Response of Salivary IL-8 to Prolonged and Interrupted Sitting

by

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A thesis submitted to the
School of Graduate and Postdoctoral Studies in partial
fulfillment of the requirements for the degree of

Master of Health Science in Kinesiology

The Faculty of Health Sciences

University of Ontario Institute of Technology (Ontario Tech University)

Oshawa, Ontario, Canada

August 2022

THESIS EXAMINATION INFORMATION

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Master of Health Science in **Kinesiology**

Thesis title: Response of Salivary IL-8 to Prolonged and Interrupted Sitting

An oral defense of this thesis took place on August 4th, 2022, in front of the following examining committee:

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The above committee determined that the thesis is acceptable in form and content and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate during an oral examination. A signed copy of the Certificate of Approval is available from the School of Graduate and Postdoctoral Studies.

ABSTRACT

The effect of prolonged sitting on inflammation, as determined by cytokine profiles, is relatively unexplored. The purpose of this work was to determine the reliability of salivary IL-8 (CXCL8) and the response of IL-8 to prolonged and interrupted sitting.

Participants completed two sessions in random order: prolonged sitting (4 hours of sitting) and interrupted sitting (prolonged sitting interrupted every 30 minutes by 3 minutes of walking). Saliva and capillary plasma samples were collected pre- and post-session. Weekly variability in salivary IL-8 and individual responses were also explored.

Prolonged sitting increased salivary IL-8 concentrations, while interrupted sitting attenuated this response. Among males, there was no increase in salivary IL-8 during interrupted sitting.

Prolonged sitting may serve as a pro-inflammatory stimulus, and movement interruptions may negate this response. Saliva sampling seems to be a reliable method in assessing IL-8 across timepoints within individuals; however, there is high variability across individuals.

Keywords: sedentary behaviour; inflammation; cytokines; Interleukin 8; sitting

AUTHOR'S DECLARATION

I hereby declare that this thesis consists of original work of which I have authored. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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_____ Nicholas O'Rourke

STATEMENT OF CONTRIBUTIONS

The manuscript included in Chapter 2 was performed in the Human Performance Laboratory at Ontario Tech University and will be submitted for publication. Co-authors of the manuscript include Dr. Shilpa Dogra, Dr. Julia Green-Johnson, Dr. Michael Jeffrey, Lin Saleem and Emmeline Means-Miller. Data collection was conducted by myself with the assistance of Emmeline Means-Miller and laboratory analysis with the assistance of Dr. Michael Jeffrey and Lin. Dr. Shilpa Dogra provided guidance as I, the first author, performed all data synthesis, statistical analyses, primary interpretations and writing of results. I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication. I have used standard referencing practices to acknowledge ideas, research techniques, or other materials that belong to others.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge the endless support I have received from my supervisor Dr. Shilpa Dogra. Dr. Dogra's support has been outstanding, and I am grateful I was able to begin my academic journey under her guidance. Academically Shilpa provided me endless opportunities and support that I am immensely grateful for and believe she has prepared me to take on any challenge. Outside of academics, Shilpa has guided us, and thankfully so, that our actions, words, and research should consider vulnerable populations and diversity. I plan to make these a mainstay as I continue my research journey. I view Shilpa as a fantastic role model and will strive to carry on many of the lessons I have learned from her. Although I am excited to continue my journey with my Ph.D., I will always remain grateful and fondly remember my time under Shilpa's supervision.

Next, I would like to thank my partner Alexandra for her support and for putting up with me during some of the stressful times. Somehow, Alex has entertained my endless ramblings about my thesis with care and compassion; completing my undergraduate and graduate degrees would not be possible without her influence. Alex is a wonderful, intelligent, and caring person, and I am glad we can graduate and pursue Ph.D. studies together.

I would also like to thank my lab mates, particularly Emmeline, who aided me in data collection. It made all the sitting a bit easier! Importantly, I would also like to thank Dr. Julia Green-Johnson, Dr. Michael Jeffrey, and Lin from the JGJ lab for their kindness and support during my cytokine analysis. Without their help, this study would not be possible. I enjoyed my time in the lab, and they helped make it a safe and engaging environment; it was a scary place initially for a kinesiology student. They have helped inspire me to pursue more lab-based work with confidence, knowing that with the right support, the lab is not such a daunting place and can be fun and rewarding.

The kind and knowledgeable support I have received has set me up for a successful career, and I hope to have the ability to continue working with many of them in the future.

Nick O'Rourke

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LIST OF ABBREVIATIONS AND SYMBOLS

BMI	Body Mass Index
CRP	C Reactive Protein
CV	Coefficient of Variation
CXCL8	Cysteine x Cysteine Ligand 8
CXCR1/2	Cysteine x Cysteine Receptor 1 and 2
ELISA	Enzyme-linked Immunosorbent Assay
G-CSF	Granulocyte Colony-stimulating Factor
GM-CSF	Granulocyte-macrophage Colony-stimulating Factor
HDL	High Density Lipoprotein
HIIT	High Intensity Interval Training
HRmax	Maximum Heart Rate
ICAM-1	Intercellular Adhesion Molecule-1
IFN	Interferon
IL	Interleukin
IL-18BP	Interleukin 18 Binding Protein
IL-1ra	Interleukin 1 Receptor Antagonist
IP-10	Interferon-gamma Inducible Protein-10
IS	Interrupted Sitting
LDL	Low Density Lipoprotein
LFA-1	Lymphocyte Function-associated Antigen-1
M-CSF	Macrophage Colony-stimulating Factor
MET	Metabolic Equivalents
MICT	Moderate Intensity Continuous Training
MIF	Macrophage Migration Inhibitory Factor
mIL-1R	Membrane Bound Interleukin Receptor
mRNA	Messenger Ribonucleic acid
MVPA	Moderate to Vigorous Physical Activity
PS	Prolonged Sitting
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
RPM	Rotation per Minute
sIL-1R	Soluble Interleukin 1 Receptor
sTNFR	Soluble Tumour Necrosis Factor Receptor
T2DM	Type 2 Diabetes Mellitus
TG	Triglycerides
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumour Necrosis Factor Alpha
VO2	Maximal Aerobic Capacity

Chapter 1. Literature Review

On a population level, people are now engaging in more sedentary behaviour than ever before, and people are sedentary for more prolonged, uninterrupted durations [1]. Prolonged bouts of sitting can negatively affect many aspects of health through various mechanisms [2-6]. Excess sedentary time can increase this health risk independent of other factors such as exercise [4, 7]. The study of sedentary physiology is an area that needs further examination to understand why sitting can become so harmful from a physiological perspective to address the ever-growing concerns of population-wide increases in sedentarism.

Research Questions:

1. What is the daily variability of IL-8 in the saliva of young, healthy males and females?
2. Do levels of IL-8 (saliva and capillary plasma) change in response to a prolonged sedentary session or a prolonged sedentary session interrupted by light intensity exercise in young healthy males and females?

1.1 The Context of Sedentarism

Studying the behavioural and physiological consequences of prolonged sedentary time is becoming a rapidly growing field of study [8]. Within this field, the emphasis of several definitions has been on behaviour. Simply put, ***sedentary behaviour*** is excess time spent in low energy expenditure activities, mainly activities such as lying down or sitting [8]. A consensus project by Tremblay et al. summarizes the various definitions of sedentary behaviour. The prevailing definition is activities such as sitting or lying that require less than 1.5 metabolic equivalents (METs) [8]. ***Sedentary time***, synonymous with ***sitting time***, is a more meaningful measure for physiological research and is studied and measured in several ways. Much of the existing research is epidemiological or cross-sectional and involves self-reported sedentary time, sometimes measured with proxies, such as T.V. viewing time [8]. Although this has provided us with extensive information and many hypotheses, it is suggested that to further the sedentary physiology field; we need to implement clear standardized procedures for measuring sedentary time [8, 9]. Device-based measurement of sedentary time is becoming more common due to accelerometers and/or inclinometers that detect movement or lack thereof [10].

Another important thing to consider is the distinction between sedentary behaviour and physical inactivity. This distinction is needed, as physical inactivity and sedentary behaviour are different behaviours and have different, although sometimes overlapping, physiological and behavioural outcomes [2, 8, 11]. For example, a person with an office job may commute to work for ~60 minutes by car and sit at their work for around 8 hours. They may spend several hours watching TV or on the internet when home. As such, this person is sedentary for a large portion of their day. However, they are also inactive, as they have not engaged in any physical activity throughout the day. Adding 30 minutes of physical activity would significantly alter their movement pattern and health; however, it would not drastically change the fact that they spend over 10 hours per day being sedentary.

As mentioned, a recurring theme in the field of sedentary physiology is the distinction between sedentary behaviour and physical inactivity [8, 11]. The nature of sedentary behaviour, the physiological mechanisms and responses, and the actual way we measure it differ from physical inactivity [12, 13]. Once more, this distinction is important because sedentary time is becoming an ever-growing health concern. Population levels of sedentary time are continuously increasing [1, 14], and more recently, the COVID-19 pandemic has caused dramatic increases in people's sedentary time [15, 16]. With increased technological use and further restrictions due to the ever-evolving pandemic, sedentary time may increase further [1, 16]. Thus, for this thesis, the term ***sedentarism*** will be used when looking at the physiological aspects of sitting.

On a population level, studies have reported substantial increases in sedentary time over the last 50 years, which seems to have coincided with the rise of obesity [7]. Additionally, occupational and recreational sitting over this timeframe has drastically increased people's daily sedentary time [3]. Increased technological use at home and work have drastically increased screen time, a standard proxy of sedentary time [8]. From 1950 to 2000, it is reported that daily television viewing time has nearly doubled from 4.5 to 8 hours per day [9]. Future enhancements in technology will likely further reduce the need to engage in light-intensity physical activity and activities of daily living in people's day-to-day lives [17].

Reports suggest that Canadian adults spend around 10 hours of their waking day (equal to 69% of the waking day) in sedentary pursuits [18]. Compounding these large amounts of sedentary time is that only 15% of Canadian adults meet the current physical activity guidelines (minimum 150min/week of moderate to vigorous physical activity) [18]. Canada is not alone in these trends. An American study reported that, on average, 54.9% of waking hours are spent in sedentary behaviour [19], and similar findings are seen in Australian adults [20].

The importance of these trends is the repeated linking of sedentarism with poorer health outcomes and greater risk of several diseases [21, 22]. Many reviews of the prospective studies have linked sedentary time to several health conditions in adults and youth [21, 22]. Prolonged, chronic exposure to sedentary time has been linked to increased body weight and body mass index [23, 24], risk of being overweight or obese [23, 25-28], waist circumference gain [29], risk of type 2 diabetes [28, 30], cardiovascular disease [17, 31-34], endometrial cancer [35, 36], and even mortality [14, 22]. The strength of the association varies, but there is moderate to strong evidence for type 2 diabetes, cardiovascular disease risk, and cardiovascular disease mortality [22].

All these associations have sparked several studies in the area of sedentary physiology. One prevailing area of research is the role of interrupting sitting, where the aim is to see if interrupting a session of prolonged sitting with a bout of movement, ranging from standing to high-intensity intervals, can reduce or attenuate the harmful effects of sitting. Several studies have used these methods (interrupted sitting) and are referenced throughout this work.

Summary: Epidemiological and cross-sectional studies suggest that sedentary behaviour is an independent predictor of several health outcomes [22]. The mechanistic association linking sedentary time to these outcomes is poorly understood; however, some metabolic and vascular mechanisms have been explored [2, 11, 37, 38].

Gaps: Other mechanisms associated with the diseases linked to sedentarism, such as inflammation, have been explored less frequently, and experimental studies in this area are lacking.

1.2. What do we know about sedentary physiology?

Sedentary physiology is the study of the body's response to any form of sedentarism or sitting [8]. The field of sedentary physiology is unique from other related fields, such as exercise physiology [39], in that it aims to assess the effects of sedentarism alone on the body. The subsections below detail the metabolic and vascular effects of prolonged sedentary time. These are the more well-explored areas in sedentary physiology, and several experimental and prospective studies have been completed.

Vascular effects

A growing body of evidence supports the concept that prolonged sitting of a minimum of 1-6 hours in a single session can impair ***vascular function***, specifically within the lower limb [40]. The femoral and popliteal arteries seem most vulnerable to these detrimental effects [40, 41]. These detrimental effects are sourced to the endothelial dysfunction that occurs in these vessels, and, importantly, this endothelial dysfunction is a critical feature in the development and progression of atherosclerosis [42]. Prolonged sitting has been associated with greater adipose tissue and inflammation across different ethnicities and is linked to a greater risk of atherosclerosis [42, 43]; these are both risk factors that can further reduce endothelial function [43]. Endothelial function is reduced after at least 1 hour of prolonged sitting, highlighting the importance of interrupted sitting and the need for experimental work to understand the effects on vascular health [41].

Prolonged sitting can also alter vascular function through changes in ***blood pressure.*** Sitting is reported to increase hypertension risk, with acute prolonged sitting bouts known to cause increased blood pressure [33]. In contrast, interrupted sitting is associated with lower systolic and diastolic blood pressure [44]. During seven hours or more of prolonged sitting, blood pressure can be increased, while interruption can attenuate this increase [44]. Relative to other sedentary positions, such as being supine, sitting increases blood pressure to a greater extent, likely due to the compensation by lower limb vasculature related to reduced blood flow [40].

Prolonged sitting also causes ***reduced blood flow***, especially in the lower limb. It is hypothesized that sitting increases the leg's hydrostatic pressure and causes blood pooling,

reducing venous return [45]. Furthermore, this coincides with a lack of muscle contraction in sitting, reducing the venous muscle pump [45, 46]. The biomechanical aspects of sitting also play a role in reduced blow flow [47] as the legs' position during sitting creates arterial bending, which can hamper flow and increase peripheral resistance [47]. In addition, the chair's pressure on the lower limbs can also compress the leg and reduce flow [41]. Reduced lower limb blood flow can be quantified by measuring calf circumference to measure lower leg swelling with a sensitive strain gauge. This method is seen in multiple studies [45, 46], including previous work from our laboratory, although the more accurate and commonly used method is flow-mediated dilation [48].

At the core of these vascular issues is the ***reduced shear stress*** associated with lower blood flow and blood pooling in the lower limbs during sitting [49]. On top of these mechanical restraints to blood flow, prolonged sitting affects the vasculature at an endothelial level, affecting many biomarkers essential in vascular health [49, 50]. These associations have been reported in two recent systematic reviews [37, 51].

It is important to note that inflammation plays a role in these processes described above and is a fundamental part of vascular health [43, 52]. Notably, it has been shown that inflammation can result from and/or cause vascular dysfunction [49, 50]. Inflammation may be more intrinsically linked to the vascular dysfunction seen during prolonged sedentary time. It may be correlated to several markers, specifically, pro-inflammatory cytokines, which may be responsible for these processes. These markers and hypotheses will be explored in later sections.

Metabolic effects

Sedentarism has been strongly implicated in altering metabolic processes and their biomarkers [2]. Most, if not all, of sedentary time effects have been linked to a worsening metabolic profile [2, 22]. Reviews of the prospective studies on sedentary time and health have repeatedly linked excess sedentary time with an increased risk of obesity and type 2 diabetes, two of the most common metabolic disorders [17, 21, 22, 26, 53]. Several acute/experimental studies have examined biomarkers within these conditions (obesity and diabetes). Several biological measures, including glucose tolerance, lipid and glucose profile, fasting insulin, and insulin sensitivity, have been examined after acute bouts of

sitting [2]. Although interpreting studies as a whole is complex due to the varying sitting protocols, ranging from a few hours in a sitting position to seven days in bed rest, they have a similar trend of a worsened metabolic profile [2].

Several studies have reported that ***lipid profiles*** are negatively affected by prolonged sitting. Fasting triglycerides are shown to be significantly increased after prolonged sedentary time [54-56], high-density lipoprotein cholesterol is shown to decrease in both sexes [54, 56], and low-density lipoprotein is shown to increase in multiple studies [55, 56].

Several biological measures of glucose homeostasis are altered during prolonged sedentary time. Frequently examined measures include fasting ***glucose***, fasting ***insulin***, glucose tolerance and insulin sensitivity [2]. Multiple studies have shown trends or significant decreases in insulin sensitivity [55, 57-59]. Fasting glucose levels tend to be higher after sedentary time, with longer durations associated with greater fasting glucose levels [55, 57, 60, 61]. Most studies show a small to moderate increase, but some inconsistencies exist between experimental studies. Insulin sensitivity is consistently shown to be reduced, and glucose tolerance shows moderate to large decreases in response to acute sedentary behaviour [2]. These metabolic effects compounded onto one another show that sedentary time can have a prominent effect on metabolic health, increasing the risk of several metabolic diseases [37, 52, 62].

Although some of the work above involved bed rest studies (an extreme version of sedentarism), it is one of the standard methods used in experimental sedentary research [2, 8, 11, 13, 37]. Several reviews have quantified acute bouts of sedentary time in varying forms and intensities, ranging from <1 day (standard sitting position) up to 7 days (bed rest) [2]. This work has brought to light many of the consequences of prolonged sedentary time, with metabolic, vascular, and inflammatory factors being the most prominent [2, 11, 37]. Building upon this work, protocols using standard sitting (in a chair) of 4-8 hours may be more feasible, and this work can allow a more translational and generalizable approach.

Similar to the vascular effects of sedentary behaviour, inflammation also has an integral role in metabolic dysfunction [52, 63]. Uncontrolled or chronic levels of inflammation have been linked to many of the metabolic conditions noted above and a

worsened metabolic profile [52, 63]. Specifically, obesity and diabetes have been extensively linked to chronic inflammation, both as a consequence and etiology [64-66]. Sitting's role as a potential pro-inflammatory stimulus in increasing metabolic disease is a growing area of interest.

Summary: In epidemiological and experimental research studies, prolonged sedentary time can negatively alter vascular and metabolic health.

Gaps: The physiological disruptions linked to sedentary time warrant further work within sedentary physiology. Work examining inflammatory markers in experimental designs is needed to elucidate mechanistic pathways between sedentary time and health outcomes.

1.3. Inflammation

1.3.1 Chronic inflammation

Inflammation is not an event that occurs in isolation; it involves a multitude of mechanisms and responses. Inflammation is an essential component of the immune system, and its effects can be broadly described by the acronym SHARP: swelling, heat, loss of function, redness, and pain [66]. The inflammatory process involves several immunological events, including chemoattraction of several white blood cells, activation of the complement proteins, increased natural killer cell activity, the release of several inflammatory mediators, heightened body temperature, increased vascular permeability and flow, and stimulation of acute-phase proteins [52, 67]. Most notably, in the context of this work, inflammation initiates the release of an assortment of cytokines and chemokines [68, 69]. Although inflammation is an essential component of the immune response critical for homeostasis, errors or alterations in this system can occur, resulting in disease [66, 68].

Although the above mainly describes acute inflammation, it is important to note its distinction from chronic inflammation. Chronic systemic low-grade inflammation is an inflammatory response that is not eliminated or controlled or when inflammatory pathways are being over/unnecessarily stimulated [66]. This chronic inflammatory state can cause permanent damage and is linked to many diseases [52, 66]. Chronic inflammation may not be as potent as some acute inflammatory responses as, in many chronic scenarios, the body is trying to control the inflammation through varying anti-inflammatory mechanisms [66,

68, 70]. There is a need for balance between pro and anti-inflammatory states, and chronic inflammation is a swing in this balance to a pro-inflammatory one.

The term “inflammaging” is an excellent way to elaborate on the concept of chronic low-grade inflammation, elaborating how inflammation may be one of the mechanisms linking aging to disease [67]. More and more conditions are linked to chronic inflammatory states through varying pathways or mechanisms [66, 67]. For example, diabetes [52] and insulin resistance [71] have been strongly linked to pro-inflammatory states or pathways associated with immune cells’ infiltration and inflamed adipose tissues, causing chronic elevation of pro-inflammatory markers [63]. Cardiovascular disease is closely linked to pro-inflammatory processes, with immune cells playing an important role, such as atherosclerosis development and progression [52, 68]. A strong link has been established between neurodegenerative diseases and chronic inflammation [72]. Neuroinflammation has become a prominent research topic associated with neurological and psychiatric conditions, such as Parkinson’s disease [52, 72]. It is vital to note that this inflammation is not always localized; it can be systemic, meaning that many of the body’s systems can be affected.

1.3.2. Measuring Inflammation: Cytokines

Cytokines' function and role in inflammation may be critical in understanding the mechanistic link between sedentary time and chronic physiological disruptions leading to disease states. An overview of cytokines is provided below before a more detailed examination of the specific link between sedentary time, and inflammation is explored.

Cytokines are released from nearly all nucleated cells, and nearly all cells can respond to them [31, 73-75]. Cytokines are small molecular proteins that vary substantially from one another and are primarily categorized by their type of function, not structure; such a subcategory includes chemokines [31, 73-75]. Primarily, cytokines are key mediators of host responses to a stressor, including disease or infection and are involved in many mechanisms involving maintaining homeostasis [75]. Cytokines differ from other messenger molecules, like hormones, as they are released by nearly all cells, not just specialized cells, with cytokines being released in response to signals, not rhythmically [31, 74, 75]. This distinction is essential, as this reactivity of cytokines provides important

information for understanding cytokine responses. Cytokines can be released acutely and chronically, and this is now a well-established area of interest in immunology and a growing area associated with the study of physical stressors. Such areas include acute and chronic responses to exercise or sitting, commonly referred to as the field of exercise immunology [31, 75-77].

Although cytokines are produced by many cells, they are primarily involved and studied in an immunologic context. They are heavily involved in the generation, management and termination of immune and inflammatory actions [74, 75]. Sixty plus cytokines have been identified, with some yet to be discovered. A list of the more commonly studied cytokines is provided in Table 1.

Table 1. List of Commonly Studied Cytokines

Name	
IL-1α	IL-14
IL-1β	IL-15
IL-2	IL-16
IL-3	IL-17
IL-4	IL-18
IL-3	IL-32
IL-4	TGF- β
IL-5	TNF- α
IL-6	TNF- β
IL-7	IFN- α
IL-8	IFN- β
IL-9	IFN- γ
IL-10	M-CSF
IL-11	G-CSF
IL-12	GM-CSF
IL-13	MIF

Table 1 Note: IL: Interleukin, TGF: Transforming growth factor, TNF: Tumour necrosis factor, IFN: Interferon, M-CSF: Macrophage colony-stimulating factor, G-CSF: Granulocyte colony-stimulating factor, GM-CSF: Granulocyte-macrophage colony-stimulating factor, MIF: Macrophage migration inhibitory factor.

Cytokines can be classified as pro-inflammatory or anti-inflammatory [73, 74, 78]. They are considered pro-inflammatory when they initiate or are part of inflammatory cascades, causing the release of several inflammatory molecules [75]. The most potent pro-inflammatory cytokines are IL-1, tumour necrosis factor-alpha (TNF- α), and IL-8, with

IL-1 (α and β) and TNF-α having an essential synergistic role in inflammatory responses [75, 79, 80].

Anti-inflammatory cytokines disrupt or diminish the intensity of inflammatory cascades [75]. The most noted anti-inflammatory cytokines are interleukin 1 receptor antagonist (IL-1ra), IL-4, IL-6, IL-10, IL-11, IL-13 and transforming growth factor-beta (TGF-β) [74, 75, 81]. These cytokines usually function to suppress or diminish pro-inflammatory cytokines' function, inhibiting various inflammatory cells and pathways (mainly IL-1, TNF- α, and IL-8 inhibition) [74]. They also promote anti-inflammatory cells/pathways and greater expression of anti-inflammatory soluble cytokine receptors (sTNFR1/R2, sIL-1R2, mIL-1R2, IL-18BP) [74, 75].

This dichotomy or distinction of pro and anti-inflammatory is not true in all scenarios, with many cytokines playing a role on either side. Cytokine function is highly dependent on the pathway, stimulus, source, target cell and more [73]. For example, IL-6, a well-known pro-inflammatory cytokine, is increased in an infection/sepsis reaction, stimulated by IL-1, and causes the release of the acute phase protein C reactive protein (CRP) in a classical acute phase response [75, 82, 83]. However, it is considered anti-inflammatory when IL-6 is released as a myokine in response to exercise. It then stimulates the release of IL-1ra and IL-10, inhibiting the inflammatory cytokine IL-1 without inducing CRP or TNF-α [81, 84]. Although this is a specific example, it details how a single cytokine can have a different role based on the physiological stimulus. As with most immunologic molecules, the key is balance. The balance between pro and anti-inflammatory cytokines plays a crucial role in several disease processes and disease risk [73-75, 85, 86]. It is this potential imbalance that can lead to chronic inflammatory states. Over-stimulated or unchecked inflammation can swing this balance to a pro-inflammatory state, potentially transforming into chronic low-grade inflammation related to many negative health implications.

Table 2 provides descriptions of the commonly examined cytokines in response to physical stress providing further context about the varied roles cytokines can play. The type (pro or anti-inflammatory), source and action/function will be described briefly. IL-8 is excluded from this table and will be discussed individually later.

Table 2. Commonly investigated cytokines in response to physical stress

Cytokine	Type	Source	Action/Function
IL-6	IL-6 is both pro-inflammatory and anti-inflammatory. This distinction mainly depends on the stimulus and mechanism of release [84].	IL-6 is produced by many immune cells, including T and B lymphocytes, macrophages, and natural killer (NK) cells. IL-6 can also be produced/released by nonimmune cells such as smooth and skeletal muscle, chondrocytes, astrocytes, and glial cells [87].	IL-6 is one of the more potent mediators of the acute phase response in a more traditional inflammatory pathway. During a sepsis or infection response, there is an initial marked increase in TNF-α and IL-1β, which are well-known inflammatory markers. This is followed closely by a significant increase in IL-6 [84]. IL-6 is an important signal for CRP to be released from the liver in the acute phase response. IL-6 and CRP are known to induce cell proliferation, differentiation and programmed cell death; it has been reported that elevated IL-6 at rest may indicate an increased disease risk [88]. IL-6 is also anti-inflammatory. When induced through exercise, IL-6 can exhibit anti-inflammatory functions on other cytokines, like IL-1β and TNF-α and promote the release of potent anti-inflammatory cytokines IL-1ra and IL-10 [84]. The increases seen in exercise-induced IL-6 can be drastic, with reports of 100-fold increases after intense exercise (marathon) [89]. Exercise-induced IL-6 does not mimic the same pathway seen in sepsis or disease. The myokine form of IL-6 functions more beneficially to combat exercise inflammation/damage by creating an anti-inflammatory cascade with IL-1ra and IL-10 [84]. IL-6 has also been indicated as a modulator of metabolism and has been reported to increase lipolysis and fat oxidation [85]. IL-6's anti-inflammatory role could be considered regulatory as it causes the release of other anti-inflammatory cytokines.
IL-1ra	IL-1ra (receptor antagonist) is an anti-inflammatory cytokine.	IL-1ra can be released through signalling from IL-6, IL-4, IL-10 and others to counteract inflammation. IL-1ra is shown to be induced by IL-6 release, especially exercise-induced IL-6 [84].	IL-1ra inhibits pro-inflammatory IL-1 (IL-1α and IL-1β) when stimulated from IL-6. IL-1ra acts to buffer the pro-inflammatory effects of other cytokines [87]. IL-1ra is considered a pure receptor antagonist. As the name implies, IL-1ra acts to block IL-1α, IL-1β, and its family members by blocking receptors and blocking their function [80]; this is important as IL-1 has been shown in several disease models. IL-1ra has received attention as a potential treatment as an anti-inflammatory agent. [80]. IL-1ra's ability to block IL-1 is also shown to lower CRP and IL-6, functioning as a block in the

			inflammation cascade; for example, the blocking of IL-1β helps protect pancreatic beta cells [90]. IL-1ra deficient mice show a state of severe chronic inflammation that develops immediately and causes extreme inflammation of joints and erosion of bone, being linked to rheumatoid arthritis (RA) in humans. The absence of IL-1ra allows IL-1 to act unopposed with extreme consequences [80].
IL-1β	Interleukin 1-beta (IL-1β) is a pro-inflammatory cytokine.	IL-1β is produced by macrophages, microglia, muscle cells, and B and T lymphocytes during the innate response [80]. The primary sources of IL-1β are blood monocytes, tissue macrophages and dendritic cells [80].	IL-1β functions as an essential cytokine in the inflammatory cascade and promotes TNF-α and IL-6 release [91]. IL-1β has been noted in many conditions, including rheumatoid arthritis (RA), and is used as a clinical biomarker in cardiovascular disease [80]. Excessive IL-1β has been reported in many cancers, with IL-1β being expressed by tumours aiding in their neoplastic nature, mainly in blood cancers [80].
TNF-α	TNF-α is a pro-inflammatory cytokine.	TNF-α is primarily released from macrophages, NK cells, microglia, T lymphocytes, Kupffer cells and endothelial cells [87].	TNF- α plays a crucial role in the inflammatory response, promoting the release of IL-1β and IL-6. It has a substantial role in the acute phase of inflammation, angiogenesis, cell differentiation and apoptosis [91]. TNF- α is also important in activating several prostaglandins (PGs) and substances involved in pain; TNF-α is noted as a primary mediator of chronic pain [92]. TNF-α is used as a marker in the clinical setting for inflammation in several autoimmune conditions [93]. TNF-α's basic role is as a tumour necrotizing factor, but it also aids in the margination of lymphocytes to a site of inflammation, increasing the expression of adhesion molecules [87]. TNF-α is usually viewed negatively (pro-inflammatory) when elevated and has been reported to play a key role in tissue wasting in cachexia in cancer patients and many other conditions [79].
IL-5	IL-5 is a pro-inflammatory cytokine based on eosinophilic inflammation.	IL-5 is secreted/produced by hematopoietic cells, including T cells, Th2 helper cells, mast cells and	IL-5 is a part of the granulocyte-monocyte colony-stimulating factor (GM-CSF) family; this description also relates to its function [95]. The primary function of IL-5 is the activation, survival and migration of eosinophils [96], as IL-5 is most involved in the maturation and activation of eosinophils [97]. IL-5 stimulation results in eosinophil

		eosinophils. IL-5 is also released from non-hematopoietic cells, such as epithelial cells and helper cells [94].	degranulation, proliferation, differentiation and survival, with many of these effects seen in the airways [98]. IL-5 directs the eosinophil response, regulating the recruitment of eosinophils to the inflammation site, and causing them to activate [98]. IL-5 in humans is reported only to affect basophils and eosinophils [99]. IL-5 is linked to asthma, as excess eosinophil activation can cause hyperreactivity and inflammation in the airways, especially in uncontrolled hypereosinophilic asthma [100].
IP-10	Interferon-gamma inducible protein-10 (IP-10) is a pro-inflammatory/regulatory chemokine.	IP-10 secretion is mainly determined by interferon-gamma release (IFN-y) and other endogenous cytokines such as TNF-α, IL-2, IL-17, IL-23, IL-27, IFN-α and IFN-β [101]. IP-10 is released by a multitude of cells depending on the stimulus. These cells include monocytes, T cells, NK cells, endothelial cells, and stromal cells, with the majority of IP-10 released from monocytes [102].	IP-10 is a chemotactic cytokine that plays a critical role in the recruitment of Th1 cells, NK cells, macrophages and dendritic cells into locations of inflammation [103]. IP-10 also binds to many G protein-coupled receptors and causes cellular effects that inhibit endothelial cell proliferation, growth factor independent hematopoiesis, and tumour necrosis [103]. IP-10 can also encourage T cell adhesion to endothelial cells, inhibit angiogenesis, and inhibit colony formation by the bone marrow [104]. IP-10 is a mediator of many pathophysiological conditions, many of which are in the lungs, including sarcoidosis, pulmonary fibrosis, emphysema, and asthma [103]. Excessive expression and production of IP-10 by T cells have been associated with smoking-induced emphysema, resulting in reduced lung elasticity [103].
CRP*	CRP is a well-known pro-inflammatory acute-phase protein. CRP is also anti-inflammatory in the immediate response, where it is elevated rapidly to high	CRP is synthesized and secreted by the liver, mainly in response to IL-6, and CRP is usually enhanced synergistically with IL-1β [106].	C-reactive protein (CRP) is heavily involved in the acute phase response. It is secreted from the liver in response to IL-6, which can elevate pro-inflammatory cytokines during infections [105]. CRP plays an anti-inflammatory role during a normal acute phase response, responding very quickly with high levels [105]. CRP is also integrated into the complement cascade pathway and acts with C5 for proper complement functioning [105]. CRP levels can influence whether the body responds in a pro or anti-inflammatory manner [105]. Even in small amounts, consistently elevated CRP can

	levels. However, CRP is mainly studied and known as pro-inflammatory [105].		indicate chronic low-grade inflammation, which is linked to several diseases [105]. Clinically, resting CRP levels are used to detect acute infections, assess treatment response, and evaluate chronic inflammation status in chronic diseases. An example of this is that elevated resting CRP is correlated with mild inflammation in vascular diseases (atherosclerosis and coronary heart disease) [107].

*CRP is not technically a cytokine (it is an acute-phase protein but highly involved in inflammatory responses). High sensitivity or highly sensitive C-reactive protein (hs-CRP) is no different from normal CRP; the difference relates to the nomenclature [108], and the "high sensitivity" refers to the assay assessment type being used to assess CRP [108].

The inflammatory (pro vs anti) balance discussed previously is why examining physical stressors, such as exercise, is essential. For example, with pro-inflammatory states associated with many diseases (levels beyond normal), exercise is proposed to be anti-inflammatory [84]. It is hypothesized that increasing the amount of exercise (the anti-inflammatory stimulus) will change this balance to an anti-inflammatory or a disease prevention state [31, 84, 109, 110]. On the other hand, prolonged sedentary time is hypothesized to be associated with increased inflammation, indicating a potential increase in pro-inflammatory cytokines [48, 111, 112]. Based on this, it can be hypothesized that repeated bouts of prolonged sitting can alter this balance to a pro-inflammatory state [112, 113]. This relationship has been explored in several studies that examined interrupted sitting, with the interruption usually being some form of exercise, with protocols (exercise and sitting protocols) varying with each study. It has been shown that this interruption can potentially reduce or attenuate inflammation, but more work is needed [32, 48, 51, 111, 114]. Much less work has been done assessing prolonged sitting alone, and many important cytokines have yet to be examined.

Summary: Inflammation, an essential immune defence and healing process, can become uncontrolled and stimulated chronically. Cytokines, which are molecular proteins, can be anti and/or pro-inflammatory and are commonly used to measure inflammation.

Gaps: Most of the existing research in the context of sedentary behaviour is from cross-sectional studies. Acute experimental studies examining inflammatory markers and sitting are rare, and several cytokines, including IL-8, have yet to be examined in detail.

1.4. Interleukin 8

IL-8, also frequently referred to as CXCL8, is considered a chemokine. Chemokines are a subgroup of cytokines that primarily function as chemotactic factors. IL-8 is within the CXC family of chemokines, and this designation is based on its protein sequence. This protein sequence represents the cysteine arrangement, with CXC meaning the cysteine residues are separated by another amino acid. CXC chemokines are an immunologic subset of proteins primarily involved in the chemotaxis of neutrophils and lymphocytes [115]. IL-8, in an immunologic context, is a potent chemotactic factor essential for homeostasis. It is a critical pro-inflammatory cytokine, heavily involved in several aspects of neutrophil

function, including changes in neutrophil shape and migration, movement of storage proteins, and respiratory burst [116]. Although IL-8's primary role is an essential chemoattractant [116-118], it also acts to strengthen the adhesion or binding of immune cells, such as monocytes, to the endothelium to improve the extravasation of immune cells [117]. This addition of IL-8 during adhesion can cause a conformational change in integrins, such as lymphocyte function-associated antigen-1 (LFA-1), to increase its ligand's binding affinity (its strength) to the endothelium surface during the high shear stress and rates of blood flow in vessels [117, 118]. IL-8 is not alone in many of these functions, as there is redundancy from other molecules. However, blockage studies indicate that IL-8 is one of the more potent mediators of these functions, especially chemotaxis [116]. Based on these functions, IL-8 meets the classical definition as a ***pro-inflammatory cytokine*** that directly stimulates inflammatory responses [115, 116].

IL-8 is released/produced from many different cells; this includes endothelial cells, monocytes, fibroblasts, macrophages, neutrophils, T lymphocytes and more. Its release can also be up-regulated by multiple stimuli, including IL-1, another potent pro-inflammatory cytokine [119]. IL-8 receptors are located on many structures, including neutrophils, monocytes, natural killer cells, and basophils and function to attract neutrophils to a site of inflammation/infection (by chemotaxis) and mediate their activation [118]. IL-8 has also been reported to recruit T cells and attach to B cell receptors [87].

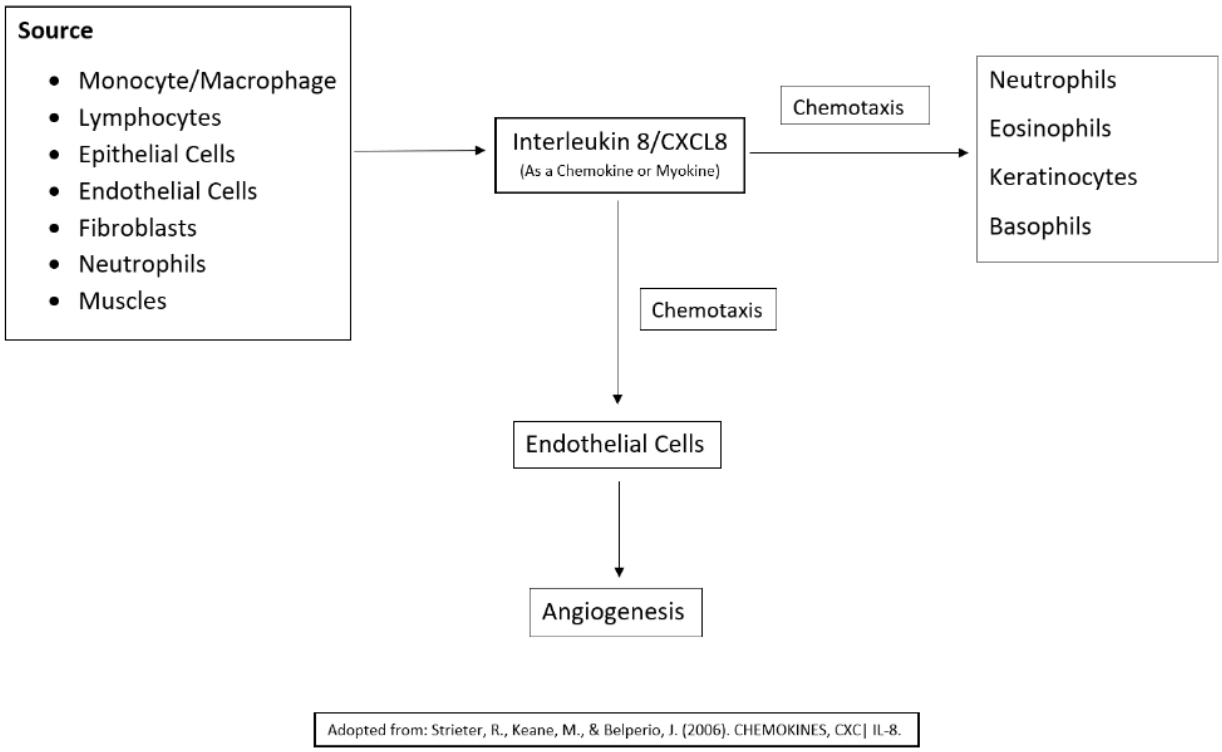
Although IL-8 is a potent chemotactic factor, it has other known functions, and importantly, it can be produced or released in ***response to physical stimuli***. One of IL-8's other primary functions is to act as an angiogenic factor, that is, to create an increase in blood vessels, which occurs in the microvascular endothelial cells in varying tissues [120-123]. The angiogenic role of IL-8 has been examined in varying contexts, such as pathology and exercise adaptations. IL-8 employs strong angiogenic effects on endothelial cells, which are highly coated in IL-8 receptors (CXCR1 and CXCR2) [120]. The potent angiogenic function of IL-8 means it plays a critical role in vascular health and function, alongside its immunologic role, and often, both of these functions work in tandem [120].

IL-8 is not only released from immunologic cells; it is also released from muscles after exercise, both concentric and eccentric [124]. Several studies have reported that IL-8

can be directly released from muscles, and in this context, IL-8 can be described as a ***myokine*** [82, 125]. IL-8 can act in an autocrine and paracrine manner in response to exercise, acting locally in angiogenesis and chemotaxis of white blood cells into the muscle; this chemotaxis is usually related to muscle damage [119]. Interestingly, the muscles themselves are also responsive to IL-8 as exercise protocols have been shown to increase IL-8 receptors (CXCR2) when assessed by muscle biopsy [126], possibly responsible for the vascular adaptations with exercise. Exercise (30 minutes of running at 75% VO_2 max) increases circulating levels of angiogenic and inflammatory markers, including IL-8 [127]. IL-8's role as a myokine is less understood than its immune effects, but the function of IL-8 as a myokine is important, as these studies show that IL-8 will respond acutely to physical stress. Plasma IL-8 has also been shown to be elevated in overweight/obese individuals, as IL-8 can be released as an adipokine (cytokine release from adipose tissue) [128].

To summarize the role of IL-8, its normal physiological functions are provided in Figure 1, adopted from Strieter, R., Keane, M., & Belperio, J. (2006) [117].

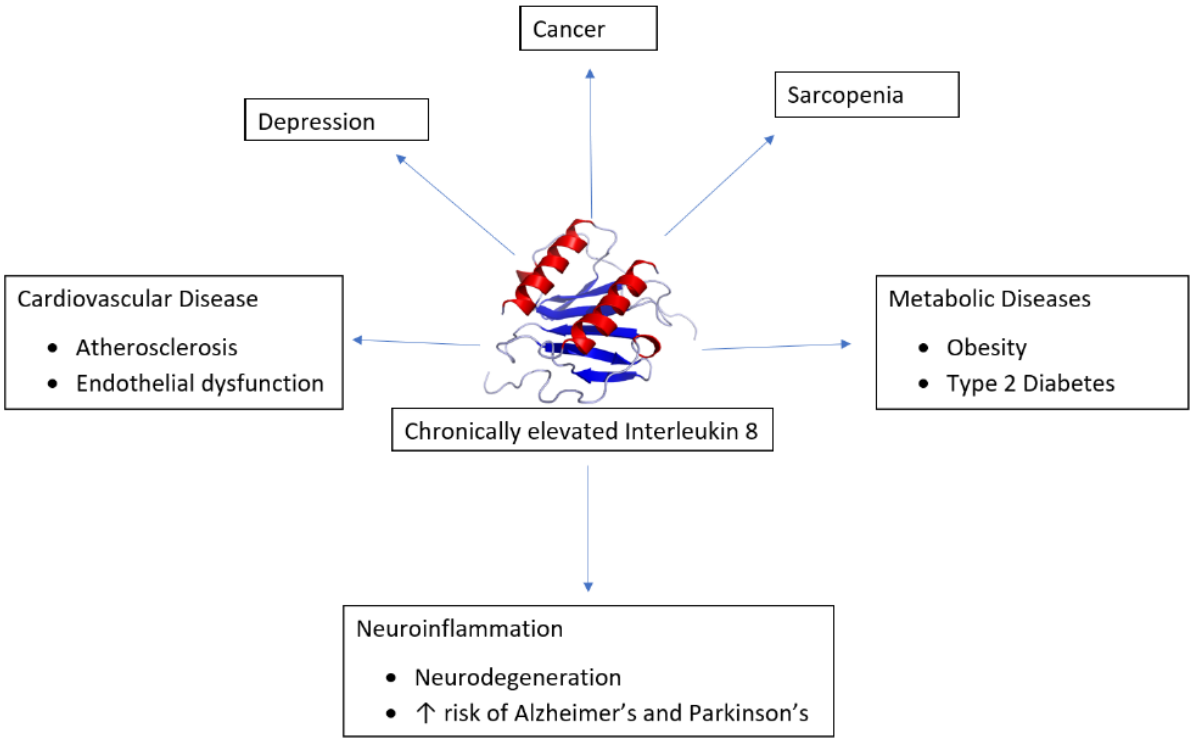
Figure 1.1 Physiological mechanisms of Interleukin 8 (CXCL8).



The ***dual function*** of IL-8 (inflammatory and angiogenic) distinguishes it from other cytokines, especially as both functions are linked to several disease processes when elevated beyond normal levels [66]. Sitting increases inflammation and reduces vascular function, both of which could involve IL-8 [11, 37]. The decision to focus on IL-8 in this thesis, as opposed to the other cytokines listed in the previous section, is based on this dual role of IL-8 and the possible mechanistic link between sedentarism and chronic physiological disruptions.

As with most immunologic markers, ensuring a balance of not too much or too little is needed for homeostasis. Being at either end of the spectrum can be pathologic. This concept applies to IL-8 as well. Because IL-8 has a role as a potent angiogenic and chemotactic factor, its chronic elevation can be inherently involved in several diseases [66]. Several conditions have been linked to chronically elevated IL-8 [52, 63, 70, 72, 129-132]. Figure 2 shows several conditions linked to elevated IL-8, and the commonality of these conditions and their relation to IL-8 is their link to chronic inflammation.

Figure 1.2. Chronically elevated IL-8 and its link to diseases
References for Figure 2: Depression [129], Cancer [130], Sarcopenia [131], Cardiovascular Disease [132], Neuroinflammation [70, 72], Metabolic Diseases [52, 63].



Our previous work [48, 133] and multiple reviews state that IL-8 is responsive to physical stress [91, 134], but little work has examined IL-8 in response to sitting. Prolonged sitting influences vascular function and the inflammatory profile [11, 37]; thus, using IL-8 to explore these phenomena could provide a potential mechanism to link both of these detrimental effects of excessive sedentarism.

IL-8 Measurement

The assessment of cytokines can be done through various means, but the most frequently used and often considered the gold standard method is to collect serum or plasma through venipuncture [91]. Collecting venipuncture samples is to draw blood and analyze biomarkers released or present in the bloodstream to gain a systemic view of pro-inflammatory markers [91]. Performing blood sampling requires special training and safety measures to ensure blood is withdrawn safely, often making it difficult logistically. Most

studies have used blood to analyze cytokine responses, but it is not without drawbacks [91]. When performing this experimental work, participants can experience acute laboratory stress, and a factor that can add to this stress includes the needles used to draw blood [91]. Furthermore, breaking the skin itself can cause a slight inflammatory reaction. The venipuncture process can cause significant stress in some individuals, both physical and psychological, leading to a potential acute increase in inflammation [91].

Saliva is a safe, non-invasive alternative to blood for assessing inflammation since taking saliva samples is less stressful for the participant; it does not involve breaking the skin, needles, or the sight of blood [91]. Slavish et al. (2015) gathered the relatively small body of work that examined changes in cytokines levels in response to stress using saliva [91]. In this review, several commonly assessed cytokines were examined. Although IL-8 was not included, this review establishes that pro and anti-inflammatory cytokines are detectable and responsive to stressful stimuli using salivary analysis [91].

A more recently published systematic review and meta-analysis by Szabo et al. (2020) expanded on this previous review. Additional studies of cytokines such as IL-8 were included in this review; this review also examined studies assessing the response of cytokines to psychological and physical stressors [134]. Importantly, this updated review found that, after post hoc sensitivity analysis, IL-8 increases from pre to post stressor exposure (psychological and physical), indicating the ability to detect changes in salivary IL-8 concentrations [134]. Although Slavish, in their 2015 review, did not include IL-8, to our knowledge, only a small number of experimental studies have examined IL-8 using saliva in response to stress. However, none of the examined studies in either review looked at sitting alone as the stressor, indicating a further gap in knowledge.

A small amount of work has shown that IL-8 is responsive to sedentary time. Two studies conducted in our laboratory have assessed and successfully detected significant changes in IL-8 concentrations using saliva [48, 133]. One study examined prolonged sitting and sitting with interruption on inflammatory and cardiometabolic markers [48]. This study will be examined in more detail later, but it noted significant differences in salivary IL-8 after a prolonged sitting session of 4 hours [48], using the same collection methods. Another study from our laboratory examined the inflammatory response to

exercise in cold-dry environmental conditions [133]. Various cytokines, including IL-8, were measured using saliva and IL-8 increased from pre to post-exercise in the normal temperature condition [133]. Although this looked at the acute response and was an exercise intervention, it again shows that IL-8 is responsive using salivary analysis with the same technique we plan to use.

Even though saliva is much easier to use for both the researcher and participant, there are some limitations. It lacks the same methodological consistency regarding sample collection (protocols, timing, stimulated vs unstimulated, collection methods etc.). Several publications that have aimed to compare venous, saliva, and capillary samples have shown that saliva can be significantly different from venipuncture and capillary samples in determining several markers (e.g., they do not always correlate) [135-140]. The lack of correlation between saliva, venous and capillary samples does not necessarily reflect the validity of salivary methods; it simply indicates that the numbers will be different, and caution is needed in comparing across studies.

Although saliva is not always correlated to venous blood samples, this does not necessarily make it invalid. The mechanisms of salivary secretion and its constituents may not and possibly should not need to be compared to blood [141, 142]. We know saliva can be used to detect changes in cytokines, including IL-8 [48, 134, 143-145], and has great potential for widespread clinical use, with its ease and speed of use, and that it could be easily used in point of care settings [146, 147]. Saliva is its own secretion, having its own set of mechanisms, and more research should aim to standardize and understand (1) the responses of salivary cytokines to several types of stimuli, (2) create standardized sample collection procedures for clinical use, and (3) understand the daily variability and fluctuations of salivary cytokines.

Interestingly, there is a strong correlation between capillary and venous samples for several markers, including cytokines. Several studies have validated capillary samples against the venous sampling in assessing several cytokines [148-150], and assays to measure capillary blood IL-8 are effective [151, 152]. Additionally, capillary measurements are suggested to be comparable to venous samples in determining general blood sample parameters (white blood cells, red blood cells, hematocrit, hemoglobin) [153,

154], drug concentrations [155], concentration and function of leukocyte subsets [156], total thrombocyte and leukocyte count [157], hemoglobin concentration [158], cortisol (in response to exercise) [159], and viral detection [160]. Thus, capillary samples can be a more practical alternative to venous samples.

Summary: IL-8 is linked to several diseases and is responsive to physical stress. It is detectable in the saliva and capillary blood.

Gap: The role of IL-8 in prolonged sitting (a form of physical stress) has yet to be explored in detail.

1.5. Movement and Inflammation

1.5.1. Sitting and inflammation

As mentioned above, sedentary time is associated with many chronic diseases and these diseases are all associated with chronic low-grade inflammation. As previously mentioned, prolonged sedentary time may increase this inflammation [48, 161, 162], one of the key mechanistic links between sedentarism and chronic physiological disruptions (alterations of homeostasis).

A standard measure used to quantify chronic inflammation is elevated levels of pro-inflammatory cytokines. Interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) have received the most attention regarding inflammatory states [52]. However, many others have been studied and play critical physiological roles. Of relevance to our work, chronically elevated Interleukin 8 (IL-8) levels have been connected with various inflammatory diseases [82, 115, 132, 163].

Moreover, some experimental work has explored this hypothesis (inflammation and sitting). Although relatively rare, these studies have examined the association between sedentary time and inflammation using pro-inflammatory cytokines and biomarkers, mainly assessing the cytokine and acute phase protein IL-6 and CRP, respectively. Sedentary time can be measured directly and indirectly. Much of the current work uses self-reported sitting time (indirect), which has its flaws [112]; several studies using self-reported behaviour have detected elevated levels of pro-inflammatory markers with

increased sedentary time [112, 164]. Several studies have shown increased IL-6 and CRP in several cross-sectional studies, mainly using pre and post measurements [112, 164]. Some studies describe the potential for sex differences in inflammatory responses to sitting, but this needs further investigation. Notably, experimental work using acute bouts of sitting is still lacking, especially in examining specific inflammatory cytokines.

Although most studies did not examine IL-8, they provide an established link between sitting and pro-inflammatory markers, and importantly, many of these inflammatory markers are associated with diseases. For example, CRP and IL-6 are implicated in many chronic diseases and are commonly examined biomarkers used in healthcare [107]. Studying other cytokines, particularly IL-8, can provide more insights into the mechanisms responsible for some of the negative consequences of prolonged sedentary behaviour.

1.5.2. IL-8 and Exercise

Before discussing IL-8 and sitting, it is valuable to contrast IL-8 as a myokine (released from muscles) or exerkine (released in response to exercise). Significantly more research has been conducted to examine changes in levels of IL-8 (and other cytokines) in response to exercise of varied intensity, duration, and frequency in several populations [82]. Using studies that performed exercise interventions provides a baseline understanding of IL-8 and its response to a stressful stimulus, particularly physical stress.

Firstly, it is important to summarize exercise's overall hypothesized role in inflammation. A recently released systematic review and meta-analysis discussed the role of exercise and chronic inflammation [165], and this review further establishes the role of exercise as a potential anti-inflammatory stimulus [165]. It was shown that CRP was the most significantly altered marker with exercise and that exercise intensity plays a role [165]. Higher intensity exercise tended to be more advantageous than lower intensity exercise in middle-aged adults in reducing inflammation [165]. Although IL-8 was not examined in this review, it is important to understand that movement of high intensity [165], coupled with previous research that notes interrupted sitting (walking, standing, fidgeting etc.) [37, 48, 111], can beneficially impact inflammatory biomarker profiles.

Focusing on IL-8, its levels tend to increase with exercise [82], with the central hypothesis for this increase being IL-8s role in angiogenesis or as an angiogenic factor [123]. Endothelial cells contain receptors for IL-8, and it is believed that the elevated IL-8 with exercise is partly responsible for the post-exercise-induced angiogenesis adaptations [161], which is one of the key vascular adaptations imposed by exercise. As a myokine, IL-8 can have autocrine and paracrine effects, acting locally, functioning in angiogenesis, and chemotaxis of white blood cells into the muscle, seemingly concerning exercise-induced muscle damage [124]. This chemotactic function of IL-8 after exercise-induced muscle damage attracts neutrophils to the site of damage, initiating an inflammatory response, intending to remove/clean the debris from the cellular damage, meeting the classical definition of an inflammatory response; at least when muscle damage occurs [82, 115]. Although this hypothesis is less robust when assessing concentric exercise [78], IL-8 can still increase, functioning in angiogenesis [82]. IL-8s overall role as a myokine is less well understood than its immunological and clinical effects, as previously discussed.

IL-8, like IL-6, has also been shown to play a role in metabolism [166, 167]. Exercising with carbohydrate ingestion reduces IL-8 and IL-6 release significantly compared to exercise with low carbohydrate intake (low muscle glycogen) [166, 167]. IL-6 and IL-8 releases are increased during a metabolic crisis in the muscle when there is low muscle glycogen or glucose availability. IL-8 mRNA expression is increased in this low carbohydrate state, acting locally in the muscle [166, 167]. These findings also highlight the importance of attempting to control diet during studies.

Table 3 and 4 below provides an array of studies that have examined IL-8 and its response to acute and chronic exercise, respectively, with exercise of varying intensity, mode, duration, and frequency. In addition, a variety of ages and clinical populations have been examined. In most of these studies, IL-8 is being measured along with many other cytokines, but only IL-8 responses are reported here.

Table 1.3. IL-8 Response to an Acute Bout of Exercise

Reference	Population/ Demographics	Type (saliva, plasma, serum)	Exercise/ Intervention Details	IL-8 Response (Direction)	Responsiveness/ Timing	Resting Values	IL-8 Response
[168]	Experienced marathon runners n=10, age 21-39	Plasma	42.2 km marathon race (avg duration 2h 34min)	Significantly increased	Pre-race samples were taken a day before, and post-race were taken within 10min after completion	Mean: 22(pg. ml $^{-1}$) SD: 9 (pg.ml $^{-1}$)	Mean: 55 (pg.ml $^{-1}$) SD: 25 (pg. ml $^{-1}$)
[78]	n=8 untrained men (students)	Plasma	90 W, 90min cycling session 3 days in a row	Increased	Samples were taken pre and post. No significant increase on day 1, but a significant increase on day 3	Session 1: 5.4 pg/ml ± 1.6 Session 3: 4.4 pg/ml ± 2.7	Session 1: 7.1 pg/ml ± 5.5 Session 3: 7.5 pg/ml ± 2.8
[169]	2 groups Lean control (n=10) and overweight/Obese group (n=12)	Serum	- High intensity interval exercise (HIIE) 10 × 60s (85–90%PMax)/75s (50%PMax) - Moderate intensity interval exercise (MIIE) 10 × 60s (70–75%PMax)/60s (50%PMax)	Increased significantly immediately after HIIE but drops below resting level 30min post-exercise. MIIE had no effect	Samples were taken pre, post and 30 min after. Increases are seen immediately after. IL-8 was decreased (from resting) 30 min post-exercise (similar for both overweight and lean groups)	Overweight: 1.77 ± 0.20 pg.ml Lean: 1.03 ± 0.11 pg.ml	Overweight: ~2.5 pg.ml Lean: ~ 1.65 (immediately post-exercise)

[171]	10 M, 10F Avg age 29 5 in control 5 in the yoga group	Saliva	Yogic breathing/TMP (a type of yoga) – one on one session with yoga instructor 10 min of breathing exercises and 10min of TMP yoga	Significantly reduced compared to control	Samples were taken before, 5min, 10, 15- and 20-min of intervention. Changed significantly over time. Lowered and continued to lower at each measurement	Yoga: 364.68 pg/mL	Yoga: 20min: 196.05
[172]	24 male long-distance runners taking high carb diet/supplement Avg age: 36.5, avg VO2 of 60	Plasma	2 sessions of treadmill running at 70% Vo2 max to exhaustion separated by 4 weeks	Increased	Samples pre and post. A Significant increase was seen when exercising to exhaustion	4.24 pg/ml	14.9 pg/ml
[89]	10 M marathon runners, avg age 31.7	Plasma	Marathon race – 42.195km	Increased	Samples were taken pre and post – with large increases post-marathon Was significant	1.16 pg/ml	11.06 pg/ml
[173]	11 Sedentary women. 6 obese and 5 without obesity Avg age of 30 Avg BMI 35 (obese group)	Plasma	60min of cycling at 60% Vo2 peak – moderate-intensity continuous cycling	Initial increase then decrease	Tested at baseline, post-intervention and up to 24h post-exercise. IL-8 peaked at 2h post but dropped 24h post below resting	OB: ~3 Control: ~2.2	OB: ~2.1 Control: ~1.6

Table 1.4. IL-8 Response to Regular Exercise

Reference	Population/ Demographics	Type (saliva, plasma, serum)	Exercise/ Intervention Details	IL-8 Response (Direction)	Responsiveness/ Timing	Resting Values	IL-8 Response
[174]	8 healthy young males	Serum	6 HIIT sessions over 2 weeks (3 per week). Progressive training, intervals increased every 2 sessions 60s at 100% VO2 and 75s rest at 50W (from 8-12 intervals)	Small but significant increase after acute HIIT session in both sessions 1 and 6	Samples were taken at the first and last sessions. Taken before, after and 15,30, and 45 min after. All significant changes from resting values	Session 1: ~ 4.5 pg/ml Session 6: ~ 4 pg/ml	Session 1: ~ 7 pg/ml Session 6: ~ 6.5 pg/ml
[175]	Women with fibromyalgia (n=125) and healthy controls (n=130) Age 20-65	Plasma	15-week (2x per week) progressive resistance training – 10 cycle warm-up, 50min of RT mainly of the lower limb – progressed from 40% MVC to 70/80%MVC or relaxation program (RCT design)	Increased	Measured baseline and post-intervention – an immediate increase was seen	FM: 1.4 (2.2) Healthy: 0.9 (1.9)	Reported change in levels using Δ Exercise: Δ -0.3
[170]	30 patients with moderate to severe COPD – avg age of 63	Plasma	2 exercise groups – ET and RT – Both groups did 3 sessions per wk for 8wks, 35min sessions	Minimal decrease – not significant in both the ET	Samples were taken 48 hr before the first and after the last training – no response	ET ~ 7.6 RT ~ 5.9	ET ~ 7.55 RT ~ 5.8

			ET: Cycling or treadmill at 14-15 RPE RT: 4 strength exercises for the major muscle groups	and RT groups			
[176]	71 sedentary adults with periodontal disease – 30 in control and 31 Tai Chi Age range: 60-74	Saliva (flow cytometry)	Tai Chi 5 days per week for 6 months	Decreased in both groups	Samples take pre and post-intervention	Control: 3560.53 ± 809 Tai Chi: 4971.24 ± 835 (pg/mL)	Control: 3215.66 ± 260 Tai chi: 2252.42 ± 330 (pg/mL)
[177]	28 men with stable, ischemic chronic heart failure Avg age 67, all on conventional medication therapy	Plasma	3-month long exercise program – 2 wks in cardiac rehab 3x/wk - 10 min warm-up, 25 min endurance training (walking or jogging) and 10 min cooldown Then exercise at home 30min on cycle 3x/wk for the rest of the time	Decreased – but not significant	Samples were taken pre and post-intervention; after the 12 weeks – Resting levels of TNFa, IL-6, and IL-8 in CHF patients are reported to be much higher than non-CHF in sex/age-matched controls	12.7 ± 9.2 pg/ml	11.2 ± 4.7 pg/ml
[128]	15 F, 12 M with severe obesity avg BMI over 45.8 kg/m2	Plasma	15wk lifestyle intervention – including a hypocaloric diet and	Decreased	Samples were taken pre and post – trends were of decreased inflammatory markers post –	5.6±0.6 ng/l	4.8±0.4 ng/l

			moderate daily PA: 203hr of MVPA 5/wk		significantly lower IL-8 was reported but related this decrease to a loss of adipose tissue, not the exercise itself		
[178]	51 women with metabolic syndrome 31 intervention 21 control Avg age: 59.8	Serum	6-month therapeutic lifestyle modification program Including health monitoring and counselling, health education, diet and exercise. 3x/wk for the first 3 months then 2x/wk the next 3 – aerobic dance class – avg 200kcal expenditure	Increased then decreased in the intervention.	Samples were taken at baseline, halfway (3 months) and postintervention IL-8 was elevated and midpoint but decreased at post, but still higher than baseline. The Control group continued to elevate with a peak post-intervention	2.6 pg/ml	3 months: 3.8 pg/ml 6 months: 2.9
[179]	424 Seniors aged 70-89 at risk for disability Avg age – 76.4 Avg BMI 30.7 2 groups, active and control (health education)	Plasma	12-month PA intervention Aerobic, strength, balance and flexibility are all trained. 3 phases – phase 1 supervised 3x/wk Phase 2 – supervised 2x/wk, home workout 3x/wk	Increased	Measure baseline, 6 months and 12 months post-intervention. The increase peaked at the midpoint and decreased slightly at 12 months, but IL8 increased from baseline	7.50 pg/mL	6months: 7.96 pg/mL 12 months: 7.77 pg/mL

			Phase 3 – 3+x/wk at home				
[180]	37 adults with T2DM Avg age of 54.9 Avg BMI 29.6	Plasma	12 weeks program of either HIIT or MICT group. Participants were then given a 40wk home exercise program HIIT – WU for 10 min at 70%HR, 4x4 INTERVALS AT 90-95% HRm, with 3min recovery at 70% between. Total of 40min 3x/wk MICT – 210min per week of unsupervised MICT on bike	Increased in HIIT and MICT	Measures were taken at baseline, 12wks, and 1 yr. IL8 levels in HIIT dropped at 12 wks but was higher than baseline at 1yr; the same trend was seen in MICT	HIIT: 2.25 pg/mL MICT: 1.95	HIIT: 12wk: 1.91 1yr: 2.29 MICT: 12wk:1.63 1yr: 2.04
[181]	17 M and F with diagnosed pre-diabetes put into a HIIT or MICT group Avg age: 26.2 Avg BMI: 31.6	Serum	Both 4x/wk for 8wks MICT – 20 min at 55-50%HRR with 5 min UP and 5 min CD HIIT – Ten 1min intervals at 75-80%HRR, with 1 min rest at 35-40%HRR	MICT – increase HIIT – decrease	Samples were taken pre and post MICT and HIIT seem to have opposing effects on IL-8; MICT increased IL8 while HIIT decreased	MICT- 8.2 HIIT- 10.7 pg/mL	MICT- 11.7 HIIT- 8.6 pg/mL

[182]	31 elderly stable COPD patients Mean age 77.2 17 in exercise and supplement and 14 in control	Serum	3-month home-based Light intensity pulmonary rehab combined with nutritional supplementation Breathing exercises, walking for 15min, respiratory muscle training also with an education program The intensity was set at 40-50%Vo2	Decrease	Samples were taken before and after 12 wk program – IL-8 was significantly decreased in the exercise group and was ns increased in controls	Ctrl- 1.50 Exercise- 1.97 pg/ml	Ctrl- 1.77 Exercise- 1.07 pg/ml
[183]	20 obese postmenopausal breast cancer survivors Put into Ex or control group Avg age – 53 BMI- 33	Plasma	16 wk supervised aerobic and RT program 3x/wk for 150mins MVPA total 5 min WU 40-50%VO2max, full-body RT in circuit training 80%1RM for lower body and 60% for the upper body all 3x10 after RT they did cycling/treadmill at 65-70%HRm for 30-50min Exercised were progressed	Decrease in EX	Samples were taken pre and post-intervention EX group showed reduced IL-8 compared to the control group which showed an increase	EX- 5.53 ng/dL Crtl- 5.46 ng/dL	EX- ~ 4.25 Ctrl- ~ 5.94 ng/dL

[184]	46 postmenopausal breast cancer survivor women put into an AT+RT group or control Avg age- 56.2	Serum	3 month, 160min/wk. 2x/wk of moderate to vigorous walking, and 2xwk of RT with bands	Decrease	Samples were before and after the intervention IL-8 decreased in the exercise group but was ns. The Ctrl group showed a minor increase	EX- 6.8 Ctrl- 6.1 pg/ml	Ex- 5.4 Ctrl- 6.3 pg/ml
[185]	58 patients with moderate or severe asthma Aged 20-59	Serum	3month/12week aerobic program 2x/wk for 35min (5min warm-up, 25min training, 5min cooldown) or educational control group	Increase	Samples were taken before and after the intervention. IL-8 increased significantly in the HIIT group.	Ex- 1564.0 Ctrl- 1713.9 fg/mL	Ex- ~1882.8 Ctrl- ~1765.6 fg/mL
[186]	49 patients with HIV infection	Plasma	Concurrent training (RT and ET in the same session) 3xwk for 16 weeks 20min of treadmill at 50-70%HRR	Decrease	Samples were taken before and after the intervention. IL-8 was decreased significantly in the training group and increased in the controls.	Ex- 8.0 Ctrl- 7.1 pg/ml	Ex- 5.4 Ctrl- 8.1 pg/ml
[187]	62 elderly postmenopausal women aged 65-85	Serum	12 week at home bench step exercise Bench step with a height of 15cm and 20cm – 20minsessions with the goal of 40	Increase	Samples were taken before and after the 12week intervention. IL-8 showed a minor decrease after the exercise intervention, but it was NS	Ex- 4.63 Ctrl- 5.22 pg/mL	Ex- ~4.54 Ctrl- ~5.13 pg/mL

			steps/min, increased by 10step/min every 4min with 2 min rest intervals				
[64]	20 obese adolescents	Serum	12-week exercise program – 2x60min sessions and 1 120min per week for 12wks 60min sessions – 30min aerobic, 20 RT 120min session – 2 bouts of 25min of aerobic and 2 20min bouts of RT	Decrease	Samples were taken at baseline and after the 12week intervention. IL-8 showed a minor decrease after the intervention, but the change was NS	~5.9 pg/mL	~ 5.7 pg/mL
[170]	30 patients with moderate to severe COPD – avg age of 63	Plasma	2 exercise groups – ET and RT – Both groups did 3 sessions per wk for 8wks, 35min sessions ET: Cycling or treadmill at 14-15 RPE RT: 4 strength exercises for the major muscle groups	Minimal decrease – not significant in both the ET and RT groups	Samples were taken 48 hr before the first and after the last training – no response	ET ~ 7.6 RT ~ 5.9	ET ~ 7.55 RT ~ 5.8

Interpretation of the above studies provides insight into how IL-8 responds to stress, particularly physical stress. To broadly summarize the above tables, regular exercise tends to lower IL-8, indicating an anti-inflammatory effect of regular exercise. However, the effects of acute exercise are unclear, with IL-8 increasing and decreasing in several studies. This variation in response is likely related to differences in exercise protocols, and the population studied. Although tables 3 and 4 are not extensive, there is an apparent lack of studies examining acute responses, and most of the acute studies involve athletes. Nevertheless, these studies can provide insights into changes in cytokine concentration in response to exercise. These studies show that IL-8 can change relatively quickly by adding or removing a stressful stimulus. Changes seem to become more significant the more prolonged, intense, and frequent the stimulus is applied. These facts are relevant to our work because when participants are exposed to our prolonged sitting protocol, we predict to see the same inflammatory progression and amplification as we progress through the protocol. We anticipate that the longer duration of sitting, the greater the inflammatory response will be over time (assessed by IL-8). By taking measurements on multiple occasions, we hope to determine a time course and the natural variability of the inflammatory response of IL-8, an area yet to be explored [188].

1.5.3. IL-8 and sitting

In order to establish context for our proposed work, a detailed look at the previous studies looking at IL-8 and sitting is warranted. Only a minimal amount of work has examined the effects of prolonged sitting on IL-8, and even less has been done using acute protocols, notably on healthy participants. To our knowledge, the response of IL-8 to sitting has only been examined in a small handful of studies, with most being cross-sectional and on various populations. Each study below, including previous work from our laboratory, has looked at IL-8 and its response to sitting; with this said, we will explore them in more detail and summarise their importance.

The first study we will be examining looked at the association between physical activity and sedentary behaviour in pregnant women [189]. These pregnant women (n=50) participated in a 1yr cross-sectional study, where a triaxial accelerometer tracked sedentary behaviour for seven days. Several immunometabolic and pro and anti-inflammatory

biomarkers were analyzed by serum. Notable findings included that meeting the activity guidelines was positively associated with a higher level of IL-8 ($p=0.06$) and that none of the inflammatory or metabolic markers, including IL-8, were associated with increased sitting time [189]. The lack of association between IL-8 and sitting time could be attributed to the findings that pregnant women seem to have greater resting IL-8 than non-pregnant women [190]. Therefore, the physical stimulus of sedentary behaviour may not be enough to cause a significant change in an already elevated inflammatory state [189]. In contrast, the increased IL-8 levels induced by exercise in their study is likely because of its release as a myokine due to muscle contraction and/or muscle damage, as discussed previously [82]. Pregnancy adds another level of complexity to the function of IL-8, and it becomes challenging to distinguish elevations due to pregnancy or the imposed physical stress, sitting or exercise.

In the study above [189], valuable insight can still be drawn even though IL-8 increased after exercise and was not associated with increased sitting time in this population. Firstly, it shows the importance of existing inflammation and how this can muddle the acute responses occurring and provides another example of controlling for as many factors as possible. Secondly, it provides another example of the proposed role of IL-8 as an exerkine, being responsive to exercise-induced stress. Based on these results, if exercise is used to interrupt sitting, it raises questions about whether an increase or decrease is expected. Similar to Table 3, regular exercise in this study lowered IL-8, but the effects of sedentary behaviour are not as clear.

The second cross-sectional study assessed the association between sitting and interrupted sitting times with many measures, including muscle mass, strength, function, and inflammation in community-dwelling older adults [111]. Again, the participant's sedentary time and activity were collected with a validated tool (ActivPAL) for seven consecutive days. These researchers examined various inflammatory cytokines using serum, including IL-8 [111], and interestingly, this study demonstrated that sitting was not associated with inflammatory marker changes. It is important to note that this study used a composite z-score for cytokines, then summed them, combining all the pro-inflammatory cytokines into one variable. In producing this composite z-score, anti-inflammatory

cytokines were subtracted from this score, giving an overall inflammatory score (sum of all pro-inflammatory cytokines minus anti-inflammatory cytokines) [111]. Using this method makes it difficult to reach a conclusion regarding any specific cytokine, such as IL-8; however, it provides a broader lens to inflammatory markers, providing an interesting way to assess the balance of pro and anti-inflammatory cytokines. Surprisingly, a correlation between inflammation and sitting did not exist, even though they reported that sitting time increased the likelihood of obesity and sarcopenia, which have been linked with states of chronic inflammation [76]. The possibility that the older adult population already has a higher inflammatory state (at rest) and that older adults are the most sedentary age group [191] could make changes from baseline to the end of the study difficult to achieve or observe.

Moving to the previous work conducted in our laboratory, which is likely the most similar to our proposed work, we examined prolonged sitting versus interrupted sitting, looking at various inflammatory markers in young, healthy adults. The prolonged sitting session was 4 hours in duration, and in the intervention session, sitting was interrupted by a short supramaximal 20sec cycling sprint every hour [48]. During the 4 hours, the total activity was 9 minutes, including warm-up and cool down, with 1min of actual supramaximal cycling. Many cytokines and biomarkers were assessed using saliva, and vascular function was also assessed. Importantly, acute prolonged sitting was associated with a significant increase in IL-8 ($p < 0.001$); this is a crucial finding we aim to build on, indicating that prolonged sitting can be a pro-inflammatory stimulus. During the interrupted sitting, IL-8 decreased, showing an anti-inflammatory effect of acute exercise. The results indicate that the short supramaximal exercise interruption can attenuate the negative sitting response, indicated by the increase in IL-8 during prolonged sitting. We also observed vascular function indirectly using a strain gauge, and interestingly vascular function improved during the interrupted session (leg swell was significantly decreased). We can hypothesize that the effects of interruption could be related to vascular improvements, since IL-8 has a vital role in endothelial function. From a myokine perspective, our exercise interruption protocol was unlikely to be a significant enough stimulus (intensity, duration, frequency) to increase IL-8 from the working muscle or cause exercise-induced adaptions (angiogenesis) and muscle damage linked to IL-8.

In summary, the most important finding is that sitting interruption can attenuate the negative increase in IL-8 seen in prolonged sitting, but more work needs to be done to clarify this. From a vascular standpoint, leg swell (assessed by strain gauge) was significantly increased in the prolonged sitting group, with swelling becoming greater the longer the duration. In contrast, the interrupted sitting protocol significantly attenuated leg swell and inflammation over the study [48].

Although this pilot study discussed above [48] provides the platform for our proposed research, it has some limitations. Limitations include that the sample size was relatively small and was underpowered to perform sex-based analysis, especially since sex differences in acute sitting protocols without interruption have yet to be addressed. Furthermore, the exercise mode (high-intensity sprints on a cycle ergometer) may not be realistic in day-to-day settings and may have caused a physiological disruption that skewed the findings. Other methods, such as short bouts of walking, which are more realistic, should be examined in the context of inflammation. With that in mind, the use of IL-8 to assess these differences in an acute protocol can allow for novel findings.

Section 5. Summary

Summary: The role of IL-8 in exercise as a myokine can provide insights into what may be expected during prolonged sitting, another form of physical stress.

Gaps: The cross-sectional nature of the existing studies makes it difficult to infer causation between the effect of sedentary behaviour on IL-8. There is a clear need for more acute experimental studies on IL-8, a mechanistically and clinically important cytokine.

1.6. Purposes and Hypotheses

To our knowledge, the studies described in the last section are the only studies that have examined IL-8 and sedentary time experimentally. Although the two cross-sectional studies did not show a significant change in IL-8, they both used a clinical population (older adults and pregnant women). The measurements were taken over a long period, making it challenging to draw definitive conclusions about IL-8's role in sedentary behaviour. Our previous work and the proposed mechanistic actions of IL-8 described in section 4 are the foundation of our proposed work. With the knowledge that IL-8 is responsive to sitting and

its potential role in inflammation during sitting, further work is needed. Sitting for 4-8 hours is typical for most Canadian individuals [18], and using walking interruption, may be more representative than our previous protocol [48]. The acute responses of pro-inflammatory cytokines to sitting are understudied, and this work can bring to light an important pathway linking sitting to chronic physiological disruptions, using IL-8 as the model.

Thus, the purpose of this work is to:

1. Determine the daily variability in concentrations of salivary IL-8.
   Hypothesis: We expect that the variability across three measures of fasting salivary IL-8 will be similar to what has been previously observed from plasma samples.
2. Examine changes in capillary plasma and salivary IL-8 in response to prolonged sitting and sitting interrupted by 3 minutes of light intensity activity every 30 minutes in young, healthy adults.
   Hypothesis: We hypothesize that sitting will increase IL-8 concentrations from baseline in the prolonged sitting session and that interrupting sitting with bouts of walking will attenuate this increase in IL-8.

With the information provided in this literature review, our hypothesis can be summarized in Figures 3 and 4. Prolonged sitting will increase the pro-inflammatory biomarker IL-8, and the associated decrease in vascular function will also increase inflammation, likely through IL-8 related mechanisms, causing an acute increase in inflammation. In opposition to this, sitting interrupted with short bouts of walking should attenuate or have opposing effects on the response of IL-8. Knowing the acute effects of sitting (increased pro-inflammatory cytokines), repeated bouts of prolonged sedentary time could theoretically cause an increased risk of chronic inflammation and its associated diseases based on these repeated acute exposures.

Figure 1.3. IL-8's hypothesized role in acute prolonged sitting.

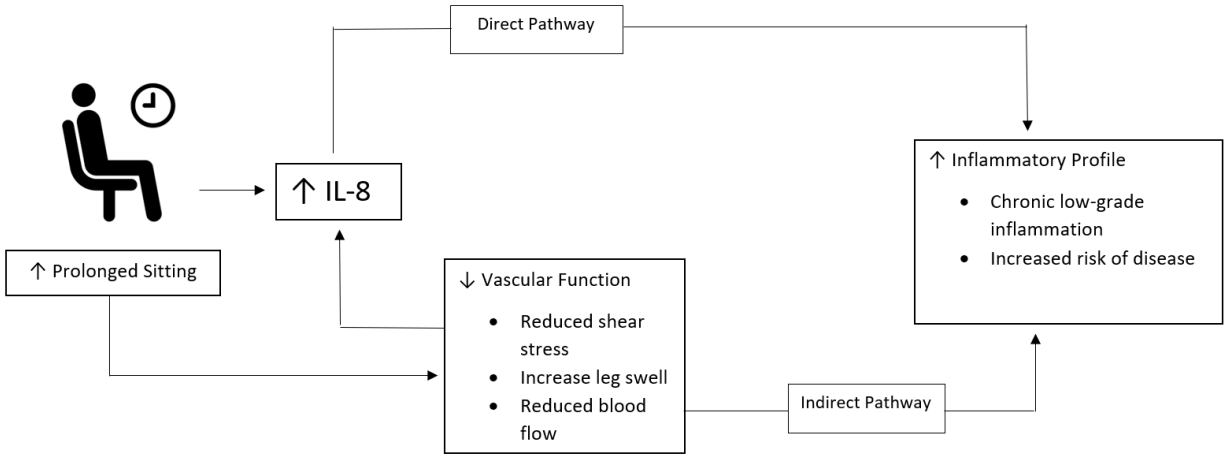
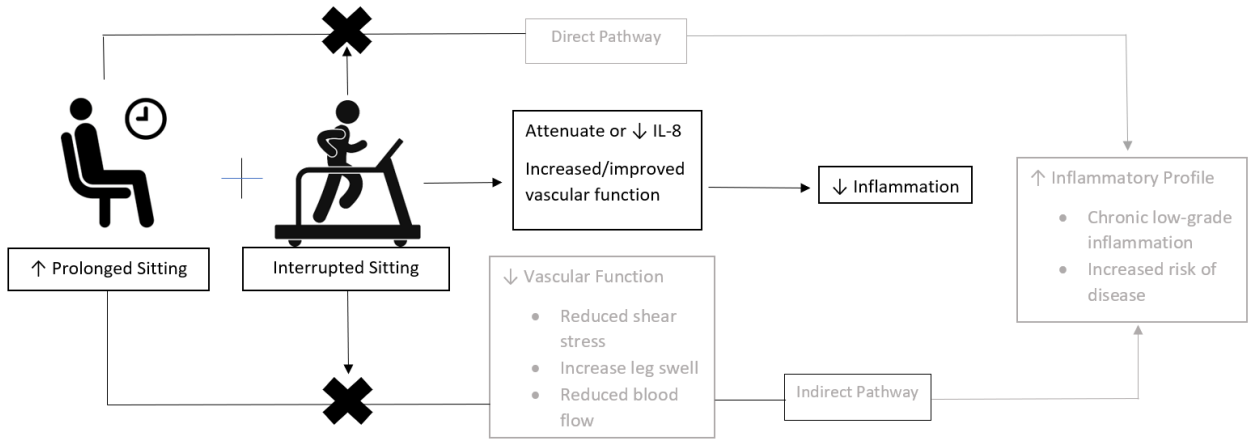


Figure 1.4. Interrupted sitting's hypothesized role on IL-8



Building on the hypotheses proposed in Figure 3 and 4, several mechanisms of IL-8 activity must also be considered (chemotaxis, angiogenesis, adhesion). As mentioned in earlier sections, prolonged sitting can reduce shear stress, and this alteration of vascular function may perturb IL-8's role in this adhesion process [45, 49]. This increase in binding activity (for adhesion) by IL-8 that happens during infection or repair typically occurs when the shear rate is relatively high (during homeostasis) in the vessels of our body. In contrast, prolonged sedentary time can reduce shear stress [45, 49], with some possible extreme scenarios and consequences, such as blood clots (i.e., those reported after prolonged air travel) [43, 192]. It can be hypothesized that the role of IL-8 in this lower shear rate

environment (during prolonged sitting) can be altered. In this reduced shear rate state, immune cells may need less support from chemokines such as IL-8 to allow or strengthen adhesion. Based on this, it can be hypothesized that an increase in systemic IL-8 due to prolonged sitting may relate to the lower IL-8 needed at the sites of chemotaxis due to the lower shear rate and shear stress in vessels. However, other cytokines, like TNF-α that up-regulate intracellular adhesion molecule-1 (ICAM-1), may also play a role in this proposed scenario.

In addition, the hypothesized increase in IL-8 in acute prolonged sitting may be related to an increased pro-inflammatory environment due to several factors discussed earlier. Nevertheless, based on our current mechanistic understanding, these changes in IL-8 may be in response to changes in vascular function. As previously mentioned, IL-8 plays a role in angiogenesis, potentially as a countermeasure to the reduced blood flow during excessive sitting, pointing to another possible way to explain its proposed increase [121-123, 127]. However, the role of IL-8 in adhesion may be another potential avenue of interest, as mentioned above. Still, it is more likely that all these processes are co-occurring, leading to an increase in IL-8 and inducing a pro-inflammatory state [116, 123]. Nonetheless, it seems that whatever the mechanism, prolonged acute sedentarism disrupts homeostasis, and using IL-8 to hypothesize these changes, based on its known functions and interactions, is worthy of investigating.

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Chapter 2. Manuscript

Response of Salivary IL-8 to Prolonged and Interrupted Sitting

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European Journal of Applied Physiology

2.1 Abstract

Background: Experimental studies examining the effects of prolonged sitting have shown physiological changes in vascular and metabolic health markers with sitting between 2-8 hours. However, changes to inflammatory markers, such as cytokines, have been poorly examined in acute studies. ***Purpose:*** We aimed to determine day to day variability of salivary IL-8 (CXCL8) and the response of IL-8 (salivary and plasma) to prolonged and interrupted sitting. ***Methods:*** Using a randomized crossover study design, healthy participants completed a prolonged and interrupted sitting session. The prolonged session consisted of four hours of continuous sitting while the interrupted session included an interruption (3 minutes of walking) in sitting every 30 minutes. Saliva and capillary plasma were collected pre and post-session. Samples were analyzed using ELISA. ***Results:*** Prolonged sitting increased salivary IL-8 concentrations (PS: T1 median: 22.09 pg/ml, T2 median: 86.18 pg/ml; p=<0.01), while interrupted sitting attenuated this response (IS: T1 median: 22.09 pg/ml, T2 median: 51.99 pg/ml; p=0.021). Females had a more pronounced salivary IL-8 response to prolonged and interrupted sitting than males. Our results indicate that within individuals, salivary IL-8 concentrations range from consistent to highly variable. No significant results for plasma were seen. ***Conclusion:*** We found that four hours of prolonged sitting significantly increased salivary concentrations of the pro-inflammatory cytokine IL-8. Interrupting prolonged sitting every thirty minutes with walking seems to blunt the increase in IL-8 seen during prolonged sitting, especially in males. Of note, individual variability should be more carefully considered as the response to the sessions varied across participants.

Keywords

Inflammation, sedentary physiology, IL-8/CXCL8, salivary cytokines, prolonged sitting, interrupted sitting

2.2 Introduction

Sedentary behaviour, defined as sitting or lying activities that require less than 1.5 metabolic equivalents (METs) [1], is a pervasive behaviour associated with adverse physiological consequences. A sedentary lifestyle has been linked to altered body composition [2], increased risk of type 2 diabetes [3], higher rates of cardiovascular disease [4], cancer [5], and all-cause mortality [6]. Notably, even short bouts of sitting for 1-6 hours can lead to impairments in vascular function, blood pressure [7], shear stress [8], lipid profiles (altered HDL, LDL, and TG) [9], glucose tolerance, and insulin sensitivity [10].

Experimental work examining such bouts of sitting has consistently shown a positive effect of physical activity on various cardiometabolic markers [11, 12]. However, there is a dearth of such experimental studies looking at pro or anti-inflammatory markers. A recent study examining the effect of sitting for 4 hours compared to sitting for 4 hours interrupted by exercise found that salivary concentrations of Interleukin-8 (IL-8) increased in response to prolonged sitting ($p < 0.001$; T1: 0.19 ± 0.32, T2: 0.50 ± 1.00) but this increase was attenuated with by a short supra-maximal 20-second cycling sprint every hour, totaling 9 minutes of activity including a short warmup and cooldown [13]. Although several cytokines were examined, IL-8 was the most responsive and consistent. IL-8 is a pro-inflammatory chemokine (chemoattractant cytokine) that has a *dual function* (pro-inflammatory and angiogenic) that distinguishes it from other cytokines [14]. Interestingly, previous research using cross-sectional data using accelerometry did not show a significant association between IL-8 and sedentary time, although a trend was observed [15, 16].

Sedentary behaviour is consistently associated with elevated levels of pro-inflammatory markers [17, 18]. Research on physical activity suggests that different types and intensities of activity can serve as a potent anti-inflammatory stimulus [19-21]. For example, acute exercise can immediately alter the immunological environment and promote the release of IL-6 as a myokine shown to promote anti-inflammatory pathways, including increasing IL-10 and IL-1ra, which are well-known anti-inflammatory cytokines [20, 22]. It is becoming clear that interrupting prolonged bouts of sedentary time with physical activity can attenuate the pro-inflammatory effects of sitting [23, 24]. However, a limited amount of experimental work has examined such acute effects. Although exercise

intensity has been shown to play a role in the proposed anti-inflammatory effects of exercise [21], sitting interruptions of lighter intensity that are more realistic, practical, and implementable are worthy of investigation. Determining the lowest level of interruption needed to disrupt the pro-inflammatory nature of prolonged sitting would allow for an effective intervention strategy.

Of note, most studies on cytokines to date have used serum samples. However, the use of salivary cytokine analysis is limited. Saliva can be considered a more feasible biomarker source than venipuncture (serum/plasma) as it is easier to collect (little training required), cheaper, less stressful for the participant and non-invasive [25, 26]. A recent review and meta-analysis found that salivary IL-8 increases in response to a stressor (psychological and physical) [26]. Thus, our study aims to examine changes in the concentration of IL-8 in capillary plasma and saliva in response to *prolonged sitting* and *interrupted sitting* using 3 minutes of light intensity activity every 30 minutes in young, healthy adults. We hypothesized that concentrations of IL-8 would increase from baseline in the prolonged sitting session and that interrupting sitting with bouts of walking would attenuate this increase in IL-8. Additionally, we aimed to determine the weekly variability in concentrations of salivary IL-8. We hypothesized that the variability across three measures of fasting salivary IL-8 would be low within individuals.

2.3 Methods

Study Design: A randomized cross-over design was used wherein a random number generator randomly allocated participants to either the prolonged sitting (PS) or interrupted sitting (IS) for their first sitting session. Randomization was balanced.

Participants: Eligible participants were those between the ages of 18-30 years who were non-smokers without any existing respiratory, cardiovascular, or metabolic conditions that could affect inflammation and who were not taking medications that would impact their inflammatory or exercise response. Individuals with an injury that would impede running were also excluded. All participants provided written informed consent prior to participation in the study. This study was approved by the Research Ethics Board at the Ontario Tech University.

Protocols: Each participant completed three laboratory sessions. Session 1 was used to collect baseline measurements and to conduct a maximal exercise test. Sessions 2 and 3 were performed in random order. Participants were seated continuously for 4 hours in the PS session without interruption. In the IS session, participants were seated for 4 hours; however, they performed 3 minutes of light intensity activity (60%HRmax) every 30 minutes (at times: 30, 60, 90, 120, 150, 180, 210, 240min). Throughout both sitting sessions, participants were instructed to perform as little lower limb movement as possible. Participants were also permitted to use their upper limbs to use a computer or a device of their choosing for the study duration.

Measures:

Baseline Measures: During session 1, resting heart rate, resting blood pressure (A&D Medical Digital Blood Pressure Monitor, Model UA -767FAM, A&D Engineering, Inc. San Jose, CA, USA), height (cm), and body mass (kg) (Detecto Weigh Beam Eye-Level, Webb City, Missouri), were measured.

Maximal exercise: An incremental to maximal exercise test using a stepwise protocol (increase every 2 minutes) was performed on a treadmill (Trackmaster, FullVision, Newton, KS). Participants were fitted with a portable HR monitor (Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland) for continuous measurement of HR. Rating of Perceived Exertion (RPE) was measured every 2 minutes at the end of each stage. Participants were connected to a metabolic cart for gas analysis (Parvo Medics 2400, USA). Using the metabolic cart, concentrations of expired O_2 and CO_2 were analyzed, and ventilation was measured. Test termination criteria included a Respiratory Exchange Ratio (RER) >1.15, HR ±10 beats per minute (bpm) of age-predicted maximal HR (220-age), a plateau in oxygen uptake (VO_2), or volitional fatigue. The highest HR recorded during the test was used for HRmax. The determination of the participant's VO_2max was done by calculating the highest VO_2 that was attained during the test. After finding the peak VO_2, VO_2max was a mean of +/-5 breaths from this value. An RER of greater than 1.15 was also used to confirm that participants have achieved VO_2max [27]**.**

Saliva Collection and Analysis: Baseline saliva samples were taken upon arrival at the start of the three sessions. Participants were instructed to refrain from alcohol, smoking, strenuous exercise and use of anti-inflammatory medications in the preceding 24 hours (regular smokers and those on routine anti-inflammatory medications were excluded). On the day of their session, participants were instructed to be fasted (overnight fast), not to have any caffeine/stimulant, supplements, or mouthwash, and were told to attempt to drink 1L of water. Saliva samples were also taken at the end of the PS and IS sessions. Nutritional intake during the sessions was controlled, as a standardized breakfast and snack were provided. (590 Calories; Fats 6.5g, Carbohydrates 127g, Proteins 11.1g)

Saliva samples were collected using oral swabs (Salimetrics SalivaBio, Salimetrics LLC, State College, PA, USA). The swab was placed under the tongue for 5 minutes and immediately centrifuged (VWR Clinical 2000, Germany) at 4000 rpm for 5 minutes and collected 1.5-3ml of saliva. Saliva samples were then placed into a -80 °C freezer. Once removed from the freezer on the day of analysis, salivary samples were centrifuged for 15 min at 1500 x g at 4C° to remove mucins and particulate matter that could potentially interfere with antibody binding. Prior to cytokine analysis, total protein content within each saliva sample was determined using the Coomassie PLUS Protein Assay Reagent (Thermo Fisher Scientific, MA, USA). IL-8 levels (R&D Systems, Catalog# DY208) were quantified by enzyme-linked immunosorbent assays (ELISA), following the manufacturer's protocols (R&D Systems, MN, USA), using 96-well high-binding microplates (Greiner Bio-One, NC, USA), and read at a wavelength of 450 nm using a Cytation 5 microplate reader (Bio-Tek Instrumentation, VT, USA). Saliva samples were tested in triplicate, and the average values were used for data analysis. An IL-8 standard curve ranging from 7.82 pg/mL to 500 pg/mL was used and saliva samples were tested at a 1:4 dilution; 1:4 dilutions have frequently been used in salivary cytokine literature [28]

Capillary Blood Collection and Analysis: Capillary plasma samples were taken upon arrival before participants began their sitting sessions. Capillary samples were also collected as a post-measurement upon completion of the IS and PS sessions. A finger prick technique (capillary sampling) using a lancet (HTL-STREFA, Inc. Marietta, GA, USA) and capillary tube (Alere San Diego Inc. San Diego, CA, USA) was used to obtain a blood

sample of 40-200μL. Before the capillary samples were collected, the hand was warmed using a disposable hand warmer for 5 minutes to arterialize the blood at the fingerpick site. Capillary samples were centrifuged (VWR Clinical 2000, Germany) at 3800 rpm for 5 minutes immediately after collection to separate plasma. Separated plasma was stored in anticoagulant-coated microcentrifuge tubes in a -80°C freezer. IL-8 concentrations in plasma samples were quantified as described for saliva samples, but plasma samples were tested in duplicate, and the average values used for data analysis. As IL-8 within plasma tends to be relatively low in healthy persons and, therefore, commonly run at no dilution [29], plasma samples were tested at a 1:1 dilution, similar to many other studies where limited sample volumes were collected [30]. Additionally, two samples were run at a 1:5 dilution due to low amounts of samples (<50ul). An additional two were run at a 1:50 dilution as their IL-8 concentration initially exceeded our detection limit. All plasma samples were run after only one freeze/thaw cycle as recommended [31]. All dilutions were completed using our diluent (for one 96 well plate): 18ml of PBS + 2ml of blocking solution (0.2g (0.1%) of BSA + 20ml of PBS)) + 10ul of Tween 20 (0.05%).

Salivary or capillary samples below the detection level were calculated based on our lowest level of detection and the dilution factor used (based on meeting the cited criteria), using the following formula: $LOD \div \sqrt{2} \times dilution\ factor$ [32]. These values were used in our calculations of means and standard deviations. It is important to note that using this calculation tends to overestimate concentrations [32]. Nine participants had plasma IL-8 concentrations that were under the level of detection for all four-time points and were calculated using this formula. Salivary IL-8 was detected more consistently, with only 5 participants (one at all four-time points) below the limit of detection requiring imputed values at varying time points. Exactly 62 samples (both saliva and plasma at varying time points) of 216 samples needed to be calculated (40 of which were plasma samples).

Additionally, although Bradford assays were completed to determine protein concentrations for saliva samples, all results are reported in pg/ml. Results adjusted for salivary protein are provided in the appendices.

Statistical Analysis

Descriptive statistics (Means ± SD) were performed on sample characteristics. Concentrations of IL-8 in saliva and serum were first assessed to determine if there were any outliers using Grubb's test or the extreme studentized deviate (ESD) (GraphPad online tool (https://www.graphpad.com/quickcalcs/Grubbs1.cfm), $p < 0.05$ (two-tailed)), and second to test for normality using a Shapiro-Wilk and Kolmogorov-Smirnov test. The data were not normally distributed.

To determine the day-to-day variation in salivary IL-8 for each participant, the coefficient of variation (CV) was calculated using each of the three fasted baseline saliva measures. Individual data, as well as the mean and standard deviation reported. A Pearson correlation was used to calculate the R^2 between saliva and plasma at each time point (T1 and T2 for each session).

To determine changes in IL-8 levels following prolonged and interrupted sitting, a Wilcoxon signed-rank test was used to compare pre- and post-data from the PS and IS sessions for saliva and plasma. Percent change in IL-8 was also determined for each session and compared using a Wilcoxon signed-rank test. To assess for sex differences, Mann-Whitney tests were used for concentration and percent change comparisons between groups, and within-group differences used a Wilcoxon signed-rank test. Statistics were performed in SPPS (v.28, IBM).

Sample Size Calculation: Using an effect size of 0.64 from previous work [13], an α of 0.05 and β of 0.8, a sample size of 21 was determined using GPower 3.1.9.7 statistical software [33]. We aimed for 24 participants (12 females and 12 males) to account for dropouts.

2.4 Results

A final sample of 24 participants (Figure 1.) was included in our analysis. Participant characteristics (Mean ± SD) can be found in **Table 2.1**. There were equal numbers of males and females in our analysis, and all participants reached their VO_2max. According to outlier analysis, one female participant had salivary IL-8 concentrations that were outliers, and another female participant had serum IL-8 concentrations that were outliers. These two

participants were only removed from the analyses in which they were deemed to be outliers.

Day to Day Variability and Correlations

Individual coefficient of variation (CV) for salivary IL-8 is reported in **Figure 2.2.** The mean CV for the sample was 0.353 ± 0.273 and ranged from 0 – 0.91.

There was no correlation between plasma and salivary IL-8 concentrations for either the prolonged (T1: R^2 = 0, p= 0.94, T2: R^2 = 0.012, p= 0.63) or interrupted (T1: R^2 = 0.002, p= 0.83, T2: R^2 = 0.012, p= 0.63) sessions.

Changes in IL-8 Concentration in Response to Prolonged and Interrupted Sitting

Figure 2.3 presents changes in IL-8 concentrations during PS and IS. Salivary IL-8 concentrations (pg/ml) increased significantly from pre to post session (PS: T1 median: 22.09 pg/ml, range: 3.95 – 901.09, T2 median: 86.18 pg/ml, range:12.15 – 939.24; p=<0.01; IS: T1 median: 22.09 pg/ml, range:3.49 – 699.12, T2 median: 51.99 pg/ml, range 3.27 – 533.49; p=0.021). Plasma IL-8 concentrations (pg/ml) were not significantly different between prolonged or interrupted sitting (p=>0.05). The percent change was 0 for both plasma samples. For salivary IL-8, a significant difference in percent change between PS (median: 134.43%, range: -43.96 – 1115.69) and IS (median: 50.80%, range: -75.5 – 356.35; p=0.011) was observed.

Sex Based Analysis

Figure 2.4 depicts differences in salivary IL-8 concentrations by sex and session. No significant differences were observed when comparing males and females. Within the female sample, salivary IL-8 significantly increased for both PS (T1 median: 32.42 pg/ml, range:13.50– 344.27, T2 median: 102.26 pg/ml, range:102.26 – 785.17; p=0.03) and IS (T1 median: 42.39 pg/ml, range: 9.96– 387.96, T2 median: 79.67 pg/ml, range: 3.272 – 479.56; p=0.013). Males showed a significant increase in salivary IL-8 after PS (T1 median: 22.09 pg/ml, range: 3.95– 901.1, T2 median: 50.06 pg/ml, range: 12.15 – 939.24; p= 0.022), but no significant changes were observed during IS (p=>0.05). No differences were observed

in plasma IL-8 concentrations between males or females (plasma values are not displayed in some figures because the mean percent change for both groups was zero).

Individual responses

Individual responses of salivary IL-8 to both sessions are depicted in **Figure 2.5**. There was a great deal of variability between individuals.

2.5 Discussion

We sought to determine the weekly variability and differences in response to prolonged and interrupted sitting of salivary concentrations of IL-8 in young, healthy adults. Our results indicate that within individuals, salivary IL-8 concentrations range from consistent to highly variable. We also found that salivary IL-8 concentration increases after four hours of sitting and that interrupting four hours of sitting with walking led to a blunted increase in salivary IL-8. These findings provide interesting insights into the role of IL-8 in sedentary physiology and the dose of activity needed to attenuate the negative consequences of too much sitting.

Our results support our proposed hypothesis that sitting would lead to an increase in IL-8 and that interrupting sitting can attenuate this response. To our knowledge, only a few studies have examined IL-8 and sedentary behaviour. Two of these studies were cross-sectional and examined special populations; pregnant women [16] and older adults [15]. Neither study found an association between sedentary time and IL-8. This may be because baseline inflammation is altered in older adults and pregnant women [34]. A previous study from our laboratory used a similar study design; however, the exercise interruption was of high intensity [13]. In our previous study, 4 hours of prolonged sitting significantly increased IL-8, and interruption using supra-maximal cycling significantly attenuated this increase. The walking protocol used in the current study also attenuated IL-8 but not to the same extent, possibly indicating a role of intensity and volume. While the number of studies examining IL-8 in this context may be limited, our findings align with previous research investigating other pro-inflammatory cytokines. For example, a study comparing prolonged sitting with sitting interrupted by light and moderate activity found that uninterrupted sitting disrupted immune function and that light intensity breaks upregulated

immune function and reduced inflammatory signals [35]. They performed five hours of uninterrupted sitting with interruptions every twenty minutes for two minutes. Notably, they also observed significant variability in the individual responses.

The individual response to the protocols was highly variable. For example, in some participants, prolonged and interrupted sitting resulted in increases in IL-8, while in others, it led to a decrease. Most participants responded in accordance with our hypothesis, i.e., increased IL-8 in response to prolonged sitting and an attenuated response to interrupted sitting; however, some of the changes may be due to randomness or technical error [36]. Nevertheless, differences in individual responses have been consistently noted across exercise physiology literature and are an important phenomenon to consider. Research using measured maximal oxygen uptake, or VO_2max, has consistently shown that the response to exercise training can vary, indicating that each individual has their own trainability or range based on genetic factors [37]. It is possible that those with a higher VO_2max would have a more favorable inflammatory biomarker profile [38-40]. Although we did not observe a consistent association between VO_2max and IL-8, this is something that should be further investigated, particularly in light of sex differences.

As hypothesized, females had a higher baseline salivary IL-8 concentration and a greater response to prolonged and interrupted sitting. Previous research has shown that females have a more potent immune system before menopause [41]. Taken together, the intensity and volume required to reduce the pro-inflammatory effects of prolonged sitting may differ between sexes. Fitness levels may also play a role in the IL-8 response as it may increase the sensitivity to exercise, i.e., those with higher fitness levels may be primed for positive adaptions/responses. For example, studies have shown increased IL-8 receptors (CXCR1/2) in muscles after training; therefore, more IL-8 may be bound rather than circulated systemically in response to a physical stressor such as sitting [42]. Future research is needed to examine characteristics or mechanisms that result in these observed sex differences.

Only a few studies have explored the variability of cytokine profiles looking at the day-to-day variability in older adults [43], monthly variability over six months in healthy adults [44], and the day-to-day variability of chemokines (including IL-8) in tear secretions

[45]. Our work provides new insights into the intra-individual variation in salivary IL-8, showing that salivary IL-8 measures are consistent week-to-week overall but can be highly variable in some. An important note is that saliva offers the same reliability as other bodily fluids for assessing cytokines and is superior with regard to ease of collection and analysis [46]. In contrast, plasma collection and analysis created challenges in the present study. Several individuals had IL-8 levels below the limit of quantification, which necessitated imputation. Therefore, it appears that small volumes of plasma may not be appropriate for assessment of cytokines such as IL-8. For example, one study comparing saliva and blood found that salivary IL-8 was detected in greater concentrations and was detected more consistently [46]. Blood collection also tends to cause acute stress in participants as it involves breaking the skin and the sight of blood. Capillary sampling also seems to be influenced by hydration and the arterialization of the hand/fingers. Although behaviours also influence saliva, it seems more consistent with respect to acute changes in certain cytokines such as IL-8 and IL-1ra [13, 47, 48].

Strengths of our work include the homogenous sample of young and healthy adults, the well-controlled environment, and the randomized design. A weakness of the study is the lack of venous samples for comparison to larger volumes of plasma and the number of samples that required imputation for plasma.

In conclusion, we found that four hours of prolonged sitting significantly increased salivary concentrations of the pro-inflammatory cytokine IL-8. Interrupting prolonged sitting every thirty minutes with walking seems to blunt the increase in IL-8 seen during prolonged sitting, especially in males. Of note, individual variability should be more carefully considered as the response to the sessions varied across participants. A precision medicine approach to understand how sedentary behaviour alters inflammation and the mechanisms of these individual differences may be needed. Future work should also explore what other cytokines or factors are elevated during prolonged sitting and what mechanisms may be responsible for these changes.

2.6 References

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Table 2.1. Participant Characteristics

	Female (n=12)	Male (n=12)	Total Sample (n=24)
Age (yrs)	20.9 ± 1.8	21.3 ± 2.5	21.1 ± 2.2
BMI ($kg \cdot m^2$)	24.4 ± 2.1	25 ± 3.6	24.7 ± 2.9
Physical Activity ($min^{-1} \cdot wk$)	154.2 ± 74.9	158.8 ± 189.6	156.2 ± 141
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	37.2 ± 4.9	45.6 ± 8.5	41.4 ± 8

Figure 2.1. Participant Flow Chart

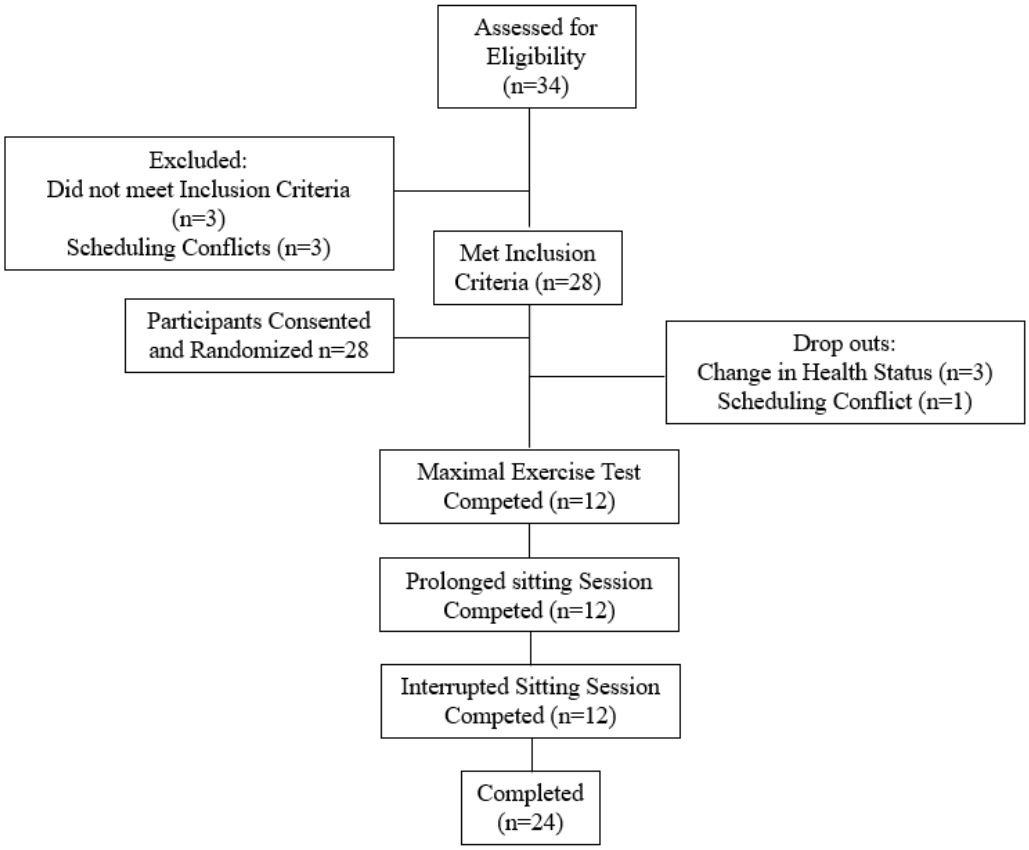


Figure 2.2. Individual Coefficient of Variation for Salivary IL-8 Concentrations

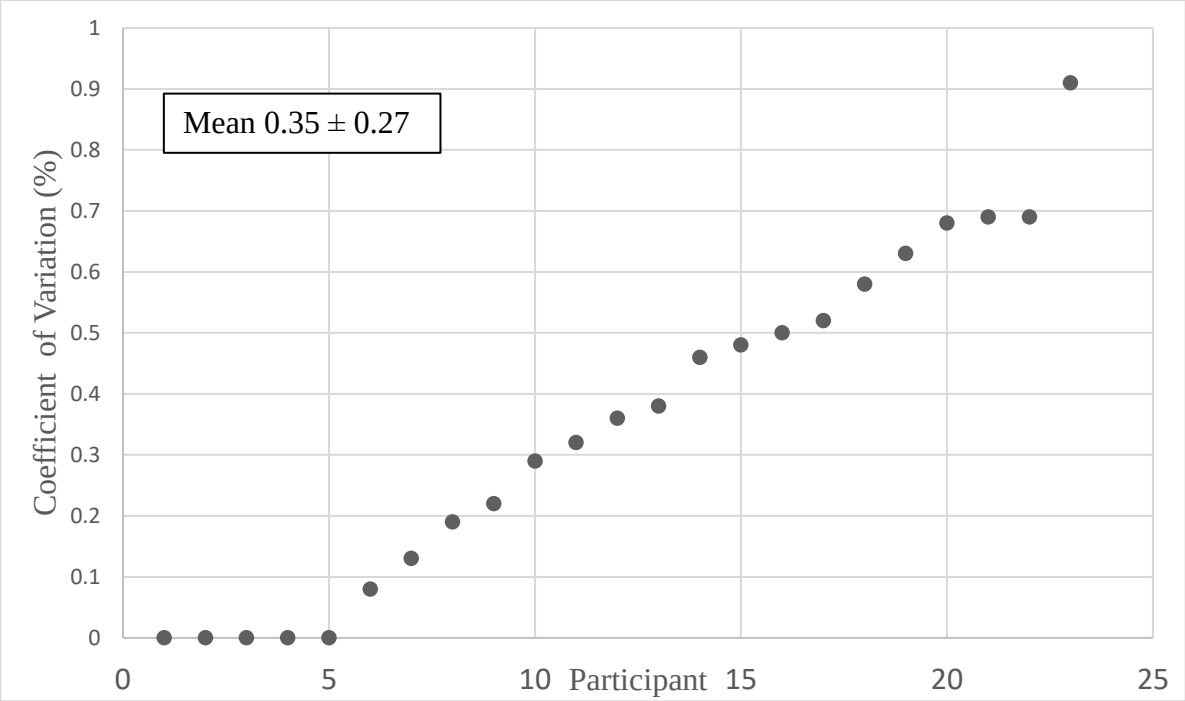
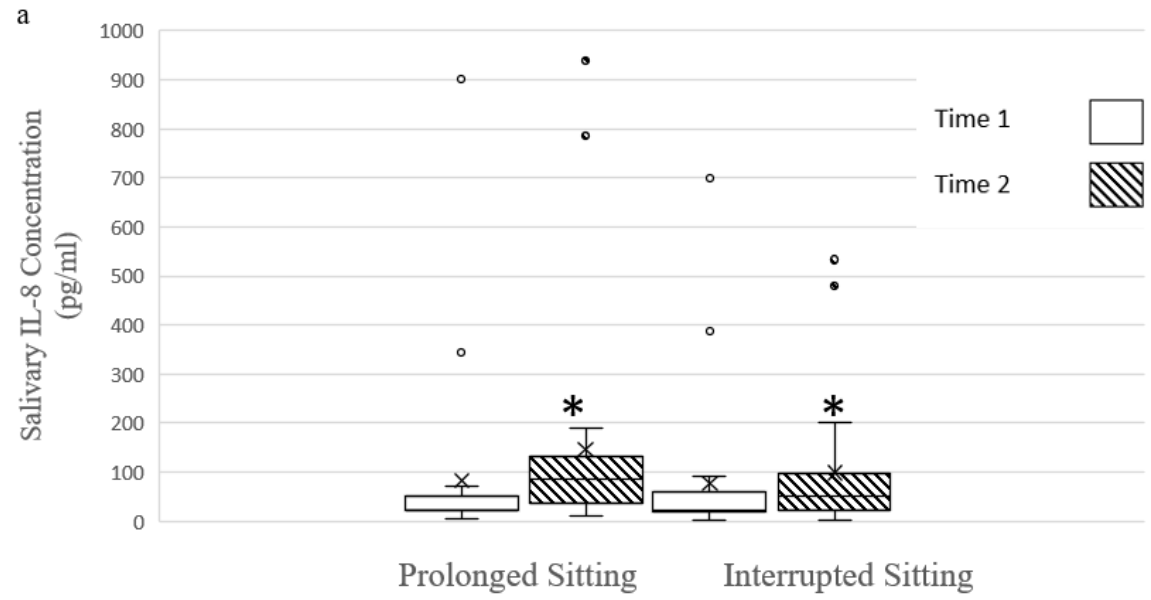
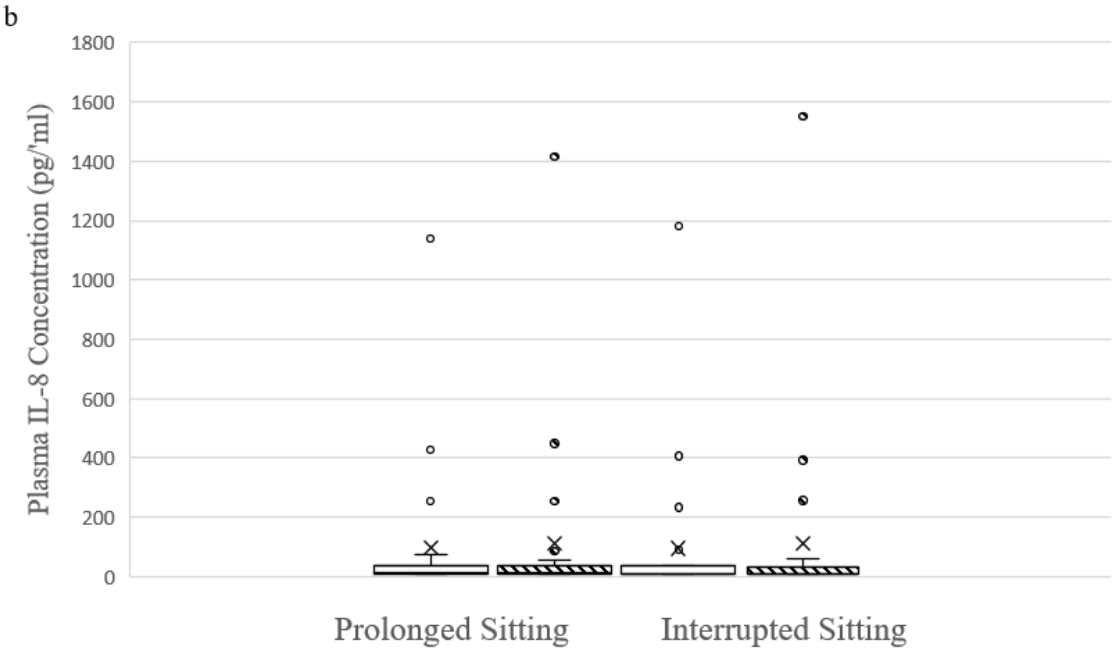
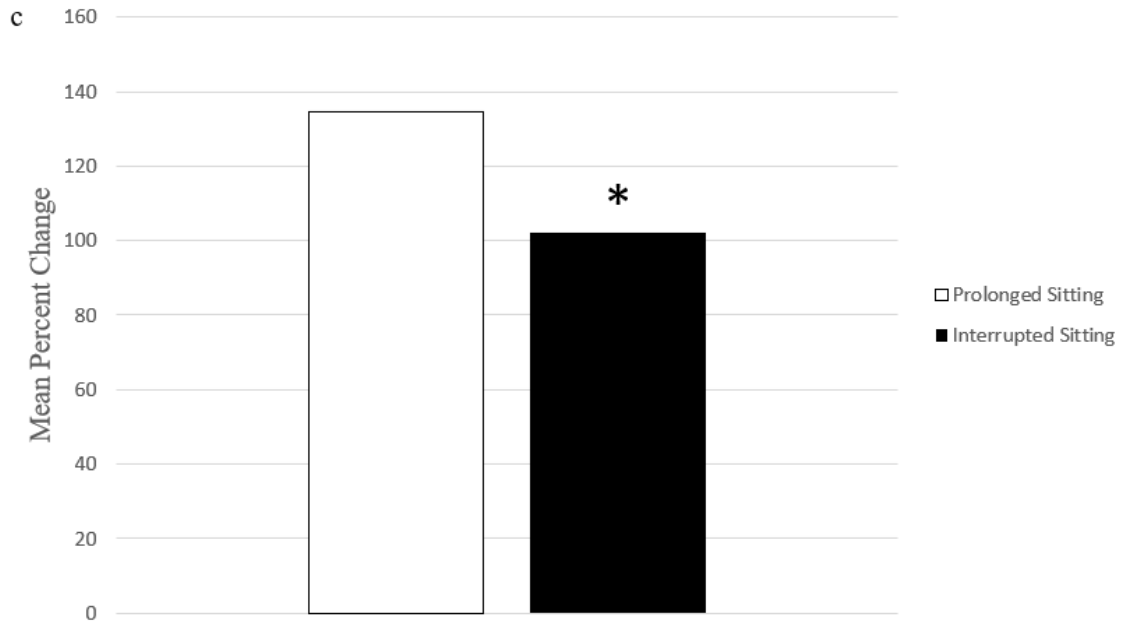


Figure 2.3. Changes in IL-8 levels in response to prolonged and interrupted sitting sessions based on salivary IL-8 concentrations (a), plasma IL-8 concentrations (b), and percent change (c) in salivary IL-8

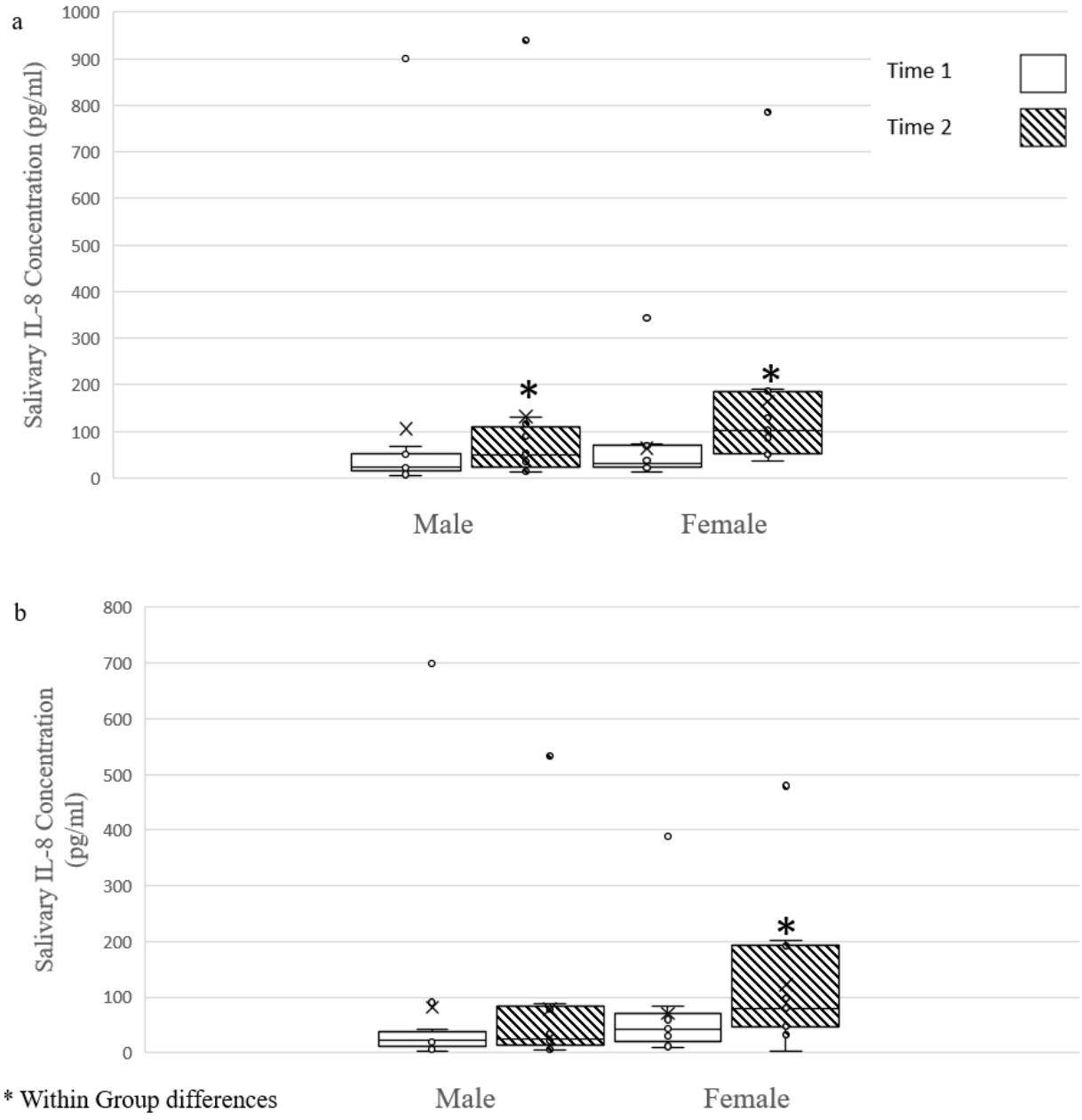




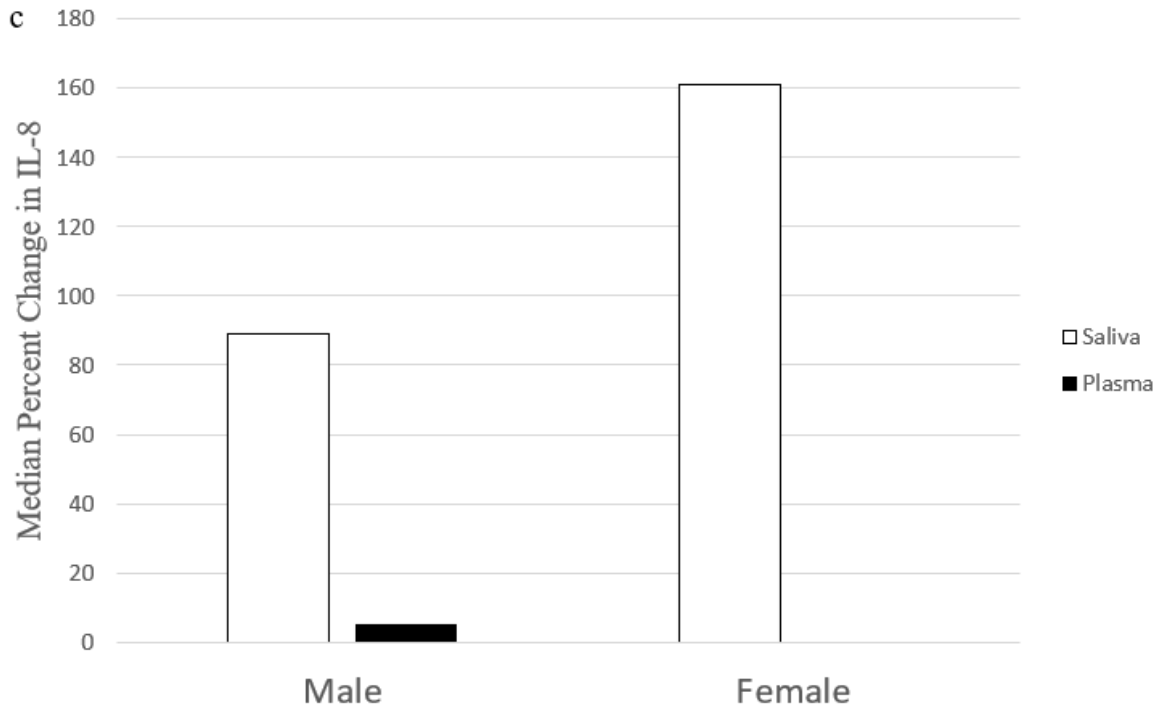


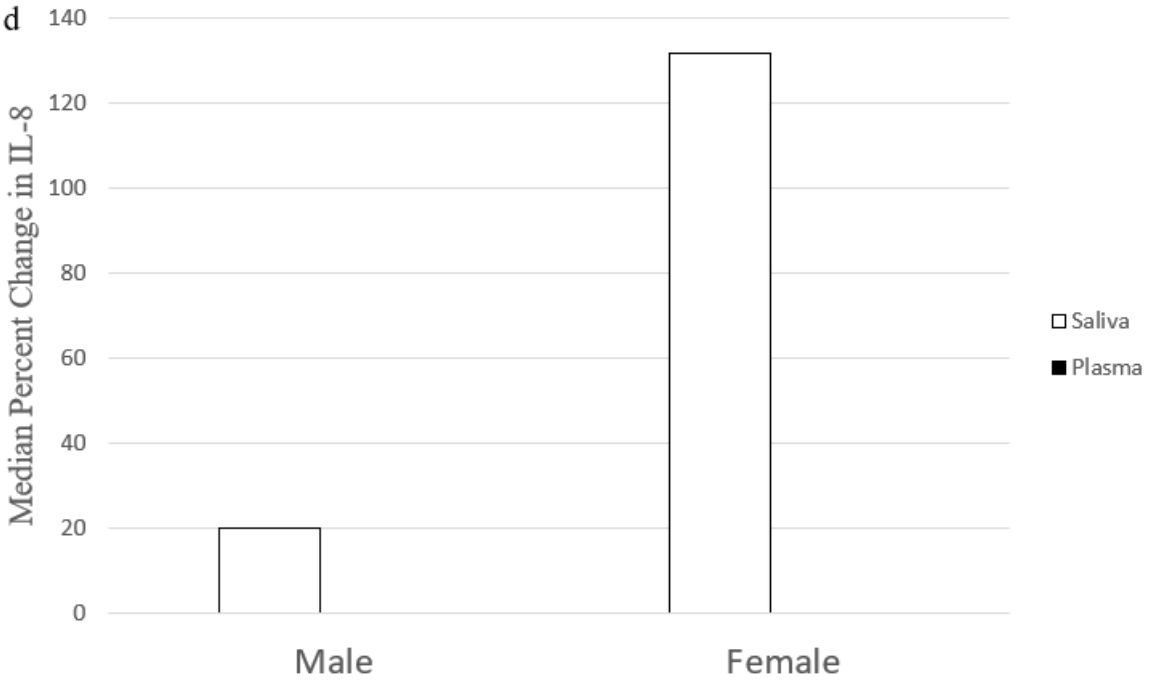
Caption: Changes in the concentration of IL-8 in response to prolonged and interrupted sitting sessions are reported. Pre-sitting (T1) and post-sitting (hour 4, T2) data are displayed in a box and whisker format where the line in the box represents the median, the x represents the mean, and the outer edges of the boxes represent the interquartile range, and the error bars represent the minimum and maximum. Figure 1a: Salivary IL-8 increased from T1 to T2 in both conditions. Figure 1b: Plasma IL-8 did not change from T1 to T2 in either condition. Figure 1c: The percent change from T1 to T2 was significantly smaller during the interrupted session, indicating an attenuated response. * Indicates significance at an alpha of 0.05

Figure 2.4. Changes in IL-8 concentrations following prolonged (a) and interrupted sitting (b) for males and females, and percent change in salivary IL-8 following prolonged sitting (c) and interrupted sitting (d)



* Within Group differences



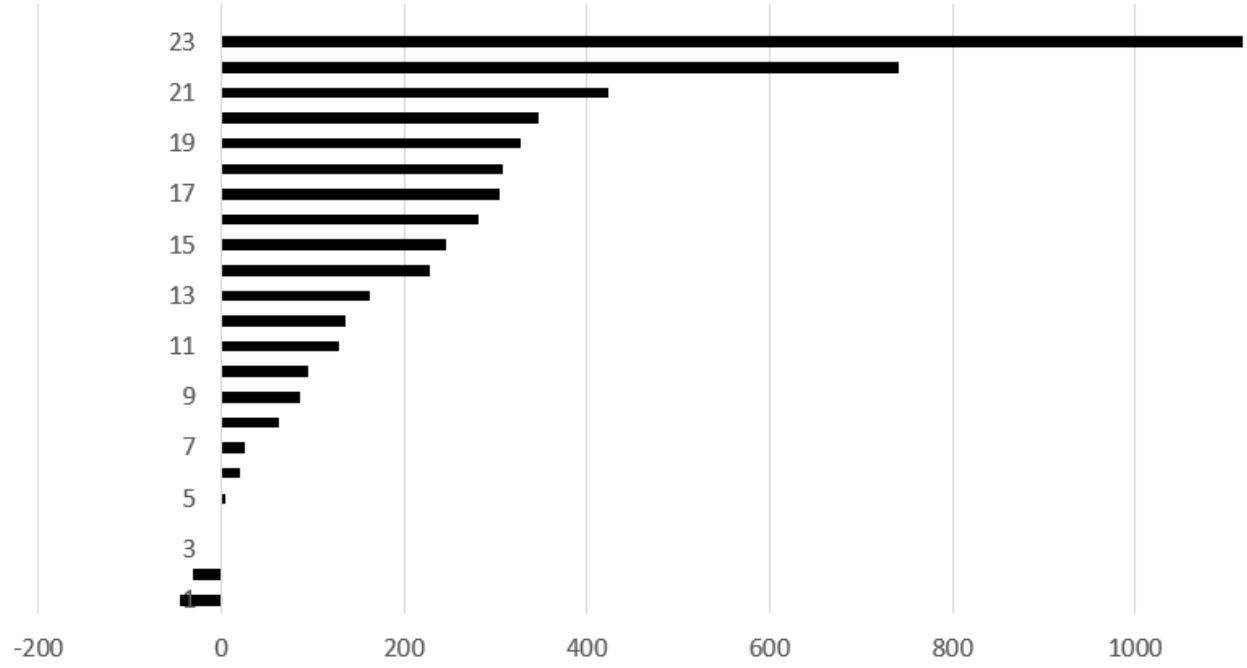


Caption: Changes in the concentration of IL-8 in response to prolonged and interrupted sitting sessions are reported by sex. Pre-sitting (T1) and post-sitting (hour 4, T2) data are displayed in a box and whisker format where the line in the box represents the median,

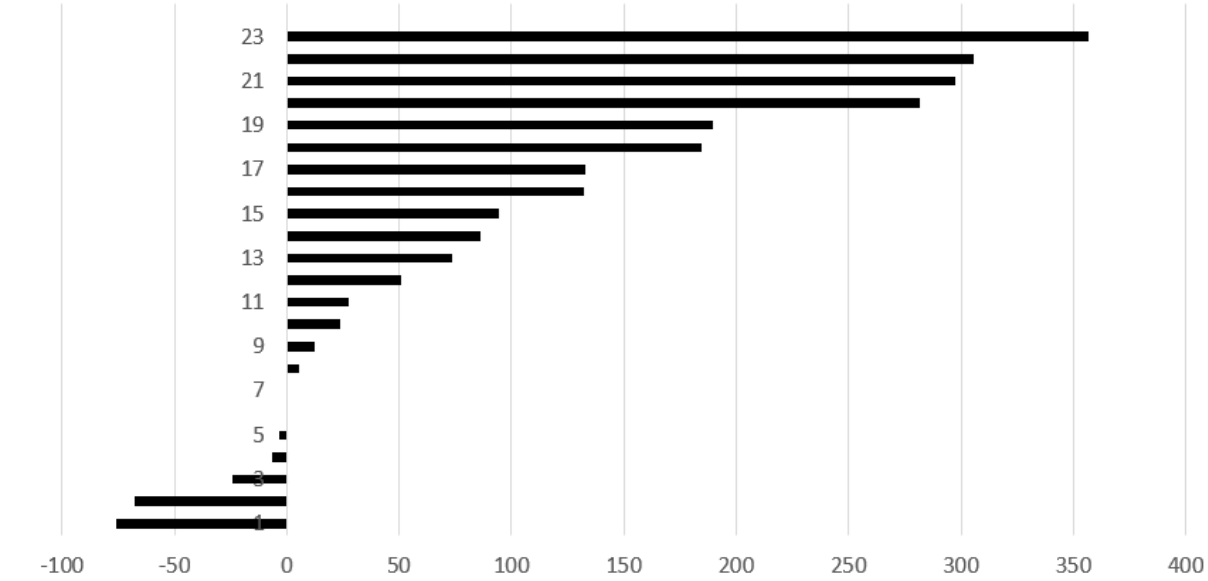
the x represents the mean, and the outer edges of the boxes represent the interquartile range, and the error bars represent the minimum and maximum. Figure 2a: Median salivary IL-8 significantly increased from T1 to T2 in males and females. Figure 2b: Salivary IL-8 concentration only increased from T1 to T2 in females. Figure 2c: No significant difference was noted in the percent change from T1 to T2 in either condition when comparing males and females. * Indicates within-group differences at an alpha of 0.05

Figure 2.5. Individual Percent Change of Salivary IL-8 to Prolonged (a) and Interrupted Sitting (b)

a



b



Caption: Percent change from T1 to T2 for each individual participant is reported. Figure 3a displays individual responses to prolonged sitting, and Figure 3b shows interrupted sitting responses. As visually displayed, there is large variability in both protocols' responses, with a larger magnitude increase during prolonged sitting.

Chapter 3. Discussion

3.1 Discussion

Hypotheses and Findings

Hypothesis 1: We expect that the variability across three measures of fasting salivary IL-8 will be consistent within individuals and indicate reliability for the analysis of salivary IL-8.

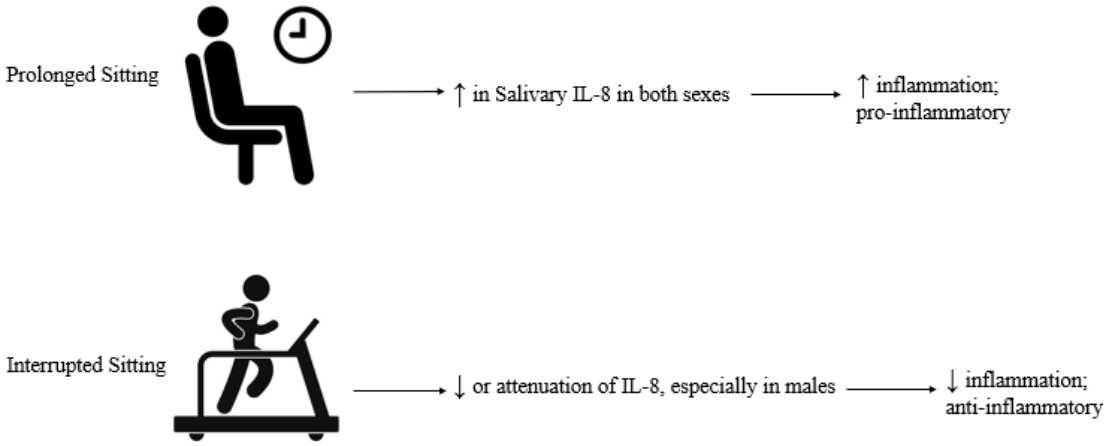
- Using a coefficient of variation analysis, we showed that the week-to-week variability within individuals across three baseline timepoints was low. However, there is considerable variation between individuals.

Hypothesis 2: We hypothesize that sitting will increase IL-8 concentrations from baseline in the prolonged sitting session and that interrupting sitting with bouts of walking will attenuate this increase in IL-8, and that sex differences may exist.

- Four hours of prolonged sitting significantly increased salivary IL-8, whereas interrupted sitting attenuated this response, increasing IL-8 to a lesser extent. Males exhibited a favourable response to interrupted sitting, showing lower salivary IL-8 levels. Females had a greater IL-8 response to prolonged and interrupted sitting, significantly increasing in both sessions.

Our results can be briefly summarised in Figure 3.1

Figure 3.1. The Effect of Prolonged and Interrupted Sitting on Salivary IL-8 concentrations.



We aimed to determine if 4 hours of prolonged sitting would increase the concentration of IL-8 and whether interrupting sitting with 3 minutes of walking every 30 minutes would attenuate the pro-inflammatory effect of sitting. We found that prolonged sitting significantly increased salivary IL-8 in our sample and that interruption attenuated this increase. Sex differences were also noted, with females tending to have greater IL-8 levels at baseline and in response to both sitting protocols. Furthermore, there was large variability within our sample, and individual responses provide an interesting insight into how prolonged sitting, similar to exercise, results in an individualistic response. However, none of our analyses with plasma IL-8 yielded significant results, mainly due to variability and the low IL-8 seen in plasma resulting in several imputations.

Insights from the Data

The field of sedentary physiology is relatively small but growing, but the primary body of knowledge comes from cross-sectional studies. Although transitioning from a dearth of cross-sectional studies to more clinical laboratory work is common, laboratory work in sedentary physiology can be considered quite challenging. Much of the acute or laboratory work has used a model of extreme sedentary behaviour using bed rest. Bed rest studies involve the participant being supine (at varying angles) and immobilized for a designated period. Bed rest allows for more control of participant behaviour and often occurs in hospital settings, with studies having participants bed-bound for hours to months. Bed rest studies have been extensively used to mimic the effects of space travel as the role of gravity changes when supine and can be altered by angling the bed (incline or decline). The extreme nature of these bed rest models makes it difficult to clearly understand the effects of prolonged sedentary time in average persons. Methods such as ours, where acute sitting sessions are used, can be considered more realistic or representative of daily living. However, they lack the same level of control and stringency as the bed rest models. Additionally, bed rest studies tend to require large amounts of funding for the equipment, staff, participant compensation, and even post-intervention rehabilitation. Whereas acute studies can be done simply with a standard chair setup, the costs mainly depend on the analysis undertaken.

Both types of work (bedrest and acute) require a prolonged time where the participant physically must be in the lab (as opposed to cross-sectional work), making recruitment without financial compensation difficult as there can be lost time (and possibly income). Of note, during our recruitment was the difficulty in recruiting female participants. Females tend to be underrepresented in research, and it was interesting that our recruitment approaches did not seem to draw their attention compared to their male counterparts. We could still reach (and exceed) our calculated sample size (n=21), but we aimed to collect 24 participants and have even numbers of males and females. Due to timeline constraints, we had to stop accepting male participants and focus solely on female recruitment for the last two months of data collection. It may be worthwhile to investigate literature that describes differences in male/female recruitment methodologies and alter our recruitment approaches in future studies.

While acute findings can be viewed in isolation, acute bouts of prolonged sitting are a prominent part of life, i.e., a sedentary lifestyle. In that case, it is possible that the cumulative effects of these repeated bouts of prolonged sitting can promote a chronic low-grade inflammatory state. Although IL-8 is just one of a vast arrays of cytokines that may be altered with prolonged sitting, it has important physiological functions such as chemotaxis and angiogenesis that create a heterogeneous environment if chronically elevated. Although rationale has been provided, it is important to note that it is currently unclear what a clinically meaningful increase in IL-8 would be and infer a direct correlation. However, several studies combined with our findings seem to support the pro-inflammatory nature of prolonged sitting. Further work from our laboratory will use multiplex cytokine analysis in response to prolonged sitting, using an explorative design, as few cytokines have been examined in this context.

Saliva and Blood as Biomarkers: Benefits and limitations

Although we have promoted using saliva as a biomarker, it is not without its unique challenges. Saliva is an easy-to-use non-invasive technique, but many aspects of salivary analysis create unique challenges compared to blood (venipuncture).

The oral cavity is not sterile and is exposed to the external environment more than blood. For example, the oral cavity is exposed to many components such as food, liquids,

damage, and more. Salivary cytokine analysis can vary due to many of these different behaviours. Salivary secretions are also heavily influenced by oral infections, food debris, alcohol, caffeine, blood contamination, etc. A detailed review from Szabo & Slavish details many methodological considerations when using saliva to measure inflammatory biomarkers [1]. These authors shed light on many aspects of salivary collection and analysis, and we frequently referred to this work for methodological considerations [1]. Compared to saliva, blood samples can be considered sterile; although it involves a more invasive procedure to collect, it tends to be less influenced by day-to-day behaviour. Certain behaviours easily influence the oral environment, and salivary secretions tend to be reactive. However, our work provides answers to some of the future directions discussed in the review by Szabo & Slavish, as we attempted to control these behaviours to the best of our ability. For example, we looked at how IL-8 varies weekly in a relatively homogenous sample and controlled environment and how IL-8 reacts to acute bouts of prolonged and interrupted sitting (a form of physical stress).

Regarding blood analysis, capillary samples were used; this was based on several studies that detailed the reliability of capillary samples to venous samples in analyzing various biomarkers, including cytokines [2, 3]. Capillary samples are quite simple to collect, relatively non-invasive compared to venipuncture, and do not require a medical directive or additional certification training. Furthermore, if we decided to use venipuncture, this would have elevated the biosafety level of the laboratory, requiring additional safety measures and would require us to hire a phlebotomist or obtain expensive training. From a practicality standpoint, we decided on capillary sampling with the addition of being validated by the literature. Although we have justified this decision, there are still cons to this decision, the most prominent being that venipuncture provides a multitude of times more sample quantity than capillary samples. For our current work, capillary sampling provided low sample quantities, in some cases just barely enough for sufficient analysis as we aimed to collect 200ul. Several participants, either by nature or influenced by their behaviours (such as hydration), provided minimal amounts of blood. The participants that provided such low samples made room for error during biochemical analysis extremely stringent. ELISA analysis of plasma requires a certain sample threshold (varies on dilution and standard used) for the analysis to be accurate. We diluted our

samples, which seemed effective, as when troubleshooting, it seemed autoblocking occurred in undiluted samples. Such a low sample volume also limited our analysis's quality because we could only run our samples in duplicate, which is less accurate than triplicate, as seen in our saliva analysis. In addition, it seems plasma IL-8 is not as sensitive to change as salivary IL-8; many plasma samples were under the limit of detection and required imputation by the above methods.

In summary, venipuncture would have provided more samples, allowing us not to dilute the samples and worry about running out of samples, and so to efficiently run triplicates, which is the gold standard. Our low sample volume required sample dilution, and even then, we could only run our samples in duplicate. Other studies have used limited capillary samples and ran them in duplicate while still showing accurate results but having ample quantities of plasma and running in triplicate would be ideal for the greatest accuracy.

Bioenergetic Considerations

Although it may not be directly considered a limitation, the standardized meal we used could have impacted our results. The nutritional values of the standardized meals we provided (590 Calories; Fats 6.5g, Carbohydrates 127g, Proteins 11.1g) included breakfast and a snack and had a relatively high carbohydrate value. Carbohydrate status and intake have been shown to alter cytokines levels, especially in the context of myokines [4-6]. The release of both IL-6 and IL-8 are altered when carbohydrate levels vary, specifically muscle glycogen levels. Cytokine/myokine concentrations are increased (in response to exercise) in a low muscle glycogen state (overnight fast) compared to a fed state [7-10]. As we provided a high-carb meal, these effects are likely muted (low muscle glycogen and therefore an increased IL-8 response).

In comparison, our previous pilot study used a maximal intensity cycling intervention at the end of each hour, likely providing a greater stimulus due to the high intensity [11]. Furthermore, concerning bioenergetics, this protocol would be considered anaerobic, using different energy systems than our current work (walking; aerobic). The bioenergetics of the exercise intensity could have interplayed with the nutrition source we provided and the IL-8 response. For example, in our previous study, high-intensity exercise (30 seconds at

supra-maximal intensity) would primarily use the fast-acting anaerobic fuel sources, such as phosphocreatine, muscle glycogen and anaerobic glycolysis [12]. However, it seems unclear if this affected our results as we saw significant changes in IL-8 in our pilot study. We likely removed this effect (if there is an effect) during our current work due to the lower intensity, duration, and type of exercise chosen. However, our results still indicate a role of our walking protocol in reducing IL-8 from prolonged sitting, especially in our male sample. It is possible that females uniquely need a higher intensity protocol to combat their more exaggerated response to prolonged sitting.

Cytokine Semantics and Mechanisms

Another aspect to consider in our work and several exercise immunology studies is the cytokines' mechanism and/or source. For example, suppose an exercise intervention is conducted, and cytokines are measured pre and post by venipuncture. The results indicate that cytokine levels are significantly increased at the post-measurement. It is commonly inferred that this increase is from the working skeletal muscle, but this cannot be definitively shown in many cases. Blood sampling is a system-wide approach, and many cell types can release cytokines. When we infer that muscles or any other specific tissue is the source, this cannot be confirmed without much more invasive measures such as biopsies. This has commonly resulted in several cytokines being labelled as myokines without true confirmation of their release source. Essentially, cytokines secreted by other tissues should not be labelled as myokines. An increased circulating level of a cytokine in response to physical exercise (or another physical source, such as sitting) does not mean that this is guaranteed to be a myokine since it could have been released from many different sources throughout the body [13]. Although we are not proposing the source of IL-8 in our study, it is important to consider this when interpreting the results.

The above discussion is also highly relevant to salivary cytokines. Many factors influence salivary cytokines, but the actual source of salivary cytokines is still widely debated. It is challenging to discern cytokine responses that are oral cavity specific or are changed systemically. Due to the consistent and acute changes seen in salivary cytokines, as detailed in the reviews by Slavish and Szabo, and our previous work [11, 14, 15], it is fair to infer systemic changes are occurring [1, 16, 17]. However, caution is warranted

when making claims about system-wide changes. It is hypothesized that certain blood-borne proteins could leak or cross over into salivary secretions or be directly released from salivary glands through local immune cells [18-20]. The complex nature of salivary secretions is beyond the scope of this work, but the mechanisms of salivary cytokine secretion from the systemic environment are not fully understood. However, the excellent work by Slavish and Szabo has clarified the use of saliva as a biomarker source to measure inflammatory cytokines [1, 17]. Although this is more methodological work, not mechanistic, several dental journals, papers and researchers continue to explore the mechanisms of salivary secretions, primarily concerning oral pathology. Saliva has excellent potential as a bedside biomarker source, and specialists in biomarker analysis, such as clinical biochemists, should work with existing experts to develop a consistent and reliable salivary analysis for use in the clinical setting. Experimental work such as ours is also needed. Our analysis of salivary IL-8 can hopefully stem further work in this area, building further foundational knowledge on the acute changes of salivary cytokines.

Limitations

Although limited research has examined prolonged sitting in young, healthy adults, it can also be considered a limitation. The inflammatory response differs with age and disease status, making our findings difficult to generalize. Additionally, due to the high variation in cytokines between individuals, our data were not normally distributed, so non-parametric testing was conducted. By performing non-parametric analysis, we lose some nuance in the individual responses. We provided individual responses for this reason, but our CV analysis shows consistency within individuals. Our analysis of our plasma samples was limited due to the low quantities of samples collected, with seemingly natural lower levels of IL-8 in plasma, as shown in studies comparing saliva to blood for IL-8 analysis [21]. IL-8 seems more consistently measured in saliva and at higher quantities than in plasma [21]. Many of our plasma samples had IL-8 levels under our detection limit, meaning we had to use our imputation method, especially when non-parametric testing was likely to affect our results. For example, the median for some plasma samples was then calculated by imputation, and this made the comparison between saliva and plasma difficult.

Future Directions

Although saliva is not always correlated to venous blood samples, likely considered the gold standard for biomarker analysis, this does not necessarily make saliva invalid. The mechanisms of salivary secretion and its constituents may not and possibly should not need to be compared to blood [19, 22]. Saliva analysis can detect changes in cytokine profiles including IL-8 [11, 17, 21, 23, 24], and has the potential for widespread clinical use. It is easy and quick to use and could be easily implemented in point-of-care settings [18, 25]. Saliva is its own secretion, having its own set of mechanisms, and more research should aim to standardize and understand (1) the responses of salivary cytokines to several types of stimuli, (2) create standardized sample collection procedures for clinical use, and (3) understand the daily variability and fluctuations of salivary cytokines.

Conclusion

In conclusion, prolonged sitting increases concentrations of salivary IL-8, a pro-inflammatory cytokine that is linked to disease when elevated chronically. However, sitting interruption with short bouts of walking can attenuate this increase in salivary IL-8 seen in acute bouts of prolonged sitting. In contrast, the changes seen in plasma remain inconclusive. There seem to be sex differences in the salivary IL-8 response to prolonged and interrupted sitting, with females showing a greater response to sitting in either condition and males responding more favourably to interruption. There are apparent individual differences in the IL-8 response of individuals, with both positive and negative responders. Future work should aim to understand what other cytokines may respond to prolonged and interrupted sitting, investigate individual responses to these physical stimuli, and take a more precision medicine approach to interruption protocols.

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Appendices

Appendix A.

A.1. Eligibility Questionnaire

Eligibility Questionnaire

1. Age: _____

2. Sex:

   Female ☐
   Male ☐

If female, are you currently pregnant?

   Yes ☐
   No ☐

3. Do you have any chronic diseases such as asthma, heart disease, irritable bowel syndrome, arthritis, or others?
   a. Yes
   b. No

   If yes, please describe:

4. What is your current:
   1. Height (cm):
   2. Weight (kg):

5. Do you currently have an infection, cold, or flu like symptoms?
   1. Yes
   2. No

   If yes, please describe.

6. Are you currently taking any prescription or over the counter medications regularly?
   1. Yes
   2. No

   If yes, please list the medications here: _____

7. Do you have any injuries that would limit your ability to run or walk on a treadmill? (e.g. knee injury)
   1. Yes
   2. No

If yes, please describe the injury here: _____

8. Have you ever been a regular smoker?
    1. Yes
    2. No

    If yes, please indicate for how long: _____

9. Do you have any dietary restrictions?
    1. Yes
    2. No

    If yes, please indicate what kind: _____

    Please note, we will be providing a standard breakfast and snack that must be consistent across participants, therefore, this information is important for us to determine your eligibility.

10. At the present time do you smoke daily, occasionally, or not at all?
    1. Daily (at least 1 cigarette per day for the last 30 days)
    2. Occasionally (at least 1 cigarette in the last 30 days but not every day)
    3. Not at all (you did not smoke at all in the last 30 days)

11. Do you have a history of dental injuries and/or infections?
    1. Yes
    2. No

    If yes, please describe the injury and/or infection and the date at which this occurred: _____

12. Please confirm that you are a student at Ontario Tech University.

    Yes

    No

A.2 Informed Consent

ONTARIO TECH UNIVERSITY

ELIGIBILITY ASSESSMENT CONSENT

Study Title: Physiological responses to prolonged and interrupted sitting

Name of Principal Investigator: Shilpa Dogra, PhD

Research Team: Nicholas O'Rourke, BHSc; Emmeline Meens-Miller, BHSc; Julia Green-Johnson, PhD.

Overview

Thank you for your interest in our research study. There are several sections to this document. First, there is a brief explanation of how we will assess your eligibility, and what we will do with the data we collect through our eligibility questionnaire. Next, you will find an information letter containing several details for the full study. You can read this to determine if you are still interested in participating, or if you have any questions for us. You will also find a COVID-19 section at the end of this document. Please read this carefully to understand the steps we are taking to minimize risks associated with the virus.

Eligibility Questionnaire

In order to determine whether you are eligible for this study, we have a short questionnaire for you to complete. This questionnaire includes personal information such as your health status, dietary restrictions, and lifestyle behaviours. Due to the nature of this data, we want to obtain your consent to collect the data.

Please note, your data will be kept confidential. If you would like, it can be sent to us via email, or, you can come to the laboratory where we can provide a printed copy. Once we have determined whether you are eligible or ineligible for the study, we will destroy this data. In other words, no print or digital copies of this information will be retained.

As a reminder, the security of e-mail messages is not guaranteed. Messages may be forged, forwarded, kept indefinitely, or seen by others using the internet. Thus, if you do return the eligibility questionnaire to us via email, we ask that you password protect the document to improve security. You can communicate this password to us by leaving a message on our laboratory voicemail. Please also be sure to delete the email you sent us.

CONSENT

I understand how the data from my eligibility questionnaire will be handled. Any questions regarding this part of the study have been answered to my satisfaction.

I understand my consent to participate, or to not participate in this study is voluntary. I also understand my right to withdraw from any part or all of this study for any reason. I waive no legal rights by participating in this study.

If I have any questions regarding my rights as a research participant, or about any issues relating to this study, I will contact Dr. Shilpa Dogra at 905.721.8668 ext. 6240 or at Shilpa.Dogra@ontariotechu.ca.

I hereby consent to participate in this study.

_____	_____	_____/_____/_____
Participant (Print Name)	Signature	Date

For a member of the research study: I have ensured the named participant above has thoroughly understood all aspects of this research study, and have answered all questions to their satisfaction.

_____	_____	_____/_____/_____
Research Member (Print Name)	Signature	Date

Participant Concerns and Reporting:

Please read this consent form carefully and feel free to ask the researcher any questions that you might have about the study. If you have any questions about your rights as a participant in this study, complaints, or adverse events, please contact the Research Ethics Office at (905) 721-8668 ext. 3693 or at researchethics@ontariotechu.ca

If you have any questions concerning the research study or experience any discomfort related to the study, please contact the researcher at 905.721.8668 ext. 6240 or shilpa.dogra@ontariotechu.net.

By signing this form you do not give up any of your legal rights against the investigators, sponsor or involved institutions for compensation, nor does this form relieve the investigators, sponsor or involved institutions of their legal and professional responsibilities

This study has been approved by the OTU Research Ethics Board REB#16473.

ONTARIO TECH UNIVERSITY

Study Title: Physiological responses to prolonged and interrupted sitting

Name of Principal Investigator: Shilpa Dogra, PhD

Research Team: Nicholas O'Rourke, BHSc; Emmeline Meens-Miller, BHSc; Julia Green-Johnson, PhD.

Study Information

Introduction:
You are invited to participate in a research study that is being conducted at Ontario Tech University (OTU). Throughout this document you will find the study purpose, procedure, benefits and risks, as well as your right to refuse to participate or withdraw from the study. Please thoroughly read and understand all sections of this document before you agree to participate in this study. This is known as the informed consent process. Should you have any questions concerning any of the information, words, or your rights, please contact the researchers above to gain full understanding before signing this consent form.

Purpose & Explanation of the Study:
The purpose of the proposed study is to compare markers of inflammation between a continuous sitting session and a session of sitting interrupted by short periods of walking. To do so, we will compare your response to 4 hours of continuous sitting with your response to 4 hours of sitting interrupted by 3 minutes of walking every half hour.

Eligibility:
In order to be eligible, you must be between the ages of 18-30 years, have a body mass index <30 kg/m^2, must be a non-smoker, and not be taking any medications (such as NSAIDs) that would affect your inflammatory levels, must not be pregnant, and not suffering any acute infections. You must also have no major cardiometabolic, respiratory, or musculoskeletal conditions that would impact your ability to run and walk on a treadmill, and you must be able to consume the standard food provided. You have already completed an eligibility questionnaire.

At the first session, we will also be measuring your resting heart rate, blood pressure, height and weight to confirm eligibility in the study.

Assessment Procedures:
As a participant in this study, you will be asked to attend 3 laboratory sessions. During the first laboratory session we will ask you to provide a saliva sample, perform a maximal exercise test, and then fit you with a small device that measures your daily activity levels. The exercise test will be conducted on a treadmill, starting with a warm-up pace, and progressively getting harder until you reach a point where you can no longer exercise; this will be followed by a cool-down. The exercise portion usually lasts between 8-12 minutes.

We will then fit you with a small device to be worn for a seven-day period. The goal is to always keep the device on, including during sleep and any water-based activities (shower, swimming etc.) The device will be removed the following week at the beginning of your second session.

For each laboratory session, you will arrive at the laboratory fasted, having had at least 1L of water that morning. We also ask that you refrain from alcohol and intense exercise at least 24 hours prior to the session. We will also confirm that no new infections or conditions have arisen between sessions and that no new medications (that impact inflammation) have been taken. We also ask that you be prepared to exercise, wearing the appropriate clothing (athletic clothing and shoes) for comfort and ease of data collection. We also recommend you bring a device, such as a laptop, to keep yourself busy during the sessions. Before beginning the experiment, we will guide you through the process to obtain your own blood via a finger-prick method, we will also collect a saliva sample, measure your resting heart rate and blood pressure, provide you with breakfast, and fit a small strain gauge on your leg. The 4-hour experimental protocol will then begin. This will either be a) 4 hours of continuous sitting or b) 4 hours of sitting interrupted by 3 minutes of walking every 30 minutes. During the 4-hour experimental procedure, you will be allowed to complete an activity of your choosing so long as you are seated (e.g. reading, computer work, watching a movie, etc.). At the midway point of the experimental condition, you will be provided with a granola bar and juice as a snack. Following the experimental procedure, a finger prick blood sample and saliva sample will again be collected. Each session should be completed by approximately 2:30 pm.

Participant Compensation:
You will not be paid for your participation in this study; however, you have much to gain! You will be sent your personal results in the form of an email at the end of the study. If you are a Kinesiology student, you are also able to obtain bonus marks in your Kinesiology courses this semester. The bonus marks will be distributed as per your selection in the Participant Pool survey sent to you at the beginning of the semester. There are 4 components to this study: Maximal exercise test session (1%), activity monitor data (2%), each of the two sitting sessions (1.5% each). If you withdraw from the study, you will receive the bonus marks for the sessions commenced.

Risks and Participant Safety:
Participation in any research study is associated with some risks. The potential risks of this study include feelings of shortness of breath, quickened heart rate, light headedness, and muscular discomfort during and following exercise, and feeling coerced in to participating in the study. You may also experience feelings of stiffness or soreness due to sitting for an extended period. Additionally, you may feel discomfort at the site of the fingertip prick, and have some skin discomfort from wearing the activity monitor. To minimize these risks and to ensure your safety throughout this study, the researchers involved with the study are highly trained and supervised by an Certified Exercise Physiologists, have current CPR training. Additionally, an emergency action plan is posted in the laboratory where sessions are taking place. We will also encourage you to follow all instructions closely, and immediately report any unusual exercise related symptoms.

COVID-19 Risks and Safety
At the end of this document, you will find a detailed description of how we will work to minimize the risks associated with COVID-19. We encourage you to contact us if you have any questions or concerns with regards to COVID-19 safety.

Benefits:
There are numerous benefits to you as a participant in this study. Engaging in the study will provide you with information related to your fitness and cardiovascular health. Participating in this study will also allow you to learn about the effects of interrupting extended sitting time on your health.

Cost of Participating:
There are no costs associated with participation in this study. There will be no reimbursement for any costs incurred for participating in this study (e.g. transportation fees etc.).

Withdrawal:
You have the right to withdraw from the study without any consequence and will be allowed to do so at any point during the study. If you would like to withdraw from the study, please contact the researcher via email at nicholas.orourke@ontariotechu.net or in person. In addition, any data collected from you can be withdrawn and destroyed. Please notify us if you would like your data to be destroyed. You have the right to withdraw your data at any point during the study or for up to 4 weeks following completion of your participation. A reminder, if you withdraw from the study, you will receive the bonus marks for the sessions commenced.

Participant Confidentiality:
At each session, the PI and an assistant will be present to collect data. Following the session, only the research team will have access to your data. Your data will be kept confidential and will be coded (therefore stored anonymously). All hard copies of your data will be stored in a locked cabinet in a laboratory at OTU. Furthermore, identifier codes will be stored in a separate office. The data will be saved on an OTU Google Drive. Your data may be used for secondary use if it is requested for use in a systematic review. In this case, identifier codes would not be provided, to ensure your confidentiality.

Conflict of Interest
You may be a student in the PIs courses or the TA's lab sections. Please note, there are no additional benefits or penalties associated participation or withdrawal related to this study. We will be following the standard protocols outlined in the Participant Pool.

Results
If you are interested in learning about the results of this study, you can read the thesis of Nicholas O'Rourke. This is expected to be published in our library within the next 12 months. Additional results will be available in the thesis of Emmeline Meens-Miller, 24 months from now.

CONSENT

I understand the procedures, potential risk and benefits of this study. Any questions regarding this study have been answered to my satisfaction.

I understand my consent to participate, or to not participate in this study is voluntary. I also understand my right to withdraw from any part or all of this study for any reason. I waive no legal rights by participating in this study.

If I have any questions regarding my rights as a research participant, or about any issues relating to this study, I will contact Dr. Shilpa Dogra at 905.721.8668 ext. 6240 or at Shilpa.Dogra@ontariotechu.ca.

Please indicate if you consent to your data being used for purposes of secondary analysis.

☐ - Yes

☐ - No

(If no, we will not use your data for future studies.)

I hereby consent to participate in this study.

_____	_____	_____/_____/_____
Participant (Print Name)	Signature	Date

For a member of the research study: I have ensured the named participant above has thoroughly understood all aspects of this research study, and have answered all questions to their satisfaction.

_____	_____	_____/_____/_____
Research Member (Print Name)	Signature	Date

Participant Concerns and Reporting:

Please read this consent form carefully and feel free to ask the researcher any questions that you might have about the study. If you have any questions about your rights as a participant in this study, complaints, or adverse events, please contact the Research Ethics Office at (905) 721-8668 ext. 3693 or at researchethics@ontariotechu.ca

If you have any questions concerning the research study or experience any discomfort related to the study, please contact the researcher at 905.721.8668 ext. 6240 or shilpa.dogra@ontariotechu,net.

By signing this form you do not give up any of your legal rights against the investigators, sponsor or involved institutions for compensation, nor does this form relieve the investigators, sponsor or involved institutions of their legal and professional responsibilities

This study has been approved by the OTU Research Ethics Board REB#16473.

COVID-19 Related Precautions

General Notes

- All researchers involved with this study are vaccinated with both shots of the COVID-19 vaccine, and will be carefully following public health guidelines to minimize their risk of exposure and transmission.
- 24h prior to each session you will be required to complete a COVID-19 self-assessment and indicate to the researcher if you have answered "yes" to any of the questions asked.
- All researchers will wear a mask and face shield/goggles at all times. You will be asked to **bring a mask or face covering from home**. It will be mandatory that you wear your mask or face covering until you enter the laboratory. In the event that you forget your mask or face covering, you will be provided with a surgical mask.

Laboratory Session:

- The research team is comprised of 4 researchers. Only two of these researchers will be present at a time during each session. Both researchers present will wear a face shield/goggles and a face mask at all times.
- You will be asked to arrive on campus dressed in active wear that you are comfortable running in. Please use the washroom prior to arrival; however, please note that a washroom is available if needed.
- One member of the research team will greet you at the ***rear entrance*** of the UA building and ask that you sanitize your hands with a 70% hand sanitizer solution. You will be required to wear your mask before entering the building. If you forget your mask a disposable mask will be provided.
- You will then be escorted to the laboratory via a short corridor. (Note: Access to this corridor is restricted to all personnel except the researchers and participants of this study)
- To maximize your safety, all surfaces and touch points will be sprayed and wiped down with a 70% alcohol solution before you enter the laboratory. Once inside the laboratory you may remove your mask or face covering.
- Once you have completed your laboratory session all contact surfaces will be sprayed and wiped down with a 10% bleach solution.
- Prior to exiting the laboratory, you will be asked to put your mask back on and use the provided hand sanitizer once more.
- A member of the research team will escort you out of the UA building using the same restricted corridor.
- **Expelled air:** During the maximal exercise test you will be exhaling through a tube that expels air into the room. At present, the nature of airborne transmission is unknown under such conditions. As such, both researchers present will always be wearing a face shield/goggles and medical style mask.

Note: Physical distancing cannot be practiced inside the lab due to the nature of the research.

Participant Confidentiality – Contact Tracing

- We will be collecting your name and phone number that we must retain in order to follow up with you and/or conduct contact tracing if you may have been exposed to COVID-19.
- In some cases, this may need to be shared with the University or Public Health, and as a result, we cannot guarantee privacy and confidentiality of your participation in the study.
- Contact information will be kept separate from data collection through the research study to allow for de-identification of the research data.
- Although your data will be kept confidential and stored anonymously, we cannot guarantee anonymity, as the personal contact information does identify you as a participant.
- In the event that you develop any COVID-19 symptoms or test positive after the study or at any point during the study, you must contact the researchers as soon as possible.

This study has been approved by the Ontario Tech University Research Ethics Board (REB) [insert REB # assigned] on [insert date].

Any questions regarding your rights as a participant, complaints or adverse events may be addressed to Research Ethics Board through the Ethics and Compliance Officer - please contact the Research Ethics Office at (905) 721-8668 ext. 3693 or at researchethics@ontariotechu.ca

Face-to-Face/In-Person Research Consent Form Addendum for COVID-19

Title of Research Study: Physiological responses to prolonged and interrupted sitting

Name of Principal Investigator (PI): Shilpa Dogra

PI's contact number(s)/email(s):

Phone: (905)721-8668 ext. 6240

Email: Shilpa.Dogra@ontariotechu.ca

Names Student Leads: Nicholas O'Rourke, BHSc; Emmeline Meens-Miller, BHSc

I agree to participate in this study taking place at Ontario Tech University during the current COVID-19 pandemic. I understand that my participation is optional. I confirm that I have read and understood the consent form and have been advised on the potential risks related to in-person face-to-face research involving human participants at this time.

If you feel that you are in a vulnerable group with respect to COVID-19 effects (e.g. senior, immunocompromised, living with individuals that may be susceptible to COVID-19), it may be best that you do not participate in the study.

Because you are coming on campus, the following safety protocols must be followed:

- Screening
- Use of non-medical masks or face covering while participating in the research study
- Follow instructions provided to you with respect to arriving at the study location, including entry points, designated waiting areas and washrooms, timing of arrival
- Hand washing
- Precautions taking public transit or travelling to the research site
- Physical distance (maintaining 2-meter distance from others)
- Personal Protective Equipment (PPE) provided to participants by research team

We will be collecting personal contact information that we must retain in order to follow up with you and/or conduct contact tracing if you may have been exposed to COVID-19 in coming to the research site. As a result, we cannot guarantee privacy and confidentiality of your participation in the study.

We cannot guarantee anonymity, as the personal contact information does identify you as a participant.

Contact information will be kept separate from data collection through the research study to allow for de-identification of the research data.

During this time, the university may request information relating to all people entering and exiting our campus. As such please be advised that it may not be possible to keep your participation in a study confidential; however, no information about the data you share with us in the study will be shared outside of the research team.

You maintain your right to withdraw from the study, including research data. If you do withdraw, we will continue to maintain your contact information and will only give it Durham Public Health and the University if required for contact tracing.

There may be additional risks to participating in this research during the COVID-19 pandemic that are currently unforeseen and, therefore, not listed in this consent form.

If you think you have COVID-19 symptoms or have been in close contact with someone who has it, use the Government of Ontario's COVID-19 self-assessment tool and follow the instructions it provides to seek further care. In addition, you must inform the Principal Investigator immediately for follow up.

By checking each of the boxes below, I acknowledge and agree with the statements as follows:

☐ I have either been fully vaccinated with an approved government vaccine, or I have chosen not to be vaccinated.

- ☐ I acknowledge and accept that there is a risk that I could be exposed to COVID-19 while participating in this research project, despite the approved precautions and protocols that have been put in place.
- ☐ I acknowledge and accept that while participating in the study, the researchers may need to be closer than the recommended social distancing guidelines in order to carry out the experimental protocols and/or procedures.
- ☐ I acknowledge and confirm that I am willing to accept this risk as a condition of attending the university to participate in research.
- ☐ I acknowledge and understand that there may be unknown risk related to COVID-19.
- ☐ I confirm that the study team has answered all my questions about the study and has advised me of all the risks related to in-person face-to-face research for this study.
- ☐ I acknowledge that participating in this study may involve third party risks to others where I may expose individuals that I live with or am in close contact with.

Consent to Participate:

1. I have read the consent form and understand the study being described;
2. I have had an opportunity to ask questions and those questions have been answered. I am free to ask questions about the study in the future;
3. I freely consent to participate in the research study, understanding that I may discontinue participation at any time without penalty. A copy of this consent form has been made available to me.

_____ _____

_____ _____

Print Study Participant's Name Signature Date

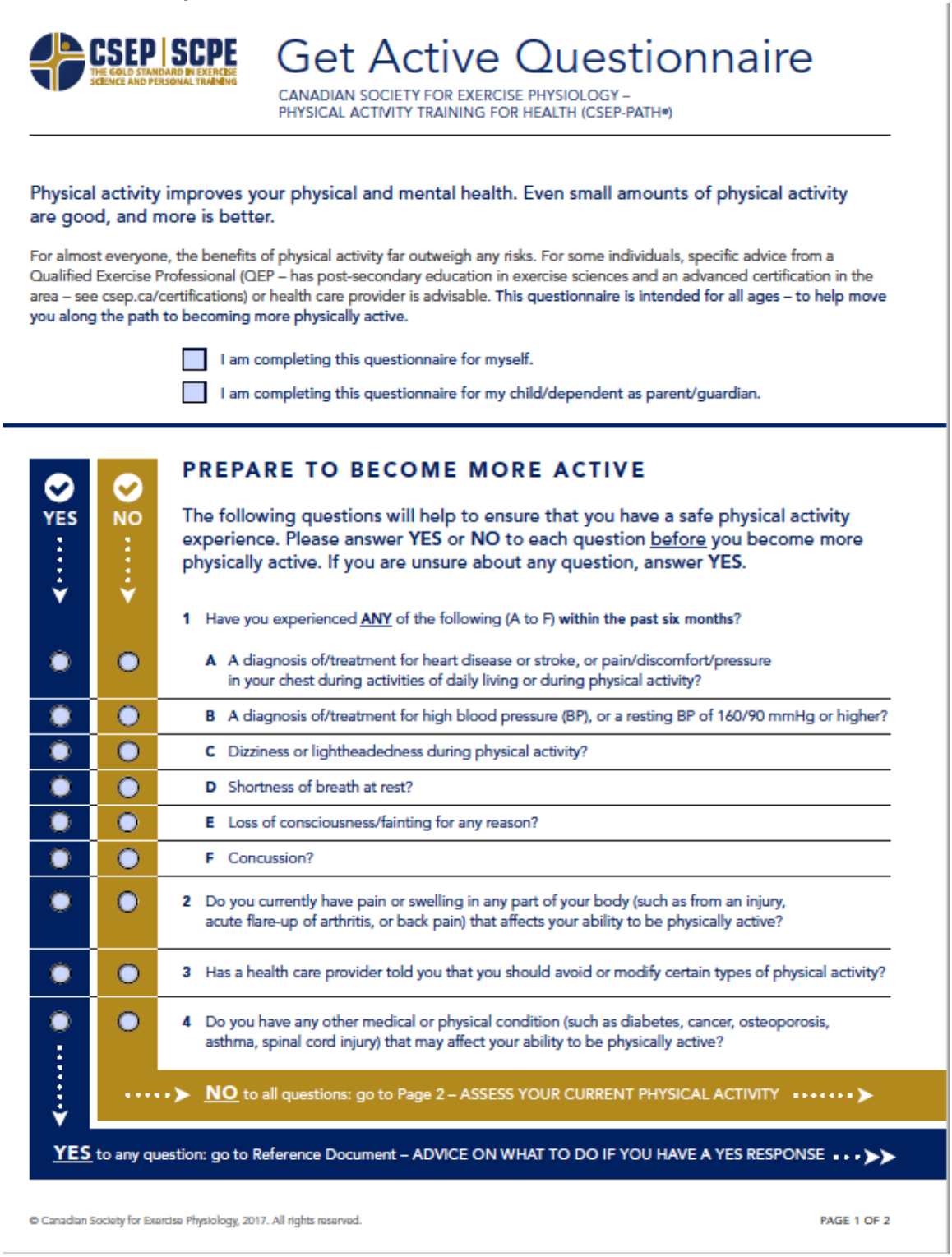
My signature means that I have explained the study to the participant named above. I have answered all questions.

_____ _____

_____ _____

Print Name of Person Obtaining Signature Date

A.3 Get Active Questionnaire



CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
		1 Have you experienced **ANY** of the following (A to F) **within the past six months**?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	**2** Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	**3** Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	**4** Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ____ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ____ MINUTES/DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ____ MINUTES/WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

- ☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.
- ☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print] | Signature (or Signature of Parent/Guardian if applicable) | Date of Birth

Date | Email (optional) | Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

A.4 Session 1 Data Collection Sheet

Session 1: Data Collection Sheet

Participant No.		**Date**	
Participant Arrival Time		**Session to be Completed Today**	1
Age		**Sex**	

☐ Informed Consent
☐ Get Active Questionnaire (*appended at end of doc*)
☐ Participant reminded right to withdraw
☐ Followed instructions

Obtain Emergency Contact information:
Name:
Relationship:
Phone Number:

Saliva Samples
☐ Saliva SOP followed
☐ Eppendorf tubes:
- Number obtained:
- ☐ Labeled

☐ Breakfast provided

Anthropometrics

Height (cm)	
Weight (kg)	

Resting Heart Rate & Blood Pressure

	Blood pressure (mmHg)	Heart rate (bpm)
Trial #1		
Trial #2		

Activity Monitor Fitting

☐ Monitor fitted, confirmed to be working
☐ Instructions provided

Maximal Exercise Test (data collection sheet below)

☐ Maximal Exercise Test SOP followed
☐ File labeled:
- Name:

End of Session

☐ Any questions answered
☐ Reminder of date for next session
☐ Instructions for next session provided

Maximal Exercise Test Data Collection Sheet

Participant Name/Id: Age: Height (cm): Date:

Time (mins)	Speed	Grade	HR	RPE
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

15				
16				
17				
18				
19				
20				

A.5 Session 2 and 3 Data Collection 3

Session 2 or 3: Prolonged or Interrupted Sitting Data Collection Sheet

Participant No.		**Date**	
Participant Arrival Time		**Session to be Completed Today**	Sitting Interrupted Sitting
Participant Has Arrived Fasted:	YES NO	**Participant Has No New Health Conditions Since Completing the Eligibility Questionnaire:**	YES NO

☐ Participant followed all other instructions
☐ Participant reminded right to withdraw

Resting Measures

Measure	**Data**	**Notes**
Heart Rate		
Blood Pressure		

Saliva Samples
☐ Saliva SOP followed
☐ Eppendorf tubes:
- Number obtained:
- ☐ Labeled

Capillary Samples
☐ Finger prick SOP followed
☐ Eppendorf tubes:
- Number obtained:
- ☐ Labeled

☐ Breakfast provided

Participant Fitted with Strain Gauge: YES

Time Chart

Enter the time at which each of the following steps took place:

Event	**Time**	**Notes**
Breakfast Provided		
Experiment Began		
Snack Provided		
Experiment Completed		

In-session Measures

Measurement	Measure	Data
1	Heart Rate Blood Pressure	
2	Heart Rate Blood Pressure	
3	Heart Rate Blood Pressure	
4	Heart Rate Blood Pressure	

Post-Session Measures

Measure	Data	Notes
Heart Rate		
Blood Pressure		

Saliva Samples

☐ Saliva SOP followed

☐ Eppendorf tubes:

- Number obtained:
- ☐ Labeled

Capillary Samples

☐ Finger prick SOP followed

☐ Eppendorf tubes:

- Number obtained:
- ☐ Labeled

Strain Gauge Removed:

Date and time of next session confirmed: YES

A.6 Saliva and Blood Samples Standard Operating Procedures

SALIVA SWAB SAMPLE COLLECTION AND HANDLING

Purpose and Background

Collection and handling of saliva swab samples used for analysis of inflammatory cytokines.

Equipment and Materials

- Oral Swab
- Swab Storage Tube

- Medical Gloves
- Centrifuge
- Freezer
- Cryogenic Gloves
- Biohazard Bag
- Lab Coat
- Paper Towel
- Disinfecting wipes
- 70% alcohol spray

Sample Collection Instructions:

1. If sample is being collected in ACE, cordon off area using long tables in the transition area outside the small chamber, and place signage in the area to indicate biohazardous materials are present.
2. Provide participant with water to rinse their mouth and wait 10 minutes before sample collection.
3. Researcher puts on lab coat, medical gloves, and mask.
4. Researcher removes Oral Swab from packaging.
5. Researcher labels sample.
6. Instruct participant to look at picture and place the swab in their cheek.
7. Instruct participant to keep the swab in place for 5 minutes and to avoid moving the swab around.
8. Instruct participant to remove cap of swab storage tube and place swab inside.
9. Instruct participant to recap swab storage tube.
10. Centrifuge sample (balanced with 2 samples, for 5 minutes, at 1200 rpm).
11. Pipette sample into Eppendorf tubes.
12. Place sample in freezer (-80°C).
13. At the end of each day, wipe down sink with disinfecting wipes.

Participant Instruction

Participants will be instructed on how to perform the saliva collection using this graphic/researcher demonstration.

***Storage**:* Samples will be collected in either UAB 346 or the ACE, and stored in a -80°C freezer in UAB 346. Samples will be transported from the basement of the Science Building to the 4th floor of the Science Building in room UA 4480. Prior to transportation, all samples will be securely sealed and double bagged with the Biohazard Symbol on the outside of the second bag. When samples are collected at the ACE, they will be double bagged and placed in a bucket of ice until the time of transportation; a Biohazard symbol will be placed on the outside of the container used to transport the samples.

***Analysis**:* All samples will be handled in a level 2 (L2) Biosafety Cabinet in UA 4480. All individuals involved with analysis have completed L2 Biosafety Training, and will be added to the appropriate biosafety permits.

***Sample Disposal**:* Saliva samples will be disposed using the UOIT pre-approved protocol as outlined in the Biosafety Manual. All collection tubes, assay plates, plastic pipettes, and gloves will be disposed of in a biohazardous waste bag before autoclaving for at least 30 minutes at 121° C. This bag will be placed into a regular garbage bag for disposal after autoclaving.

***Personal Protective Equipment**:* Gloves will be worn while collecting the sample. New gloves will be worn when analyzing the sample. Hands will be washed each time after gloves are removed. Cryogenic gloves will be used to place samples in the -80°C freezer. The researcher will wear a lab coat and gloves when analyzing samples.

Emergency Response Plan

In the event of a biological spill, section 10.0 of the UOIT biosafety manual will be followed. A hard copy of this manual is available in the laboratory and will be taken to the ACE.
The protocol outlined in section 10.2 in the UOIT biosafety manual will be followed in the event of a small spill of saliva. Relevant procedures are outlined below:

- Immediately notify other individuals in the area that there has been a biohazard spill.
- If necessary, block access to the area.
- Individuals involved in the spill should check for possible contamination of clothing, footwear and skin. Any potentially contaminated clothing should be left in the area until cleaned.
- Before beginning cleanup of the spill put on appropriate protective clothing (gloves, lab coats, mask, etc.)
- Identify the area requiring clean up and decontamination.
- Set up a disposal bag to allow easy discarding of contaminated cleanup materials.
- Use absorbent materials (paper or cloth towels) to dry the area where the saliva has spilled. Wipe the area with disinfecting wipes, and then spray with alcohol spray, and wipe again.
- Place the absorbent material into the disposal bag and repeat the decontamination procedure.
- Remove gloves and place them in a clearly marked biohazard disposal bag with the other contaminated materials. Seal the bag and place it inside another marked biohazard bag for disposal.
- Hand hygiene must be performed at the end of a biohazard spill clean up.

It should be noted that in the event of a medical emergency, the researcher has a hard copy of an emergency action plan posted in the laboratory; this will also be taken to the ACE. There is

also a phone in the laboratory and the ACE, so that security can be contacted to initiate emergency procedures.
Of note, a trained researcher will always be present for data collection and analysis.

Incident and Emergency Response
All individuals working with biohazardous materials will report all security incidents to the Certificate holder, Biosafety Officer and Campus Security as soon as possible. Security incidents include, but are not limited to, breach of containment, unauthorized removal of pathogens, and unauthorized personnel in restricted areas. Please refer to the UOIT Biosafety Manual for information on biohazard spill response procedures and other emergency procedures (e.g., earthquake, fire).

Emergency Medical Procedure
Emergency medical procedures apply when any person is exposed to body fluids, infections or communicable disease. Exposure may be via a cut or puncture wound, mucous membrane contact or non-intact skin contact.
• Wash the exposed site immediately.
• If mucous membrane contact (eyes, nose, mouth), or non-intact skin contact (cuts, rash or dermatitis), flush with water and the nearest faucet or eye wash station (available in the lab).
• Immediately inform the laboratory supervisor.
• Seek prompt medical attention, giving the medical provider details on the agent.
• As soon as possible, the PI **MUST** complete a UOIT Accident/Injury report. This is to be done electronically. This form can be found at http://www.uoit.ca/forms/accidentinjury/

Biosafety Manual
A hardcopy of this SOP and the UOIT Biosafety Manual is available in the laboratory at all times and will be taken to the ACE when data collection is occurring there.

FINGER PRICK BLOOD SAMPLE COLLECTION AND HANDLING
Purpose
Finger prick blood sampling will be used to collect 40-200 µL of blood for storage and analysis.

Scope and Applicability
1. This protocol does not apply to venous or arterial blood sampling.
2. This operating protocol refers to all instances in which capillary blood micro-samples are taken following pricking of one or more fingers or other skin sites (e.g., fleshy part of the ear lobe). Typical analytical determinations from the sampled blood include, but are not limited to, blood glucose or blood lipids via a point of care system (Alere, Cholestech LDX), and cytokines via ELISA or flow cytometry.
3. The micro-analyzers and sampling devices (lances, lancing units) are commercial and approved devices that do not require medical prescription or training. As such, similar finger/ear blood sampling is currently performed by the general public with no specific training.
4. Sampling will only occur in a laboratory setting. The procedures described here are based on those detailed for disposal of liquids and solids containing biohazardous agents in the UOIT Biosafety Manual.

Responsibilities

The main aim of this protocol is to ensure the safety of the participants as well as the researchers, and anyone who might inadvertently come in contact with the associated equipment and/or materials. More specifically, the aim is to ensure that blood from a tested individual is not carried over, coming in contact with another individual during or following the testing procedure.

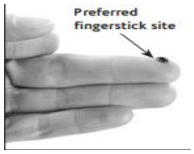
As per the permit in the researcher's lab, the researcher (and her graduate students) are responsible for ensuring this. First, they will follow outlined cleaning procedures, and don appropriate personal protective equipment as indicated below. Second, they will place a sign on the laboratory door to ensure that no cleaning staff enter the room to clean or clear garbage. Biohazard bags will be taken to UA 4405 to be autoclaved weekly, and will be transported in a leak-proof container. Finally, it should be noted that the lancets, pipettes, and microtainers are one-time use materials. Thus, there is no risk of transmission of blood-borne pathogens to the participants. According to Ekwueme et al (2002), the risk to the researcher is also negligible.

Materials and Equipment

1. Medlance Plus Universal Lancets
2. MiniPet Pipettes (40µL) / Capillary Tubes or microtainer
3. Eppendorf tubes
4. Alere Cholestech LDX Analyzer or Elisa or cytometry
5. Test Cassettes
6. Centrifuge
7. Hand warmers (disposable)
8. Gloves
9. Lab Coat
10. Goggles
11. Alcohol Swab
12. Alcohol Spray (70%)
13. Bleach Spray (10% hypochlorite bleach)
14. Dry Cotton Swabs
15. Gauze
16. Paper Towel
17. Biohazard waste/sharps disposal container

Procedures

1. Perform hand hygiene and put on gloves. Wear goggles and lab coat.
2. Provide hand warmer to participant for 3 minutes.
3. Choose a finger that is not cold, cyanotic, or swollen. Point to a place on the finger where the participant will make the prick.
4. Apply a massaging motion to the fleshy portion of the finger five or six times. 



5. Cleanse the ball of the finger with an alcohol swab.
6. Allow for finger to air dry.
7. Demonstrate the finger pricking procedure, and blood collection procedure.

8. Ask the participant to prick themselves, and provide step by step instructions as they go.

8. Ask them to twist off tab from lancet and position the lancet firmly against the puncture site.

8. Ask them not remove the device from the site until an audible click is heard. Ask them to discard the lancet into a biohazard sharps container.

8. Hand them a piece of cotton to wipe away the first drop. If blood does not flow freely, ask them to gently massage towards the puncture to induce bleeding. If blood will not flow – ask them to make another puncture on a new finger with a new lancet.

8. Give them the capillary tube or microtainer and ask them to position it directly beneath the puncture site. Ask them to apply gently, intermittent pressure along finger capillaries up to the puncture site (strong repetitive pressure (milking) must be avoided) to allow them to refill with blood and to help ensure continuous blood flow.

8. If the cut is made across the finger prints and the area dried, the blood should well up into a rounded drop.

8. Ask the participant to maintain the finger at a horizontal level to avoid venous stasis. Have them hold the capillary tube in a nearly horizontal position and collect the blood by touching the edge of the capillary tube or the top of the microtainer repeatedly to the drops of blood as they form.

8. Have them fill the capillary tube or microtainer until the blood has reached the required level. For anticoagulated samples, invert the tube manually, approximately 10 times to ensure adequate mixing.

8. Have them wipe the site dry and apply direct pressure with clean gauze or cotton ball until bleeding has stopped.

8. For capillary tube samples:

i. Use the plunger in the capillary tube to empty the blood sample on to the sample well in the cassette.
ii. Load the cassette in to the analyzer drawer and run the sample through the Alere, Cholestech LDX. Discard the capillary tube into a biohazard sharps container.
iii. Remove the cassette and discard in the biohazard container.

8. For microtainer samples:

i. Centrifuge the sample
ii. Pipette the plasma into Eppendorf tubes labelled with the participant ID number and session information.

iii. Transfer Eppendorf tubes to -80 freezer.
iv. Discard microtainer and pipette in the biohazard container.

8. Remove gloves, goggles, and lab coat. Perform hand hygiene.
8. Clean area down with 10% hypochlorite bleach.

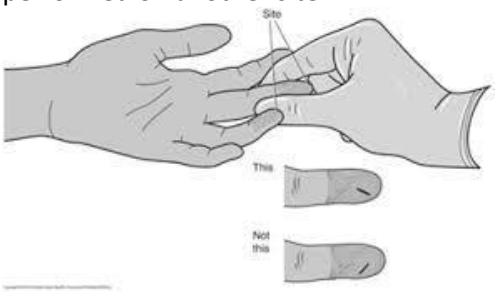
Participant Safety

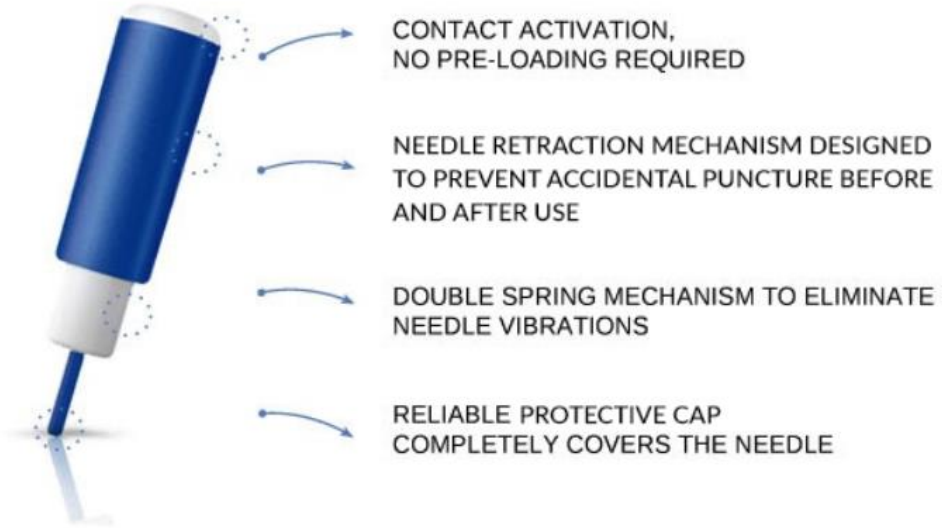
In addition to following cleaning procedures, and sample handling outlined herein, the researcher will ask whether the participant is comfortable and feeling well. These measures will help to prevent fainting.

The researcher will also ensure that the participant is seated comfortably and is not showing any signs of discomfort that could lead to fainting (e.g. loss of facial colour, excessive sweating, distress). If any of these signs are present, the procedure will be terminated immediately. If the participant feels light headed or demonstrates any other signs, they will be asked to remain seated, and will be given a juice box. Their heart rate and blood pressure will also be monitored. If the participant faints, the lab assistant will immediately call security and ask for the CERT, while the researcher will stay with the participant.

Finger Prick and Lancet Information

The researcher is required to have reviewed the video and PDF instructions on how to perform finger prick blood sampling (acquired from MEDLANCE plus website, included below) in order to adequately demonstrate the procedures to the participant. The following image displays the sites at which the finger prick can occur. Illustrating the tips of each finger excluding the thumb and index finger. No finger will be punctured twice, if another puncture is required it will be performed on another site.





https://www.clockmedical.com/customer/docs/skudocs/583_Instructions.pdf
http://htl-strefa.com/products/safety-lancets/medlance-plus/

Personal Protective Equipment and Handwashing

As noted above in point 1, the researcher will begin with washing their hands, then don a lab coat, then wear gloves and goggles. After the sample has been collected, analyzed, and discarded, the researcher will remove gloves and lab coat, and then wash their hands (point 21).

Waste Management Plan

As part of the procedure to collect and analyze the sample, the following waste materials will be generated: cotton, gauze, lancets, capillary tubes or microtainer, pipettes, gloves, and cassettes. All of these materials will be handled by the trained researcher, and will be discarded into biohazards containers and sharps containers accordingly, and be disposed of weekly. Only the PI and her graduate students have access to the laboratory. The laboratory is locked when none of these individuals are in the laboratory. A sign will be placed on the door to ensure cleaning staff do not enter the laboratory.

Disposal of Blood Samples:

- Any residual blood samples will be collected into leak-proof containers able to withstand autoclaving temperatures. They will not be sealed.
- Containers will be placed into an autoclavable pan with sufficient capacity to contain all liquid in case of breakage and autoclaved for 60 minutes at 121˚C (waste cycle).
- Following steam sterilization, innocuous liquids may be disposed of via the laboratory drainage system. Flush with sufficient clean water to purge the drain immediately after disposal of all liquids.

Disposal of Solid Materials Contacting Blood Samples:

- Any solid items which have contacted blood samples will be collected in orange autoclavable bags which display the biohazard warning symbol. These items may include gloves, cassettes, pipette tips, serological pipettes, absorbent tissues, etc.
- The autoclavable bags will be closed but not sealed airtight to allow steam penetration, and situated in a leak-proof container before they are placed into the autoclave chamber. The solid waste will be autoclaved for 60 minutes at 121˚C (waste cycle).
- Following steam sterilization and cooling, the biohazard symbols on the autoclave bags will be covered with blackened autoclave tape to deface the biohazard label as well as indicate that the contents have been sterilized. The entire autoclave bag will be placed into a black plastic garbage bag for disposal and set out for residual waste pick up.

Disposal of Sharps Container:
Once the fill line is reached, the sharps container will be closed, placed in an autoclavable bag, autoclaved and then disposed of in regular garbage.

Transportation and Storage of Microtainer samples*:* Samples will be collected in UAB 346, and stored in a -80°C freezer in UA 4480. Samples will be transported from the basement of the Science Building (UAB 346) to the 4th floor of the Science Building in room UA 4480. Prior to transportation, all samples will be securely sealed and double bagged with the Biohazard Symbol on the outside of the second bag.

Analysis*:* All samples will be handled in a level 2 (L2) Biosafety Cabinet in UA 4480. All individuals involved with analysis have completed L2 Biosafety Training, and will be added to the appropriate biosafety permits. Samples will be analyzed using enzyme-linked immunosorbent assays (ELISA) or cytometric bead array-based flow cytometry to determine concentrations of cytokines. For these procedures, sample dilutions will be carried out in a BSL2 cabinet, as will washing steps. Analysis of cytokine concentrations by ELISA will be carried out using a BioTek plate reader, and analysis by flow cytometric bead array will be carried out using an Accuri C6 benchtop flow cytometer. Samples for flow cytometry are routinely fixed with paraformaldehyde.

Emergency Response Plan
In the event of a biological spill, section 10.0 of the UOIT biosafety manual will be followed. A hard copy of this manual is available in the laboratory.
It should be noted that in the event of a medical emergency, the researcher has a hard copy of an emergency action plan posted in the laboratory. There is also a phone in the laboratory so that security can be contacted to initiate emergency procedures.
Of note, a trained researcher will always be present for data collection and analysis.

Below is an emergency action plan that will be posted in the laboratory:
12.7 Incident and Emergency Response
All individuals working with biohazardous materials will report all security incidents to the Certificate holder, Biosafety Officer and Campus Security as soon as possible. Security incidents include, but are not limited to, breach of containment, unauthorized removal of pathogens, and unauthorized personnel in restricted areas. Please refer to the UOIT Biosafety Manual for

information on biohazard spill response procedures and other emergency procedures (e.g., earthquake, fire).

12.8 Management of Exposure to Blood and Body Fluids

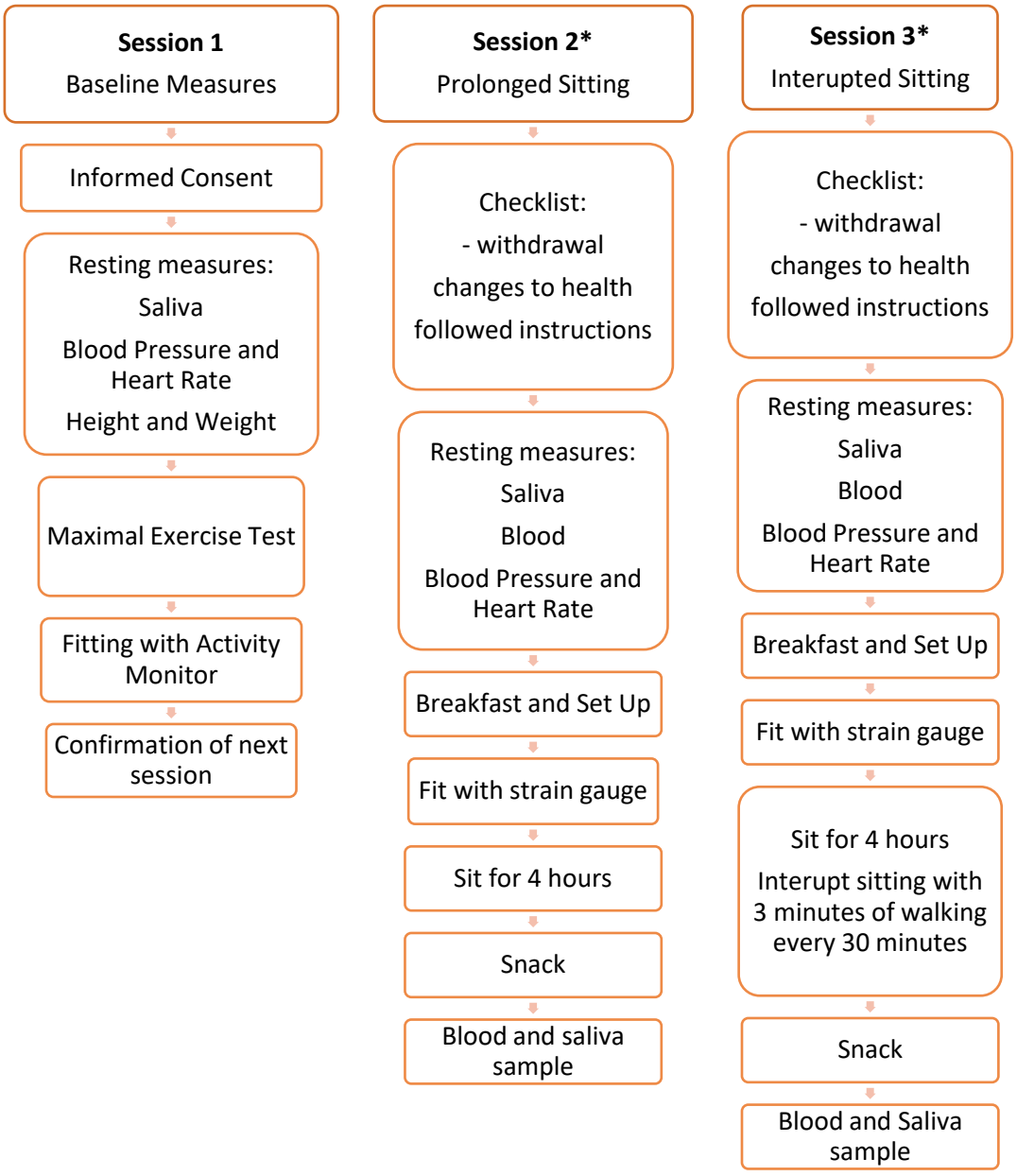
- Immediately on exposure, administer First Aid.
- As soon as possible, the PI **MUST** complete a UOIT Accident/Injury report. This is to be done electronically. This form can be found at http://www.uoit.ca/forms/accidentinjury/.
- Copies are to be sent to the Manager of Insurance and Risk Management and a copy will be kept in the PI's office. A copy will also be sent to Jamie Coad.
- If needed, the PI may visit one of the following emergency departments:
    - **Lakeridge Health Oshawa,** 1 Hospital Court, Oshawa, ON; Tel: 905-576-8711 x 3214/4560 & Fax: 905-721-4749
    - **Lakeridge Health Ajax**, 580 Harwood Avenue, Ajax, ON; Tel: 905-683-2320 x 1210 & Fax: 905-428-8277
- Transportation should be via Blueline Taxi Services. Blueline can be contacted at 905-440-2000, state that this is an emergency and that the taxi is to proceed to the Avenue of Champions by the UA building. Because the maximum benefit of immunoprophylaxis is achieved the sooner it is initiated, (preferably 1 or 2 hours post exposure).
- Call Security, extension 2400, so that they are aware that a taxi is arriving to take the PI to the hospital. Then proceed to the UOIT UA entrance.

Biosafety Manual

A hardcopy of this SOP and the UOIT Biosafety Manual is available in the laboratory at all times.

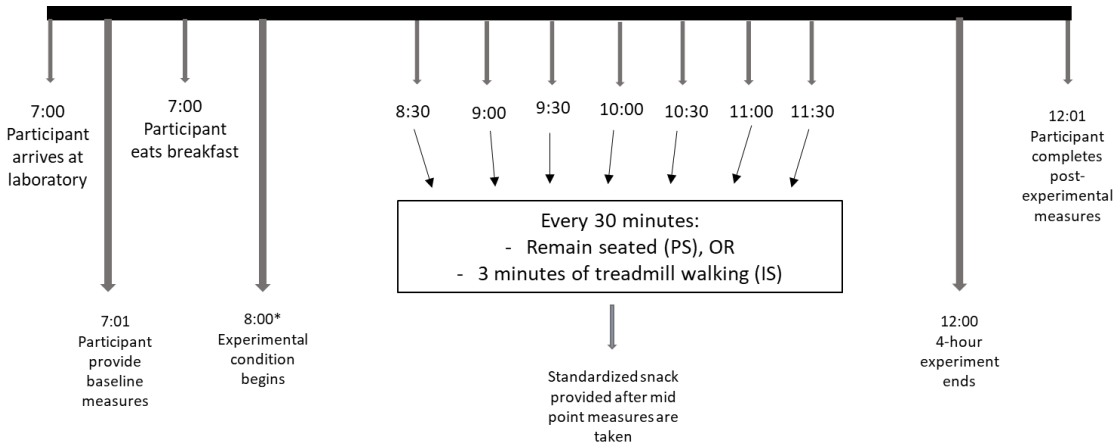
A.7 Study Timeline Overview

Experimental Protocols: Overview



***Note:** session 2 and 3 will be performed in random order

Closer look at Session 2 and 3:



* Participants will be able to start the sitting protocol at 8am or 9am, pushing the timeline ahead an hour, finishing at 1pm. This is up to the participant and will be consistent for all that individual's sessions.

Appendix B.

B.1 Figure 6. Salivary IL-8 concentrations before and after Prolonged (a) and Interrupted Sitting (b) Sessions, and percent change in salivary IL-8 (c) adjusted for to salivary protein concentrations

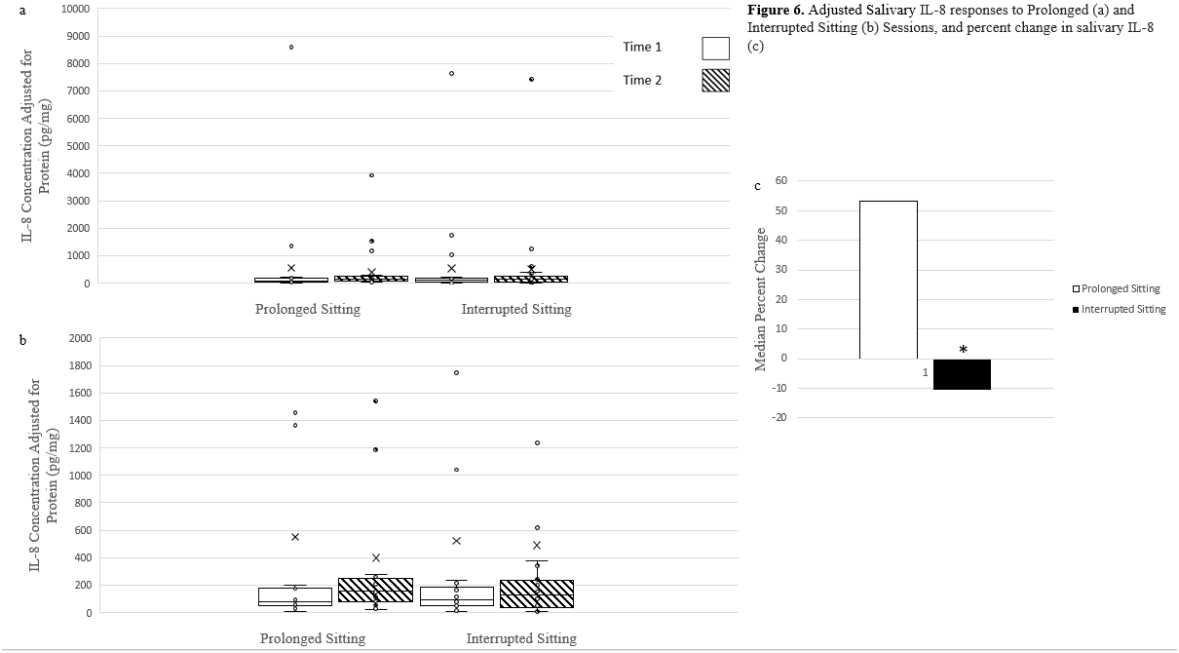
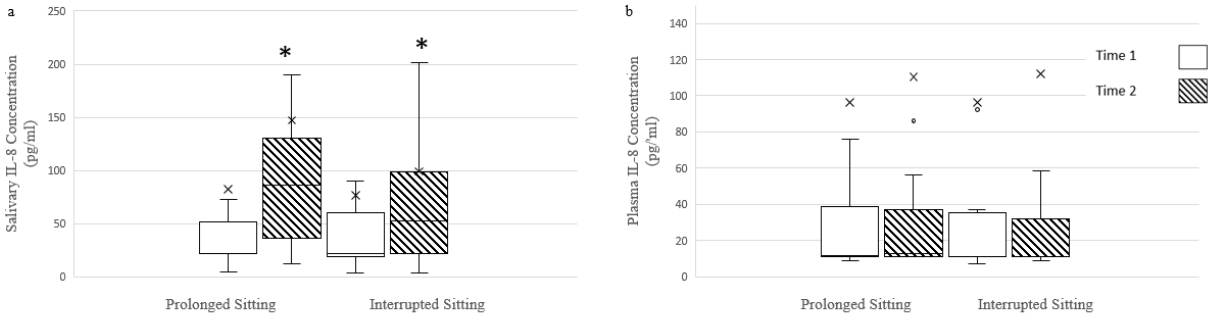


Figure 6. Adjusted Salivary IL-8 responses to Prolonged (a) and Interrupted Sitting (b) Sessions, and percent change in salivary IL-8 (c)

B.2 Figure 7**.** Salivary (a) and Plasma IL-8 Concentrations (b) Response to Prolonged and Interrupted Sitting with Outliers Removed

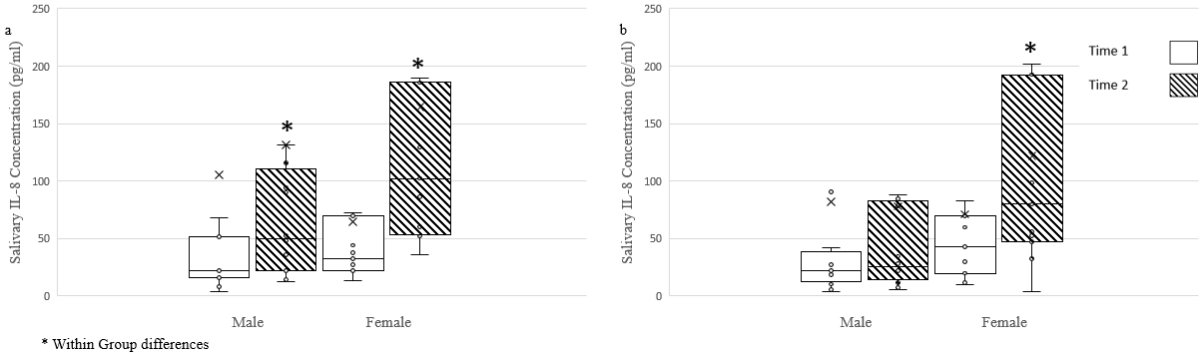
Figure 7. Salivary (a) and Plasma IL-8 (b) Response to Prolonged and Interrupted Sitting with Outliers Removed



Outliers are removed from figures There were two outliers for each saliva measure and three outliers for each plasma measure.

B.3 Figure 8. IL-8 concentrations in response to Prolonged (a) and Interrupted Sitting (b) for Males and Females, Adjusted for Protein and with Outliers Removed

Figure 8. IL-8 responses to Prolonged (a) and Interrupted Sitting (b) for Males and Females, Adjusted for Protein and with Outliers Removed



* Within Group differences