrointestinal disease. Participants were required to be somewhat active or active, quantified by an average of 7,000 – 13,000 steps per day,[22] but not engaged in a structured training program or purposefully exercising more than 3 times per week. Participants were blind to the step-based inclusion criteria of 7,000 to 13,000 steps per day. Exclusion criteria included current or recent injury, acute illness, or infection within the last 14 days, a body mass index of >30 $kg \cdot m^{-2}$, hypertension (SBP > 140 mmHg; DBP > 90 mmHg), or on any type of hypertension or lipid-modifying medication. All participants provided written informed consent, and the study was reviewed and approved by the University of Lethbridge Human Subject Research Committee.

Preliminary assessment. During each participant's initial visit, a medical history

screening form and 12-month physical activity recall questionnaire were completed. Participants then completed a 7-day preliminary assessment of daily step count, sedentary time, and physical activity. A Piezo Rx pedometer (Deep River, ON) was used to measure step count, and an ActiGraph GT3X accelerometer (ActiGraph LLC, Pensacola, FL) was used to assess sedentary time and physical activity. Participants were instructed to wear both devices on the same hip all day except for during sleep or water activities (e.g.: showering, swimming). A daily log sheet was used to confirm when the devices were worn. If the average daily step count was between 7,000 and 13,000 steps per day then height, weight, resting blood pressure and heart rate were measured to confirm eligibility. Eligible participants were matched based on age and BMI since both are known to affect CRP concentrations.[7 21] After matching they were randomly assigned to one of two intervention groups (Fig. 2.1). A saliva sample was collected during the preliminary assessment from 17 of the 19 participants, to allow comparison of salivary CRP between the preliminary assessment and day 0 of the intervention.

Intervention. Participants in both groups were instructed to change their behaviour for 10 days. Participants in the active group were instructed to increase their daily step count by 3,000 steps above their preliminary average. Evidence shows that 30 min of moderate intensity activity equates to 3,000-4,000 steps,[23] thus adding 3,000 steps/day to the preliminary step count in non-exercising adults is approximately equivalent to adding 30 min/day of moderate intensity activity. Participants in the sedentary group were instructed to eliminate all structured exercise and reduce their daily step count to < 5000 steps per day, in order to mimic an inactive and sedentary lifestyle.[23] All participants were given a

list of domain-specific tips on ways to increase either their sedentary time or physical activity at work, home, and leisure. Participants were also directed to maintain their normal diet, report any illness or injury, and refrain from any anti-inflammatory drug use for the duration of the intervention. The research team maintained regular contact with each participant via email and/or phone to promote adherence. A timeline of the study protocol is shown in Figure 2.1.

Step count, sedentary time, and physical activity. During the 10-day intervention, participants were given a pedometer to self-monitor their daily step count so they could reach their specific target. To assess changes in sedentary time or physical activity, participants also wore an ActiGraph GT3X accelerometer (ActiGraph, Pensacola, FL). The ActiGraph recorded in 10-s periods, and a minimum wear time of 10 h per day was required. Non-wear time was identified by 90 consecutive minutes of zero counts with a 2-min spike tolerance.[24] Counts refer to the magnitude of gravitational force produced over a given time (1 count is equal to 0.01664 g of force per second) and reflect movement intensity. Sedentary time is estimated by a lack of movement counts. Sedentary time was defined as <100 counts/min, and moderate to vigorous physical activity (MVPA) was defined as >1951 counts/min.[25] Accelerometer data were reduced using ActiLife version 6.1 software (ActiGraph, LLC, Pensacola, Fla., USA). Outcome variables included time spent sedentary, in light physical activity (LPA), and in MVPA.

CRP. Saliva samples were collected during the preliminary assessment in 17 of the 19 participants and at day 0 and Day 10 of the intervention for all participants. Two preliminary samples were not collected because of deviation from collection protocol

(alcohol intake) and potential contamination (recent dental work). CRP concentrations were measured via saliva as it is less invasive than blood sampling, moderately to strongly correlated with serum concentrations,[15] and routinely used in research.[16 17]

Samples were collected using the SalivaBio Oral Swab (Salimetrics, LLC., State College, PA) and according to manufacturer's instructions. Participants were directed to avoid eating for 1 hour before sample collection and avoid drinking alcohol for 12 hours prior. Participants thoroughly rinsed their mouth with water directly before saliva samples were collected and samples were immediately frozen at -80°C until analysis. CRP concentrations were assessed using a commercially available high sensitivity enzyme-linked immunosorbent assay (ELISA) (Salimetrics, LLC., State College, PA). Average inter- and intra-assay variability was 3.4 ± 0.33% and 9.1%, respectively. All samples from each participant were analyzed in the same assay to minimize the effects of inter-assay variability.

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, N.Y., USA). Salivary CRP concentrations did not meet the criteria of a normal distribution so logarithmic transformations were applied prior to analyses. Comparisons of the preliminary assessment data between groups were performed using independent sample t-tests (two-tailed). Difference in CRP between groups and across time were examined using a two-way repeated measures ANOVA (group x time). Where significant interactions were found follow-up analysis was done using separate one-way ANOVAs for each group. Statistical significance was set at $p < 0.05$. All values are reported as mean ± SD.

RESULTS

Forty-five women responded to the call for subjects and 20 completed the study. Seven women were screened out by the medical history questionnaire due to known inflammatory issues and 12 did not meet the preliminary activity criteria (i.e.: <7,000 steps or >13,000 steps per day). Two women dropped out during the preliminary assessment for personal reasons; and four women started in the sedentary group but failed to comply with study protocol. One participant in the sedentary group was injured during the intervention and was omitted from analyses, leaving a final sample of 19; 10 in the active groups and 9 in the sedentary group. Table 2.1 shows the anthropometric and physiological characteristics of both groups, as well as the preliminary activity profile. There was no difference in age, BMI, waist circumference, CRP concentration, or activity profile between groups after matching and randomization (Table 2.1).

Change in activity and sedentary time. During the intervention, the sedentary group significantly decreased their average daily step count from 9392 ± 702 to 3854 ± 147 ($p < 0.001$) and the active group increased their daily step count from 8772 ± 427 to 12815 ± 454 ($p < 0.001$) (Fig. 2). Table 2.2 shows the accelerometer data for both groups. During the intervention, the sedentary group increased their daily sedentary time by 69.8 min, which was 85% of their day, compared to 76% during the preliminary assessment. The sedentary group also significantly decreased both their LPA and MVPA ($p < 0.001$). Only MVPA changed significantly in the active group during the intervention, as they added 19 minutes per day ($p < 0.001$). Sedentary time did not change in the active group; it was 74%

of wear time during the preliminary assessment and 72% during the intervention. During the 10-day intervention, there were significant differences in step count, and proportion of time spent sedentary and in MVPA between the two groups ($p < 0.001$) (Figure 2.2). There was no statistical difference in LPA between the two groups during the intervention period ($p = 0.090$).

Change in C-reactive protein. From the preliminary assessment period to the start of the intervention, CRP did not change significantly ($p = 0.987$). The change in CRP from day 0 to 10 of the intervention is shown in Figure 2.3. There was a significant time by group interaction ($p < 0.001$, partial $\eta^2 = 0.548$, observed power (β) = 0.99), but no main effect for time ($p = 0.867$) or group ($p = 0.806$). Follow-up analyses showed a significant time effect in both the sedentary and active groups. CRP concentration in the sedentary group increased an average 31%, from 0.376 ± 0.199 $\mu g \bullet L^{-1}$ to 0.487 ± 0.264 $\mu g \bullet L^{-1}$ ($F(1, 8) = 8.7$, $p = 0.019$, partial $\eta^2 = 0.52$, $\beta = 0.732$). In the active group CRP decreased an average of 22%, from 0.413 ± 0.202 $\mu g \bullet L^{-1}$ to 0.259 ± 0.090 $\mu g \bullet L^{-1}$ ($F(1, 9) = 12.2$, $p = 0.007$, partial $\eta^2 = 0.874$, $\beta = 0.874$).

DISCUSSION

This study examined the effects of 10 days of altered daily movement behaviour on CRP in women. We observed an average 22% decrease in CRP among women after 10 days of increased physical activity, while 10 days of decreased physical activity and increased sedentary time resulted in a 31% increase in CRP. The present study sheds light on the extent to which even short-term changes in daily activity may influence inflammation and disease risk among healthy female adults.

The pattern of behaviour change that resulted from the interventions differed between the two groups of women. By decreasing their daily step count to less than 5,000 steps, the sedentary group increased their daily sedentary time by more than an hour per day and decreased both their LPA and MVPA. While the decrease in activity in this study was experimentally induced, the daily step count was not unrealistic as the average American adult accumulates only 5117 to 5756 steps per day.[22 26] Furthermore, participants remained in a free-living environment and attended to activities of daily living, including jobs and child care. The increase in CRP in this group supports current cross-sectional data that have shown an association between sedentary time and CRP.[3 13 27] The active group significantly increased their total daily MVPA, however, there was no significant change in sedentary time. The decrease in CRP in this group is consistent with previous studies that have shown exercise to have an anti-inflammatory effect.[10]

There is some debate over whether or not the health risks associated with sedentary time are truly independent of physical activity. The fact that there was no change in sedentary time in the active group suggests that CRP is sensitive to changes in MVPA independent of sedentary time. The sedentary group significantly increased sedentary time, but they also engaged in very little MVPA, therefore, it is possible that the removal of daily MVPA, rather than the increased amount of sedentary time, caused the increase in CRP. This is supported by Henson et al.[27] who found that the association between sedentary time and CRP was no longer significant after adjusting for MVPA, and other studies that have found CRP to be positively affected by MVPA.[12] It is important to note that the majority of experimental research that has reported a decrease in CRP with

physical activity used interventions of several months.[10 12] The increase in systemic CRP we observed after only 10 days of increased activity is a novel finding that suggests inflammatory responses may be even more sensitive to physical activity than previously thought, especially in women.

The mechanisms by which physical activity or sedentary time influence CRP are unclear and potentially differ in response to physical activity versus sedentary time.[28] Previous studies have suggested that physical activity reduces inflammation by reducing body fat (4,7,28),[10 11 19] increasing insulin sensitivity,[11] and improving endothelial function.[29] The relationship between physical activity and systemic, low-grade inflammation is well established in the literature [12] and some combination of these 3 mechanisms likely explains the 22% decrease in CRP in the active group.

Body composition, insulin action, and endothelial function also change rapidly in response to increased sedentary time. For example, Krogh-Madsen et al.[30] showed significant decreases in lower body lean mass following 14 days of reduced ambulatory activity, and Stephens et al.[31] reported a 39% reduction in insulin-stimulated glucose uptake following 1 day of sitting when compared to 1 day of minimal sitting in healthy young adults. Others have observed short term changes in endothelial function in human [32] and animal models [33] in response to sedentary time.

Lipoprotein lipase activity and lipid metabolism may also differentially respond to sedentary time compared to physical activity [28]. After 7 days of reduced ambulatory activity, Dixon et al.[19] observed a significant increase in total fasting triglyceride concentration in lean and overweight middle-aged men. They also observed increases in insulin and glucose

area-under-the-curve using an oral glucose tolerance test.[19] Elevated levels of insulin and triglycerides have both been implicated in atherosclerotic and inflammatory processes.[8] Thus the observed increase in CRP with increased sedentary time may have been caused by some combination of altered body composition,[30] decreased peripheral insulin sensitivity,[19 30 34] reduced endothelial function,[32] and altered lipid metabolism.[19 28] Future research is needed to examine changes in these variables during periods of increased sedentary time.

Other groups have examined the effect of reduced ambulatory activity on markers of inflammation.[19 30 35] Dixon et al. reported no change in serum CRP levels after 7 days of reduced stepping in lean and overweight middle-aged men and Krogh-Madsen et al.[30] showed no post-intervention difference in 6 markers of inflammation compared to baseline values in young, healthy males. It is important to emphasize that both studies focused exclusively on men and the majority of existing experimental research does not include women. Breen et al.[35] reported a 25% increase in CRP concentration following 14 days of reduced stepping in 10 healthy older adults (5 males and 5 females). Male and female data were not presented separately, however, sex differences in cardiovascular [20] and immune response [36 37] may explain why male-specific trials did not find a change in immune markers, but Breen et al.[35] did. Previous research has demonstrated that females differentially respond to inflammatory stimuli, such as acute exposure to a lipopolysaccharide infusion [37] and short-term sleep deprivation [36] compared to males. Both studies found females to exhibit larger increases in IL-6, TNF-α, and CRP, suggesting females respond to short-term inflammatory stimuli to a greater extent than males. The

number of women living with and dying of CVD exceeds those of men,[38] as does the number of women suffering from multiple lifestyle-related chronic conditions.[39] Given the associations between inflammation and chronic disease, there is a need for more experimental data in women to develop our understanding of the relationship between sedentary time, activity, and inflammation.

The primary limitation of this study is the lack of a control group. However, we did collect preliminary saliva samples prior to the intervention from 17 of the participants. We found no significant difference in CRP concentrations between the initial meeting and the starting day of the intervention (range of 9 to 24 days apart). This suggests that the significant differences observed after the intervention can be attributed to the changes in activity behaviour and did not occur by chance. Another limitation is that we did not control for the length of sedentary bouts or number of breaks in sedentary time, and the pattern in which sedentary time is accumulated can influence health outcomes.[27 40] Future studies that intervene on the patterns of sedentary behaviour as opposed to just total sedentary time are needed to determine if it will influence inflammation.

In conclusion, these results suggest that 10 days of either increased sedentary time or MVPA is a sufficient stimulus to increase or decrease CRP, respectively, and point to inflammation as a potential mechanistic link between movement behaviour and health outcomes in healthy middle-aged women. The decrease in CRP concentration in the active group despite no significant change in sedentary time or LPA challenges current cross-sectional data that show sedentary time impacts health independently of MVPA. Further exploration to confirm sedentary time as an independent risk factor is warranted. Future

trials should also emphasize behavioural and physiological sex differences in response to experimentally modified sedentary time by including data from both males and females.

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Table 2.1. Preliminary anthropometric, physiological, and behavioral characteristics of sedentary and active groups.

	Sedentary (n = 9)	**Active** (n = 10)	P
Age (yr)	49.6 ± 5.6	49.9 ± 5.2	0.891
Height (m)	1.64 ± 5.3	1.66 ± 5.2	0.413
Weight (kg)	76.5 ± 7.7	73.6 ± 10.5	0.518
BMI ($kg \bullet m^{-2}$)	28.4 ± 3.5	26.6 ± 3.7	0.296
WC (cm)	90.7 ± 10.4	87.0 ± 8.3	0.399
CRP ($\mu g \bullet L^{-1}$)	0.45 ± 0.78	0.53 ± 1.04	0.857
Steps per day	9392 ± 2106	8772 ± 1350	0.451

All values are presented as mean ± SD

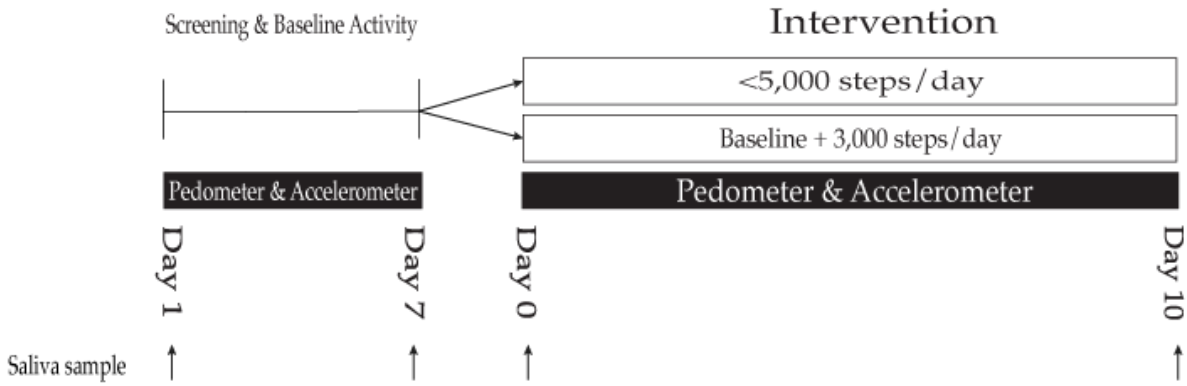

Figure 2.1. Study timeline (sedentary group, n = 9; active group, n = 10).

Table 2.2. Change in activity profile from the 7-day preliminary assessment to the 10-day intervention in the sedentary and active group.

	Sedentary			Active			
	Preliminary	Intervention	P (within)	Preliminary	Intervention	P (within)	P (between §)
Avg. wear time (min)	900 ± 48	885 ± 47	0.323	878 ± 81	933 ± 33	0.101	0.019
Avg. sedentary per day (min)	682 ± 44	752 ± 45	< 0.001	648 ± 92	669 ± 32	0.428	< 0.001
Avg. LPA per day (min)	164 ± 31	117 ± 31	0.001	178 ± 47	193 ± 57	0.229	0.001
Avg. MVPA per day (min)	54 ± 16	16 ± 7	< 0.001	52 ± 22	71 ± 21	0.003	< 0.001
% of time spent sedentary	75.8 ± 3.0	84.9 ± 1.5	< 0.001	73.7 ± 6.5	71.9 ± 4.7	0.192	< 0.001
% of time spent in LPA	18.2 ± 3.2	13.3 ± 1.6	< 0.001	20.4 ± 5.5	18.4 ± 8.5	0.432	0.090
% of time spent in MVPA	6.0 ± 1.7	1.8 ± 0.7	< 0.001	5.9 ± 2.5	7.6 ± 2.2	0.016	< 0.001

All values are presented as mean ± SD; § difference between groups during the intervention period.

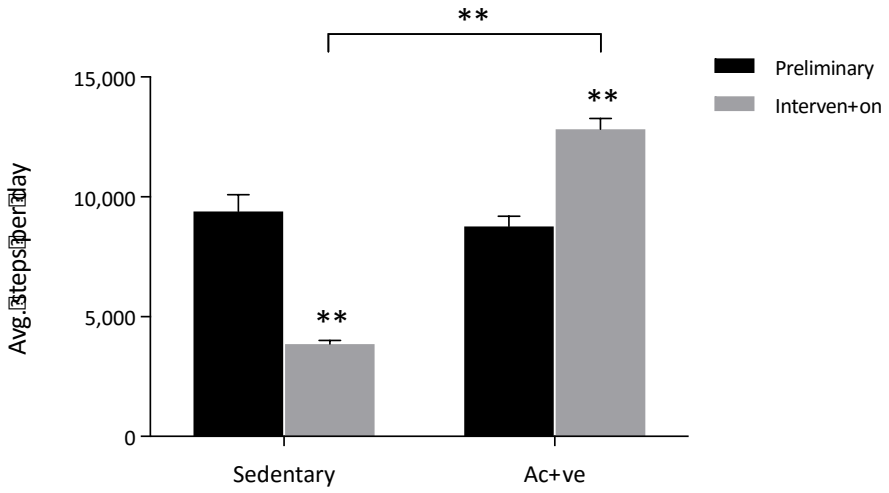

Figure 2.2. Change in average daily steps from 7 days of preliminary activity to 10 days in the sedentary and active group. Values are presented as mean ± SD; **p < 0.001.

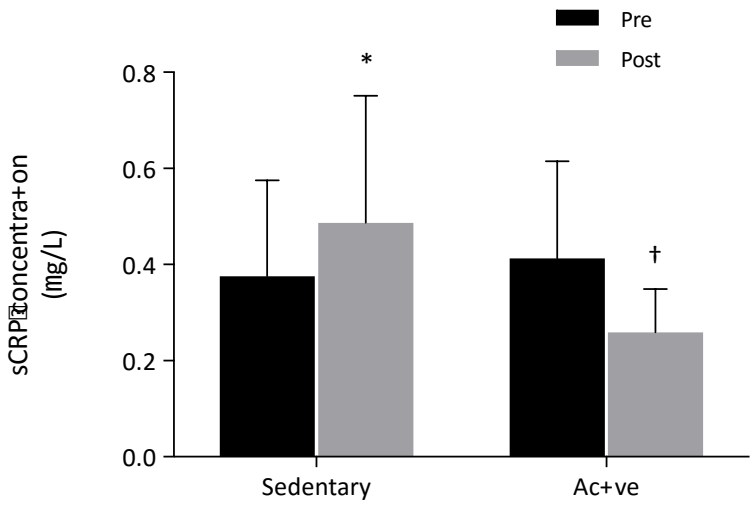

Figure 2.3. Salivary CRP concentration on days 0 and 10 of the intervention period. Values are presented as mean ± SD; *p < 0.05, †p < 0.01.

CHAPTER 3: CHANGE IN DAILY MOVEMENT PATTERNS IN RESPONSE TO SHORT-TERM CHANGES IN ACTIVITY BEHAVIOUR

ABSTRACT

Along with total time spent sedentary, the pattern of sedentary time accumulation is important, as regular interruptions to prolonged bouts of sedentary time have been shown to benefit health. The purpose of this study was to explore the change in activity behaviour, including the patterns of sedentary time, in response to 10 days of (a) increased sedentary time and (b) increased physical activity (PA). Twenty healthy females aged 40-60 years participated in the study. After completing a preliminary assessment of daily step count, sedentary time and physical activity (PA), participants were randomly assigned to one of two 10-day interventions, either sedentary or active. The sedentary group reduced their step count to <5000 steps/day and the active group added 3,000 steps/day to their preliminary average. During both the preliminary assessment and intervention period, participants wore a pedometer to monitor their daily step count and an accelerometer to objectively assess sedentary time and PA. The sedentary group increased their sedentary time by 9.3% ($p < 0.001$), and decreased light PA (LPA) and moderate-to-vigorous PA (MVPA) by 5.2% and 4.1%, respectively ($p < 0.001$). There was an increase in the number of prolonged sedentary bouts ($p = 0.004$), and a decrease in break rate (breaks • hr-sed^{-1}) ($p = 0.006$). In the active group, there was no change in sedentary time or LPA, however, the proportion of time spent in MVPA significantly increased by 1.7% ($p = 0.016$). There was no change in the pattern of sedentary time. Our results suggest that middle-aged women differentially alter their daily movement behaviour and patterns in response to short-term

increases in either PA or sedentary time, and these changes are not simply opposite of one another. Moreover, interventions focused on increasing PA may not be an effective way to reduce sedentary time.

INTRODUCTION

Regular engagement in physical activity (PA) is well-known to both preserve and improve health. However, despite overwhelming support, only 15% of Canadian adults reach the minimum recommendation of 150 min per week [1] and long-term adherence is difficult to maintain.[2] In addition to lack of physical activity, high amounts of sedentary time have been shown to negatively affect cardiometabolic health, [3-14] even in individuals who are physically active. [7 8 14-18] For example, a recent meta-analysis published by Ekelund et al. [15] reported that even for individuals engaged in 25-35 minutes per day of moderate activity, accumulating 8-10 hrs of sedentary time per day increased the risk of cardiovascular disease by 17-20% compared to those that were sedentary for < 4 hrs per day.

In addition to total time spent sedentary, research also suggests that the pattern within which sedentary time is accumulated is an important factor.[19 20] Large population-based studies have shown an attenuated association between sedentary time and health markers, such as fasting triglycerides and C-reactive protein, after controlling for the number of interruptions or 'breaks' to sedentary time.[6 21 22] Moreover, several experimental studies have shown that acute exposure to prolonged, uninterrupted bouts of sedentary time influence insulin sensitivity and glucose tolerance to a greater extent than shorter, regularly interrupted bouts, even when total sedentary time is the same.[23-25] Peddie et al. [25] elegantly

demonstrated this effect using a randomized crossover design with 3 conditions, each lasting 9 hours: (1) a prolonged sitting-only condition, (2) an activity condition consisting of 30 minutes of walking followed by 8.5 hrs of uninterrupted sitting, and (3) a regular activity-break condition that included regular 1 min 40 s walk breaks every 30 min. They found that regular activity breaks lowered insulin incremental area under the curve (iAUC) by 26% when compared with prolonged sitting and by 18% when compared with physical activity. Regular activity breaks also lowered plasma glucose iAUC values by 39% compared to prolonged sitting and by 37% compared to continuous physical activity.[25]

A recent meta-analysis reviewed how overall activity behaviour (e.g. proportion of time spent sedentary, in light PA (LPA), and in moderate-to-vigorous PA (MVPA) changes in response to three different intervention strategies. They reported that interventions focusing exclusively on sedentary time led to large and clinically significant reductions in sedentary time (~91 min • day^{-1}), while PA-only interventions and interventions simultaneously targeting both PA and sedentary time led to much smaller decreases in total sedentary time (~19 and 35 min reduction per day, respectively).[26] However, despite our understanding that the pattern of activity behaviour may be an important moderating variable, the pattern of behaviour change in response to PA and sedentary interventions in a free-living environment has been poorly characterized. One study [27] recently described the pattern of behaviour change in response to 7 days of increased sedentary time in younger adults (aged 25.2 ± 5.7 years). Compared to baseline, they observed a significant decrease in MVPA and LPA, increased time in both prolonged sedentary bouts and in total sedentary time, as well as a significant reduction in the number of interruptions to

sedentary time.[27] Given the potential importance of interrupting sedentary time, more data are needed to confirm the results presented by Lyden et al. [27] and address the nuances of how a PA intervention affects the pattern of movement behaviour. The purpose of this study was to explore the overall change in activity behaviour, and in patterns of sedentary time in response to 10 days of purposeful changes in daily movement behavior (a) increased sedentary time and (b) increased physical activity.

METHODS

Participants. Twenty healthy females aged 40-60 years were recruited via list-serves, online postings, and word of mouth. Inclusion criteria mandated that participants be free of any known chronic or inflammatory diseases including diabetes, cardiovascular disease, arthritis, asthma, or any inflammatory gastrointestinal disease. Exclusion criteria included current or recent injury, acute illness, or infection within the last 14 days, a body mass index of >30 $kg \bullet m^{-2}$, hypertension (SBP > 140 mmHg; DBP > 90 mmHg), or on any type of hypertension or lipid-modifying medication. All participants provided written informed consent, and the study was reviewed and approved by the University of Lethbridge Human Subject Research Committee.

Preliminary assessment. During each participant's initial visit, a medical history screening form was completed. Participants then completed a 7-day preliminary assessment of daily step count, sedentary time, and physical activity. An ActiGraph GT3X accelerometer (ActiGraph LLC, Pensacola, FL) was used to assess sedentary time and physical activity. Participants were instructed to wear the accelerometer all day except for during sleep or water activities (e.g.: showering, swimming). A daily log sheet was used to

confirm when the accelerometer was worn. After preliminary testing, height, weight, resting blood pressure and heart rate were measured to confirm eligibility. Participants were then matched by age and BMI. After matching, participants were randomly assigned to one of two intervention groups.

Intervention. Participants in both groups were instructed to change their behavior for 10 days. Participants in the sedentary group were instructed to eliminate all structured exercise and reduce their daily step count to < 5000 steps per day, in order to mimic an inactive and sedentary lifestyle.[28] Participants in the active group were instructed to increase their daily step count by 3,000 steps above their preliminary average. Evidence shows that 30 min of moderate intensity activity equates to 3,000-4,000 steps,[28] thus adding 3,000 steps/day to the average preliminary step count in non-exercising adults is approximately equivalent to adding 30 min/day of moderate intensity activity. Additionally, participants were asked to incorporate the added activity throughout their day and to avoid adding the steps in dedicated bouts of activity. All participants were given a list of domain-specific tips on ways to increase either their sedentary time or physical activity at work, home, and leisure (Table 3.1). The research team maintained regular contact with each participant via email and/or phone to promote adherence.

Sedentary time and physical activity. To assess changes in sedentary time or physical activity, participants wore an ActiGraph GT3X accelerometer (ActiGraph, Pensacola, FL). The ActiGraph recorded in 10-s periods, and a minimum wear time of 10 h per day was required. Non-wear time was identified by 90 consecutive minutes of zero counts with a 2-min spike tolerance [29]. Counts refer to the magnitude of gravitational force produced over

a given time (1 count is equal to 0.01664 g of force per second) and reflect movement intensity. Sedentary time is estimated by a lack of movement counts. The following definitions were used: [30]

- Sedentary time: < 100 counts • min^{-1},
- LPA: 100 – 1951 counts • min^{-1},
- MVPA: >1951 counts • min^{-1},
- Sedentary bout: ≥ 20 consecutive minutes at < 100 counts • min^{-1}, with a 2 min spike tolerance,
- Sedentary break: any instance where a sedentary bout of ≥ 2 min was interrupted by a period identified as not sedentary (e.g. ≥ 100 counts • min^{-1}),
- Break rate (breaks • $hr\text{-}sed^{-1}$): total number of sedentary breaks divided by total hours spent sedentary,
- Bout of MVPA: 10 consecutive minutes at > 1951 counts • min^{-1}, with a 2 min drop tolerance.

Accelerometer data were reduced using ActiLife version 6.1 software (ActiGraph, LLC, Pensacola, Fla., USA). All outcome variables are presented in Table 3.

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, N.Y., USA). Independent sample t-tests (two-tailed) were used to analyze differences between groups during the preliminary assessment. Difference between groups and across time were examined using a two-way repeated measures ANOVA (group x time). Where significant interactions were found,

follow-up analysis was done using repeated measures and one-way ANOVAs (two-tailed). Statistical significance was set at $p < 0.05$.

RESULTS

Participant characteristics at the preliminary assessment are summarized in Table 3.2. There was no difference in age, anthropometry, activity profile, or pattern of sedentary time between the two groups at the start of the intervention. Of the 20 women who participated, 7 (or 35%) met current PA guidelines of 150 min of MVPA per week in bouts of 10 mins or longer.[31] Average time spent sedentary during the preliminary assessment was 11.1 ± 1.2 hrs per day and daily MVPA was 53 ± 19 min.

Changes in outcome variables within the two groups, as well as differences between the groups during the intervention period are shown in Table 3.3.

Proportion of time spent sedentary. There was a significant group by time interaction ($p < 0.001$, partial $\eta^2 = 0.73$, observed power (β) = 1.0), and a main effect for time ($p < 0.001$, partial $\eta^2 = 0.55$, $\beta = 0.99$) and group ($p = 0.001$, partial $\eta^2 = 0.49$, $\beta = 0.97$). Follow-up analysis showed that the sedentary group increased the proportion of time spent sedentary by 9.3% ($p < 0.001$, partial $\eta^2 = 0.93$, $\beta = 1.0$), but there was no significant change in the active group ($p = 0.19$). During the intervention, the sedentary group spent a larger proportion of their time sedentary than the active group ($p < 0.001$).

Proportion of time spent in LPA. There was no significant interaction effect ($p = 0.21$) or group effect ($p = 0.08$). However, there was a main effect for time ($p = 0.01$, partial $\eta^2 = 0.31$, $\beta = 0.77$). Follow-up analysis showed that the sedentary group decreased the proportion of time spent in LPA by 5.2% ($p < 0.001$, partial $\eta^2 = 0.83$, $\beta = 1.0$), but there

was no significant change in the active group ($p = 0.43$). There was a trend towards a difference between the two groups during the intervention ($p = 0.064$).

Proportion of time spent in MVPA. There was a significant group by time interaction ($p < 0.001$, partial $\eta^2 = 0.79$, $\beta = 1.0$), and a main effect for time ($p = 0.003$, partial $\eta^2 = 0.40$, observed power (β) = 0.91) and group ($p = 0.001$, partial $\eta^2 = 0.45$, $\beta = 0.95$). Follow-up analysis showed that the sedentary group decreased the proportion of time spent in MVPA by 4.1% ($p < 0.001$, partial $\eta^2 = 0.91$, $\beta = 1.0$), and the active group significantly increased the proportion of time spent in MVPA by 1.7% ($p = 0.016$, partial $\eta^2 = 0.50$, $\beta = 0.75$). During the intervention, the sedentary group spent a smaller proportion of their time in MVPA than the active group ($p < 0.001$).

Sedentary bouts per day. There was a significant interaction effect ($p = 0.03$, partial $\eta^2 = 0.24$, $\beta = 0.61$) and a main effect for time ($p = 0.002$, partial $\eta^2 = 0.43$, $\beta = 0.93$). A trend towards a group effect ($p = 0.058$) was also observed. Follow-up analysis showed an increase in the number of prolonged sedentary bouts in the sedentary group ($p < 0.01$, partial $\eta^2 = 0.62$, $\beta = 0.92$), but no change in the active group ($p = 0.31$). During the intervention, the sedentary group had more sedentary bouts than the active group ($p = 0.02$), and spent more of their sedentary time in prolonged, uninterrupted bouts.

Average length of sedentary bouts. There was no interaction effect ($p = 0.49$), or main effect for time ($p = 0.97$) or group ($p = 73$).

Sedentary breaks per day. There was no interaction effect ($p = 0.99$), or main effect for time ($p = 0.58$) or group ($p = 0.55$).

Break rate. There was no interaction effect ($p = 0.45$) or group effect ($p = 0.31$). However, there was a main effect for time ($p = 0.011$, partial $\eta^2 = 0.31$, $\beta = 0.76$). Follow-up analysis showed a decrease in break rate in the sedentary group by 0.7 breaks per hour of sedentary time ($p = 0.006$, partial $\eta^2 = 0.59$, $\beta = 0.90$), but no change in the active group ($p = 0.27$). During the intervention, there was no difference in break rate between the two groups ($p = 0.15$).

Bouts of MVPA per day. There was a significant interaction effect ($p < 0.001$, partial $\eta^2 = 0.73$, $\beta = 1.0$) and a main effect for group ($p = 0.003$, partial $\eta^2 = 0.39$, $\beta = 0.89$). No main effect for time was observed ($p = 0.93$). Follow-up analysis showed that the sedentary group decreased the number of MVPA bouts per day from 0.9 to 0.05 ($p = 0.001$, partial $\eta^2 = 0.72$, $\beta = 0.99$), and the active group significantly increased the number of MVPA bouts per day from 0.9 to 1.7 ($p = 0.001$, partial $\eta^2 = 0.74$, $\beta = 1.0$). During the intervention, the sedentary group had fewer bouts of MVPA per day than the active group ($p < 0.001$).

Average length of MVPA bouts. There was no interaction effect ($p = 0.49$), or main effect for time ($p = 0.97$) or group ($p = 0.73$).

DISCUSSION

The purpose of this study was to explore changes in activity behaviour and patterns in response to an intervention aimed at increasing either sedentary time or physical activity. Our results suggest that both the overall activity profile and the pattern of sedentary behaviour differentially respond to experimental interventions. In the sedentary group, we observed significant changes in all three categories of activity intensity (increased sedentary time, decreased LPA and MVPA). While the number of sedentary bouts increased by 2.5

per day, the average and maximum length of sedentary bouts did not change. This suggests that sedentary time was increased via an increase in the number of sedentary bouts, and not an increase in the length of the bouts. Our results from the sedentary group are consistent with Lyden et al. [27] who also showed an increase in the amount of sedentary time spent in prolonged sedentary bouts when they had young healthy adults reduced daily steps from > 10,000 to < 5,000 for 7 days. However, whether the increase in sedentary time observed by Lyden et al. [27] came from longer or more frequent sedentary bouts is unclear as neither the average nor the maximum length of sedentary bouts were reported. Similar to the current study, they also found decrease in the break rate from 5.8 to 4.6 breaks per hour of sedentary time.[27] These data suggest that young and middle-aged adults similarly alter activity behaviour, as well as the pattern of activity, in response to experimental reductions in ambulatory activity.

In the active group, the only variables that changed were related to MVPA. Total MVPA increased via more frequent, but not longer, bouts of MVPA. In fact, the 19 min increase in MVPA from the preliminary assessment to the intervention period can be explained by the addition of one average length bout of MVPA. The lack of change in the active group's sedentary time or LPA was observed despite instructions to integrate the added 3,000 steps throughout participants' day rather than in longer bouts of dedicated walking/activity. Additionally, there was also no change in the number of breaks in sedentary time or the average length of sedentary bouts. These results imply that interventions focused on increasing MVPA may have little effect on sedentary time and LPA in a free-living environment. This presumption is supported by Prince et al. [26] who found PA-focused

interventions to be less effective in reducing sedentary time than those focused on PA & sedentary time, or those focused exclusively on reducing sedentary time. Our results are also in agreement with previous reviews that have reported short-term increases in MVPA during PA interventions.[32 33] However, long-term PA adherence is notoriously difficult to maintain,[34] thus alternative means to improving health outcomes should be explored (e.g. reducing sedentary time and/or increasing LPA). Reducing sedentary time may be an achievable short- and long-term goal for most individuals, and is likely to be more accessible to low income individuals, those with limited time, and/or those apathetic toward structured physical activity involvement.[12]

Interruptions or breaks in sedentary time have been cross-sectionally and experimentally shown to offset the negative effects of excessive sedentary time on health.[23-25] Healy et al. [20] were one of the first groups to report that, independent of total time spent sedentary, the number of breaks in sedentary time imparted beneficial effects on plasma triglycerides, waist circumference, and 2-hr plasma glucose (a measure of glucose metabolism). In fact, those in the highest quartile of breaks in sedentary time had, on average, a 5.95 cm lower waist circumference ($p = 0.025$) and a 0.88 mmol/L lower 2-h plasma glucose ($p = 0.019$) compared to those in the lowest quartile. Their results also showed that the majority of sedentary breaks were < 5 minutes in length and spent in LPA. It was speculated that a potential mechanism for the detrimental association was the absence of low-intensity skeletal muscle contractions, and thus a reduction in daily energy expenditure in those with the least number of breaks.[20] An increase in the number of sedentary breaks has been observed to increase energy expenditure throughout the day.[35]

The beneficial association between breaks in sedentary time and metabolic markers may therefore reflect higher total energy expenditure in those with more frequent breaks, an effect that has been previously explored and demonstrated.[25 35-37] Even activities as minimal as standing, rather than sitting, have been shown to result in substantial increases in total daily energy expenditure and resistance to fat gain.[35 37 38] Intervention studies have also shown that breaking up prolonged bouts of sedentary time improves acute glucose control and insulin action,[23-25 39] which is likely to contribute to lower incidence of obesity, dyslipidemia, and diabetes.[40]

During the preliminary assessment, the participants in this study accumulated above-average amounts of both sedentary time (~11.1 hrs • day^{-1}) and MVPA (~53 min • day^{-1}). This is compared to 9-10 hours of sedentary time and 20-30 minutes of MVPA in other Canadian women of similar age.[1 41] This discrepancy might be explained by the fact that 17 of the women in the present study were working full-time and occupational sitting is a large contributor to overall sedentary time.[42] Furthermore, participation in this study required that women be able to complete 10 days of reduced stepping, which means anyone employed or working in a non-sedentary or somewhat active setting (e.g. grade school teachers, day home attendants, massage therapists, physiotherapists, etc.) were unable to participate. The above-average amounts of daily MVPA might be explained by high educational attainment in the sample. Over 90% of the sample had completed at least 2 years of post-secondary education, and it is established that educational attainment and socio-economic status and positively associated with physical activity.[43-45] High levels

of both sedentary time and MVPA also support evidence showing that the categorization of 'active' and 'sedentary' are independent and not mutually exclusive.[46]

The primary strength of this study was the objective measurement of activity via accelerometry. This allowed us to gather more detailed information about the activity profile and patterns from the preliminary assessment to intervention than could have been gathered from self-report methods. That said, one limitation of this study and indeed, any study that relies on accelerometers, is that we have no information about the context of sedentary time, and studies have shown that different types of sedentary time are associated with greater health risks than others (i.e.: television versus reading).

Much of the research focused on sedentary time is based on the premise that it affects health even in physically active individuals, and that the two areas of study (exercise physiology and sedentary physiology) are not simply opposite of one another.[12] The classic example of this is lipoprotein lipase kinetics, which have been shown to be affected differently by sedentary time compared to exercise.[47 48] The results of the present study support this paradigm and extend it beyond just physiological outcomes. Our results suggest that middle-aged women differentially alter their daily movement behaviour and patterns in response to short-term increases in either PA or sedentary time, and these changes are not simply opposite of one another. Further investigation into the pattern of activity in response to PA and sedentary behaviour interventions is warranted.

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Table 3.1. List of tips and strategies given to participants to help them reach their daily step goal.

Sedentary group

General:

- *Enlist family, friends, and co-workers to support you.* Explain what you're doing, why you're doing it, and how they can help.
- Drive to your destination and park as close as possible.
- Avoid stairs whenever possible.
  - Use the elevator instead!
- Choose to sit rather than stand whenever possible (ex: stay seated while talking on the phone).
- Recruit friends or a significant other to walk any pets that need walking.
- Avoid housework. Ask your significant other and/or kids to help out.
- Go to the grocery store with a list and plan to minimize your steps.
  - Recruit your significant other and/or kids to pack in the groceries.
- Prepare meals in bulk.

Recreation:

- Find a TV series on Netflix and see how many episodes you can watch without getting up.
  - Recommended series from the Active Healthy Aging Lab: Sherlock, The Good Wife, House of Cards, and Suits.
- Go to a movie (or 2). Tuesday is cheap movie night!
- Read a book.
- Go for a scenic drive instead of a walk or bike ride.
- Use this time to meet friends for lunch, dinner, coffee, or just a visit (no walks allowed).
- Finish that craft project you have been putting off!

At Work:

- Drive to work instead of walking or biking.
  - Better: have someone drop you off outside your door and pick you up.
- Use a rolling chair to move around your office.
- Email or call co-workers instead of walking to their work space.
- Avoid stairs and take the elevator whenever possible.
- Eat lunch somewhere nearby your office and drive when possible.

Active group

General:

- *Enlist family, friends, and co-workers to support you.* Explain what you're doing, why you're doing it, and how they can help.
- Bike or walk to your destination when possible.
- Park as far away from the door as possible.
- Take the stairs whenever possible.

- Choose to stand rather than sit whenever possible (ex: stand while talking on the phone).
- Take advantage of nice weather and attend to outdoor maintenance (i.e. fertilize your yard, mow the lawn, prep soil for planting, de-clutter the garage, etc.)
- Prepare more meals at home.
- Walk down every aisle at the grocery store.
    - Do this at Costco for the biggest bang.
    - Go with a list to avoid over-filling your cart.

Recreation:

- Go for a walk with family or friends instead of a movie or Netflix.
- Volunteer at the soup kitchen for a couple hours (http://www.soupbridge.org/ ;(403) 320-8688).
- Go to the park for a picnic instead of out for dinner.
- Visit local museums.
- Go bowling.
- Opt for a scenic bike ride instead of drive.
- Take the dog for an extra-long walk.
- Spend some time at the mall shopping.
- Volunteer to mow or rake a neighbour's yard (bonus points for being a good neighbour).

At Work:

- Walking or biking to work instead of driving.
    - Park far from the door if you drive.
- Get up and walk around your office instead of using a rolling chair to get around.
- Walk to colleagues' work space instead of calling or sending an email.
- Take the stairs and avoid the elevator whenever possible.
- Eat lunch somewhere further away from your office than usual.

Table 3.2. Participant anthropometric characteristics and preliminary activity profile (n = 20).

	Sedentary (n = 10)	**Active** (n = 10)	P
Age (yr)	50.4 ± 5.9	49.9 ± 5.2	0.84
Height (m)	1.65 ± 5.1	1.66 ± 5.2	0.48
Weight (kg)	75.7 ± 7.7	73.6 ± 10.5	0.62
BMI ($kg \bullet m^{-2}$)	28.0 ± 3.5	26.6 ± 3.7	0.39
WC (cm)	90.0 ± 10.1	87.0 ± 8.3	0.48
Wear time ($min \bullet day^{-1}$)	909 ± 53	878 ± 81	0.33
Sedentary time ($min \bullet day^{-1}$)	690 ± 47	648 ± 92	0.22
LPA ($min \bullet day^{-1}$)	166 ± 29	178 ± 47	0.49
MVPA ($min \bullet day^{-1}$)	53 ± 15	52 ± 22	0.90
% of time spent sedentary	75.9 ± 2.8	73.7 ± 6.5	0.33
% of time spent in LPA	18.2 ± 3.0	20.4 ± 5.5	0.29
% of time spent in MVPA	5.9 ± 1.6	5.9 ± 2.5	0.94
Avg. number of sedentary breaks per day	13.7 ± 3.8	11.2 ± 3.8	0.16
Avg. length of sedentary bouts (min)	31.6 ± 3.8	32.6 ± 3.7	0.92
Max. length of sedentary bout (min)	66.7 ± 16.1	69.7 ± 22.0	0.73

BMI, body mass index; CRP, C-reactive protein; LPA, light physical activity; MVPA, moderate to vigorous physical activity. Data presented as mean ± SD.

Table 3.3. Pattern of activity in the sedentary and active group during the preliminary assessment and intervention periods.

	Sedentary			Active			
	Preliminary	Intervention	P (within)	Preliminary	Intervention	P (within)	P § (between)
Wear time (min • day^{-1})	908 ± 53	895 ± 54	0.311	878 ± 81	933 ± 33	0.101	0.074
Sedentary time (min • day^{-1})	690 ± 47	763 ± 55	**<0.001**	648 ± 92	669 ± 32	0.428	**<0.001**
LPA (min • day^{-1})	166 ± 29	116 ± 11	**<0.001**	178 ± 47	193 ± 57	0.229	**0.002**
MVPA (min • day^{-1})	53 ± 15	16 ± 7	**<0.001**	52 ± 22	71 ± 21	**0.003**	**<0.001**
% of time spent sedentary	75.9 ± 2.8	85.2 ± 1.6	**<0.001**	73.7 ± 6.5	71.9 ± 4.7	0.192	**<0.001**
% of time spent in LPA	18.2 ± 3.0	13.0 ± 1.7	**<0.001**	20.4 ± 5.5	18.4 ± 8.5	0.432	0.064
% of time spent in MVPA	5.9 ± 1.6	1.8 ± 0.7	**<0.001**	5.9 ± 2.5	7.6 ± 2.2	**0.016**	**<0.001**
Number of sedentary breaks per day[a]	79.6 ± 9.1	78.5 ± 12.2	0.679	74.3 ± 12.7	75.7 ± 8.2	0.750	0.561
Break rate (breaks • hr-sed^{-1})	6.9 ± 0.8	6.2 ± 1.0	**0.006**	6.9 ± 0.8	6.8 ± 0.8	0.577	0.150
Number of sedentary bouts[b] per day	4.0 ± 1.9	6.5 ± 2.7	**0.004**	3.2 ± 2.0	3.7 ± 2.2	0.312	**0.020**
Time per day in sedentary bouts (min • day^{-1})	125 ± 60	211 ± 94	**0.004**	108 ± 74	122 ± 77	0.479	**0.033**
% of sedentary time spent ≥ 20 min bouts	18.0 ± 7.9	27.3 ± 11.2	**0.007**	15.7 ± 10.0	17.9 ± 10.7	0.392	0.070
Avg. length of sedentary bouts (min)	31.6 ± 3.8	32.2 ± 2.9	0.580	32.6 ± 3.7	32.1 ± 3.1	0.666	0.971
Max. length of sedentary bouts (min)	66.7 ± 16.1	75.3 ± 18.7	0.225	72.6 ± 20.5	74.0 ± 19.3	0.674	0.877
Number of MVPA bouts[c] per day	0.9 ± 0.6	0.05 ± 0.08	**0.001**	0.9 ± 0.7	1.7 ± 0.7	**0.001**	**<0.001**
Time per day in MVPA bouts (min • day^{-1})	17.9 ±11.7	0.5 ± 0.9	**0.001**	17.7 ± 14.3	35.0 ± 16.0	**0.001**	**<0.001**
% of MVPA spent in ≥ 10 min bouts	31.9 ± 17.0	2.8 ± 5.3	**<0.001**	34.0 ± 24.8	49.1 ± 17.5	**0.033**	**<0.001**
Avg. length of MVPA bouts (min)	19.7 ± 7.2	3.2 ± 5.2	**<0.001**	22.0 ± 10.6	20.7 ± 4.4	0.703	**<0.001**
Max. length of MVPA bouts (min)	28.0 ± 12.6	3.3 ± 5.3	**<0.001**	31.5 ± 16.6	42.1 ± 15.4	0.072	**<0.001**

LPA, light physical activity; MVPA, moderate-to-vigorous physical activity. §, difference between groups during the intervention. [a] Sedentary break, any instance where a sedentary bout of ≥ 2 min was interrupted by a period identified as not sedentary; [b]Sedentary bout, ≥ 20 consecutive minutes at < 100 counts • min^{-1}; [c] MVPA bout, >10 consecutive minutes in MVPA (> 1951 counts • min^{-1}).

CHAPTER 4: GENERAL DISCUSSION

While modest amounts of physical activity (PA) are known to preserve and improve health,[1] it has become apparent that sedentary time, specifically prolonged, uninterrupted bouts of sedentary time, increase the risk of abnormal cardiometabolic function, even in physically active individuals.[2-6] Despite growing awareness of the health consequences of excessive sedentary time, the underlying physiological mechanisms are poorly understood. C-reactive protein (CRP) is a marker of systemic inflammation that represents a potential link between sedentary time and adverse health outcomes.[7-10] Therefore, the primary purpose of this thesis was to explore the effect of (a) increased sedentary time, and (b) increased physical activity on salivary CRP in women. A secondary objective was to examine how these two behavioural interventions would influence overall movement patterns. This is important since the pattern of sedentary time accumulation is thought to mediate the effects of excessive sedentary time on health. It has been shown that bouts of prolonged sedentary time detrimentally impact health, while frequent breaks in sedentary time appearing to mitigate these deleterious effects.[8 11-14]

The study presented in Chapter 2 showed that salivary CRP increased significantly after 10 days of increased sedentary time, and decreased after 10 days of increased physical activity (PA) in healthy middle-aged women, providing evidence that daily movement behaviour affects inflammation. The analysis in Chapter 3 showed that an intervention focused on increasing physical activity had no effect on the amount or pattern of sedentary time, suggesting that individuals do not simply replace one behaviour with another.

The unique properties of CRP have led to its use in several areas of clinical application, such as identifying acute infection, detecting sepsis in critically ill patients, and monitoring disease progression. In autoimmune diseases, such as rheumatoid arthritis,[15] CRP is used to monitor the severity of the disease, as well as assess the risk for future disease-related developments (i.e. the development of arthritis in previously unaffected joints). Additionally, even when within a normal range, epidemiological studies have repeatedly shown a strong predictive relationship between CRP concentration and future cardiovascular events.[16-18] Comparable associations between CRP and various other metabolic abnormalities (i.e. obesity, insulin resistance, hypertension, etc.) have also been identified.[16 18 19]

Because of its utility, there is a demand for more practical ways to quantify CRP. Salivary diagnostics represent a promising new technique that is cost effective, easy to use, and less invasive than blood or tissue sampling,[20] and evidence supports an association between serum and salivary CRP concentrations.[21-26] Moreover, correlations have been established between salivary CRP and subclinical atherosclerosis,[24] BMI,[27] chronic obstructive pulmonary disease,[26] and oral health,[27] suggesting it is sensitive to known inflammatory conditions. Salivary CRP has been shown to increase in response to a 104-km ultramarathon [28] and a minimally invasive heart surgery,[22] while Jamshidpour et al. [23] observed a decrease following a 6-8 wk exercise trial. These data suggest salivary CRP may be a valid and reliable measure of low grade inflammation.

Like blood, saliva is a dynamic and complex fluid that contains an array of enzymes, hormones, antibodies, cytokines, and antimicrobial constituents that can reflect the

physiologic state of the body.[29 30] However, there has been little discussion surrounding the mechanism by which CRP moves from the serum to the saliva. There are several ways serum proteins reach the saliva, including intracellular (e.g. passive diffusion) and extracellular routes (e.g. ultrafiltration through tight junctions), as well as through the gingival crevicular fluid (GCF).[31] Given its relatively large molecular mass (approx. 115 kDa),[32] CRP is unlikely to reach the saliva through passive diffusion or ultrafiltration.[33 34] Salivary CRP likely enters the oral cavity via the gingival crevicular fluid (GCF), which can be either a transudate or inflammatory exudate of serum, that mixes to becomes a constituent of whole saliva.[35] Given the previously demonstrated association between salivary and serum CRP [21-26 36], current evidence points to salivary CRP as a potential alternative to serum measures in research and clinical settings.

Quantifying CRP, as well as other biochemicals, via saliva represents a particularly useful advancement in bioanalysis techniques for researchers who are often constrained by budget and willingness of volunteer participants. This is especially true when multiple blood draws over a short period of time are required or in working with vulnerable populations, such as children or the elderly. The results of this study suggest that salivary CRP is sensitive to changes in physical activity, and more work is needed to determine if it is also sensitive to changes in the pattern of sedentary time, which has been established as important to health.[12 37-40]

As expected, the sedentary intervention in chapter 3 resulted in an increase in total sedentary time and decrease in the number of sedentary breaks per hour of sedentary time. It is unclear whether either or both of these changes are responsible for the observed increase in

CRP. Numerous studies suggest that along with total sedentary time, prolonged, uninterrupted bouts of sedentary time detrimentally affect cardiometabolic health markers, such as insulin sensitivity, serum triglyceride concentration, and serum CRP.[11 12 40] Most of the epidemiological research in this area has focused on lipid concentration and glucose control/insulin resistance. This has resulted in a limited number of studies exploring the moderating effects of sedentary breaks on the association between serum CRP and total sedentary time.[7 8 11] In 2011, Healy et al. [7] reported a positive association between sedentary time and CRP in 4757 US adults, even after controlling for potential cofounders such as age, sex, ethnicity, medical history, exercise, and socio-demographic variables. After adjusting for total sedentary time, they also found a significant inverse association between breaks in sedentary time and CRP.

Not all studies have shown an association between sedentary time and CRP. Henson et al. [8] observed no association between serum CRP and sedentary time after adjusting for moderate-to-vigorous physical activity (MVPA), although they did note a trend towards an association between CRP and breaks in sedentary time in their "inactive" group of high risk adults.[8] Carson et al. [11] also found no correlation between CRP and daily sedentary time, or with the amount of time spent in prolonged sedentary bouts (≥ 20 minutes) in a sample of 4935 Canadian adults. However, similar to the trend identified by Henson et al. [8] they did find a significant negative correlation between the number of sedentary breaks and CRP. Although the cross-sectional evidence surrounding the association between sedentary breaks and CRP is limited, the existing research points to a more consistent relationship between CRP and

sedentary breaks when compared to total time spent sedentary.

The results from Chapter 3, summarized in Table 4.1, show that in the sedentary group the proportion of sedentary time that was spent in prolonged, uninterrupted bouts increased from the preliminary assessment to the intervention. Sedentary breaks per hour of sedentary time and LPA also decreased, and MVPA was dramatically reduced. As shown in Chapter 2 these changes in movement were associated with a 31% increase in CRP. There are two possible reasons for the increased CRP in the sedentary group. First, it is feasible that the *combination* of increased sedentary time, reduced LPA and the frequency of sedentary breaks, concomitant with more prolonged sedentary bouts (≥ 20 minutes) explains the increase in CRP. Second, it is also possible that increased inflammation results from removing MVPA, which has been shown to have anti-inflammatory effects.[41 42] This hypothesis is supported by the 22% decrease in CRP in the active group, despite no change in in the proportion of sedentary time they spent in prolonged bouts, sedentary breaks, or LPA.

More experimental evidence exploring the effect on short-term alterations in daily activity patterns is needed to understand the biological connection between sedentary time and inflammation. Some work in this area has been done, but similar to observational evidence, the majority of experimental data exploring this phenomenon are focused on glucose metabolism and insulin sensitivity. [12 13 38 40 43-45] Outside of glucose control and insulin sensitivity, very little is known about the physiological mechanisms driving this maladaptation to prolonged sitting. Healy et al. [40] proposed that a reduction in the number of sedentary breaks results in lower daily energy expenditure. However, even after matching for total

physical activity, and thus energy expenditure, Peddie et al. [13] still found blunted glucose tolerance and insulin sensitivity in response to prolonged sitting relative to regularly interrupted sitting. To address this gap in knowledge, two studies have compared skeletal muscle gene expression and molecular signaling pathways during different sedentary conditions to provide novel insight into what might be underlying the beneficial effect of breaking up sedentary time.[39 46] To identify the acute transcriptional events in skeletal muscle that occur during breaks in sedentary time, Latouche et al. [46] used three 5 hr experimental conditions. The three conditions consisted of: (1) a 5-hr uninterrupted sitting bout; (2) a sitting bout that was interrupted every 20 minutes with 2 min of light walking (3.2 km • hr^{-1}); and (3) a sitting bout that was interrupted every 20 minutes with 2 min of moderate walking (5.8 - 6.4 km • hr^{-1}). They took hourly blood samples during the 5-hr conditions, as well as a vastus lateralis muscle biopsy 40 - 50 minutes following the last activity bout. Using advanced transcriptional techniques, they identified 71 statistically significant biological pathways and functions. Their results showed that the main biological pathways that were differentially expressed across the conditions were related to cellular development, growth and proliferation, carbohydrate metabolism, and CVD risk. One such example is nicotinamide N-methyltransferase (NNMT), which showed increased expression following the interrupted sitting conditions compared to the prolonged sitting bout. NNMT is a cytosolic enzyme that catalyzes the N -methylation of nicotinamide, producing 1-methylnicotinamide (MNA).[47 48] Experimental studies have shown that MNA has both anti-inflammatory and anti-thrombotic properties, scavenges oxygen radicals, as well as lowers plasma triglyceride levels. [47 48] Along with an increased expression of NNMT, Latouche et al. [46] identified 2 other genes related to

CVD, legumain (LGMN) and dylein light chain (DYNLL1), that were differentially expressed following the interrupted sitting conditions compared to the prolonged sitting bout. DYNLL1 expression increased with the intensity of activity breaks [46] and is thought to play a role in glucose metabolism via GLUT-4 translocation [49] and to have downstream effects on TNF-α related regulation of inflammation and apoptosis.[50] These new findings represent a potential link between prolonged bouts of sedentary time and an inflammatory response.[46]

A follow-up study by the same group explored potential molecular mechanisms accounting for the improved glucose tolerance that is observed with frequent interruptions to prolonged sitting.[39] They used the same protocol as Latouche et al. [46] but added a 3-day intervention that consisted of prolonged uninterrupted sitting for 6 hours per day and a interrupted sitting condition (2 min of light walking every 20 minutes). They examined contraction- and insulin-mediated glucose uptake signaling pathways, as well as changes in proteins related to oxidative phosphorylation. They found that acute interruptions to sitting over one day stimulated the contraction-mediated glucose uptake pathway and that both acute (5-hr condition) interruptions to sitting, with moderate-intensity activity over one day, and light-intensity activity over three days, induced a modulation of the insulin-signaling pathway, in association with increased capacity for glucose transport. More simply, there was a shift towards contraction-mediated glucose uptake after short-term exposure to interrupted sedentary bouts when compared to uninterrupted bouts, pointing to improved peripheral insulin sensitivity and glucose disposal following regularly interrupted sedentary bouts.

When applied to our results, these data might suggest that the observed increase in CRP in

the sedentary group, along with a reduced sedentary break rate and increase in the number of prolonged sedentary bouts, may be the result of a shift towards insulin-mediated glucose uptake in skeletal muscle (i.e. reduced peripheral insulin sensitivity). This speculation is supported by other experimental data from Krogh-Madsen et al. [44] who found two weeks of reduced stepping resulted in significant changes in the metabolic response to an infused glucose load. In addition to a reduced rate of insulin-stimulated glucose disappearance (a marker of peripheral insulin sensitivity), subjects experienced an increased insulin response to both a glucose tolerance test and an oral fat tolerance test.[51] Using a hyperinsulinemic-euglycemic clamp technique, the measured glucose infusion rate was slower post-intervention. A slower glucose infusion rate combined with no change in hepatic glucose turnover suggests reduced peripheral insulin sensitivity and a diminished ability to utilize blood glucose in skeletal muscle. Insulin resistance is intimately tied to an inflammatory state, specifically through TNF-α, a pro-inflammatory cytokine responsible for the activation of numerous signal transduction cascades.[52 53] Furthermore, TNF-α has been shown to stimulate CRP production in human coronary artery smooth muscle cells similar to an IL-1β and IL-6 combination.[54] Therefore, breaking up sitting time with frequent interruptions can alter skeletal muscle gene expression, modify molecular signaling to improve glucose control, and potentially mitigate inflammatory processes.

Advances in activity monitoring and statistical analysis procedures have allowed researchers to consider how 24 hours of activity, including sleep, sedentary behaviour, LPA, and MVPA, affect health outcomes.[55-58] Recent evidence suggests that increasing LPA may be a feasible way

to improve the health of those disinterested, unwilling, or unable to participate in regular MVPA.[12 13 38 55 56] Using isotemporal substitution modelling, data presented by Buman et al. [55] showed that for every 30 min reallocation of sedentary time to MVPA, there was a 2-25% improvement in cardiometabolic biomarkers (e.g. waist circumference, insulin, triglycerides). Comparatively, for every 30 min of sedentary time reallocated to LPA, there was a 2-4% improvement. It is clear that, minute-for-minute, MVPA far exceeds LPA in terms of its potential to improve biomarkers of health. However, given that 85% of the Canadian population fails meet daily PA recommendation of 150 minutes per week of MVPA,[59] it is becoming increasingly important to explore alternate means to improving population health. Interventions aimed at reducing sedentary time have been shown to result in large and clinically significant decreases in total time spent sedentary by approximately 90 mins.[60] Reallocating that 90 mins to LPA could mean significant reductions in cardiometabolic risk factors by ~6-12%,[55 60] resulting in clinically meaningful reductions to health risk. This is supported by data from Ekblom-Bak et al. [58] who showed replacing 30 minutes of sedentary time with LPA resulted in 3% lower fasting glucose and 3.1% lower homeostatic model assessment for insulin resistance (HOMA-IR) [61] values. HOMA-IR is a measure of insulin resistance as is a calculated from fasting plasma glucose and insulin concentrations. Ekblom-Bak et al. [58] also stratified participants by WC, cardiorespiratory fitness, and fasting glucose concentration before using an isotemporal substitution technique to analyse the potential effect of replacing sedentary time with LPA, and sedentary time for MVPA. Although PA substitutions produced improvements in most groups, HOMA-IR values improved to a greater extent in individuals with a higher risk stratification than those with a lower risk stratification.

Notably, there was little change in HOMA-IR values when substituting sedentary time with LPA in the low risk stratification group. Therefore, the benefit of reducing sedentary time and increasing LPA is likely greatest in higher risk individuals.[58] This conclusion is supported by a number of studies.[41 62-64]

In Chapter 3, we showed that a successful 10-day PA intervention had no significant effect on sedentary time or LPA (Table 3.1). The participants increased their activity by slightly decreasing both their LPA and their sedentary time, but ultimately physical activity makes up such a small amount of daily time, that sedentary time remains very high. In fact, despite 9 of the women in that group meeting Canada's minimum physical activity recommendations they still accumulated more than 11 hours of sedentary time per day. These results echo the conclusions of a review by Prince et al. [60] who found significant but small reduction in sedentary time in response to PA-only interventions. It is plausible that an increase in voluntary exercise is accompanied by a decrease in LPA and an increase in sedentary time in response to perturbations in energy balance that result from increased PA.[65] This speculation is also supported by Prince et al. [60] who showed that interventions focused on both PA and sedentary time reduction resulted in about a 30-minute reduction in sedentary time, while sedentary-only interventions resulted in a 90-minute reduction. Notably, 5 of the 6 studies examined by Prince et al. [60] were carried out in an occupational setting. This is meaningful because occupational sitting is a significant contributor to overall sedentary time,[66] and the majority of the women in the present study were engaged in full-time work.

While the participants in this study were very sedentary (both during the preliminary assessment and during the intervention), they were also more active when compared to other

women of similar age.[59 67] A recent meta-analysis published by Ekelund et al. [3] suggests that the MVPA accumulated by the active group (71 ± 21 minutes per day) may be sufficient to off-set the detrimental effects of sedentary time. Using more than a million participants, they found that individuals with the highest amount of sedentary time (> 8 hours per day) did not have an increased risk of mortality relative to those with the least amount of sedentary time (< 4 hours per day) if they were engaged in > 60 minutes per day of moderate intensity PA. In this study, 3 women in the active group met this requirement during the preliminary assessment week and this number increased to 6 during the intervention. For some women, but not all, increasing MVPA may be a viable option to offset the health risks associated with high sedentary time. However, long-term adherence to exercise is difficult to maintain [68] and the perceived barriers are plentiful.[69] Together, these results imply that interventions focused on reducing sedentary time, especially in an occupational setting, may be the most effective way to reduce sedentary time and improve health. A recent randomized controlled trial suggests interventions focused on reducing sedentary time in the workplace may be effective.[70] More research needs to be done to confirm the long-term feasibility of reducing sedentary time and expand to other populations.

LIMITATIONS

Although accelerometers are the current gold standard in objectively quantifying patterns of physical activity and sedentary time in free-living individuals, they do have limitations., one of which is the lack of contextual information they provide. This is important as we are beginning to understand that not all sedentary behaviours affect health to the same extent. For example, leisure sedentary time is more closely associated with adverse health outcomes compared to

occupational sedentary time[71-73] and mentally stimulating/challenging sedentary activities, such as reading or socializing, impact health differently than passive activities, such as watching TV or playing video games.[74 75] More research and development is needed to advance current technologies to include contextual information. A potential option is wearable cameras,[76] although the feasibility of this in large studies is questionable.

The external validity of this study may be limited as the sample of women were of a specific age (40-60 yrs) and in good general health, free of chronic or inflammatory diseases. The majority were also working full-time (85%) and had completed at least 2 years of post-secondary education (90%). The specific inclusion criteria of these studies limits the generalizability of the results.

FUTURE DIRECTIONS

There is very little experimental evidence exploring whether or not the health implications of sedentary time are truly independent of physical activity. Data from chapter 2 suggests that CRP, and possibly other markers of inflammation, are more sensitive to changes in MVPA rather than sedentary time. However, a small proportion of Canadian adults meet current PA recommendations. Thus, future work in this area should focus on (a) establishing the efficacy of interventions focused on reducing sedentary time, both inside and outside of the workplace,[60] (b) examine the dose-response relationship of LPA on health, and (c) integrate controlled amounts of MVPA into intervention trials to establish how much PA is needed to off-set the detrimental health effects of sedentary time. Peddie et al. [13] found that even after matching for physical activity, regular interruptions to prolonged sitting beneficially affected

glucose tolerance and insulin sensitivity. More studies using this design are needed, especially those exploring other physiological responses beyond glucose tolerance/insulin action.

Finally, serum CRP as a marker of systemic inflammation and predictive of future cardiometabolic disease is highly reliable. In fact, seminal work by Ridker et al. [17] showed serum CRP to be the best univariate predictor of future cardiovascular events compared to 12 other biomarkers. However, the reliability of salivary CRP has not been well documented and needs to be further explored. The results in Chapter 2 suggest that it is stable across short periods of time (e.g. 7 to 24 days) and that it is sensitive enough to detect short-term changes in activity, which is consistent with previous data.[23]

CONCLUSION

Though our understanding of how sedentary time affects health is evolving, more work is needed to understand the physiological mechanisms and how behavioural interventions affect activity patterns. The change in systemic CRP we observed after only 10 days of altered activity suggests that inflammatory responses may be even more sensitive to physical activity than previously thought, especially in women. The research exploring the underlying mechanism are lacking, however, current evidence suggests skeletal muscle gene transcription and molecular sequencing may partially explain the observed changes in CRP. More evidence is needed to understand the physiological underpinnings of sedentary time and the patterns within which it is accumulated. Our results also suggest that simply adding more PA to an individual's day does little to reduce sedentary time, especially in those with predominantly sedentary jobs. However, in some instances, engagement in additional PA could serve as a potential way to mitigate the ill-effects of excessive sedentary time and thus

preserve health. Future research should focus on establishing salivary biomarkers as a reliable alternative to serum markers, and continue to explore the nuances of sedentary time, such as how interventions affect sedentary patterns and how the different types of sedentary time impact health.

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Table 4.1 Summary of chapter 2 & 3 results.

	Active	**Sedentary**
CRP	↑	↓
% of time spent sedentary	↑	↔
% of time spent in LPA	↓	↔
% of time spent in MVPA	↓	↑
Number of sedentary breaks per day[a]	↔	↔
Break rate (breaks • hr-sed^{-1})	↓	↔
Number of sedentary bouts[b] per day	↑	↔
Time per day in sedentary bouts (min • day^{-1})	↑	↔
% of sedentary time spent ≥ 20 min bouts	↑	↔
Avg. length of sedentary bouts (min)	↔	↔
Max. length of sedentary bouts (min)	↔	↔
Number of MVPA bouts[c] per day	↓	↑
Time per day in MVPA bouts (min • day^{-1})	↓	↑
% of MVPA spent in ≥ 10 min bouts	↓	↑
Avg. length of MVPA bouts (min)	↓	↔
Max. length of MVPA bouts (min)	↓	↔

LPA, light physical activity; MVPA, moderate-to-vigorous physical activity. §, difference between groups during the intervention. [a] Sedentary break, any instance where a sedentary bout of ≥ 2 min was interrupted by a period identified as not sedentary; [b] Sedentary bout, ≥ 20 consecutive minutes at < 100 counts • min^{-1}; [c] MVPA bout, >10 consecutive minutes in MVPA (> 1951 counts • min^{-1}).