Understanding the combined effects of exercise and sedentary behaviour on cognitive, metabolic, and vascular function in older adults

Mr Michael J. Wheeler BSc (Hons)
Thesis Declaration

I, Michael Wheeler, certify that:

This thesis has been accomplished during enrolment in this degree.

This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

In the future, no part of this thesis will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

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The research involving human data reported in this thesis was assessed and approved by The University of Western Australia Human Research Ethics Committee (RA/4/1/6990) and from The Alfred Hospital Ethics Committee (181/14) and (51/16). Written informed consent has been received and archived for the research involving patient data reported in this thesis.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

This research was supported by an Australian Government Research Training Program (RTP) Scholarship.

Signature: [Embedded Image]

Date: 24-July
Abstract

Background
Cardiovascular disease, type 2 diabetes (T2D) and dementia, are leading causes of death and disability worldwide. In the context of population ageing, their increasing prevalence represents a major global challenge. The development of these diseases and their risk factors are further compounded by low levels of physical activity, particularly among older overweight and obese adults. In addition to physical inactivity, sedentary behaviour has recently emerged as a distinct, and highly prevalent, behavioural consideration for public health. Indeed, it is possible for a person to achieve the guideline-recommended daily amount of exercise (30 minutes at moderate-vigorous intensity), while also devoting a large portion of the same day to sedentary behaviours, typically prolonged sitting. The idea that the health benefits of exercise could be mitigated by prolonged sitting is controversial, and provides a compelling focus for current research.

Experimental evidence demonstrates that interrupting prolonged periods of sitting with intermittent light-intensity activity ‘breaks’ may improve markers of cardiometabolic risk. However, there is a lack of experimental evidence on the effect of prolonged sitting, and breaks in sitting, on aspects of brain health, including brain blood flow and cognition. This is an area of interest as many ‘cardiometabolic’ risk factors relating to glucose metabolism, insulin resistance, blood pressure and vascular function, are also risk factors for cognitive decline and dementia. Moreover, experimental studies have traditionally investigated the effects of exercise, or the effects of sedentary behaviour in isolation, but have rarely considered the most ecological scenario: the combination of these behaviours. Specifically, it is unknown whether exercise-induced effects on markers of cardiometabolic and cognitive health are influenced by a subsequent period of prolonged sitting, or breaks in prolonged sitting. In acknowledgement of these evidence gaps, this thesis sought to provide experimental evidence on the combined effects of exercise and sedentary behaviour on cognitive, metabolic and vascular function in the acute setting.

Methods
The new evidence presented in this thesis is the result of a randomised crossover trial designed and completed during a three-year period of data collection. In a controlled experimental setting, older overweight to obese adults (n=67; 67±7 years; BMI 31.2±4.1 kg/m²), completed three 8-hour conditions in random order, separated by a minimum 6-
day washout (Figure 1). These conditions were designed to reflect realistic patterns of behaviour such that the results may have reasonable real-world validity.

![Figure 1. Randomised crossover trial conditions. SIT: Uninterrupted sitting (8 hr, control). EX+SIT: Sitting (1 hr), moderate-intensity walking (30 min), uninterrupted sitting (6.5 hr). EX+BR: Sitting (1 hr), moderate-intensity walking (30 min), sitting interrupted every 30-minutes with 3-minutes of light-intensity walking (6.5 hr).](image)

**Thesis structure**

**Chapter 1 – Introduction and literature review.**


This chapter begins by outlining the public health context underlying the research in this thesis. It describes how epidemiology has revealed associations between sedentary behaviour and cardiometabolic health. Specifically, increased sedentary time is consistently associated with poorer glycaemic control, and increased risk of cardiovascular disease, T2D and all-cause mortality. In addition, emerging evidence suggests an association between sedentary behaviour and cognitive health. The aim of this chapter is to integrate evidence on the cardiometabolic impact of sedentary behaviour, with what is known about the physiology of the brain; that is, to review the evidence describing how sedentary behaviour may have a detrimental impact on brain health. It concludes that ‘cardiometabolic’ mechanisms, broadly defined, likely play an important role in this context. This narrative synthesis of literature informed the outcomes which were subsequently tested in an experimental setting (Chapters 2-5).
Chapter 2 – The effects of exercise with and without breaks in sitting on cognitive function and serum brain derived neurotrophic growth factor.


While there is an established role for exercise in improving cognition in the acute setting, results of large randomised trials are mixed. This is counterintuitive since an acute bout of exercise is the substrate of an exercise intervention when repeated multiple times, over a period of months or years. However, no exercise intervention aiming to improve cognitive health has targeted both exercise and sedentary behaviour concurrently. This chapter investigates whether the cognitive benefits of an exercise bout are influenced by subsequent sedentary behaviour. The results demonstrate that a morning bout of exercise improves cognitive performance over an 8-hour period, relative to prolonged sitting. However, the aspect of cognition that improves, depends on whether-or-not subsequent sitting is interrupted. The results demonstrate that exercise improves executive function during a subsequent period of prolonged sitting. However, when exercise is combined with breaks in sitting, working memory improves, but executive function does not improve relative to prolonged sitting. In addition, serum brain derived neurotrophic growth factor (BDNF) increases following exercise, with or without subsequent breaks in sitting. This suggests that different patterns of physical activity and sedentary behaviour may enhance distinct aspects of cognition.

Chapter 3 - The effects of exercise with and without breaks in sitting on postprandial glucose, insulin and triglycerides.

Based on Wheeler et al. (2019). Prepared for publication.

The previous chapter demonstrated that both continuous exercise and breaks in sitting influence cognitive performance. However, there was no clear ‘best’ condition in terms of overall cognition. Therefore, it remains unclear whether a strategy that combines these behaviours would be superior to a strategy that targets exercise alone. However, insight in this regard may be gained by investigating cardiometabolic outcomes, given the interplay between exercise, cardiometabolic function and cognition described in Chapter 1. Building on the previous chapters, this chapter reports the finding that exercise-induced
reductions in postprandial insulin and triglycerides are amplified by subsequently interrupting prolonged sitting, relative to exercise followed by prolonged sitting. This suggests that the benefits of exercise on cardiometabolic function can be amplified by regular interruptions to sitting, which may have implications for the design of future exercise interventions.

Chapter 4 – The effects of exercise with and without breaks in sitting on blood pressure and plasma catecholamines.

Based on Wheeler et al. (2019). Effect of morning exercise with or without breaks in prolonged sitting on blood pressure in older overweight/obese adults: Evidence for sex differences. Hypertension. DOI: 10.1161/HYPERTENSIONAHA.118.12373

Continuing with the theme of cardiometabolic health, this chapter reports on sex differences in the blood pressure and catecholamine response to exercise and breaks in sitting. Previous research demonstrates that relative to aged matched males, pre-menopausal women are protected from the vascular impacts of prolonged sitting due to increased vascular β-adrenergic sensitivity and estrogenic upregulation of nitric oxide. Since all females in the current study were post-menopausal, this degree of protection from prolonged sitting may not be present. The findings reveal that exercise-induced reductions in blood pressure are amplified by subsequent interruptions to prolonged sitting, but only in females. Males had equal blood pressure lowering effects following exercise, regardless of whether subsequent sitting was interrupted with walking breaks. We also observed sex-differences in the adrenaline response, whereby females demonstrated reduced adrenaline, but males demonstrated increased adrenaline following exercise, with or without breaks in sitting. This underscores the important role of exercise in blood pressure control for men and women. It also suggests that women may achieve optimal blood pressure control by considering both exercise and sedentary behaviour.

Chapter 5 – The effects of exercise with and without breaks in sitting on cerebral blood flow.

Based on Wheeler et al. (2019). Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older adults. Journal of Applied Physiology. DOI:10.1152/japplphysiol.00001.2019
This penultimate chapter investigates a vascular outcome with direct relevance for brain health, cerebral blood flow. The findings reveal differences in the 8-hour pattern of cerebral blood velocity between the respective experimental conditions. During the uninterrupted sitting condition, a pattern of decline in cerebral blood velocity in bilateral middle cerebral arteries was observed. Specifically, the sum of bilateral velocities declined by 20% following breakfast, and stayed lower than baseline for remainder of the condition. However, when a morning bout of exercise was performed, there was an initial dip in cerebral blood velocity of approximately 20%, followed by a subsequent recovery in the afternoon, such that afternoon time points were not significantly different to baseline. This highlights the important role of morning exercise for the daily maintenance of brain health.

Chapter 6 – Conclusions
This thesis contributes a novel set of integrated experimental findings that advance our understanding of exercise and sedentary behaviour by highlighting their complimentary effects on cardiometabolic and cognitive health. Outcomes more directly related to brain health such as cognitive performance, BDNF and cerebral blood flow were all improved following a morning bout of exercise, with or without subsequent breaks in sitting. This suggests that uninterrupted sitting should be avoided, and moderate-intensity exercise encouraged, for the daily maintenance of brain health. However, additional benefits were observed for outcomes of postprandial insulin, triglycerides and blood pressure when exercise was combined with subsequent breaks in sitting. This represents an opportunity to optimise improvements in these outcomes by considering sedentary behaviour in addition to planned exercise. Moreover, improved cardiometabolic health may have implications for cognitive health over time. Future studies should test whether similar strategies, when repeated over longer periods, result in sustained improvements in cardiometabolic and cognitive outcomes. In addition, more studies in the acute setting are needed to determine how to optimise patterns of exercise and sedentary behaviour to inform the design of longitudinal studies and interventions. Nonetheless, the research in this thesis has established that physiologically distinct changes occur in the acute setting as a result of different patterns of behaviour involving continuous exercise, intermittent light-walking and prolonged sitting. This knowledge may lead to improved non-pharmacological strategies to reduce the risk of cardiovascular disease, T2D and dementia in older overweight to obese adults.
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Associated publications, presentations, and awards

Publications by the candidate produced during candidature which are directly relevant to the thesis


Additional publications produced during candidature


International conference presentations


National conferences


Other abstracts where the candidate was not the presenting author


Invited presentations/ seminars

1. Invited presentation: “Understanding the role of sedentary behavior within the triad of physical activity, cardiometabolic function and cognition”. 74th Japanese Society of Physical Fitness and Sports Medicine Congress, Tsukuba, Japan. September 2019
2. **Invited webinar**: “Disseminating research output to increase audience and outreach”. *International Society for Physical Activity and Health Webinar*. September 2018. [webpage link]

3. **Invited presentation**: “Practical science communication skills”. *Edith Cowen University 3-minute thesis launch*. May 2018


Community engagement/science outreach – non peer reviewed publications

The following publications with “*The Naked Scientists*” are the result of support from The University of Western Australia (UWA) to travel to Cambridge in the UK to spend three months working as a science journalist. This opportunity arose after winning the UWA 2016 three-minute thesis competition, when *The Naked Scientists* offered me a three-month internship making science radio shows and writing articles about scientific research.


**Significant awards during candidature**

1. Honourable mention on the Dean’s list (2019). Awarded by The Board of the Graduate Research School in recognition of an outstanding thesis at The University of Western Australia.

2. Young Investigator Award - 2018 (3rd place) & 2019 (1st place). European College of Sport Science (ECSS).


5. Quarterly research prize awarded by The Baker Heart and Diabetes Institute for publication excellence in the journal Hypertension (2019).

6. Travel Award (2018). From The University of Western Australia to attend an international conference. $1850

7. First place at the 3-Minute Thesis Competition (2016). The University of Western Australia. [presentation link]


9. Travel Award (2016). From The Baker Heart and Diabetes Institute to attend an international conference. $800

Authorship declaration: Co-authored publications

This thesis is presented as a series of papers and contains work that has been published.

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<th>Chapter</th>
<th>Publication Title</th>
<th>Status</th>
<th>Student Contribution</th>
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<td>1</td>
<td>Sedentary behavior as a risk factor for cognitive decline? A focus on the influence of glycemic control in brain health</td>
<td>Published</td>
<td>Performed literature review; created figures; wrote the paper; critical revision; corresponding author</td>
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<tr>
<td>2</td>
<td>Distinct effects of acute exercise and breaks in sitting on cognition in older adults: a randomised crossover trial</td>
<td>Published</td>
<td>Study design; data collection; data analysis and interpretation; wrote the paper; created figures; critical revision; corresponding author</td>
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<td>3</td>
<td>Combined effects of exercise and interrupting sitting time on postprandial glucose, insulin and triglycerides in older overweight/obese adults</td>
<td>Prepared for publication</td>
<td>Study design; data collection; data analysis and interpretation; wrote the paper; created figures; critical revision; corresponding author</td>
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<td>4</td>
<td>Effect of morning exercise with or without breaks in prolonged sitting on blood pressure in older overweight/obese adults: Evidence for sex differences</td>
<td>Published</td>
<td>Study design; data collection; data analysis and interpretation; wrote the paper; created figures; critical revision; corresponding author</td>
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<td>5</td>
<td>Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older Adults</td>
<td>Published</td>
<td>Study design; data analysis and interpretation; wrote the paper; created figures; critical revision; corresponding author</td>
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Student signature: [signature]
Date: 24-JUNE-19

I, Prof Daniel Green certify that the student’s statements regarding their contribution to each of the works listed above are correct.

As all co-authors’ signatures could not be obtained, I hereby authorise inclusion of the co-authored work in this thesis.

Coordinating supervisor signature: [signature]
Date: 24-JUNE-19
Acknowledgements

I thought it would be easy to write my acknowledgements. If writing a thesis is the intellectual equivalent of running a marathon, surely I have crossed the finish line? Writing the acknowledgements must be similar to ordering a beer after the race and telling everyone what a challenging, but great race it was; and that I couldn’t have done it without help along the way. But it’s harder than that. It’s hard because I’m not really sure what the purpose of the acknowledgements is.

Maybe the purpose is to capture a moment in time, so when I read this again, future Michael will remember. Maybe my future self is contemplating doing another PhD? In that case I should read this and remember all the early morning alarms that woke me up for testing. All the hours spent labelling tubes and weighing out food. All the moments of squinting at the screen, the deep breaths, the late nights and the weekend work. The logical conclusion for my future self should be not to do another PhD, and go travelling instead.

But maybe the purpose is to offer future students a glimpse of PhD life. In which case I would honestly say it is amazing. You get to spend hours reading, designing, writing and presenting about what fascinates you. You get to ask questions nobody yet knows the answer to, and do cool research to learn something new. Then there are all the smart, hardworking, and fun people you meet along the way.

I know the purpose of the acknowledgements is to thank those people, but I hope anyone who has helped me get across the line knows who they are, and knows that I am deeply grateful. However, there are a few special people I would like to mention.

David, you were my entry into PhD life. I want to thank you for always being so supportive, positive and encouraging. You welcomed me into the lab and gave me responsibility during an 8-month work placement, even before you realised I looked like Jobe Watson. I had such a good experience then that I couldn’t wait to come back out to do a PhD.
Danny, I remember the first time I saw you present. It was at Kuopio, in Finland. Your presentation was as hilarious as it was fascinating, and I hoped that as my supervisor you would teach me your ways. I really value my connection to you and the lab at UWA. It was there I won the 3MT comp and prepared my winning talk for ECSS 2018, thanks to your guidance. You have cultivated both an academic and family environment within the lab, and I always look forward to coming back.

Paddy, I don’t think I could have asked for a better PhD companion. It must have been the combination of an Irish name with a New Zealand accent. Your hard work, dedication, critical thinking and down to earth approach to science has inspired me. I know these characteristics permeate to success in non-academic aspects of your life too, most notably in the slippery pig arena; proof you are a great guy as well as a great scientist.

Caitlyn, thank you for putting up with me over the last few months. It must have been hard to deal with all the late nights, me being distracted, and my refusal to ever cook by declaring “desperate times”. To continue the marathon analogy, you have been the person on the sidelines cheering the most. I am grateful for all the love and support you have shown me and I look forward to doing the same for you as you enter the final 6 months (fingers crossed) of your PhD.

Mam, Dad & Alan, although I have been living away for the past few years I have felt your love and support as strong as ever. I know it would not have mattered what I ended up doing. You have always encouraged me to seek happiness wherever it may be. I would not have been able to take advantage of the opportunities that arose along the way without the values of respect for others, self-sufficiency and optimism that you gave me. I could not have done any of this without you.

In addition, many others have helped me along the way. Back here in Ireland (I’m writing this in Skerries) those people are my friends and family, including but not limited, to Cian Hurley and the Hams; Enda, John, Barry, Heidi, and Donal O’Gorman; the Augheneys and the O’Callaghans.

In Melbourne, the Gourlays and the Wheelers. Through The Baker Institute I’m lucky to have met and worked with Neville Owen, Bronwyn Kingwell, Megan Grace, Beth Howard, Julian Sacre, Tara Boelsen-Robinson, Gavin Lambert, Aarup Dhar, Glen

Then in Perth, more Wheelers. Through UWA and Danny’s lab I’m lucky to have met and worked with, Louise Naylor, Kurt and Brianne Smith, Howard Carter, Andy Haynes, Sarah Hissen, Lauren McKeown, Hannah Thomas, Channa Marsh, Treya Long, Elisa Robey, Jaye Lewis, Keith George, Helen Jones, Dave Oxborough, Phil Ainslie, Michael Rosenberg, Tim Cable, Bomba, Giovanna Biagioni, Robyn Owens, Krystyna Haq, Daria Shkredova, Anna Scheer, Arga, Grant Landers and Tim Ackland. Through extension of the UWA 3MT, I’m lucky to have met and worked with Chris Smith, Izzie Clarke, Katie Haylor, Stevie Bain and Georgia Mills.

If you were hoping to see your name here but were disappointed, don’t worry. There are 200+ pages in this thesis and I may have decided to insert your name somewhere in there. You will just have to read on to find out!
Preface

Sedentary behaviour – put simply, too much sitting – has recently emerged as an important contributor to poor health. From a public health perspective, excessive sedentary behaviour is now considered as being distinct from not getting enough exercise, suggesting that these behavioural parameters represent two levers which may both influence population health. Epidemiological research has consistently demonstrated that a high volume of sitting, which is highly prevalent, is adversely associated with markers of cardiometabolic risk, cardiovascular disease and type 2 diabetes (T2D) incidence and mortality.\(^1\)\(^-\)\(^3\) A current focus for research in this area has been to determine what level of exercise is required to mitigate the health risks posed by high volumes of sitting.

These observations are important in the context of global trends of population ageing and rising obesity rates. Older overweight and obese adults not only represent a group in which low physical activity and high volumes of sitting are ubiquitous; they are also at an increased risk of developing a multitude of chronic diseases, including but not limited to cardiovascular disease and T2D. For example, ageing and physical inactivity are also major risk factors for dementia, which is fast becoming one of the leading causes of death and disability globally. Interventions that could delay or prevent its onset are urgently needed.

Strong evidence supports the positive effects of acute exercise on markers of cognitive health, yet evidence is inconclusive as to whether exercise training can delay the onset of dementia. However, when viewed through a public health lens, exercise is one component of the spectrum of physical activity which includes sedentary behaviour, light-activity and moderate-to-vigorous exercise. Sedentary behaviour and light-intensity activity represent a substantial contribution of time, with some estimates suggesting they comprise 95% of behaviour that occurs during waking hours.\(^4\) Reducing and breaking up prolonged bouts of sitting with intermittent walking improves markers of cardiometabolic risk,\(^5\)\(^,\)\(^6\) yet the implications of this approach for brain health are not well understood.

The experimental work presented in this thesis originated from practical questions about how different patterns of physical activity and sedentary behaviour influence cardiometabolic and cognitive health. For example, a person can exercise regularly but also engage in high volumes of sitting throughout the day. However, it is unknown
whether the benefits of exercise are mitigated by subsequent prolonged sitting. Given how widely applicable such scenarios are to everyday life, it seems imperative to investigate the combined effects of exercise and sedentary behaviour on cognitive performance and markers of cardiometabolic risk. Such evidence may inform and improve interventions targeting the prevention of cardiometabolic disease, T2D and dementia by providing rationale to target the whole spectrum of human movement.

This thesis by publication aims to investigate two knowledge gaps.

In Chapter 1, this thesis will address the implications of sedentary behaviour for brain health. This is done through a synthesis of literature which provides the context and rationale for subsequent experimental investigation.

Secondly, through Chapters 2-5, this thesis will address the knowledge gap relating to the combined effects of exercise and sedentary behaviour by providing experimental evidence and interpretation on the combined effects of these behaviours on cognitive performance, postprandial glucose and insulin metabolism, blood pressure, and cerebral blood velocity (Chapters 2, 3, 4 and 5 respectively).

Finally, an overall discussion in Chapter 6 will contextualise these findings and what they mean for both public health and future research.

As this is a thesis by publication, each paper will be linked by a concise summary section to minimise excessive repetition of material from the published papers. These summary sections will highlight key messages of each publication, and signpost their contribution to the overall narrative of the thesis.

Publications will be provided in their final copy-edited format and citations for those papers will appear within the paper. Other citations pertaining to the additional aspects of the thesis will be provided in the reference section at the end.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Aβ</td>
<td>Amyloid beta</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>iAUC</td>
<td>Incremental area under the curve</td>
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<td>tAUC</td>
<td>Total area under the curve</td>
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<td>BDNF</td>
<td>Brain derived neurotrophic growth factor</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
<td>Blood pressure</td>
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<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CT</td>
<td>Computerised tomography</td>
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<tr>
<td>DALY</td>
<td>Disability adjusted life year</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>EX+BR</td>
<td>Exercise plus breaks</td>
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<td>EX+SIT</td>
<td>Exercise plus sitting</td>
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<td>FMD</td>
<td>Flow-mediated dilatation</td>
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<td>GLUT</td>
<td>Glucose transporter type</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<td>HKII</td>
<td>Hexokinase II</td>
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<td>HOMA2-%β</td>
<td>Homeostasis model assessment of beta cell function</td>
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<td>HOMA2-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>MCAv</td>
<td>Middle cerebral artery velocity</td>
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<td>MET</td>
<td>Metabolic equivalent of task</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MVPA</td>
<td>Moderate-to-vigorous physical activity</td>
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<td>NO</td>
<td>Nitric oxide</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>RPE</td>
<td>Borg Rate of Perceived Exertion</td>
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<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SIT</td>
<td>Uninterrupted sitting</td>
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<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
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<td>T2D</td>
<td>Type 2 diabetes</td>
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CHAPTER 1
Introduction and Literature Review

This introductory chapter provides a detailed account of the scientific rationale for investigating the cognitive and metabolic effects of exercise and sedentary behaviour in subsequent chapters.

Specifically, Section 1.1 aims to provide context around the development of a public health perspective on physical activity and sedentary behaviour. Epidemiological studies in this area have been influential, not only for informing current public health guidelines, but also in stimulating experimental work to investigate physiological and mechanistic questions around exercise and sedentary behaviour. For example, observational research has consistently described an association between sedentary behaviour, cardiovascular disease and T2D. Experimental studies have since investigated the effects of sedentary behaviour on glycaemic control, a key risk factor for these diseases.

Section 1.2 is based on a published review article and it discusses experimental evidence pertaining to the effects of sedentary behaviour on glycaemic control, which is the strongest extant evidence base on the physiology of sedentary behaviour. However, it is widely accepted that glycaemic control is central to many other biological processes. For example, glycaemic control in conjunction with cerebral blood flow regulate fuel supply to the brain and thus form the foundations of brain health. The objective of this section is to describe the implications of sedentary behaviour for these aspects of brain health.

Finally, Section 1.3 provides an overall summary and specific rationale leading to the primary research objectives of this thesis.
1.1 The demographic perspective on physical activity and sedentary behaviour

There is increasing acceptance that the future landscape of population health will be shaped by current global trends. Population ageing, rising obesity rates and low levels of physical activity have a compelling universal health impact. Recent data from the United Nations indicates that between 2010 and 2015, for the first time in history, the number of older adults (>65 years) in developed regions grew to outnumber children younger than 15 years. These data suggest that by 2100, there will be twice as many older adults as there are children (Figure 2). The implications of this for population health are profound, as ageing is a major risk factor for many of the leading causes of death and disability globally. In 2016, deaths from diabetes, cardiovascular disease and dementia were 1.4 million, 17.6 million and 2.4 million, respectively, representing increases over a ten-year period of 31.1%, 14.5% and 44.7%, respectively. Perhaps even more concerning are the years of healthy life lost (i.e. disability-adjusted life year; DALY), with adults living longer, the burden of these diseases increases. Global data indicates that from 1990 to 2016, DALYs attributable to diabetes, cardiovascular disease and dementia increased 108.3%, 32.4%, 120.8%, respectively. With greater proportions of the population ageing into their sixth decade and beyond, there should be an emphasis on maintaining health into these later years.

Figure 1. Actual and projected numbers of children and older adults in developed regions of the world. Data is from the United Nations Department of Economic and Social Affairs, revised 2017 world population prospectus. Developed regions include Europe, North America, Australia/New Zealand and Japan.
Demographic impacts on physical (in)activity and cardiometabolic health

While ageing is not a modifiable risk factor, it is associated with a cluster of other risk factors, many of which are modifiable. Physical inactivity, for example, has been implicated as a key modifiable risk factor linked to the pathophysiological processes of insulin resistance, dyslipidaemia, abdominal obesity, systemic inflammation, hypertension, atherosclerosis and neurodegeneration.\textsuperscript{10,11} Moreover, the total burden of disease attributable to physical inactivity has been estimated to be 13.4 million DALY’s globally in 2013.\textsuperscript{12} Therefore, there is general agreement that through the promotion of regular physical activity, multiple pathophysiological processes can be ameliorated and disease burden reduced. However, the proportion of individuals meeting the minimum recommended level of physical activity (150 minutes per week of moderate intensity) is alarmingly low among adults, and decreases with age. For example, it is estimated that 19.4\% of adults aged 18-29 years do not meet this minimum level globally, but that figure rises to 55.3\% by 80 years and older.\textsuperscript{13}

These low adherence rates reflect changes in our society that have decreased the necessity for physical activity as part of the daily routine. A salient example of this trend is the dramatic shift in the types of occupations that people are employed in. Over the last fifty years, there has been a declining prevalence of physically active occupations such as agricultural and goods producing jobs, but increases in the number of physically inactive ‘desk’ jobs.\textsuperscript{14} It has been estimated that occupation-related energy expenditure has declined by approximately 100 calories per day from 1960 to 2010 (Figure 2).\textsuperscript{14} To give this figure some context, it has been argued that that an extra 10 calories per day could explain an increase in average weight of ~3-5 kgs over a generation.\textsuperscript{15} This decline in activity is likely driven by an increased reliance on automation. For example, it is estimated that in the U.S. from 1950 to 2000, there was a 224\% increase in the daily amount of vehicle miles travelled per person.\textsuperscript{16} Understanding the health implications of these societal changes has been a focus for modern research.
One of the earlier studies to document the health implications of exposure to an environment that discourages physical activity was the London Transport Workers Study.\textsuperscript{18} Published in \textit{The Lancet} in 1953, Morris and colleagues demonstrated in a prospective analysis of 31000 men (35-64 years) between 1949-1952, that bus drivers, whose job required a lot of sitting, had a higher annual incidence of mortality from coronary heart disease (1.5 cases per 1000 workers) than bus conductors (0.8 cases per 1000 workers), whose job allowed considerably more movement.\textsuperscript{18} The authors also provided a retrospective analysis of ~2.6 million deaths that occurred between 1930-1932, where the last occupation before death was independently classified by how physically demanding it was. The authors were then able to demonstrate that the coronary mortality
rate among workers was lowest in those with “heavy work”, such as construction and manufacturing, and highest in those with “light work”, such as clerical desk jobs (Figure 3). Moreover, this finding was consistent across social classes, suggesting an effect that was independent of socioeconomic status. In addition to coronary heart disease, the authors also documented a trend of increasing mortality due to diabetes from heavy to light workers.

![Figure 3. Coronary heart disease mortality among workers by occupational physical activity level and social class. Social class III - skilled workers; IV- semi-skilled workers; V- unskilled workers.](image)

There are, however, some caveats worth considering. Firstly, by taking the last occupation before death, the authors cannot account for those workers who may have changed jobs. It is possible that the less physically ‘strenuous’ jobs were reserved for the infirm, which may explain the increased mortality in this group. In addition, the pathophysiology of cardiovascular disease is a gradual process that can take many years to manifest. In the case of their prospective analysis, the authors only studied bus drivers and conductors from 1949 to 1952.

In 1975, Paffenbarger and colleagues attempted to address these issues with a study published in *The New England Journal of Medicine*. The authors followed 6351 dockworkers in the San Francisco Bay Area for 22 years. The energy output associated with 49 different dock-jobs were estimated from heart rate and oxygen consumption data and jobs were assigned as high, medium and low activity. Importantly, job assignments were reclassified every year to account for the effect of job transfers. The authors found that the coronary heart disease mortality was almost double in the moderate and light activity categories, compared to the high activity category.
Since then, numerous studies have demonstrated that higher physical activity levels,\textsuperscript{20} or higher fitness levels,\textsuperscript{21} are associated with reduced cardiovascular and all-cause mortality. Generally, fitness level is more predictive of cardiovascular disease than activity level,\textsuperscript{22} although fitness has been considered as a more accurate, albeit indirect, measure of physical activity than self-report.\textsuperscript{23} Both of these lines of evidence were influential in shaping public health recommendations on physical activity.

**The development of public health guidelines on physical activity**

The currently adopted minimum level of physical activity advised by governments and peak body organisations around the world is at least 150 minutes of moderate intensity or 75 minutes of vigorous intensity per week.\textsuperscript{24–26} In the 1970’s, both the American Heart Association,\textsuperscript{27,28} and the American College of Sports Medicine,\textsuperscript{29,30} released guidelines on exercise testing and prescription. Strong evidence on the health benefits of exercise, at that time, came from exercise training trials which focused on endurance exercise to increase the maximum volume of oxygen consumption.\textsuperscript{31–33} As such, the recommendations reflected this. For example, the 1975 guidelines by the American College of Sports Medicine promoted 20-45 minutes of exercise at between 70%-90% heart rate reserve 3-5 days a week.\textsuperscript{30}

A different picture began to emerge from prospective cohort studies investigating the dose-response relationship between physical activity levels, fitness levels and cardiovascular/all-cause mortality (Figure 4).\textsuperscript{34} Studies emerged demonstrating that high levels of training are not necessarily required to gain health benefit,\textsuperscript{35–37} and, in fact, the largest reductions in mortality occur in sedentary individuals who become moderately active.\textsuperscript{38,39} Such evidence led to the revision of guidelines to promote more moderate levels of exercise.\textsuperscript{40}
Figure 4. Relative risk of all-cause mortality associated and leisure time moderate-vigorous physical activity. Adapted from Powell et al. Data represent the median dose-response curve from 11 prospective including 268,521 men and women. Note the largest reductions in mortality occur by small increases in physical activity in the least active.

However, even with a focus on more moderate levels of exercise, many people do not achieve the minimum recommended dose (150 minutes of moderate intensity/week), and are classified as ‘inactive’. A recent study by Guthold and colleagues provides the most comprehensive estimates of the prevalence and trends of physical inactivity. This study analysed survey data from 1.9 million people in 146 countries (which is 96% of all countries), finding that although, on average, trends of physical inactivity between 2001 to 2016 have not changed, the prevalence of those not meeting the guidelines remains high at 27.5%. However, by placing a target on achieving a minimum level of moderate-to-vigorous intensity physical activity (MVPA), the health benefits of lower intensity activities may be overlooked.

The physical activity spectrum
Given the dose-response relationship between physical activity level and mortality risk, it became clear that a more nuanced understanding of the health implications of exercise that may occur below the “minimum threshold” was needed. The development of devices to objectively measure physical activity facilitated this understanding. Besides the obvious advantage of objective versus self-report assessment of physical activity, the introduction of objective devices has also allowed the validation of self-report measures to improve their accuracy. The result is an improved capacity to do population
surveillance of physical activity and to understand the health implications of behaviours along a spectrum of intensity.

It is now acknowledged that physical activity behaviour exists along a spectrum of intensity, and all behaviours across this spectrum carry implications for health. One important detail that has been clarified by accelerometer-derived measures of physical activity is that people devote only a small proportion of the waking day to activities performed at the moderate-to-vigorous end of the intensity spectrum; while a much larger amount of time is devoted to behaviours at the lower end. For example, some estimates indicate that 95% of the waking day is comprised of sedentary behaviour and light-intensity physical activity. Sedentary behaviour, which is defined by a low energy expenditure (<1.5 metabolic equivalent tasks) in a sitting or reclining posture during waking hours, has become a public health concern, in addition to too little exercise.

Observational studies have revealed that high volumes of sitting can increase the risk of cardiovascular and all-cause mortality, after statistical adjustment for MVPA. This suggests that sedentary behaviour may confer health risks that are not fully mitigated by the level of physical activity observed in the majority of the population. This idea has led to an important distinction; that a person can achieve the daily-recommended amount of exercise, while still accumulating high amounts of sitting in the same day, i.e. ‘the active couch potato’ (Figure 5).
Figure 5. Sedentary behaviour and physical activity as distinct behavioural considerations for public health. This figure depicts the continuum of time spent in sedentary behaviour (vertical axis) and MVPA (horizontal axis) during one day. Plus signs denote the healthy end of the spectrum, and vice versa for minus signs. Note it is possible for a person to accumulate high volumes of sitting while still achieving enough MVPA to meet physical activity guidelines (the active couch potato scenario). Adapted from Dempsey and colleagues (2014).46 MVPA; moderate to vigorous physical activity.

The dose-response relationship between physical activity, sedentary behaviour and all-cause mortality is of great interest to public health experts. To address this, Ekelund et al. conducted a meta-analysis of prospective cohort studies including over 1 million men and women who were followed for 2.0 to 18.1 years.44 The results demonstrated that high levels of MVPA (60-75 minutes per day) are required to offset the mortality risk associated with high levels of prolonged sitting (>8 hours per day). This relationship is illustrated below (Figure 6).
Figure 6. Joint associations of sitting time with physical activity level. Adapted from Ekelund et al. Data is from a meta-analysis of 13 prospective studies including 1,005,791 men and women who provided self-reported sitting time and physical activity level and who were followed up for 2-18 years. Note how a high level of exercise (>35.5 MET-hours per week or about 60-75 minutes of moderate-intensity activity per day) is required to offset the increased all-cause mortality risk associated with >8 hours per day of sitting. MET; metabolic equivalent of task, physical activity guidelines currently recommend 16 MET-h/week.

The above figure demonstrates that at the highest level of sitting (>8 hours per day), increasing MVPA exercise from 5 minutes to 25-35 minutes per day (2.5-16 MET-h/week) begins to lower mortality risk; but 60-75 minutes per day (35.5 MET-h/week) is required to offset this risk. It also demonstrates that for those achieving low levels of MVPA (5 minutes per day or 2.5 MET-h/week) additional risk reductions can be obtained by reducing sitting time. This idea is especially relevant to older adults, who can find it difficult to exercise. Accelerometer-derived measurements indicate that older adults spend between 65%-80% of their waking day sedentary. Indeed, sedentary time increases, and time spent in MVPA decreases with age (Figure 7).
Figure 7. Time spent in sedentary behaviour and moderate to vigorous intensity activity during the waking day by age. Calculated using accelerometer data from 7000 US adults, adapted from Sparling et al. The dotted lines represent a daily level of sedentary time considered harmful (top) and the recommended daily amount of exercise (bottom).

**New guidelines that consider the physical activity spectrum**

Evidence such as that described above has led some health agencies to also recommend reducing sedentary time. For example, Australia’s current Physical Activity and Sedentary Behaviour Guidelines state: “Even if you do more than the recommended amount of physical activity every week, you will still benefit from minimising time spent sitting each day, and from regularly interrupting periods of sitting”; and the guidelines advise to: “Break up long periods of sitting as often as possible”. The Australian guidelines also recognise that older adults often do not achieve the 30 minutes per day of MVPA and advise; “If you can’t do 30 minutes now, start with 10 minutes once or twice a day”; older Australian are also advised: “If you can, also try to reduce the time you spend sitting for long periods.” In this way, these guidelines reflect the idea that all behaviour along the physical activity spectrum may influence health and small increases in movement are considered a stepping stone towards achieving 150 minutes of MVPA per week.
The type of evidence that supports these guidelines has high applicability to real world behaviours, such as epidemiological evidence, which, by its very nature seeks to estimate the effects of real-world behaviours that are representative of the population. However, observational evidence cannot infer causation and often lacks mechanistic insight. In the case of sedentary behaviour research, observational evidence has been highly beneficial for hypothesis generation, leading to more definitive experimental trials. Specifically, the observed associations between sedentary time and markers of cardiometabolic risk,\textsuperscript{1,49–51} have spurred experimental trials investigating the effects of interrupting prolonged sitting on glucose, insulin and lipids.\textsuperscript{5,6} This experimental evidence has also been crucial to informing physical activity and sedentary behaviour guidelines.

**Evidence gaps in experimental research**

One important distinction between the ‘exercise’ and ‘sedentary behaviour’ lines of experimental evidence is that one evidence base is more established than the other. While experimental evidence relating to the effects of acute and chronic exercise has been accumulating since the 1950’s, it is only relatively recently that evidence on the effects of sedentary behaviour has emerged.\textsuperscript{5,6} Since exercise and sedentary behaviour have often been studied in isolation, experimental evidence on their combined effects is lacking. From a practical perspective it makes sense to study their combined effects; both behaviours co-exist in reality, and they may exert opposing influences on health. It seems worthwhile to investigate whether the beneficial effects of exercise can be attenuated by sedentary behaviour, testing in an experimental setting the ‘active couch potato’ scenario (sufficiently active, but also high amounts of sedentary time) that was described through epidemiological research.

One specific area that this focus could be applied to is the interface between cardiometabolic and cognitive health. Through evolution, there is a conserved link between physical activity, cardiometabolic function and brain health (Figure 8). Indeed, for survival, our ancestors had to both out-run and out-plan their peers in the hunt for food, and utilise those gained food resources effectively in order to pass on their genes.\textsuperscript{52} This evolutionary perspective can help inform current research on physical activity and sedentary behaviour. It is reasonable to propose a nexus between the contemporary decrease in MVPA and increase in sedentary time that occurs with the demographic and societal change described above, and the increasing prevalence of cerebrovascular disease and dementia apparent in older adults.\textsuperscript{53} Given that older adults often find it difficult to
achieve public health targets for MVPA, it raises the pertinent question; can sedentary behaviour also be targeted to improve brain health?

Figure 8. The triad of physical activity, cardiometabolic function and cognition. This schematic represents evolutionarily conserved links that may help focus the perspective of current physical activity research. Of particular interest is the role that prolonged periods of sitting play within the triad, given that it is a recent behaviour relative to the millions of years over which these links were forged.

The next section of this chapter integrates experimental evidence on the cardiometabolic effects of interrupting sedentary time, with current understanding of the physiology of the brain, to outline a case describing how sedentary behaviour may influence brain health. This synthesis of literature thus provides the rationale for investigating the effects of exercise and sedentary behaviour on cognitive, cardiometabolic and metabolic function, which are described later (Chapters 2 - 5).
1.2 The influence of sedentary behaviour on brain health

This section seeks to address the evidence gap on the role that sedentary behaviour plays within the triad of physical activity, cardiometabolic function and brain health, through a published review article written during the early part of the candidature. This review outlines how sedentary behaviour may influence brain health by describing the importance of glucose and cerebral blood flow in maintaining a fuel supply to the brain. With this understanding, the review then postulates how dynamic interactions between glycaemic control and cerebral blood flow may work to damage the brain over time under conditions of repeated glucose excursions. These dynamic interactions may occur as a result of mechanisms that exist to protect the brain from prolonged exposure to hyperglycaemia, or the contrasting extreme, prolonged exposure to hypoglycaemia. However, when both these mechanisms act in concert under conditions of brief repeated glucose excursions; rather than protecting the brain, the result is an exacerbation of damage over time.

The link to sedentary behaviour is then made, as research demonstrates that prolonged sitting facilitates exaggerated postprandial excursions of glucose and insulin, relative to sitting that is regularly interrupted by brief light-activity breaks. Thus, there exists a potential practical strategy for most older adults which has biologically plausible benefits for brain health; regularly interrupting postprandial sedentary behaviour.

This review article establishes the rationale to test this strategy, and considers the outcomes needed to do so. This forms the basis for the remainder of the thesis, which provides unique experimental evidence on whether sedentary behaviour versus breaks in sitting can attenuate or enhance the benefits of acute exercise on cognitive and cardiometabolic outcomes in older adults.
Sedentary behavior as a risk factor for cognitive decline? A focus on the influence of glycemic control in brain health

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Abstract

Cognitive decline leading to dementia represents a global health burden. In the absence of targeted pharmacotherapy, lifestyle approaches remain the best option for slowing the onset of dementia. However, older adults spend very little time doing moderate to vigorous exercise and spend a majority of time in sedentary behavior. Sedentary behavior has been linked to poor glycemic control and increased risk of all-cause mortality. Here, we explore a potential link between sedentary behavior and brain health. We highlight the role of glycemic control in maintaining brain function and suggest that reducing and replacing sedentary behavior with intermittent light-intensity physical activity may protect against cognitive decline by reducing glycemic variability. Given that older adults find it difficult to achieve current exercise recommendations, this may be an additional practical strategy. However, more research is needed to understand the impact of poor glycemic control on brain function and whether practical interventions aimed at reducing and replacing sedentary behavior with intermittent light intensity physical activity can help slow cognitive decline.

Keywords:

Dementia; Alzheimer’s disease; Cognitive function; Diabetes; Glucose metabolism; Exercise; Physical activity; Light-intensity activity; Sitting; Sedentary behavior; Breaks in sedentary time

1. Introduction

Dementia, which is an umbrella term for conditions characterized by cognitive decline, is a growing global health issue. Combined projections from a meta-analysis predict that global dementia prevalence will double every 20 years [1]. Dementia prevalence also represents a huge global economic cost, estimated to be US $604 billion in 2010 [2]. Strategies that can delay or prevent dementia are urgently needed given the burden it places on individuals, families, and the wider community. It has been estimated
that if interventions could delay by 1 year the onset of Alzheimer’s disease (AD), the main cause of dementia worldwide, compared with no change in onset, there would be 11.8 million fewer cases of the disease by 2050 [3]. Because there is currently no targeted pharmacotherapy to reduce the risk of dementia in older adults, there is a need to investigate modifiable behavioral risk factors that can attenuate cognitive decline.

Physical activity acts on multiple mechanisms to elicit improvements in brain health [4]. Most randomized controlled trials supporting the benefits of physical activity for brain function have focused on moderate to vigorous intensity physical activity (MVPA) [4,5]. This focus is embodied within current public health guidelines, which are based on achieving a minimal level of MVPA. For adults (18–64 years) and older adults (≥65 years), this is set at 150 minutes/week, accumulated in bouts >10 minutes [6]. However, nearly one-third of people worldwide do not achieve this minimum recommended level of MVPA [7]. Moreover, adherence is lowest in older adults, with some estimates indicating that 55% to 70% do not achieve the minimum recommended level of MVPA [8]. Fig. 1 highlights the small volume of time during waking hours, which is spent in MVPA, based on accelerometer data from a sample of older adults [9]. In contrast, a considerably larger volume of time is spent in sedentary behavior and light-intensity activity, but little is known about the implications of these behaviors for brain health.

Time spent in sedentary behavior and light-intensity activity may not be benign. Evidence suggests that excessive sedentary time can increase risk for all-cause mortality and chronic disease such as type 2 diabetes (T2D), even in the presence of regular MVPA to the level advocated within current public health guidelines [10,11]. Extending these investigations to brain function is a fascinating topic of current research, with early evidence hinting that sedentary behavior may also be detrimental to cognitive function [12]. However, more studies investigating this association are needed, specifically high-quality studies attempting to tease out the independent effects of sedentary behavior from physical activity using objective measures. Also of importance is understanding how sedentary behavior might affect brain function. Controlled experiments suggest that

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**Fig. 1.** This figure is based on accelerometer data from the U.S. National Health and Nutrition Examination Survey, which shows how 1367 older, overweight adults (mean age = 70.5 years; mean body mass index = 29.7 kg/m²) allocate their time on average throughout the day [9]. Most research on physical activity and brain health focuses on MVPA. However, only a very small proportion of the day is spent in MVPA. Emerging evidence suggests that replacing time spent in sedentary behavior with light-intensity physical activity can improve glycemic control. However, little is known about the implications of this for brain health. Abbreviation: MVPA, moderate to vigorous intensity physical activity.
excessive sedentary time has deleterious effects on glycemic control, but reducing and replacing sedentary behavior with intermittent light-intensity activity can ameliorate this effect [13]. In this perspectives article, we outline the potential implications of this for brain function. To do this we first establish how hyperglycemia, hypoglycemia, and cerebral blood flow (CBF) affect brain physiology. We next discuss how dynamic interactions between glycemic control and CBF may influence brain health. Finally, we propose a hypothesis that reducing and replacing sedentary behavior with light-intensity physical activity may protect against cognitive decline by reducing glycemic variability and increasing CBF. In doing so, we highlight future opportunities for both researchers and health practitioners. We argue that not only have current public health approaches failed to motivate a large proportion of the population to reach targets for MVPA, but focusing solely on MVPA ignores the potential health benefits of regular engagement in activities of a lighter intensity. Such evidence may be especially useful in the context of older adults who find it difficult to achieve targets for MVPA.

2. Glycemic control and brain health

The human brain, which accounts for about 2% of body weight, consumes about 20% of the energy required for resting metabolic rate—the minimum energy expenditure required to sustain life [14]. Most of this energy demand is met by utilizing glucose as a fuel, which places importance on glycemic control for maintaining brain health. At the cellular level, glycemic control is dependent on glucose transporters. The transport of glucose across the blood-brain barrier into the extracellular space and subsequent uptake by brain parenchymal cells is facilitated by a concentration gradient, as the transporters responsible are insulin-insensitive transporters such as glucose transporters 1 and 3 (GLUT1 and GLUT3) [15]. In this way, circulating glucose concentration regulates central glucose levels, a mechanism which may play a role in the neuropathology associated with chronic perturbations of circulating glucose [16].

Indeed, older adults with T2D are some 50% more likely to develop dementia relative to those with normal glucose metabolism [17]. Even in individuals without diabetes, higher fasting glucose and HbA1c, which is a measure of average glucose levels over 2 to 3 months, are associated with an increased risk for dementia [18]. Imaging techniques in cognitively normal adults with both prediabetes and T2D indicate that functional change related to glucose metabolism may be an early indicator of AD risk [19]. To help understand the potential link between glucose levels and dementia risk, we explore the dynamics of hyperglycemia, hypoglycemia, and CBF. We acknowledge that this is a glucose-centric view and that other mechanisms including insulin resistance, chronic low-grade inflammation, and oxidative stress contribute to neuropathology, but these aspects are beyond the scope of the present article.

3. Hyperglycemia and cerebral glucose kinetics

Exposure to hyperglycemia can result in decreased blood to brain glucose transport. This concept was first put forward in 1958 when Wyke described “relative cerebral hypoglycemia” [20]. This condition was identified in patients who displayed symptoms of hypoglycemia such as headache, confusion, and motor seizure in the presence of a normal circulating glucose concentration, where symptoms were relieved after an increase in plasma glucose. The first experimental evidence of altered blood to brain glucose transport after prolonged hyperglycemia was published in 1981 [21]. It was reported that in rats prolonged exposure to hyperglycemia followed by a return of glucose levels to normal values inhibited glucose transport into the brain by some 20%, compared with controls [21]. In cognitively normal humans with prediabetes and T2D, insulin resistance—a marker of disease severity—was associated with reduced cerebral glucose metabolism [19]. Taken together, this indicates that hyperglycemia may reduce cerebral glucose metabolism, which could serve initially as a protective mechanism. However, we hypothesize that this protective mechanism may work to exaggerate the effects of subsequent hypoglycemia and ultimately damage brain parenchymal cells by disrupting vital energy supply.

4. Hypoglycemia and brain function

Exposure to hypoglycemia can impair cognition, especially for complex tasks, which may be more sensitive to hypoglycemic impairment. For example, participants with type 1 diabetes who were tested in a driving simulator made more errors when blood glucose was 3.4 to 4.0 mmol/L, compared with driving in the range of 5.6 to 8.3 mmol/L [22]. However, repeated exposure to hypoglycemia may have more serious implications as there is a dose-dependent relationship between a higher number of severe hypoglycemic episodes and increased risk for dementia [23]. The biological mechanisms underpinning the detrimental impact of hypoglycemia on the brain are not fully understood, although insights may come from evidence which highlights a role for glucose concentration in regulating apoptosis, the process of cell death.

Glucose concentration may regulate apoptosis in the brain via a key enzyme of glucose metabolism, hexokinase II (HKII). In neurons and other cells, HKII catalyzes the formation of glucose-6-phosphate, which is the first step in most glucose metabolism pathways. However, HKII has also been described as a metabolic switch capable of turning on and off apoptosis [24]. Under conditions of glucose deprivation, HKII activates apoptosis. This places neurons, with their heavy reliance on glucose, as being particularly susceptible to apoptosis when fuel supply is disrupted [24]. Neuronal apoptosis has serious consequences, as this process is activated in neurodegenerative diseases like AD [25]. Importantly, controlled regulation of glucose protects neurons from apoptosis [24]. The exact concentration of
glucose that represents the threshold for initiation of apoptosis is unknown. However, given the brain’s high demand for energy, it is logical to suspect that repeated exposures below such a threshold may induce an energy crisis and allow progressive damage to accumulate over time.

5. Defense against neuroglycopenia

Because of the danger posed by a lack of glucose in the brain, known as neuroglycopenia, humans have evolved defensive mechanisms that are initiated when blood glucose falls below the range of 3.6 to 3.8 mmol/L [26]. As glucose levels fall, there is a decrease in secretion of insulin and increased secretion of glucagon and epinephrine to increase glucose levels [26]. However, this defense can be blunted by a single antecedent exposure to hypoglycemia in healthy people [27]. In those with type 1 diabetes and advanced T2D, this compromised defense can be more exaggerated as it occurs in a setting of relative pancreatic β cell failure and failure of endocrine regulation of hypoglycemia. This compromising milieu has been called “hypoglycemia-associated autonomic failure,” which has several purported mechanisms, the details of which can be found elsewhere [28].

Although antecedent hypoglycemia blunts the endocrine defense against hypoglycemia, a backup mechanism exists that acts to increase glucose transport into the brain. This is likely achieved via increases in CBF and upregulation of glucose transporters (GLUT1 and GLUT3), although human evidence for the latter is lacking. However, this backup mechanism takes time to manifest. For example, in healthy participants, 56 hours exposure to interprandial hypoglycemia (2.9 mmol/L) effectively blunted endocrine counter regulation to subsequent hypoglycemia [29]. However, this occurred in the presence of adaptive increases in CBF and brain glucose uptake which preserved cognitive function, relative to hypoglycemic exposure following normal glucose levels, in the same participants [29]. In another study of healthy participants, three exposures to a 30-minute bout of hypoglycemia (2.8 mmol/L) over a 24-hour period effectively blunted endocrine counter-regulation without any adaptive change in blood to brain glucose transport or cerebral glucose metabolism [30]. Taken together, these findings indicate that brief exposures to hypoglycemia may be more conducive to neuroglycopenia than prolonged exposure, because endocrine counter-regulation is compromised faster than the brain can increase blood to brain glucose transport.

6. Blood flow: supplying vital energy to the brain

Brain function is subserved by CBF as the mechanism of substrate delivery. In turn, energy demand in the brain can tightly regulate CBF. The mechanisms that match local neuronal energy demand to glucose and oxygen delivery are dynamic, to protect the brain from potentially hazardous declines in blood glucose. Neuronal energy demand is communicated to the vasculature by vasoactive neurotransmitters, particularly glutamate and by-products of synaptic signaling [31]. Regional CBF is regulated at the level of arterioles via smooth muscle cells and at the capillary level via the pericyte cells, which surround capillaries and can induce constriction or dilation. A comprehensive review of the mechanisms involved is available elsewhere [32]. Because of the tight coupling of CBF to brain function, impaired CBF can have serious consequences for brain health.

Hypoperfusion of the brain may be both a consequence and a cause of early neurodegeneration in both vascular dementia and AD. In healthy participants, ingestion of glucose has been shown to reduce regional CBF [33]. This hints that CBF is acutely sensitive to glucose levels. Both prolonged exposure to hyperglycemia and repeated exposure to hypoglycemia can induce microvascular damage and impair endothelial function leading to cerebral hypoperfusion [34,35]. In a rat model of chronic hypoperfusion, oxygen and nutrient supply to the brain is reduced, damaging the blood brain barrier, neurons, astrocytes and microglial cells, as well as impairing learning and memory [36]. Reduced CBF also slows the clearance of proteins like amyloid β from the perivascular space [37]. Amyloid β accumulation, which is implicated in the pathogenesis of AD, is toxic to the pericytes surrounding capillaries [38]. Amyloid β accumulation may also eventually affect larger blood vessels causing endothelial cell damage, vascular constriction, and a decrease in CBF [39]. In those with AD, faster deterioration in regional CBF is positively associated with a more rapid decline in cognition [40]. Thus, once established, hypoperfusion may result in a vicious cycle leading to further decreases in perfusion conducive to neurodegenerative disease. Taken together, these findings highlight the importance of protecting the brain from declines in CBF.

7. Brain exposure to glucose excursions

On a daily basis there are dynamic interactions among hyperglycemia, hypoglycemia, and CBF, the severity of which may have implications for brain health. Clarifying how these dynamic physiological states interact with each other may help us understand the effects of poor glycemic control on the brain. We hypothesize that under repetitive conditions of hyperglycemia and hypoglycemia, such that when one follows the other in a continued pattern, a negative feedback loop is established, which can shift brain physiology toward pathophysiology. Fig. 2 illustrates brain exposure to circulating glucose excursions at an early stage of damage in a hypothetical individual.

In dysglycemic individuals, the damage sustained by the brain from poor glycemic control may be exacerbated. Fig. 3 depicts both circulating and central glucose levels in a hypothetical individual with an increased fasting glucose level. The increased time spent in hyperglycemia results in adaptation to inhibit blood to brain glucose transport. This adaptation works to lower central glucose concentration.
relative to circulating concentration; therefore, under these circumstances, it is conceivable that at a normal peripheral concentration of glucose the brain can experience hypoglycemia. This phenomenon is termed relative cerebral hypoglycemia. Ensuing exposure to hypoglycemia can cause an energy crisis, which can induce neuroglycopenia and further impair CBF, paving the way for a recurring pattern of hypoglycemia and associated damage. This accumulating damage may

Fig. 2. The effects of circulating glucose on the brain at an early stage of damage. This schematic illustrates circulating glucose excursions in response to meals in a hypothetical individual. (1) Acute hyperglycemia in this scenario causes a reduction in regional CBF and a spike in insulin levels to facilitate glucose clearance. (2) These two factors combine to result in a glucose nadir. This glucose nadir can act to impair endocrine counter-regulation to a subsequent dip in glucose, exaggerating the hypoglycemic episode. As this happens over the space of a day, there is not enough time for the brain to compensate via increased CBF or upregulation of glucose transporters. (3) The result is an exaggerated hypoglycemic episode, which can impair endothelial function. This hypoglycemic episode may also be mirrored in the central concentration, depriving neurons of glucose, resulting in an energy crisis. Such a pattern, if continued, may progressively damage the brain. Abbreviation: CBF, cerebral blood flow.

Fig. 3. Brain exposure to glucose excursions at a late stage of damage. This schematic illustrates both circulating and central glucose excursions in response to meals in a hypothetical individual with increased fasting glucose level. (1) The increased time spent in hyperglycemia induces damage to pericytes and endothelial dysfunction of brain arterioles, resulting in chronic hypoperfusion and decreased blood to brain glucose transport. Downregulation of glucose transporters may also contribute to decreased glucose transport, although human evidence for this is lacking. (2) This protective mechanism works to lower central glucose relative to circulating concentration. This means that the brain may experience hypoglycemia at a normal circulating glucose level, a phenomenon known as relative cerebral hypoglycemia. (3) The ensuing exposure to hypoglycemia can disable endocrine counter-regulation to subsequent hypoglycemia. (4) Exposure to subsequent hypoglycemia is exaggerated and the ensuing energy crisis may induce neuroglycopenia and the accumulating damage could move the brain toward neuropathology.
ultimately result in measurable cognitive dysfunction and neuropathology.

Taken together, in an acute setting, switching between hyperglycemia and hypoglycemia allows for an exaggerated glucose nadir and insufficient time for the brain to induce protective mechanisms. This effect is inflated in those with increased fasting glucose levels who may experience relative cerebral hypoglycemia. Given the damage associated with neuroglycopenia, a continued and repeated pattern of excessive glucose excursions could shift brain physiology toward pathophysiology. In the following sections, we will discuss how interventions might manipulate glycemic control with the aim of preserving cognitive function.

8. Implications of glycemic control for brain health

Evidence from a recent systematic review supports the notion that over time, poor glycemic control can impair brain structure and function [41]. However, randomized controlled trials which have investigated this show mixed results. For example, a large trial did not support the idea that intensive glycemic control (HbA1c <6.0%) was associated with improved cognitive function relative to standard therapy (HbA1c < 7.0%–7.9%) after 40 weeks for those with T2D [42]. However, glycemic control was achieved in this trial using antidiabetic drugs (physician guided, individually tailored to reach target HbA1c). Therefore, the adverse effects of this pharmacotherapy may have influenced cognitive function directly. Moreover, glycemic control was defined by HbA1c—a measure of average glucose—which does not capture daily glucose excursions or variability. In a cross-sectional study of 121 older adults with T2D, a measure of glucose variability called the mean amplitude of glycemic excursions (average amplitude of glucose excursions, upward and downward, that are >1 standard deviation) was associated with measures of cognitive function (Mini–Mental State Examination and a composite score of attention and executive functioning) [43]. Importantly, this association was independent of fasting glucose, postprandial glycemia and HbA1c—suggesting that glycemic variability itself may be worthy of consideration. Although glycemic variability is more difficult to measure than HbA1c, future trials investigating the effects of glycemic control on brain health should attempt to include it as a measure.

This evidence highlights the potential complications associated with pharmacotherapy for improving cognition by targeting glycemic control. Achieving glycemic control via pharmacotherapy may even be counterproductive, resulting in an increased number of hypoglycemic events, weight gain, and increased mortality following intensive glycemic control, relative to standard therapy [42]. Physical activity, on the other hand, has multiple benefits including better glycemic control and improved cognitive function, and may represent a superior treatment option for protecting brain health. However, there has been increasing attention given to the notion that physical activity behaviors exist across a spectrum of intensity, which could have important challenges and practical implications for older adults.

9. Intensity of physical activity: implications for older individuals

Physical activity behaviors exist across a spectrum of energy expenditure, from sedentary (e.g., quiet sitting), to light-intensity (e.g., gentle walking), through to highly intense (e.g., sprinting) activities. Each of these behaviors may have different implications for glycemic control and brain health.

10. Reducing sedentary behavior: implications for glycemic control

Observational and experimental evidence highlights the detrimental associations that exist between sedentary behavior and glycemic control. A meta-analysis of observational studies indicates that excessive sitting time can
increase the risk of developing T2D, an association that remains after controlling for currently recommended levels of MVPA [11]. Moreover, a number of experimental studies in healthy participants and those with T2D have demonstrated that regularly interrupting prolonged sitting with brief light-intensity activity breaks can improve postprandial glycemic control [13]. Potential mechanisms for this improvement are likely to involve muscle contraction and localized increases in skeletal muscle glucose uptake, mediated by both the insulin and contraction-mediated (insulin-independent) glucose uptake pathways [46]. Fig. 4 depicts the acute effects of sedentary behavior and light-intensity activity on glycemic control in response to a meal in two hypothetical scenarios.

The greatest improvements in glycemic control in response to reducing and breaking up sitting time have been observed in participants with T2D [47]. This makes sense as T2D is characterized by disordered regulation of glucose levels. In one study of 19 overweight/obese participants with T2D, the effects of three different activity regimens were compared [48]. Each regimen lasted 4 days in duration and were performed in a randomized order, with a 10 day washout between regimens that were as follows: (1) Sitting: 4415 steps/day with sitting 14 hours/day; (2) Exercise: 4823 steps/day with 1.1 hour/day of sitting replaced with three consecutive 20-minute bouts of moderate to vigorous cycling, performed at least 2 hours after breakfast; and (3) “Sit Less”: 17,502 steps/day with 4.7 hours/day of sitting replaced with intermittent standing (2.5 hours) and light-intensity walking (2.2 hours) across the day. All meals and snacks were standardized for days 3 and 4 of each regimen and the primary outcome was 24-hour glycemic control measured on day 4 of each regimen. Results demonstrated that the “Sit Less” regimen reduced mean 24-hour glucose, 24-hour glucose excursions, and duration of hyperglycemia (glucose >10 mmol/L) compared with the “Sitting” regimen. In addition, an estimate of insulin resistance called the homeostatic model assessment for insulin resistance (HOMA-IR), was also reduced following the “Sit Less” compared with the “Sitting” regimen. The “Exercise” regimen also tended to improve these outcomes, albeit to a lesser magnitude than “Sit Less” despite a comparable energy expenditure. The results of this study have generated some important discussion around potential implications [49]. The implications pertaining to glycemic control are that: (1) exercise bouts performed in the morning may not fully compensate for the negative effects of prolonged sitting for the rest of the day; and (2) the duration and frequency of physical activity, aided by timing around meals, may be more important than the intensity of physical activity.

Fig. 4. The effects of sedentary behavior versus light-intensity activity on postprandial glucose profile. This figure illustrates circulating glucose and insulin levels in response to a meal, in two hypothetical scenarios. Dashed lines represent the optimal glucose range between hyperglycemia and hypoglycemia. During prolonged sitting, a lack of contraction-stimulated glucose uptake leads to more extreme glucose excursions. In the presence of intermittent light-intensity activity, glucose levels are more likely to stay within the optimal range.
Thus, when it comes to glycemic control, reducing and breaking up sitting time with intermittent light-intensity activity may offer an additional option to structured bouts of MVPA. Considering that the kinetics of central glucose is determined mainly by a concentration gradient, it could be speculated that to some extent, changes in circulating glucose levels may be mirrored in the central concentration, especially in the acute setting. With this in mind, we now discuss some evidence for the effects of light-intensity activity on central glucose kinetics and brain function.

11. Light-intensity activity: implications for central glucose and brain function

Light-intensity activity may elicit beneficial effects on central glucose kinetics, which, in turn, could serve to protect the brain from excessive glucose excursions. In one study of healthy male participants, a 35-minute bout of light-intensity cycling (30% of the maximum volume of oxygen uptake, which is a measure of exercise intensity) resulted in 30% more brain glucose uptake compared with higher intensity cycling (75% of the maximum volume of oxygen uptake) [50]. This is likely because of the preferential use of lactate by the brain in place of glucose during higher intensity activity. In addition, light-intensity cycling has been shown to increase CBF relative to seated baseline in healthy male participants [51]. This suggests that light-intensity activity could increase delivery of glucose to the brain and may be protective during hypoglycemia. However, research investigating whether light-intensity activity has a meaningful protective effect on the brain during hypoglycemia and whether this has implications for cognitive function is lacking.

There is a paucity of evidence on the acute effects of light-intensity activity on cognitive function. A recent trial in 19 healthy but overweight adults (mean age = 57.9 years, mean body mass index = 31.7 kg/m²) compared two 7-hour conditions separated by a 6-day washout: (1) prolonged sitting; and (2) sitting interrupted by intermittent light-intensity walking breaks (3 minutes walking every 30 minutes, total of 30 minutes walking) [52]. Cognitive function, neuroendocrine biomarkers, and subjective fatigue were measured at multiple time points across the day. Although a trend for improvement in episodic memory was observed in the light-intensity walking condition, the difference was nonsignificant. However, there was a significant improvement in subjective fatigue. The study was likely underpowered to detect any effect on cognition attributable to the 30 minutes of total accumulated walking. In another study, healthy but overweight adults performed 2.5 hours of accumulated walking breaks across an 8-hour day, the results demonstrated improved scores on a battery of cognitive tests (z-score effect size of $d = 0.71$), relative to 8 hours of sitting [53].

This said, it may be that the greatest benefit of light-intensity activity for the brain does not manifest in the acute setting, but rather over a longer period of time following protection from repeated glucose excursions. However, longer term trials investigating whether light-intensity activity can protect against cognitive decline are lacking. In one study of dementia patients in a nursing home setting, a 9-month tai chi intervention successfully preserved cognitive function relative to a control group who performed simple handicrafts [54]. However, tai chi may be different to other forms of light-intensity activity, as it requires memorization of complex moves, making it difficult to generalize to other forms of light-intensity activity. A recent meta-analysis examined randomized controlled trials of walking interventions in sedentary older adults with executive function as an outcome, showing a small overall benefit but only for those without cognitive impairment [55]. However, low adherence in those with cognitive impairment may have confounded results and the intensity of all walking interventions was not reported and likely contained a mix of light and moderate intensity interventions.

Taken together, the evidence to date hints that excessive sedentary time and thus insufficient light-intensity activity could be detrimental to both glycemic control and cognition, however, more research is needed. Experimental models in the acute setting have highlighted the benefits of replacing sedentary behavior with light-intensity activity for glycemic control, especially after meals. Given the potential damage to the brain caused by poor glycemic control over time, future research should investigate whether chronic interventions aimed at increasing intermittent light-intensity activity may also protect from cognitive decline. From a public health perspective, this is likely to have many advantages, most notably due to broad opportunities to imbed more light-intensity activity throughout the day. This may be especially important for older adults who find it difficult to engage in MVPA.

12. Conclusion

There is a clear need for effective and feasible intervention strategies to help preserve brain health and cognition in older adults. Evidence-based public health messages have emphasized MVPA for its ability to improve cognitive function. Despite this, older adults spend very little time doing MVPA on a daily basis. The greatest proportion of time is spent in activities of a lower intensity, which may have implications for glycemic control and ultimately brain health. The take home message is that reducing and breaking up sitting time with intermittent light-intensity activity may play a role in maintaining glycemic control and optimal brain health. While structured MVPA retains distinct physiological adaptations important for improving cognitive function, reducing and replacing sedentary behavior with intermittent light-intensity activity may be important in forestalling cognitive decline. However, the evidence-base supporting this idea is currently lacking and should be a target for future research. Such evidence would provide an additional option, alongside structured MVPA, in the arsenal of targeted interventions, policies, and programme development aimed at preventing dementia.
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RESEARCH IN CONTEXT

1. Systematic review: We reviewed the literature relating to sedentary behavior, glycemic control, and cognitive decline on relevant databases (Medline and Embase).

2. Interpretation: Older adults spend most of time in sedentary behavior and this may contribute to cognitive decline via effects on glycemic control. We propose a hypothesis that reducing and replacing sedentary behavior with light-intensity physical activity may protect against cognitive decline by reducing glycemic variability and increasing cerebral blood flow.

3. Future directions: Specifically future research should focus on understanding: (1) the mechanisms underpinning neuroglycopenia; (2) whether reducing and replacing sedentary behavior with intermittent light-intensity activity has any additive benefit to glycemic control or cognition over and above exercise at a moderate to vigorous intensity; (3) whether engaging in intermittent light-intensity activity is a feasible intervention, which can forestall cognitive decline in those who struggle to engage in moderate to vigorous intensity physical activity.

References


[22] Cox DJ, Gonder-Frederick LA, Kovatchev BP, Julian DM, Clarke WL. Progression hypoglycaemia’s impact on driving


[34] Ng JOY, Tate DB, Young LM, Davis SN. Effects of acute and antecedent hypoglycemia on endothelial function and markers of atherothrombotic balance in healthy humans. Diabetes 2015;64:2571–80.


1.3 Summary

As outlined in Section 1.1, a public health perspective has developed around physical activity and sedentary behaviour. This perspective is reflected in consensus-based guidelines promoted by governments and peak body organisations. Such guidelines represent an evolving perspective based on current best evidence. However, the evidence base is still evolving and this introductory section outlines two evidence gaps around which a better understanding may help to inform future guidelines.

One gap pertains to the need for experimental work on sedentary behaviour to move beyond cardiometabolic outcomes and address outcomes of brain health. This is important to elucidate, since advancing age is associated with both increasing sedentary time and an increasing prevalence of dementia. Section 1.2 outlined why there is precedence to suspect that high volumes of sedentary time may be detrimental for brain health. This synthesis of the literature formed the basis for the selection of outcomes in subsequent experimental investigation.

Another evidence gap identified in this introductory chapter is the need for experimental work to consider prolonged sitting and exercise as distinct behaviours that can coexist in the space of one day. Epidemiological approaches have begun to recognise this. Compositional data analysis for example, seeks to model the combined effect of multiple behaviours along the physical activity spectrum that occur in a 24-hour period.\(^{54}\) However, this advanced statistical technique is often based on crude measures which are proxies for the actual behaviours of interest. Robust experimental evidence on the combined effects of these behaviours is also required to answer practical questions. For example, is it enough to be an active commuter, or is it better to be an active commuter who also regularly interrupts prolonged periods of sitting? In addition, it would be desirable to know how these different patterns of behaviour influence outcomes important for health, productivity and quality of life.

Considering these evidence gaps, the experimental work presented in this thesis specifically aimed to determine whether the acute effects of exercise on the following outcomes are influenced by subsequent exposure to prolonged sitting or breaks in prolonged sitting:

1) Cognitive performance and brain derived neurotrophic growth factor (BDNF) (Chapter 2)
2) Postprandial glucose, insulin and triglycerides (Chapter 3)
3) Blood pressure and plasma catecholamines (Chapter 4)
4) Cerebral blood flow (Chapter 5)

The specific rationale, methods and findings for the respective outcomes are described in Chapters 2-5. Further discussion around the strengths and limitations, potential mechanisms underpinning the results, additional context and future research directions are provided in Chapter 6.

The findings of specific outcomes presented in Chapters 2-5 are all from the same study – *The Brain Breaks Study* – which included 67 older overweight/obese men and women. The methods of this trial as they relate to specific outcomes are detailed within the four outcomes papers (Chapters 2-5). In addition, a detailed published methods paper is provided in Appendix A. However, an expanded diagram of the study design is provided here as a point of reference (Figure 9).

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Figure 9. Expanded study design diagram. (A) Study flow diagram: Participants completed a phone screening and blood test prior to familiarisation. Following familiarisation, participants completed three conditions in a random order, separated by a minimum of six days. (B) Experimental day protocol: The three experimental conditions were as follows; SIT: uninterrupted sitting (8hrs, control); EX+SITE: sitting (1hr), moderate intensity walking (30mins, denoted by walking figure) followed by uninterrupted sitting (6.5hrs); EX+BRI: sitting (1hr), moderate intensity walking (30mins) followed by sitting (6.5hrs) interrupted every 30 min with 3 min of light intensity walking. Walking breaks are denoted by vertical lines in the Exercise + Breaks condition, a total of 12 breaks were accumulated with the first break beginning 30 minutes after exercise. During each condition, participants consumed a standardised breakfast and lunch meal and study outcomes were measured at multiple time points across the day. FMD; flow mediated dilation.
CHAPTER 2
The effects of exercise with and without breaks in sitting on cognitive performance and brain derived neurotrophic growth factor

2.1 Introduction
As outlined in Chapter 1, experimental evidence for the effects of sedentary behaviour on brain health is lacking. Chapter 1 also outlined evidence describing the mechanisms through which sedentary behaviour may influence brain health. It should be noted that the term “brain health” has a broad span of potential meanings. There is a plethora of mechanisms relevant to brain health such as brain blood flow, glucose metabolism and growth factors, to name a few. However, all of these mechanisms subserve cognitive function in one way or another. Conversely, cognitive performance can be considered the consolidated outcome or sum of all mechanisms relevant to brain health. Direct measurement of cognitive performance, therefore, has immense practical and clinical relevance, as it is an essential determinant of productivity and quality of life, as well as being an indicator of pathology in the case of mild cognitive impairment and dementia.

In the acute setting, moderate-to-vigorous intensity exercise appears to be a potent stimulus for improving cognition, and upregulating growth factors such as BDNF which is important for long term brain health. However, the findings from longer-term exercise interventions are mixed, with some, but not all, showing benefit. This mismatch suggests that during the course of an exercise training study, behaviours outside of the planned exercise might be exerting a negative influence, possibly ‘diluting’ the benefits for cognitive performance. Chapter 1 outlined a hypothesis describing how sedentary behaviour might be detrimental to brain health. In light of this, it is possible that the benefits of planned exercise on cognitive performance are attenuated by exposure to prolonged sitting. Understanding the combined effect of these behaviours in the acute setting may therefore better inform the design of intervention studies seeking to improve cognitive performance over longer periods of time.

The first experimental paper of this thesis, presented in Section 2.2 below, focusses on the combined effects of exercise and sedentary behaviour on cognitive performance in older adults. This study was published by the British Journal of Sports Medicine in 2019, and a copy of this paper is presented in Section 2.2 with further discussion in Section 2.3.
Distinct effects of acute exercise and breaks in sitting on working memory and executive function in older adults: a three-arm, randomised cross-over trial to evaluate the effects of exercise with and without breaks in sitting on cognition

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ABSTRACT

Background Sedentary behaviour is associated with impaired cognition, whereas exercise can acutely improve cognition.

Objective We compared the effects of a morning bout of moderate-intensity exercise, with and without subsequent light-intensity walking breaks from sitting, on cognition in older adults.

Methods Sedentary overweight/obese older adults with normal cognitive function (n=67, 67±7 years, 31.2±4.1 kg/m²) completed three conditions (6-day washout): SIT (sitting); uninterrupted sitting (8 hours, control); EX+SAT (exercise + sitting): sitting (1 hour), moderate-intensity walking (30 min), uninterrupted sitting (6.5 hours); and EX+BR (exercise + breaks): sitting (1 hour), moderate-intensity walking (30 min), sitting interrupted every 30 min with 3 min of light-intensity walking (6.5 hours). Cognitive testing (Cogstate) was completed at four time points assessing psychomotor function, attention, executive function, visual learning and working memory. Serum brain-derived neurotrophic factor (BDNF) was assessed at six time points. The 8-hour net area under the curve (AUC) was calculated for each outcome.

Results Working memory net AUC z-score-hour (95% CI) was improved in EX+BR with a ∆z-score of +28 (−26 to +81), relative to SIT, −25 (−79 to +29, p=0.04 vs EX+BR). Executive function net AUC was improved in EX+SAT, −8 (−71 to +55), relative to SIT, −80 (−142 to −17, p=0.03 vs EX+SAT). Serum BDNF net AUC ng/mL-hour (95% CI) was increased in both EX+SAT, +171 (−449 to +791, p=0.03 vs SIT), and EX+BR, +139 (−481 to +759, p=0.045 vs SIT), relative to SIT, −227 (−851 to +396).

Conclusion A morning bout of moderate-intensity exercise improves serum BDNF and working memory or executive function in older adults, depending on whether or not subsequent sitting is also interrupted with intermittent light-intensity walking.

Trial registration number ACTRN12614000737639.
Figure 1 Experimental design. Participants completed three conditions in a random order separated by a minimum of 6 days. The conditions were sitting (SIT): uninterrupted sitting (8 hours, control); exercise + sitting (EX+SIT): sitting (1 hour), moderate-intensity walking (30 min, denoted by walking figure), followed by uninterrupted sitting (6.5 hours); and exercise + breaks (EX+BR): sitting (1 hour), moderate-intensity walking (30 min), followed by sitting (6.5 hours) interrupted every 30 min with 3 min of light-intensity walking. Walking breaks are denoted by vertical lines in the EX+BR condition. BDNF: brain-derived neurotrophic factor.

Study design
Participants completed three conditions, in random order, with a minimum 6-day washout between conditions: SIT (sitting): uninterrupted sitting (8 hours, control); EX+SIT (exercise + sitting): sitting (1 hour), moderate-intensity walking (30 min), uninterrupted sitting (6.5 hours); and EX+BR (exercise + breaks): sitting (1 hour), moderate-intensity walking (30 min), sitting interrupted every 30 min with 3 min of light-intensity walking (6.5 hours). A familiarisation session was completed 3–5 days prior to testing, where participants were familiarised with cognitive testing and treadmill walking. During the 48 hours prior to testing, participants were instructed to avoid caffeine, alcohol and moderate-to-vigorous physical activity. Food was controlled from the night before testing, where participants consumed a standardised dinner at home between 19:00 and 21:00 in place of their regular dinner. This meal was tailored for each participant to meet 33% of estimated daily energy requirements with a macronutrient profile of 55%–58% carbohydrate, 29%–31% fat and 12%–15% protein as previously described.25

Exercise
Participants were instructed to avoid getting out of the chair except to void, or to complete the predetermined treadmill walking in EX+SIT and EX+BR. The 30 min moderate-intensity exercise bout in EX+SIT and EX+BR was performed on a treadmill at the same predetermined speed and incline for both conditions. The speed was set at 3.2 km/hour, which was a walking pace for all participants, and the incline was tailored to induce a heart rate (HR) response indicative of moderate-intensity (HR between 65% and 75% of age predicted maximum HR). This incline was determined during the familiarisation session. The 3 min light-intensity walking breaks were 3.2 km/hour with no incline for all participants. HR (Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE scale 6–20; light-intensity 9–11 RPE; moderate-intensity 12–15 RPE) were collected at 5 min intervals during the 30 min bout of exercise and at the end of each 3 min walking break.

Experimental day protocol
Participants reported to the laboratory at ~07:00 following an overnight fast (>10 hours), and an indwelling cannula was inserted into an antecubital vein. The experiment began at ~08:00 with a 1-hour steady-state sitting period where participants completed baseline measures of cognitive performance. Thereafter, a fasting blood sample was obtained prior to administration of a standardised breakfast meal. Participants were given 20 min to consume breakfast and lunch, which were standardised in the same way as the standardised dinner. All meals remained the same for a given participant throughout the study. After breakfast the protocol was followed according to randomisation (figure 1). In all conditions, blood for analysis of BDNF was collected at six time points and cognitive testing was assessed at four time points throughout the day. Blood samples were collected immediately prior to the meals and immediately following the final cognitive testing session. While sitting, participants were instructed to read or work quietly on a laptop and avoid activities which may influence arousal levels, such as watching television or making non-essential phone calls. Participants were supervised to ensure consistent behaviour across each of the study conditions.

METHODS
The detailed methods of this study have previously been described,25 and full inclusion and exclusion criteria and participant medication usage are provided in online supplementary tables S1 and S2. Men and postmenopausal women (≥55 to ≤80 years; body mass index ≥25 kg/m2 to <45 kg/m2) were recruited from the local community and tested at two sites: the Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia; and the Human Cardiovascular Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia, Perth, Australia. Recruitment occurred between February 2015 and July 2017. Participants gave informed consent prior to taking part.
Cognitive performance
Cognitive performance is the primary outcome and was measured on a laptop using a computerised test battery (Cogstate, Melbourne, Australia) developed for repeated testing with minimal practice effects.26–28 The content of each task was randomised and the test battery was administered in the following order each time: Groton Maze Learning Test (executive function); Detection Test (psychomotor function and speed of processing); Identification Test (attention); One Card Learning Test (visual learning); and a composite of the One Back Test and Two Back Test (working memory). Total administration time was approximately 20–25 min. Participants were familiarised with the full test battery during the familiarisation session 3–5 days prior to the first experimental condition.

Blood sampling
Venous blood samples were collected using an indwelling cannula inserted in an antecubital vein. Serum was collected for the analysis of BDNF, which is the secondary outcome. The samples were coagulated for 1 hour at room temperature (22°C–24°C), prior to centrifuging at 2000 revolutions per minute (931×g) for 15 min at 4°C. Supernatants were removed and frozen immediately at −20°C and subsequently moved to a −80°C freezer at the end of the condition. Concentration of BDNF was determined by technicians blinded to the study conditions. All samples were assayed by the same lab technician who was blinded to the conditions. Intra-assay and interassay coefficients of variation were 3.2% and 11.3%, respectively. Haematocrit and haemoglobin were determined from whole blood using a Beckman Coulter LH 785 according to standard methods, to calculate the per cent change in plasma volume from before to after 8 hours.

Statistical analysis
Power calculations were made in relation to cognitive performance. We estimated based on recent evidence an effect size of −0.40 (Cohen’s d for repeated measures) for exposure of light-intensity walking breaks on executive function task performance. Sample size calculations estimated that 48 participants would be required to detect this difference with a power of 0.80 and a two-tailed probability of 0.05 and allowing for an attrition rate of 20%. The order of conditions was block-randomised and stratified by sex by an independent third party using a computer-generated random sequence and stored in sealed envelopes as previously outlined. Researchers were unblinded to the order of conditions when familiarisation was complete, and participants were unblinded to the condition after the 1-hour steady-state period for experimental visits 1 and 2. Raw cognitive scores were standardised to the mean and SD of baseline values to create a standardised z-score. A higher standardised z-score denotes a better performance and a lower z-score denotes a worse performance. A time-by-condition interaction term was included in regression models to examine the effect of conditions on the 8-hour time course. Marginal means from these models were used to plot the time course for each outcome. Change across the 8-hour condition was quantified by calculating the net area under the curve (AUC). Specifically, this net change over the day is the area above the baseline minus the area below the baseline calculated using the trapezoidal method. Therefore, a negative net AUC value represents a net decrease across the day relative to baseline, and vice versa for a positive net AUC value. Following recommendations on data analysis of cross-over trials, linear mixed models with random intercepts were used to test the effect of the condition on each outcome. Data analysis was performed by technicians blinded to the study conditions. All models were adjusted for age, sex, waist circumference, treatment order, testing site and baseline values. Cognitive performance was adjusted for years of education as a categorical variable, and BDNF was adjusted for change in plasma volume, calculated from haematocrit and haemoglobin using the Dill and Costill method.30 Models with a time-by-condition interaction term comparing individual time points between conditions were adjusted for multiple comparisons using a Šidák correction. A probability level of 0.05 was adopted. The hypothesis that an acute bout of exercise would improve cognitive performance, relative to prolonged sitting, was tested by combining EX+SIT and EX+BR as one treatment to compare with SIT. All combinations of individual conditions were compared to test the hypothesis that the magnitude of improvement in cognition following moderate-intensity exercise would be greater when combined with intermittent light-intensity breaks from sitting, relative to uninterrupted sitting. Pearson’s correlations were used to test the association between cognitive performance and BDNF. Statistical analyses were performed using Stata V.15 for Windows.

RESULTS
Initially, 69 participants were randomised; however, 67 completed at least one condition and 65 completed all conditions, due to dropout (figure 2). Pseudo-intention-to-treat analysis was performed on the full data set of 67 participants. Participants were older (67±7 years) adults who were overweight to obese (31.1±4.1 kg/m²), with normal cognitive function (Mini Mental State Exam 29±1). Participant characteristics are included in table 1. Participant characteristics by treatment order sequence are included in online supplementary table S3.

Exercise responses
The initial 30 min exercise bout induced similar HR and RPE responses (mean±SD) under each condition (EX+SIT: 109±12 beats per minute (bpm), 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE, 71%±8% HRmax, 11±2 RPE, 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE; 71%±8% HRmax, 11±2 RPE;
EX+BR: 109±12 bpm, 71%±8% HRmax, 11±2 RPE). The average HR and RPE across all 12 walking breaks were 94±2 bpm, 61%±1% HRmax and 9±0.4 RPE.

Cognitive performance outcomes

When both exercise conditions (EX+SIT and EX+BR combined) were compared with SIT, no significant differences were observed in the net AUC for tests of attention, psychomotor function and visual learning. Working memory net AUC z-score–hour marginal mean (95% CI) was increased in EX+SIT and EX+BR combined, +39 (−2 to 79, p=0.06), and trended towards significance relative to SIT. Executive function net AUC was increased in EX+SIT and EX+BR combined, +47 (−5 to 99, p=0.08), relative to SIT, although the difference was not significant. When conditions were compared individually, no between-condition differences in net AUC were observed for tests of attention, psychomotor function or visual learning (figure 3D, E and F). However, working memory net AUC was improved in EX+BR, +53 (3 to 102, p=0.04), relative to SIT (figure 4C). Conversely, executive function net AUC was improved in EX+SIT, +72 (9 to 135, p=0.02), relative to SIT (figure 4D). In addition to the analysis of the standardised z-scores, similar results were observed in the supplementary analyses of the unstandardised data (online supplementary figures S1 and S2).

Brain-derived neurotrophic growth factor

Serum BDNF, analysed as 8-hour net AUC ng/mL-hour marginal mean (95% CI) without correction for change in plasma volume, was significantly higher in both EX+SIT, +471 (134 to 808, p=0.01), and EX+BR, +514 (175 to 853, p=0.003), relative to SIT. Correcting these values for change in plasma volume attenuated differences between conditions. However, the corrected net AUC was still higher in EX+SIT and EX+BR relative to SIT (figure 5B). No significant difference was observed between EX+SIT and EX+BR. There were no significant correlations between BDNF and cognitive performance outcomes.

DISCUSSION

We examined the effects of a morning bout of moderate-intensity exercise, with or without subsequent light-intensity walking breaks from sitting, on cognitive performance in older adults who were overweight to obese. Our principal finding is that both activity conditions conferred some cognitive benefit across an 8-hour period, relative to uninterrupted sitting.

While no impact was apparent in terms of attention, psychomotor function or visual learning, we observed that a bout of moderate-intensity exercise improved executive function scores over a subsequent period of prolonged sitting. However, when exercise was combined with subsequent breaks from sitting, improvements in executive function were attenuated while working memory scores improved. Taken together, these findings suggest that different patterns of physical activity may improve distinct aspects of cognition.

Prolonged sitting, exercise and cognition

Improvements in both executive function and working memory have previously been observed following acute and chronic exercise. Conversely, it has been demonstrated that...
breaks in sitting. This represents an opportunity to optimise
improved most when exercise was combined with subsequent
experimental setting. For working memory, we found scores
breaks in sitting on cognitive performance in a controlled
extended the combined effects of exercise, prolonged sitting and
executive function and working memory scores are impaired by
prolonged sitting and improved by intermittent breaks in
pursuing new activities. However, no previous study has exam-
med the effects of exercise, prolonged sitting and
executive function tests, respectively. In all panels, a positive value
on the y-axis denotes an improved score relative to baseline and vice
versa for negative values. The shaded area represents the moderate-
intensity exercise bout performed in EX+SIT and EX+BR. Dotted lines
represent the timing of the standardised meals. Data are marginal
means and 95% CI, adjusted for age, sex, waist circumference, years
of education, baseline, treatment order and testing site. Panels A and B
are additionally adjusted for multiple comparisons. *P<0.05 versus SIT.
EX+BR, exercise + breaks; EX+SIT, exercise + sitting; SIT, sitting.

executive function differently, and future studies may gain further
insight by testing these subcomponents separately.

Potential mechanisms
Central to mediating the benefits of exercise on synaptic plasticity,
learning and memory is BDNF.33 34 While the brain is a major
source of BDNF production following exercise,35 other circu-
lating factors secreted from exercising muscle such as irisin36 and
cathepsin B,37 or liver-derived β-hydroxybutyrate,38 may contribute
to BDNF expression in the brain. Therefore, BDNF seems to play
a central role in a coordinated response to exercise from multiple
organs. In the current study, increased concentrations of BDNF
in both exercise conditions, relative to the sitting condition,
were observed. However, BDNF changes were not significantly
correlated with cognitive outcomes. Disparity exists among studies
investigating whether the effects of acute exercise on cognition are
mediated in part by BDNF. In animal models, acutely increasing37 or
decreasing37 BDNF improves or impairs cognition, respectively.
However, in humans, memory-related cognitive tasks have been
 correlated with changes in BDNF, but non-memory-related tasks
are less likely to exhibit such a relationship.39 Moreover, peripheral
concentrations of BDNF likely do not reflect changes that occur
centrally. We chose to assess BDNF in serum, rather than plasma,
since the former provides an index of both free and platelet-stored
BDNF.40 41 Given the short half-life of free BDNF, secreted BDNF
may be stored by the platelet before it is measured in the plasma.

Figure 4  Cognitive test scores for working memory and executive
function. Panels A and B represent the working memory composite
and executive function z-scores, respectively, displayed as a change
from baseline across 8 hours. Panels C and D represent the 8-hour net
area under the curve (AUC) for the working memory composite and
executive function tests, respectively. In all panels, a positive value
on the y-axis denotes an improved score relative to baseline and vice
versa for negative values. The shaded area represents the moderate-
intensity exercise bout performed in EX+SIT and EX+BR. Dotted lines
represent the timing of the standardised meals. Data are marginal
means and 95% CI, adjusted for age, sex, waist circumference, years
of education, baseline, treatment order and testing site. Panels A and B
are additionally adjusted for multiple comparisons. *P<0.05 versus SIT.
EX+BR, exercise + breaks; EX+SIT, exercise + sitting; SIT, sitting.

Figure 5  Serum brain-derived neurotrophic growth factor (BDNF).
Panel A represents the concentration of BDNF across 8 hours. Panel
B represents the concentration of BDNF as the 8-hour net area under
the curve (AUC), relative to the baseline measure in that condition.
The shaded area represents the moderate-intensity exercise bout
performed in EX+SIT and EX+BR. Dotted lines represent the timing of
the standardised meals. Data are marginal means and 95% CI, adjusted
for age, sex, waist circumference, change in plasma volume, baseline,
treatment order and testing site. Panel A is additionally adjusted for
multiple comparisons. ***P<0.001 versus SIT. EX+BR, exercise +
breaks; EX+SIT, exercise + sitting; SIT, sitting.
We also measured haematocrit and haemoglobin at the first and last blood draw to calculate and adjust for change in plasma volume, which is important in the context of exercise. However, a more accurate measurement of change across the day could be obtained by more frequent measures of haematocrit and haemoglobin. The increases in BDNF over 8 hours demonstrates an effective ‘whole of day’ strategy that could be repeated over weeks or months with implications for learning and memory. It is also emphasised that such a strategy has other plausible benefits throughout the body, such as in the heart and vasculature, and even for respiration. This may indirectly benefit brain health by supported increased capacity to exercise.

**Implications and future directions**

Our findings have several potential implications. First, it seems likely that prolonged uninterrupted sitting should be avoided to maintain cognition across the day in older adults. In addition, our findings may have implications for the design of longer term exercise interventions seeking to improve aspects of cognitive performance. Such interventions sometimes demonstrate improved cognition, but not always. If it were possible to optimise cognitive performance over a whole day period using different exercise strategies, this may translate to improved design of exercise interventions.

We demonstrated that for working memory, the benefit of a single bout of exercise was enhanced by subsequent breaks from prolonged sitting, although to the detriment of executive function. Future studies should focus on whether modifying the volume, frequency or intensity of active breaks can identify how best to maximise cognitive benefit.

**Strengths and limitations**

The strengths of our study design include the investigation of exercise and sitting patterns that reflect three different ‘real life’ behaviours. While many people accumulate high amounts of sitting, some proportion of these individuals also engage in a daily exercise routine to a level recommended in public health guidelines. In addition, some guidelines also recommend reducing and breaking up sitting. The current study aimed to test different combinations of these behaviours, as there have been calls for more evidence in this area. Another strength lies in the selection of multiple cognitive tests reflecting different aspects of cognition using a validated instrument.

Limitations include not testing a condition involving breaks in sitting only. As previous evidence strongly supports the cognitive benefits of an acute 30 min bout of MVPA, our goal was to ascertain whether the addition of intermittent active breaks, to a single bout of exercise, further enhanced cognitive outcomes. Practice effects are a potential limitation of cognitive assessment. However, participants were familiarised with cognitive assessment on a separate occasion 3–5 days prior to the first experimental condition. In addition, the order of conditions was random, spreading any remaining practice effect across all conditions. Sample size calculations were performed to detect treatment effects and not to assess carryover effects, which can require a greater sample size. Finally, our study did not reveal a definitive mechanistic explanation for the observed changes in cognition. Future studies using techniques such as functional MRI or electroencephalogram may offer more insight in this regard.

**SUMMARY**

For older adults, engaging in a morning bout of moderate-intensity exercise increases BDNF and improves cognitive performance (working memory and executive function) over 8 hours, relative to prolonged sitting. However, the specific aspect of cognition that improves following exercise may be influenced by whether or not breaks in sitting are also performed. This suggests that various patterns of physical activity may be used for the daily maintenance of brain health.
et al.


52 Department of Health, Australia’s Physical Activity and Sedentary Behaviour Guidelines For Adults (18-64 years). Canberra, Australia: Australian Government, Department of Health; 2014.


**Table S1.** Inclusion and exclusion criteria for the Brain Breaks study

<table>
<thead>
<tr>
<th><strong>Inclusion criteria</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men and post-menopausal women</td>
<td></td>
</tr>
<tr>
<td>≥55 to ≤80 years of age</td>
<td></td>
</tr>
<tr>
<td>BMI ≥25 kg·m⁻² to &lt;45 kg·m⁻²</td>
<td></td>
</tr>
<tr>
<td>English speaking</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Exclusion criteria</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-reported sitting &lt; 5 hours per day</td>
<td></td>
</tr>
<tr>
<td>Self-reported MVPA ≥150 min/week for &gt; 3 months</td>
<td></td>
</tr>
<tr>
<td>Cognitive impairment (MMSE score &lt;24)</td>
<td></td>
</tr>
<tr>
<td>Probable dementia (TICS score of &lt;19)</td>
<td></td>
</tr>
<tr>
<td>Depressive symptoms (GDS score &gt;6 or HADS-D score &gt;8)</td>
<td></td>
</tr>
<tr>
<td>Diagnosed diabetes</td>
<td></td>
</tr>
<tr>
<td>Beta blockers, lipid lowering, anti-anxiety or antidepressant medication</td>
<td></td>
</tr>
<tr>
<td>Excessive alcohol consumption (AUDIT score &gt;8)</td>
<td></td>
</tr>
<tr>
<td>Abnormal resting ECG (determined by study doctor)</td>
<td></td>
</tr>
<tr>
<td>High blood pressure (systolic &gt; 160 mmHg or diastolic&gt;100 mmHg)</td>
<td></td>
</tr>
<tr>
<td>Exercise limiting illness or physical problem</td>
<td></td>
</tr>
</tbody>
</table>

AUDIT: alcohol use disorders identification test. BMI, body mass index; ECG, electrocardiogram; GDS, Geriatric Depression Scale; HADS-D, Hospital Anxiety and Depression Scale depression subscale; MMSE, Mini Mental State Exam; MVPA, moderate-to-vigorous physical activity; TICS, Telephone Interview of Cognitive Status.
**Table S2.** Participant medication usage in the Brain Breaks study

<table>
<thead>
<tr>
<th>Medication</th>
<th>Baseline</th>
</tr>
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<tbody>
<tr>
<td>Angiotensin II receptor blockers, n (%)</td>
<td>12 (18%)</td>
</tr>
<tr>
<td>Calcium channel blockers, n (%)</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitors, n (%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Proton pump inhibitor, n (%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory, n (%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Thyroxine, n (%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Diuretic, n (%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Bronchodilator, n (%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>5-alpha reductase enzyme inhibitor, n (%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Alpha-blocker, n (%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Hormonal replacement therapy, n (%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Anticonvulsant, n (%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Inhaled corticosteroid, n (%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>
**Table S3.** Participant characteristics by sequence group in the Brain Breaks study.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Sequence group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>Sex (F/M)</strong></td>
<td>6/4</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>65±8</td>
</tr>
<tr>
<td><strong>Waist (cm)</strong></td>
<td>110±12</td>
</tr>
<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>119±17</td>
</tr>
<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>71±7</td>
</tr>
<tr>
<td><strong>Mini Mental State Exam</strong></td>
<td>30±1</td>
</tr>
<tr>
<td><strong>Years of education</strong></td>
<td>15±2</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td>5.1±0.6</td>
</tr>
<tr>
<td><strong>Fasting insulin (pmol/L)</strong></td>
<td>50.7</td>
</tr>
<tr>
<td></td>
<td>[47.4-80.0]</td>
</tr>
<tr>
<td><strong>Fasting triglycerides (mmol/L)</strong></td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>[1.0-1.6]</td>
</tr>
<tr>
<td><strong>Fasting total cholesterol (mmol/L)</strong></td>
<td>5.2±0.9</td>
</tr>
<tr>
<td><strong>Fasting HDL cholesterol (mmol/L)</strong></td>
<td>1.2±0.2</td>
</tr>
<tr>
<td><strong>Fasting LDL cholesterol (mmol/L)</strong></td>
<td>3.3±0.8</td>
</tr>
</tbody>
</table>

Data are mean±SD, skewed variables are median [IQR]; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IQR, interquartile range.

Sequence 1: SIT (1<sup>st</sup>), EX+SIT (2<sup>nd</sup>), EX+BR (3<sup>rd</sup>)
Sequence 2: SIT (1<sup>st</sup>), EX+BR (2<sup>nd</sup>), EX+SIT (3<sup>rd</sup>)
Sequence 3: EX+SIT (1<sup>st</sup>), SIT (2<sup>nd</sup>), EX+BR (3<sup>rd</sup>)
Sequence 4: EX+SIT (1<sup>st</sup>), EX+BR (2<sup>nd</sup>), SIT (3<sup>rd</sup>)
Sequence 5: EX+BR (1<sup>st</sup>), EX+SIT (2<sup>nd</sup>), SIT (3<sup>rd</sup>)
Sequence 6: EX+BR (1<sup>st</sup>), SIT (2<sup>nd</sup>), EX+SIT (3<sup>rd</sup>)
Figure S1. Unstandardised cognitive scores for attention, psychomotor function and visual learning. Panels A-C represent scores for the Identification Test, Detection Test and One Card Learning Test, respectively, displayed as a time course across 8 hours. Panels D-F represent the 8-hour net area under the curve (AUC) for the Identification Test, Detection Test and One Card Learning Test, respectively. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Dotted lines represent the timing of the standardised meals. Data are marginal means and 95% CI, adjusted for age, sex, waist circumference, years of education, baseline, treatment order and testing site. Panels A-C are additionally adjusted for multiple comparisons.
Figure S2. Unstandardised cognitive scores for working memory and executive function. Panels A-C represent scores for the One Back Test, Two Back Test and Groton Maze Learning Test, respectively, displayed as a time course across 8 hours. Panels D-F represent the 8-hour net area under the curve (AUC) for the One Back Test, Two Back Test and Groton Maze Learning Test, respectively. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Dotted lines represent the timing of the standardised meals. Data are marginal means and 95% CI, adjusted for age, sex, waist circumference, years of education, baseline, treatment order and testing site. Panels A-C are additionally adjusted for multiple comparisons. *p<0.05 versus sitting (SIT).
2.3 Summary

The findings presented in Section 2.2 provide a novel contribution to the literature, demonstrating that the effects of an exercise bout on cognition can be influenced by whether-or-not subsequent post-exercise sitting is also interrupted. There appeared to be a combined benefit of exercise plus breaks in sitting on working memory. This provides a rationale for future studies to optimise the cognitive benefits of an acute bout of exercise by also regularly interrupting prolonged sitting. Conversely, exercise improved executive function scores during a prolonged period of sitting, but combining exercise with breaks in sitting appeared to impair executive function. These results highlight the idea that no behaviour occurs in isolation and future studies should consider the effects of both exercise and sedentary behaviour on cognition.

The results suggest that prolonged sitting should be avoided, and morning exercise encouraged, to increase cognitive performance and levels of brain derived neurotrophic growth factor (BDNF) over an 8-hour period. As discussed further in Chapter 6, there are many contexts in which improved executive function and working memory would be beneficial such as reducing road traffic accidents, improving decision-making in the workplace and reducing injury risk due to mental fatigue during dangerous tasks. In addition to these short-term benefits of improved cognition, there may be longer-term benefits. Primarily, if the observed effects were to be sustained, this may help mitigate the progression of cognitive decline with ageing. Future studies should test similar strategies to determine the longer-term effects of interventions which combined exercise and breaks in sitting on cognition and mechanisms relevant to long term brain health.

Chapter 1 highlighted the link between sedentary behaviour, cardiometabolic function and brain health. Indeed, insight on the combined effects of exercise and sedentary behaviour for brain health may be gleaned by investigating the combined effects of these behaviours on cardiometabolic and cardiovascular risk. Chapter 2 demonstrated that the effects of exercise on cognitive performance are influenced by subsequent prolonged sitting versus breaks in sitting. However, no clear ‘best’ strategy for overall brain health was identified. Measuring outcomes such as postprandial glucose, insulin and triglycerides (Chapter 3); blood pressure (Chapter 4); and cerebral blood flow (Chapter 5); could inform a more integrated understanding of the role of exercise and sedentary behaviour within the triad of physical activity, cardiometabolic function and cognition.
CHAPTER 3

The effects of exercise with and without breaks in sitting on postprandial glucose, insulin and triglycerides

3.1 Introduction

Chapter 2 reported the combined effects of exercise and sedentary behaviour on cognitive performance and BDNF. For overall cognitive performance, it was not clear whether combining exercise with breaks in sitting was superior to exercise plus prolonged sitting. However, these results left questions unanswered. Specifically, it appeared to matter whether exercise was followed by prolonged sitting, or breaks in prolonged sitting, when considering executive function or working memory individually. This indicates that sedentary behaviour may indeed influence mechanistic pathways which link physical activity, metabolism, blood flow and cognition. A better understanding of this role may be gained from investigating the combined effects of exercise and sedentary behaviour on metabolic function.

It has previously been demonstrated that interrupting sitting with intermittent bouts of light-intensity activity reduces postprandial markers of cardiometabolic risk such as glucose, insulin and triglycerides. The impetus for experimental work in this area is a consequence of observational research which has revealed associations between high volumes of sedentary time and several biomarkers of cardiometabolic risk. However, it is unknown whether prolonged sitting or breaks in prolonged sitting would influence postprandial glucose, insulin or triglycerides in the presence of an antecedent bout of moderate intensity exercise. This is a knowledge gap with practical relevance to scenarios such as active commuting. If the cardiometabolic benefits of a morning bout of exercise can be amplified by subsequent regular interruptions to sitting, this may have widespread implications for how people structure their day in order to improve their cardiometabolic risk profile.

Section 3.2 examines the combined effects of an exercise bout with subsequent prolonged sitting or breaks in prolonged sitting on postprandial glucose, insulin and triglycerides in older adults. This manuscript has been prepared for publication and is presented in Section 3.2, with further discussion in Section 3.3.
Combined effects of exercise and interrupting sitting time on postprandial glucose, insulin and triglycerides in older overweight/obese adults: A randomized crossover trial

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h Institute of Metabolic Science, University of Cambridge, United Kingdom
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Email: michael.wheeler@baker.edu.au
Abstract

Aims:
To assess the effects of exercise plus breaks in sitting on postprandial glucose, insulin and triglycerides, and to determine whether the degree of underlying insulin resistance and hyperlipidaemia predicts responses.

Materials and methods:
Sedentary overweight/obese adults (n=67; 67yr SD±7; 31.2kg·m⁻² SD±4.1), completed three conditions (≥6-day washout): SIT: uninterrupted sitting (8hr, control); EX+SIT: sitting (1hr), moderate-intensity walking (30min), uninterrupted sitting (6.5hr); EX+BR: sitting (1hr), moderate-intensity walking (30min), sitting interrupted every 30-minutes with 3-minutes of light-intensity walking (6.5hr). Participants consumed standardized breakfast and lunch meals and blood was sampled at 13 time-points.

Results:
Total area under the curve (tAUC) for glucose was increased by 2% [0.1%-4.1%] in EX+SIT and 3% [0.6%-4.7%] in EX+BR, versus SIT (all p<0.05). Insulin and insulin:glucose ratio tAUC were decreased by 18% [11%-22%] and 21% [8%-33%] in EX+SIT and by 25% [19%-31%] and 28% [15%-38%] in EX+BR, versus SIT (all p<0.001 vs SIT, all p<0.05 EX+SIT-vs-EX+BR). Triglyceride tAUC was decreased by 6% [1%-10%] in EX+BR versus SIT (p=0.01 vs SIT, p=0.047 EX+SIT-vs-EX+BR). The magnitude of reduction in insulin tAUC from SIT-to-EX+BR was greater in those with increased basal insulin resistance. No reduction in triglyceride tAUC from SIT-to-EX+BR was apparent in those with high fasting triglycerides.

Conclusions:
Additional reductions in postprandial insulin-glucose dynamics and triglycerides may be achieved by combining exercise with breaks in sitting. Relative to uninterrupted sitting, this strategy may reduce postprandial insulin more in those with high basal insulin resistance, but it may not reduce postprandial triglycerides in those with high fasting triglycerides.

Keywords
Exercise, sedentary behavior, postprandial, insulin resistance, lipids, glucose
Introduction

Population ageing and rising obesity rates are placing a large number of the population at increased risk of developing T2D and cardiovascular disease [1,2]. While exercise can be an effective therapeutic strategy for reducing key risk factors including postprandial glucose, insulin and triglycerides, its benefits in the context of other common activity-related behaviors remains unclear. Sedentary behavior, for example, is highly prevalent among older adults. Objective measurements indicate that older adults (>60 years) spend 65%-80% of their waking day in sedentary behavior (i.e. sitting) [3]. This time spent in sedentary behavior may not be benign.

Both total sedentary time and sedentary bout duration have been prospectively associated with increased mortality after adjustment for moderate-to-vigorous physical activity [4]. In experimental studies, prolonged periods of sitting have been demonstrated to cause greater postprandial rises in glucose, insulin and triglycerides, relative to sitting which is interrupted with brief bouts of physical activity (typically 2-3 minutes every half hour) [5,6]. Excessive rises in these postprandial markers can promote lipid and glucose storage [7,8], increase adhesion molecule expression and elevate oxidative stress [9], and have been prospectively associated with the development of T2D and cardiovascular disease [10–12]. Indeed, prolonged sitting is now recognized as a distinct behavioral consideration for postprandial markers of cardiometabolic risk [13].

To date, many experimental studies have attempted to isolate the separate effects of continuous exercise, prolonged sitting, and regular interruptions to sitting time on postprandial metabolism. However, a person can achieve the recommended daily amount of exercise while also accumulating a high level of sitting in the same day. From a practical perspective, and in light of current evidence, there is a need for research to investigate whether the effects of exercise on postprandial metabolism are influenced by subsequent exposure to either prolonged uninterrupted sitting or regular breaks in sitting. In the current study we examine, relative to prolonged sitting, the effect of a morning bout of moderate-intensity exercise with and without subsequent light-intensity interruptions in sitting, on postprandial glucose, insulin and triglycerides in older overweight/obese adults. In addition, secondary analyses were performed to determine whether the degree of underlying insulin resistance and hyperlipidaemia predicts responses.
Materials and methods

Participants

Men and postmenopausal women (≥55 to ≤80 years; body mass index ≥25 kg·m⁻² to <45 kg·m⁻²) were recruited from the local community and tested at two sites: the Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia; and, the Human Cardiovascular Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia Perth, Australia. Recruitment occurred between February 2015 and July 2017. Participants gave informed consent prior to taking part. The outcomes reported here are pre-specified secondary outcomes of a randomized crossover trial (ACTRN12614000737639), and the detailed methods, rationale, and design sections have been published independently [14]. Full inclusion/exclusion criteria and participant medication usage are provided in the supplemental material (Table S1, S2).

Study Design

Participants completed three conditions, in random order, with a minimum six day washout between them: SIT: uninterrupted sitting (8hr, control); EX+SIT: sitting (1hr), moderate-intensity walking (30min), uninterrupted sitting (6.5hr); EX+BR: sitting (1hr), moderate-intensity walking (30min), sitting interrupted every 30 minutes with 3 minutes of light-intensity walking (6.5hr). A familiarisation session was completed 3 to 5 days prior to testing, where participants were familiarized with treadmill walking. During the 48 hours prior to testing, they were instructed to avoid caffeine, alcohol and moderate-to-vigorous physical activity. Food was controlled from the night before testing where participants consumed a standardized dinner at home between 7PM and 9PM in place of their regular dinner. This meal was tailored for each participant, to provide 33% of estimated daily energy requirements with a macronutrient profile of 55–58% carbohydrate, 29–31% fat and 12–15% protein as previously described [14].

Exercise

The morning exercise performed in EX+SIT and EX+BR was a 30-minute bout of moderate-intensity walking. The speed was set at 3.2km·h⁻¹ which was a walking pace for all participants, and the incline was tailored to induce a heart rate (HR) response indicative of moderate-intensity (HR between 65-75% of age predicted maximum HR). This incline was determined during the familiarisation session. The three-minute light intensity walking breaks were 3.2km·h⁻¹ with no incline for all participants. Heart rate
(Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE scale 6-20; light intensity 9-11 RPE; moderate-intensity 12-15 RPE) were collected at 5-minute intervals during the 30-minute bout of exercise and at the end of each three-minute walking break. Participants were instructed to avoid getting out of the chair except to void, or to complete the predetermined treadmill walking in EX+SIT and EX+BR.

**Experimental day protocol**
Participants reported to the laboratory at ~7AM, following an overnight fast (> 10hr) and an indwelling cannula was inserted into an antecubital vein. The experiment began at ~8AM with a 1-hour steady state sitting period where a fasting blood sample was obtained prior to administration of a standardized breakfast meal. Participants were allocated 20 minutes to consume breakfast and lunch, which were standardized in a manner identical to the standardized dinner. All meals remained the same for a given participant throughout the study. After breakfast the protocol was followed according to randomization (Figure 1). Methods for analysis of blood samples are included in the supplemental material.

**Biochemical analysis**
Haematocrit and haemoglobin were determined from whole blood using a Beckman Coulter LH 785 according to standard methods, to calculate percent change in change plasma volume from pre to post each condition. Coded samples were sent to an independent National Association of Testing Authorities/The Royal College of
Pathologists of Australasia -accredited laboratory on the day of testing for the determination of glucose and triglycerides. Blood was collected into fluoride/oxalate tubes for analysis of plasma glucose using the hexokinase method. Blood was collected in lithium heparin tubes for analysis of triglycerides using a COBAS Integra 400 + analyser (Roche Diagnostics, Indianapolis, IN). Serum was collected for the analysis of insulin. Serum samples coagulated for 1 hour at room temperature (22–24 °C) prior to centrifuging at 2000 rpm (931 x g) for 15 min at 4 °C. Supernatants were removed and frozen immediately at −20 °C and subsequently moved to a −80 °C freezer at the end of the condition. Insulin was analysed from thawed samples using a chemiluminescent microparticle immunoassay (Architect ci16200; Abbott Diagnostics, Santa Clara, CA) with all samples from each participant analyzed in the same batch.

**Statistical analysis**

Sample size calculations for this study were performed in relation to the pre-specified primary outcome of this trial (cognitive function), and the details of this have previously been reported [14]. The order of conditions was block-randomized and stratified by sex by an independent third party using a computer-generated random sequence and stored in sealed envelopes. Researchers were unblinded to the order of conditions when familiarization was complete, and participants were unblinded to the condition after the 1-hour steady-state period for experimental visits 1 and 2. Postprandial glucose, insulin and triglycerides were summarized over the 8-hour period as total area under the curve (tAUC) and positive incremental area under the curve (iAUC). Specifically, tAUC and iAUC represent the total area above a baseline of zero and the incremental area above a baseline of fasting, respectively, calculated using the trapezoidal method. Skewed variables were transformed by natural logarithm prior to analysis. Skewed variables are also presented in Table 1 as median and interquartile range, and normally distributed variables are presented as mean and standard deviation. Following recommendations on data analysis for cross-over trials [15], linear mixed models with random intercepts were used to test the effect of the condition on each outcome. A time-by-condition interaction term was included in regression models to examine the effect of time course on the conditions. The time course was plotted using back-transformed marginal means. Back-transformed marginal means were calculated by exponentiation of model output. Models were adjusted for potential confounders including age, sex, waist circumference, treatment order, testing site, baseline values and change in plasma volume calculated from haematocrit and haemoglobin using the Dill and Costill method [16]. The Homeostasis
Model Assessment (HOMA2) was used to calculate surrogate indices of insulin resistance (HOMA2-IR) and \( \beta \)-cell function (HOMA2-%\( \beta \)) from fasting values of glucose and insulin on the morning of each condition. The average value across three conditions was obtained for HOMA2-IR, HOMA2-%\( \beta \) and fasting triglycerides, and these average values were examined as predictors of intervention-induced change in tAUC. Intervention-induced change in tAUC was calculated for each comparison as SIT minus EX+SIT, SIT minus EX+BR and EX+SIT minus EX+BR. Linear, quadratic and cubic relationships between each predictor variable (centered around its mean) and each dependent variable (i.e. intervention-induced change) were investigated using appropriate polynomial terms entered in mixed models, controlling for potential confounders. The best fitting polynomial equation was used to plot significant associations of the predictor variables with intervention-induced change in the dependent variables. A probability level of 0.05 was adopted. Statistical analyses were performed blinded to the study conditions using Stata 15 (StataCorp LP).

**Results**
A total of 301 individuals were telephone screened, 81 individuals were invited to have a blood test performed and 75 individuals were invited to take part in the familiarization session. Following familiarization, 69 participants were randomized. Due to dropout, 67 completed at least one condition and 65 completed all conditions. The full Consolidated Standards of Reporting Trials flow diagram for this trial has previously been published [17]. Reporting Analysis was performed on the 67 participants who completed at least one condition. Participants (35 females, 32 males) were older adults, 67 years (SD 7), and were overweight to obese, 31.1 kg\( \cdot \)m\(^{-2}\) (SD 4.1). A small proportion (16%) were classified as having impaired fasting glucose (5.6 mmol\( \cdot \)L\(^{-1}\) to 6.9 mmol\( \cdot \)L\(^{-1}\)) based on current recommendations [18]. Participant characteristics are displayed in Table 1.

**Exercise responses**
The initial 30-minute exercise bout induced identical HR and RPE responses between exercise conditions (mean±SD); EX+SIT and EX+BR: 109 bpm (SD 12), 71 %HR\(_{\text{max}}\) (SD 8), 11 RPE (SD 2). Average HR and RPE (6-20 scale) across all 12 walking breaks was 94 bpm (SD 2), 61 %HR\(_{\text{max}}\) (SD 1) and 9 RPE (SD 0.4).
Postprandial responses

There was a small increase in glucose tAUC [95% CI] in EX+SIT 2% [0.1%-4.1%, p=0.04] and EX+BR 3% [0.6%-4.7%, p=0.01] relative to SIT (Figure 2D). Relative to SIT, insulin tAUC (back-transformed; pmol·hr·L⁻¹) was decreased by 18% [11%-22%, p<0.001] in EX+SIT and by 25% [19%-31%, p<0.001] in EX+BR. Insulin tAUC decreased by 9% [3%-20%, p=0.02] in EX+BR relative to EX+SIT (Figure 2E). Relative to SIT, the insulin-to-glucose ratio (tAUC) decreased by 21% [8%-33%, p<0.001] in EX+SIT and by 28% [15%-38%, p<0.001] in EX+BR. Insulin-to-glucose ratio tAUC decreased by 8% [1%-14%, p=0.03] in EX+BR relative to EX+SIT (Figure 2F). Triglyceride tAUC was reduced by 6% [1%-10%, p=0.01] in EX+BR relative to SIT and was reduced by 5% [0.2%-9%, p=0.047] in EX+BR relative to EX+SIT (Figure 3B). Positive incremental area under the curve data is reported in the supplemental material (Figures S1, S2).
Figure 2. Postprandial glucose, insulin, insulin:glucose ratio and triglycerides. Panels A-D represent glucose, insulin, insulin:glucose ratio and triglycerides, respectively, displayed as a time course over 8 hours. The shaded area represents the timing of the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Panels E-H represent glucose, insulin, insulin:glucose ratio and triglycerides, respectively, displayed as the total area under the curve (tAUC). For EX+SIT and EX+BR, the percentage change relative to sitting (SIT) is displayed within the bar. Data are marginal means and SEM, adjusted for age, sex, waist circumference, baseline values, change in plasma volume, testing site and treatment order. * P<0.05 SIT vs EX+BR; # P<0.05 SIT vs EX+SIT.
**Predictors of intervention-induced change**

Insulin resistance (HOMA2-IR) and beta cell function (HOMA2-%β) were both associated with intervention-induced change in insulin tAUC between SIT and EX+BR (Figure 3A, 3B). These were positive quadratic (J-shaped) associations, suggesting that those with high basal insulin resistance or β-cell function had a larger magnitude of reduction in insulin tAUC between SIT and EX+BR. Fasting triglyceride concentration was associated with the magnitude of change in triglyceride tAUC between SIT and EX+BR (Figure 3C). This was a negative quadratic association (inverted J-shape), suggesting that those with high fasting triglycerides were resistant to intervention-induced reductions in triglyceride tAUC. None of the cubic regression coefficients were significant and differences in tAUC between SIT vs. EX+SIT or EX+SIT vs. EX+BR were not significantly associated to any of the baseline measures.
Figure 3. Associations between intervention-induced change and fasting baseline variables. Panels A-C represent the curvilinear relationship between intervention-induced change in insulin tAUC and HOMA2-IR, insulin tAUC and HOMA2-%β, triglyceride tAUC and fasting triglycerides, respectively. Fasting baseline variables were taken as the average across three conditions. The curved black line represents the line of best fit, calculated from a mixed model controlling for age, sex, waist circumference, change in plasma volume, testing site and treatment order. The shaded area represents the 95% confidence bands; ln indicates natural logarithm.
Discussion

This is the first study to demonstrate that post-exercise reductions in postprandial insulin and triglycerides are amplified by the addition of physically-active interruptions to prolonged sitting. This represents an opportunity to optimize current exercise strategies to reduce the risk of cardiovascular disease and T2D in older overweight/obese adults.

Reduced postprandial glucose and insulin levels are a consistent observation of most [5,6], but not all [19–21], studies examining the effects of interrupting sitting with light-intensity intermittent activity breaks. Studies which demonstrate no reductions in glycemic markers have generally investigated healthy, normal weight and/or younger participants; suggesting that the metabolic phenotype of the participants may determine the magnitude of reduction [5]. In the current study of older overweight/obese adults, small increases in glucose tAUC of 2% and 3% were observed during EX+SIT and EX+BR, respectively. These increases equate to a 0.2 mmol·L⁻¹ increase in average glucose over 8-hours in these conditions relative to SIT. While the intermittent light-intensity activity breaks could be expected to reduce postprandial glucose due to contraction-mediated glucose uptake [22], participants in the current study also exercised at a moderate-intensity which would be expected to increase hepatic glucose production [23,24]. Such increases are important for muscle substrate provision, but are unlikely to have adverse clinical consequences [25]. The small increases in glucose should be considered alongside the concurrent and large decreases in insulin tAUC of 18% and 25%. This suggests a net increase in muscle insulin sensitivity following exercise. The clinical consequences of this are worth considering.

Clinical implications

Insulin is a dynamic hormone that supports cellular energy production. In response to food, increased insulin provides circulating glucose and free fatty acids to cells for both respiration and storage. In the absence of food, low insulin levels permit the release of stored glucose and free fatty acids to be available for respiration [24]. Owing to the complex and integrated functions of insulin, impaired actions of this hormone, known as insulin resistance, are central to the development of multiple metabolic diseases [24]. The development of T2D, for example, is characterized by a decline in insulin-stimulated glucose disposal, compensatory increases in fasting and postprandial insulin, and in later stages impaired β-cell function and insulin secretion [26]. Indeed, both fasting, and
postprandial hyperinsulinemia have been associated with the development T2D and cardiovascular disease [26–29]. As insulin resistance progresses, the demand on insulin secretion increases in order to control glucose levels [26]. In the current study, the observed reductions in insulin during both exercise conditions suggest an increase in insulin sensitivity. Specifically, acute exercise has previously been demonstrated to increase muscle insulin sensitivity via AMPKα2β2γ3 mediated signalling [30]. The additional insulin reductions in EX+BR compared to EX+SIT may also reflect increased contractile-stimulated glucose uptake [22]. Therefore, it is likely that there was a reduced demand on insulin secretion during both exercise conditions. This suggests that these strategies could help curb the compensatory hyperinsulinemia associated with the progression of insulin resistance by reducing demand on insulin secretion.

Insulin also has direct effects on lipid metabolism which can have significant clinical consequences. This includes promotion of de novo lipogenesis and inhibition of lipolysis, decreased export of triglyceride-rich very-low density lipoproteins (VLDL) from the liver and chylomicrons from the gut, as well increased triglyceride-rich lipoprotein clearance from the circulation [24,31]. Therefore, insulin resistance results in reduced lipogenesis, increased lipolysis in peripheral adipose tissue, increased production of triglyceride-rich VLDL and dietary chylomicrons, and reduced clearance of lipoproteins from circulation [24,31]. The resulting hyperlipidemia can further contribute to insulin resistance in a vicious cycle, by promoting storage of lipids in muscle, liver and visceral adipose tissue [8,32].

Potential mechanisms
A significant proportion of fatty acid uptake occurs during the postprandial period, and is associated with uptake from the vascular splanchnic bed as opposed to the lower limbs [7]. Blood flow may be an important determinant of regional differences in lipid accumulation, with increased lower limb blood flow associated with increased fatty acid uptake from the thigh, as opposed to the abdominal region [33]. Conversely, prolonged sitting can create mechanical bends in lower limb blood vessels and reduce lower limb blood flow [34,35]. However, this effect can be mitigated by interrupting sitting with regular, brief bouts of physical activity. For example, we have previously demonstrated that such interruptions to sitting can increase femoral artery blood flow by an average of 46% over five hours, relative to uninterrupted sitting [36]. The current finding of reduced postprandial triglycerides during EX+BR is relevant in light of previous evidence that
lower limb blood flow increases in response to regular interruptions to prolonged sitting [36,37]. This suggests a potential dual benefit of this strategy. First, the reduction in triglycerides may be a result of directing fatty acids to the lower limb working muscles, which can act as a ‘sink’ for triglycerides due to activity of lipases and subsequent fatty acid utilization for energy production [38]. Secondly, the remaining triglycerides are also directed away from the visceral adipose tissue of the abdominal region, and towards the subcutaneous adipose tissue of the lower limbs. Future studies investigating the combined effects of exercise and breaks in sitting on regional uptake of fatty acids in the postprandial state would be highly informative.

There are several other potential mechanisms worth discussing in order to inform future investigations. In the current study, the reduction in postprandial triglycerides during EX+BR occurred during the 8-hour intervention. Conversely, in a recent meta-analysis of experimental studies comparing prolonged sitting to sitting interrupted with light to moderate-intensity activity, postprandial triglycerides were only reduced 12-16 hours after the interventions had ended [5]. Similarly, one previous study demonstrated that regular interruptions to sitting followed by an exercise bout at the end of the day, reduced postprandial triglycerides the next day [39]. The suggested reason for this delayed effect is exercise-induced activation of lipoprotein lipase (LPL) which peaks 8-16 hours after exercise [5]. In the postprandial state, LPL plays an important role in clearing triglycerides from circulation by liberating free fatty acids from triglyceride-rich lipoproteins [40]. The same day reductions in triglycerides observed in the current study may be the result of having the exercise bout first, followed by regular interruptions to sitting, resulting in reduced postprandial insulin while LPL was increasing. Insulin can stimulate LPL activity in adipose tissue while inhibiting activity in skeletal muscle [41]. Therefore, the reduced insulin concentration observed during EX+BR could have facilitated a shift in the site of triglyceride clearance, away from adipose tissue and towards the working muscles. Acute exercise can also increase muscle LPL activity which may partly explain the reduction in triglycerides during EX+BR [42]. However, the reduction could also be explained in the context of reduced insulin, as insulin can inhibit the production of dietary chylomicrons [43]. Therefore, it is unclear from the current study what proportion of the reduction in triglycerides during EX+BR relates to an increased clearance, versus a decreased production, of lipoproteins.
Baseline measures as predictors of intervention-induced change

While the precise mechanism of the observed reduction in postprandial triglycerides is unknown, our analyses offered insight on what may predict the magnitude of reduction. We observed a curvilinear relationship suggesting that those with a high fasting triglyceride level (above 0.6 ln or ~1.8 mmol-L^{-1}) at baseline may be resistant to intervention-induced decreases in postprandial triglycerides when comparing SIT to EX+BR. A higher fasting triglyceride level could indicate hepatic insulin resistance, which would promote hepatic triglyceride production [24]. In addition, insulin resistance has been associated with increased production of intestinal lipoproteins in the postprandial state [44], and has also been associated with decreased muscle lipoprotein lipase activity [45]. This may partly explain the current findings, although HOMA2-IR was not significantly associated with intervention-induced change in postprandial triglycerides (data not shown). However, HOMA2-IR is not the gold standard method to assess insulin resistance. Future studies that more accurately measure insulin resistance are needed to corroborate the current finding. One alternative is the oral glucose minimal model technique with a tracer which measures the selective effect of insulin on glucose disposal under physiological conditions [46]. Similar to previous research [47], we observed that those with higher baseline HOMA2-IR and HOMA2-%β had larger reductions in postprandial insulin when comparing the response during EX+BR to the response during SIT. However, our analyses indicate that this may be a curvilinear relationship. This suggests there may be a threshold past which greater underlying insulin resistance or β-cell function is exponentially associated with greater intervention-induced reductions in postprandial insulin.

Strengths and limitations

Strengths of our study include the large sample size and randomized crossover design, where participants serve as their own controls. In addition, food intake was controlled from the evening before each experimental condition and on the day of each experiment with the provision of standardized mixed meals. The use of real food mixed meals also represents a more ecologically valid nutrient stimulus compared to liquid meals. Another strength is the assessment of both linear and non-linear relationships between intervention-induced change and their predictors within a mixed model controlling for potential confounders. Since the relationships were found to be non-linear, this aids the interpretation suggesting that the observed relationships with intervention-induced change are only present when the value of the predictor is past a certain threshold.
There are also some limitations to consider. For example, it is unknown whether the benefits observed during EX+BR relative to EX+SIT are due to increased energy expenditure and/or muscle activation of the walking breaks or due to their intermittent nature. This mechanistic question may be particularly important in relation to postprandial triglycerides, as the role of muscle LPL activity is less clear in acute exercise studies of a shorter duration (≤8-hours) [5,48]. Future studies will need to tease apart the role of energy expenditure, muscle activation and timing of activity breaks in order to optimize strategies to reduce postprandial triglycerides. In addition, these results cannot necessarily be extrapolated to younger, leaner participants. Although we had a large sample size, our participants did not span a broad variation of metabolic phenotypes (i.e. dysglycemia and dyslipidemia). Thus, our analyses of predictors of intervention-induced change offer insights on associations only within the range of glycaemia and lipidaemia exhibited by the current study population. Future studies employing similar analysis techniques would benefit from including participants across a wider spectrum of health and disease. In addition, future studies are required to determine whether the effects observed in the acute setting are sustained when such strategies are repeated over longer periods of time.

**Conclusions**

In conclusion, we have demonstrated for the first time in an experimental setting that post-exercise reductions in postprandial insulin, insulin-to-glucose ratio and triglycerides are amplified by subsequent breaks in sitting. These results are informative from a practical perspective, since it is possible for a person to achieve the guideline-recommended level of daily exercise, while also sitting for prolonged periods in the same day (i.e. the active commuter with a sedentary job). In addition, we observed that past a certain threshold, those with a greater degree of underlying insulin resistance or β-cell function had greater intervention-induced reductions in postprandial insulin. We also observed that those past a threshold of high fasting triglycerides were resistant to intervention-induced reductions in postprandial triglycerides.

Taken together, these findings offer both a unique practical insight on the combined effects of exercise and sedentary behavior on postprandial markers of cardiometabolic risk, as well as highlighting clear areas for future investigation. These insights may assist
in optimizing future strategies to reduce the risk of cardiovascular disease and T2D in overweight/obese older adults.

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Disclosures
NC has had advisory board roles and/or speaker engagements for Novo, Lilly, Boehringer Ingelheim, Abbott, Medtronic, Astra Zeneca, Merck, Roche and Sanofi.

References


**Figure S1.** Whole-day incremental responses. Panels A-D represent the whole-day insulin, glucose, insulin:glucose ratio, and triglyceride response, respectively, displayed as the positive incremental area under the curve (iAUC). For EX+SIT and EX+BR, the change relative to sitting (SIT) is displayed within the bar. Data are marginal means and SEM, adjusted for age, sex, waist circumference, baseline values, change in plasma volume, testing site and treatment order.
Figure S2. Meal-specific incremental responses. Panels A-D represent the meal-specific insulin, glucose, insulin:glucose ratio, and triglyceride response, respectively, displayed as the positive incremental area under the curve (iAUC). The breakfast response represents the increase above the pre-breakfast time point until the pre-lunch time point. The lunch response represents the increase above the pre-lunch time point until the final time point. For EX+SIT and EX+BR, the change relative to sitting (SIT) is displayed within the bar. Data are marginal means and SEM, adjusted for age, sex, waist circumference, baseline values, change in plasma volume, testing site and treatment order.
3.3 Summary

The key findings of Section 3.2 were that exercise-induced reductions in postprandial insulin, insulin:glucose ratio and triglycerides were amplified by the addition of breaks in sitting. These results offer insight into the role of sedentary behaviour within the triad of physical activity, cardiometabolic function and cognition. The findings provide initial evidence in an acute experimental setting that the ‘active couch potato’ type scenario - i.e. exercise plus prolonged sitting - may be associated with increased cardiometabolic risk, relative to a scenario involving exercise (active commuting) followed by brief regular breaks in sitting. However, it also emphasises that prolonged sitting in the absence of exercise should be avoided to maintain optimal cardiometabolic health.

In agreement with our hypothesis, large differences between each condition were observed in the 8-hour insulin response. The implications of this are potentially widespread, since insulin is central to multiple pathophysiological processes.63 Indeed, hyperinsulinemia has been prospectively associated with the development of hypertension, stroke and cardiovascular disease.64 However, contrary to our hypothesis, postprandial glucose was not reduced by exercise with or without breaks in sitting. Since Chapter 1 hypothesised that sedentary behaviour may be detrimental to brain health by facilitating excessive glucose excursions; this raises the question whether avoiding hyperinsulinemia has both metabolic and cognitive benefits?

Indeed, the brain is an insulin-sensitive organ and evidence suggests that both central and peripheral insulin resistance may contribute to neurodegeneration and the development of dementia.65–68 Hyperinsulinemia may also have indirect impacts on brain health through other mechanisms such as changes in blood flow and blood pressure, a key risk factor for cognitive decline and dementia.69,70 In light of this, the observed reductions in insulin may have implications for brain health; which will be discussed further in Chapter 6.

Taken together, these findings suggest that by targeting both exercise and sedentary behaviour, there are potentially broad implications for both cardiometabolic and cognitive health. Chapter 4 seeks to further understand the integrative impacts of these combined behaviours on another clinical measure that straddles both cardiometabolic and cognitive health; blood pressure.
CHAPTER 4
The effects of exercise with and without breaks in sitting on blood pressure and plasma catecholamines

4.1 Introduction
The previous chapter reported on the combined effects of exercise and breaks in sitting on postprandial glucose, insulin and triglycerides. The results demonstrated that the beneficial effects of exercise on postprandial markers of cardiometabolic risk can be amplified by breaking up sitting with intermittent light-intensity walking breaks. This supports the notion, introduced in Chapter 1, that no behaviour occurs in isolation; exercise and breaks in prolonged sitting are both relevant targets. By studying the combined effect of these behaviours in the acute setting, it may be possible to optimise their effects on other important health outcomes.

Similar to the metabolic outcomes reported in the previous chapter, blood pressure is a modifiable risk factor that can have downstream impacts on both metabolic and cognitive health. Indeed, blood pressure reduction remains an important target for physical activity interventions seeking to prevent the onset of T2D and dementia. However, prolonged sitting has recently emerged as a distinct behavioural consideration for blood pressure control, in addition to exercise. It is currently unknown if the effects of exercise on blood pressure are influenced by subsequent exposure to prolonged sitting or breaks in prolonged sitting.

Section 4.2 reports on the third empirical paper of this thesis, which aims to examine the combined effects of exercise plus prolonged sitting or breaks in prolonged sitting on blood pressure. In order to better understand how such an intervention may influence blood pressure, the effects on plasma catecholamines were also examined.

This peer-reviewed paper was published in the journal Hypertension in 2019. A copy of this paper is provided in Section 4.2, with further discussion in Section 4.3.
Effect of Morning Exercise With or Without Breaks in Prolonged Sitting on Blood Pressure in Older Overweight/Obese Adults
Evidence for Sex Differences


Abstract—Both exercise and breaks in prolonged sitting can reduce blood pressure (BP) in older overweight/obese adults. We investigated whether there is an additive hypotensive effect when exercise is combined with subsequent breaks in sitting. Sex differences and changes in plasma catecholamines as a potential candidate mechanism underlying BP responses were also examined. Sedentary older adults (n=67; 67±7 years; 31.2±4.1 kg/m²) completed 3 conditions in random order—sitting (SIT): uninterrupted sitting (8 hours, control); exercise+sitting (EX+SIT): sitting (1 hour), moderate-intensity walking (30 minutes), uninterrupted sitting (6.5 hours); exercise+breaks (EX+BR): sitting (1 hour), moderate-intensity walking (30 minutes), sitting interrupted every 30 minutes with 3 minutes of light-intensity walking (6.5 hours). Serial BP and plasma epinephrine/norepinephrine measurements occurred during 8 hours. The 8-hour average systolic and diastolic BP (mm Hg 95% CI) was lower in EX+BR relative to EX+SIT (−4.5 to −2.3, −0.8 to −0.4, respectively, relative to SIT (all P<0.05). There was an additional reduction in average systolic BP of −1.7 (−2.8 to −0.6) in EX+BR relative to EX+SIT (P=0.003). This additional reduction in systolic BP was driven by women −3.2 (−4.7 to −1.7; P<0.001 EX+BR versus EX+SIT). Average epinephrine decreased in EX+SIT and EX+BR in women (−13% to −12%) but increased in men (+12% to +23%), respectively, relative to SIT (P<0.05). No differences in average norepinephrine were observed. Morning exercise reduces BP during a period of 8 hours in older overweight/obese adults compared with prolonged sitting. Combining exercise with regular breaks in sitting may be of more benefit for lowering BP in women than in men.

Clinical Trial Registration—URL: https://www.anzctr.org.au. Unique identifier: ACTRN12614000737639.

Key Words: blood pressure ◼ exercise ◼ obesity ◼ sedentary behavior ◼ sex characteristics

Blood pressure (BP) reduction remains a key consideration in exercise training interventions seeking to prevent cardiovascular and related diseases in those at increased risk, particularly in sedentary, older overweight to obese adults.1,2 Such interventions typically involve deliberate planned bouts of moderate-intensity exercise on a weekly basis.3 However, recent evidence indicates that sedentary behaviors—defined by low energy expenditure (<1.5 metabolic equivalents) in a sitting or reclining position4—may also be a distinct (and highly prevalent) behavioral consideration for BP control.5 Indeed, a number of experimental studies in overweight/obese and hypertensive adults have noted reductions in BP when sitting is interrupted with intermittent light-intensity activity.6–9 However, no previous study has examined whether the acute BP-lowering effects of exercise are attenuated by subsequent exposure to prolonged sitting or enhanced by subsequent exposure to breaks in sitting. In addition, factors that may modify or explain exercise-induced reductions in BP, such as sex differences and catecholamine levels,10–12 remain largely unexplored in the context of various patterns of exercise and prolonged sitting. We, therefore, investigated the BP-lowering effects of a morning bout of moderate-intensity exercise with and without subsequent light-intensity walking breaks from sitting, relative to prolonged sitting alone. In exploratory analyses, we investigated sex differences and concurrent changes in plasma catecholamines as a potential candidate mechanism underlying BP responses. We hypothesized that an acute bout

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of exercise would reduce BP during an 8-hour period, relative to prolonged uninterrupted sitting and that the BP reduction after acute exercise would be further enhanced by subsequent exposure to intermittent breaks in sitting.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request. The outcomes reported here are prespecified secondary outcomes of a randomized crossover trial (ACTRN12614000737639), and the detailed methods, rationale, and design sections have been published independently. In addition to the outcomes reported here, cognitive performance (primary outcome) was also assessed at 4 time points using a battery of cognitive tasks performed on a laptop (Cogstate, Ltd, Melbourne, Australia).

Participants

Men and postmenopausal women (n=67; 35 women; age, ≥55 to ≤80 years; body mass index, ≥25 to ≤45 kg/m²; English speaking) were recruited from the local community and tested at 2 sites: the Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia, and the Human Cardiovascular Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia, Perth, Western Australia. Recruitment occurred between February 2015 and July 2017 (see Table S1 in the online-only Data Supplement for full inclusion/exclusion criteria). Ethical approval was obtained from the Alfred Hospital Ethics Committee (181-14) and the University of Western Australia Human Ethics Committee (RA/4/11/6990). Participants gave written informed consent before taking part.

Study Design

Participants completed 3 laboratory trial conditions in a random order, each separated by a minimum of 6 days (Figure 1). These conditions involved the following: sitting (SIT): uninterrupted sitting (8 hours, control); exercise+sitting (EX+SIT): sitting (1 hour), moderate-intensity treadmill walking (30 minutes) followed by uninterrupted sitting (6.5 hours); exercise+breaks (EX+BR): sitting (1 hour), moderate-intensity treadmill walking (30 minutes) followed by sitting (6.5 hours) interrupted every 30 minutes with 3 minutes of light-intensity treadmill walking. A familiarization session was completed 3 to 5 days before testing, in which participants were familiarized with all testing equipment and procedures, including treadmill walking and BP measurements performed under the same conditions as each experimental visit. The speed and incline of the treadmill were standardized during the familiarization session and remained the same across subsequent conditions. Study outcomes were measured at multiple time points across the day (Figure 1). Throughout the day, participants were instructed to remain seated apart from leaving the chair to void or to perform predetermined treadmill walking in the EX+SIT and EX+BR conditions. While sitting, participants were instructed to read or work quietly on a laptop and avoid activities that may raise BP, such as watching television or making nonessential phone calls. Participants were supervised to ensure consistent behavior across each of the study conditions.

Resting BP and Heart Rate

Resting BP and heart rate were measured by the automated oscillatory method (HEM-907; Omron, Kyoto, Japan). Participants remained in a seated upright position after a period of quiet rest, and measurements were obtained using an appropriately sized cuff with the participants’ arm supported, according to current guidelines. The average of 3 serial measurements taken 1 minute apart was used to calculate BP at each time point. All measures of resting BP and heart rate were taken during steady-state sitting periods across the day, such that in the EX+BR condition, the resting measures were collected at least 25 minutes after the most recent activity break. Measurements were taken on the same arm for all conditions, contralateral to the arm with an indwelling cannula.

Blood Sampling

Venous blood samples were collected using an indwelling cannula inserted in an antecubital vein. Plasma samples for analysis of epinephrine and norepinephrine were collected into tubes containing ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, and reduced glutathione. Fluoride/oxalate, lithium heparin, and serum tubes were used to collect fasting samples for analysis of glucose, lipids, and insulin, respectively. Serum samples clotted for 1 hour at room temperature (22–24°C) before centrifuging. Plasma samples were centrifuged immediately after collection. All samples were centrifuged at 2000 rpm (931g) for 15 minutes at 4°C and stored at −80°C. Plasma concentrations of epinephrine and norepinephrine were determined from thawed samples by high-performance liquid chromatography with coulometric detection, after extraction from plasma using alumina absorption.

Samples were batch analyzed such that all conditions for a given participant were analyzed in the same run by a technician blinded to the study conditions. Fasting glucose was analyzed using the hexokinase method. Fasting insulin was analyzed using a chemiluminescent microparticle immunoassay (Architect ci8200; Abbott Diagnostics, Santa Clara, CA). Fasting lipids were analyzed using a COBAS Integra 400+ analyzer (Roche Diagnostics, Indianapolis, IN).
Statistical Analysis

Generalized linear mixed models with random intercepts were used to evaluate the differential effects of the experimental conditions on the selected outcomes. A treatment by time interaction term was included in regression models to calculate the marginal means at individual time points, which were used to graph the time course for different outcomes. Treatment by sex and treatment by time by sex interaction terms were included in regression models to examine sex differences between conditions in the 8-hour average and time course, respectively. Associations between epinephrine, norepinephrine, and BP variables were assessed using Spearman rank correlation coefficients. Outcome variables were adjusted for baseline, age, sex, waist circumference, treatment order, and testing site. Unadjusted data for participant characteristics were compared by independent-samples t test. A probability level of 0.05 was adopted. Statistical analyses were performed blinded to the study conditions using Stata 15 for Windows (StataCorp LP).

Results

A total of 69 participants were randomized. However, because of dropout, 67 completed at least 1 condition and 65 completed all conditions (see Figure S1 for the full CONSORT [Consolidated Standards of Reporting Trials] flow diagram). Intention-to-treat analysis was performed on the full data set of 67 participants. Participants were older (67±7 years of age) adults who were overweight to obese (31.1±4.1 kg/m²), and 37% were hypertensive. In addition, women had a slightly higher body mass index (31.9±4.4 versus 30.4±3.7 kg/m²; P=0.01), resting heart rate (63±1 versus 60±1 bpm; P=0.002), fasting serum insulin (66.2±37.0 versus 53.0±26.2 pmol/L; P=0.007), and fasting plasma cholesterol (5.7±1.0 versus 4.8±0.8 mmol/L; P<0.001), respectively, than men. Full participant characteristics are included in the Table, and participant medication usage is included in the online-only Data Supplement (see Table S2 for participant medication usage).

BP and Heart Rate Responses

Average systolic BP and diastolic BP during 8 hours were lower in both EX+SIT and EX+BR relative to SIT (Figure 2D and 2E; all P <0.05). Average mean arterial BP mm Hg (95% CI) during 8 hours was also lower in both EX+SIT 84 (83–86) and EX+BR 84 (82–85) relative to SIT 86 (84–88; P<0.01). There was an additional reduction in average systolic BP of −1.7 (−2.8 to −0.6) in EX+BR relative to EX+SIT (Figure 2D; P=0.003), demonstrating a significant benefit of intermittent walking breaks in addition to exercise for systolic BP relative to exercise followed by prolonged sitting. These reductions in BP occurred in the context of a higher average heart rate in both EX+SIT and EX+BR relative to SIT (Figure 2F; all P <0.001). A higher average heart rate was also observed in EX+BR relative to EX+SIT (Figure 2F; P<0.001).

Sex Differences

Significant treatment by sex interactions were observed for systolic BP (P=0.02) and mean arterial BP (P=0.04) but not diastolic BP (P=0.18). Only women demonstrated a reduced systolic BP (95% CI) of −3.2 mm Hg (−4.7 to −1.7) and mean arterial BP −1.6 mm Hg (−2.7 to −0.5) in the EX+BR condition relative to EX+SIT (Figure 3A and 3B; all P <0.01). In addition, only women demonstrated a reduced diastolic BP in EX+BR relative to SIT (Figure 3D; P=0.001). In supplementary analysis, baseline BP was predictive of net 8-hour BP, but no significant differences were observed between men and women (see Table S4 for the effect of baseline BP on net 8-hour BP). Significant treatment by sex interactions were observed for epinephrine (P<0.001) but not norepinephrine (P=0.65). Women demonstrated decreased epinephrine (95% CI) in both EX+SIT −31.6 pmol/L (−58.8 to −4.4) and EX+BR −29.3 pmol/L (−57.3 to −1.3) relative to SIT (Figure 3C; all P <0.05). Conversely, men demonstrated increased epinephrine in both EX+SIT 28.8 (4.1–53.4) and EX+BR 55.7 (31.4–80.0) relative to SIT (Figure 3C; P<0.05). An additional increase in epinephrine was observed in EX+BR 26.9 (2.3–51.6) relative to EX+SIT in men (Figure 3C; P=0.03). No differences were observed in the average norepinephrine during 8 hours for men or women (Figure 3F).

Sex Differences in Temporal Variation

Significant time by sex interactions were observed for systolic BP (P<0.001) and diastolic BP (P=0.04). A significant treatment by sex interaction was observed for systolic BP (P=0.02) but not diastolic BP (P=0.18). Treatment by time by sex interactions were not significant for systolic BP (P=0.40) or diastolic BP (P=0.73). The temporal pattern for systolic BP seemed to be different between men and women. In all 3 conditions, the pattern for women was that of a dip after breakfast followed by a rise before lunch, then another dip after lunch followed by a rise toward the end of the day (Figure 4A). Conversely, the pattern for men was relatively more stable across the day during SIT, and during EX+SIT and EX+BR, the pattern was that of an initial dip after exercise with a gradual rise across the rest of the day (Figure 4D). The temporal pattern in diastolic BP was more similar between men and women with postmeal

### Table. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=67)</th>
<th>Women (n=35)</th>
<th>Men (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>67±7</td>
<td>66±6</td>
<td>67±7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.2±4.1</td>
<td>31.9±4.4</td>
<td>30.4±3.7*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>105.4±11.8</td>
<td>103.0±11.8</td>
<td>108.0±11.3*</td>
</tr>
<tr>
<td>Resting heart rate, bpm</td>
<td>62±1</td>
<td>63±1</td>
<td>60±1*</td>
</tr>
<tr>
<td>Systolic BP, mm Hg†</td>
<td>125±14</td>
<td>126±15</td>
<td>124±14</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg†</td>
<td>74±10</td>
<td>73±9</td>
<td>74±12</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>25 (37%)</td>
<td>15 (43%)</td>
<td>10 (31%)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L†</td>
<td>5.2±0.5</td>
<td>5.0±0.4</td>
<td>5.3±0.5*</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L†</td>
<td>59.8±32.8</td>
<td>66.2±37.0</td>
<td>53.0±26.2*</td>
</tr>
<tr>
<td>Fasting cholesterol, mmol/L†</td>
<td>5.2±1.0</td>
<td>5.7±1.0</td>
<td>4.8±0.8*</td>
</tr>
<tr>
<td>Fasting triglycerides, mmol/L†</td>
<td>1.3±0.7</td>
<td>1.4±0.6</td>
<td>1.3±0.8</td>
</tr>
<tr>
<td>Fasting HDL cholesterol, mmol/L†</td>
<td>1.3±0.3</td>
<td>1.5±0.3</td>
<td>1.2±0.2*</td>
</tr>
<tr>
<td>Fasting LDL cholesterol, mmol/L†</td>
<td>3.3±0.8</td>
<td>3.6±0.8</td>
<td>3.0±0.6*</td>
</tr>
</tbody>
</table>

Data are mean±SD. BP indicates blood pressure; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

†Average of 3 baseline measures across each condition, hypertension defined as ≥130 mm Hg systolic or ≥80 mm Hg diastolic according to new US clinical thresholds.¹⁴
dips after breakfast and lunch in all conditions (Figure 4B and 4E). Time by sex interactions were not significant for epinephrine ($P=0.17$) or norepinephrine ($P=0.07$). A significant treatment by sex interaction was observed for epinephrine ($P<0.001$) but not norepinephrine ($P=0.56$). Treatment by time by sex interactions were not significant for epinephrine ($P=0.53$) or norepinephrine ($P=0.80$). The female pattern of response in epinephrine was that of a decrease during EX+SIT and EX+BR and an increase during SIT, whereas the male pattern of response was an increase during EX+SIT and EX+BR and decrease in SIT (Figure 5A and 5C). The pattern of response in norepinephrine was that of an initial increase immediately post-exercise relative to SIT, followed by a subsequent decline (Figure 5B and 5D; $P<0.05$ EX+SIT or EX+BR versus SIT).

**Discussion**

Our study is the first to demonstrate that postexercise reductions in BP can be further enhanced by subsequently breaking up prolonged sitting with light-intensity walking breaks during a period of 8 hours. This finding expands the evidence base around the potential of a combined approach of exercise and breaks in sitting and has several clinical and public health implications for BP control in older overweight/obese adults. Specifically, the additional BP-lowering effect occurred on top of the effect of an exercise bout commensurate to a level recommended in public health guidelines (30 minutes at moderate intensity). In addition, this finding may inform the design of future exercise interventions seeking to optimize BP responses across the whole day in a population at increased risk for cardiovascular disease. Optimizing BP...
reductions in such interventions is likely to lead to improved clinical outcomes.

We observed a 3.4- and 0.8-mmHg reduction in 8-hour average systolic and diastolic BP, respectively, during the exercise plus sitting condition, which increased to a 5.1- and 1.1-mmHg reduction in 8-hour average BP, respectively, during the exercise plus breaks condition. If these effects were to be sustained for a longer period of time, they would be comparable with the effects of monotherapy from many common antihypertensive drugs.16–19 In context, pharmacological intervention to reduce resting systolic BP by 10 mmHg or diastolic BP by 5 mmHg approximates to a 22% and 41% reduction in coronary heart disease and stroke mortalities, respectively.19 Exercise training, therefore, may supplement the role of antihypertensive medication because it directly lowers BP in addition to improving other risk factors.3 Future studies should be directed at understanding whether repeated exposures to a combined approach of exercise plus breaks in sitting lead to improved BP control during longer periods of time.

A body of literature exists pertaining to the acute hypotensive effect exercise.20–23 Postexercise hypotension may persist for between 12 and 22 hours.23,24 Despite this, few studies have measured postexercise hypotension in a controlled setting beyond 2 to 3 hours and in the context of other activity-related behaviors.10 Although previous studies have characterized a BP-lowering benefit from intermittent breaks in prolonged sitting compared with uninterrupted sitting,6,7,9 no previous study has examined the additive beneficial effects of combining exercise with breaks in sitting. The present study was designed to reflect different patterns of exercise and sedentary behavior that may occur in society, but in the context of a controlled and supervised experimental setting. The differential effects of

![Figure 3. Average blood pressure and catecholamine responses stratified by sex.](https://ahajournals.org/doi/fig/image/10.1161/01.hyp.0000098690.86707.65)
Figure 4. Time course of blood pressure and heart rate responses stratified by sex. A–C, Systolic, diastolic, and heart rate responses, respectively, in women. D–F, Systolic, diastolic, and heart rate responses, respectively, in men. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Data are marginal means and 95% CI. *P<0.05 sitting (SIT) vs EX+BR; †P<0.05 SIT vs EX+SIT.

Exercise with and without breaks in prolonged sitting on BP could be because of the beneficial effects of increased frequency or overall activity levels or because of the detrimental effects of subsequent prolonged sitting in the context of prior exercise. Both perspectives highlight the idea that no behavior occurs in isolation and that a comprehensive approach to understanding the health implications of multiple behaviors is likely required.

A number of mechanisms have been proposed to explain the effects of both acute exercise and prolonged sitting on BP. Reductions in BP after acute exercise are attributed primarily to changes in arteriolar function and tone, rather than decreased cardiac output. Reductions in resting BP may also stimulate the baroreflex to increase cardiac output, which would implicate reduced peripheral resistance if BP remained lowered. Our findings concur, as heart rate remained elevated for ≈2 hours post-exercise, whereas BP remained lower during this time in EX+SIT and EX+BR relative to SIT. Given that decreased cardiac output in the postexercise conditions is unlikely, there is strong reason to implicate the vasculature and total peripheral resistance in the hypotensive effect. Vascular resistance is controlled at the level of arterioles or so-called resistance vessels, which are intricately regulated by a combination of compensatory neural, endocrine, paracrine, and localized biomechanical mechanisms. In the current study, postexercise increases in blood flow and shear stress and subsequent NO-mediated vasodilation may have acted to lower total peripheral resistance. Conversely, prolonged sitting may reduce flow and shear stress via mechanical bending of the lower limb arteries, reducing metabolic demand, and increasing peripheral fluid accumulation (and reducing venous
In addition, biological variation likely evokes different pathways of physiological causation among participants in relation to BP control, as has been described for sex differences in the vascular response to exercise.31,32

Previous sex differences have been reported on the relationships between neural control of the heart and vasculature in humans,11 with associations dependent on age and hormonal status. For example, postmenopausal women exhibit larger reductions in BP after exercise relative to premenopausal women.33 This diminished response in premenopausal women is likely because of improved endothelial function at baseline.33 In 1 study, premenopausal women maintained a preserved flow-mediated dilation response in the popliteal artery after 3 hours of sitting, relative to men, despite similar reductions in blood flow and shear rate.31 In the current study, the female participants were all postmenopausal. Therefore, a protective vascular effect from prolonged sitting is less likely because of a reduced vasodilator capacity of vascular β-adrenergic receptors11 and reduced estrogenic NO upregulation.32 Our results support this idea because exposure to prolonged sitting after exercise in the EX+SIT condition resulted in a diminished systolic BP response relative to EX+BR in women but not in men.

The different pattern of response in systolic BP across the day warrants notice. Women demonstrated a fluctuating pattern with postmeal decreases in systolic BP, whereas men demonstrated a relatively more stable pattern. Because the baroreflex is responsible for correcting acute fluctuations in BP,26 it is possible that greater baroreflex sensitivity in men stabilized potential postmeal decreases in systolic BP. Interestingly, diastolic BP fluctuated similarly in both sexes, implicating cardiac function in the discrepancy between temporal patterns. Indeed, the baroreflex corrects changes in BP primarily by affecting cardiac output over peripheral resistance at rest and during lower exercise intensities and vice versa during higher exercise intensities.34

Although not measured, it is possible that men had a larger muscle mass and metaboreflex response to the exercise bout and intermittent walking, potentially explaining the sex differences in BP. This is supported by the slightly higher noradrenaline response to the exercise bout observed in men. Although more direct measures of sympathetic outflow, such as microneurography, would have compromised the present study design, such measures would be highly informative in future studies to determine whether men have a higher sympathetic response to breaks in sitting. If this was the case, sex differences in BP reduction could be viewed as differences in the balance between activity-related vasodilation and compensatory increases in cardiac output. This is supported by the observation in the current study that men had a slightly higher heart rate response to the walking breaks than women.

We observed increases in epinephrine after exercise in men but decreases after exercise in women. Because the exercise bout was performed in the postprandial state, perhaps competing influences involving postprandial decreases and exercise-induced increases in epinephrine explain this disparity. Previous work has demonstrated rapid postprandial

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**Figure 5.** Time course of plasma epinephrine and norepinephrine responses stratified by sex. A and B, Epinephrine and norepinephrine response, respectively, in women. C and D, Epinephrine and norepinephrine response, respectively, in men. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Data are marginal means and 95% CI. *P<0.05 sitting (SIT) vs EX+BR; †P<0.05 SIT vs EX+SIT.
decreases in epinephrine after the consumption of a mixed meal.35 Although the mechanism is not clear, it could be partly explained by shifts in blood flow through tissues responsible for epinephrine clearance, such as the liver.36 Conversely, exercise may acutely increase epinephrine levels to varying degrees, depending on factors such as exercise intensity, cardiorespiratory fitness, and body fat accumulation.37 In the current study, the relative exercise intensity was similar between sexes, based on heart rate data expressed as a percentage of age-predicted maximum heart rate (see Table S3 for heart rate response during exercise). Other factors such as a reduced cardiorespiratory fitness and increased body fat may blunt exercise-induced increases in epinephrine37 and would seem more plausible in the context of the current study. Although not direct measures of fitness or body fat, women had a higher resting heart rate and higher body mass index compared with men. Taken together, it is possible that the postprandial decrease in epinephrine was counteracted by exercise-induced increases in men. Conversely, women may have had a postprandial decrease in epinephrine coupled with a blunted exercise-induced increase, resulting in a net decline of epinephrine during postprandial exercise. To what extent differences in catecholamines explain the BP reductions, however, remain unclear because changes in epinephrine or norepinephrine were not correlated to changes in BP. It is also interesting to note that in a previous study of patients with type 2 diabetes mellitus, our group demonstrated that relative to prolonged sitting, norepinephrine was reduced during light-intensity breaks in sitting. However, key differences in the current study are that participants did not have type 2 diabetes mellitus and performed a postprandial bout of moderate-intensity exercise. Higher intensity exercise is known to induce a larger sympathetic response and those with type 2 diabetes mellitus are known to demonstrate autonomic dysfunction, which may explain the disparity.

The well-controlled randomized crossover design is a strength of this study because it provides control for person-specific factors and affords smaller sample sizes. Trial conditions were also well standardized, with strict but pragmatic control for potential confounder variables, such as diet, physical activity, medications, and baseline values. There are also some limitations to our study. Gold standard measures of exercise capacity and relative exercise intensity using oxygen consumption were not performed, and conditions were not matched for energy expenditure. This should be investigated in future studies to better elucidate mechanisms of BP lowering in relation to continuous versus fractionized activity. More mechanistic investigation may also lead to further elucidation of potential sex differences. We did not include a fourth condition involving breaks in sitting alone. This was intentional because we considered the evidence that an acute 30-minute bout of exercise impacts BP responses to be strong, and there are a number of studies that support the BP-lowering effect of active breaks compared with uninterrupted sitting alone.6-9 We, therefore, focused on a superiority approach to determine whether the addition of active breaks to an exercise bout enhanced the BP benefit. Finally, because cognitive performance was also assessed (data not reported), it is possible that this assessment influenced BP. However, any effect would be spread equally across conditions as the timing of cognitive assessment was standardized, the order of conditions was random, and participants were familiarized with cognitive assessment before the first experimental visit.

**Perspectives**

Previous experimental research has sought to separate the effects of exercise and sedentary behavior on BP. However, these behaviors coexist in society and likely interact in ways that have important implications for health. We have studied the combined effects of exercise with prolonged sitting and exercise with breaks in sitting to better understand how BP may be affected by various patterns of these behaviors. We found that a morning bout of exercise combined with subsequent breaks in sitting lowered BP more than exercise followed by prolonged sitting. Moreover, this additional BP-lowering effect was driven by female participants. Although longer term studies are required to corroborate our findings, this line of evidence may inform clinical and public health discussions around tailored strategies to optimize BP targets in older adults with increased cardiovascular disease risk.

**Sources of Funding**

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**Disclosures**

None.

**References**


What Is New?

- The blood pressure-lowering effect of exercise is attenuated by subsequent exposure to prolonged sitting.

- Female participants demonstrated an additional blood pressure-lowering effect after exercise plus breaks in sitting, relative to exercise plus prolonged sitting.

- Men demonstrated equal blood pressure-lowering effects after exercise with or without subsequent breaks in sitting.

What Is Relevant?

- These findings are relevant to clinical and public health discussions around tailored strategies to optimize blood pressure targets in older adults with increased cardiovascular disease risk.

Summary

These findings highlight the idea that no behavior exists in isolation, and studying the combined effects of different behaviors may lead to a comprehensive understanding of their health implications.
## Supplementary material- *Hypertension*

Table S3. Heart rate and ratings of perceived exertion during exercise and breaks in sitting.

<table>
<thead>
<tr>
<th>Measurement of exercise intensity</th>
<th>Condition</th>
<th>Females (n=35)</th>
<th>Males (n=32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise heart rate (bpm)</td>
<td>EX+SIT</td>
<td>111±11</td>
<td>107±12</td>
<td>0.13</td>
</tr>
<tr>
<td>Exercise heart rate (bpm)</td>
<td>EX+BR</td>
<td>111±11</td>
<td>107±13</td>
<td>0.12</td>
</tr>
<tr>
<td>Walking breaks heart rate (bpm)</td>
<td>EX+BR</td>
<td>96±10</td>
<td>91±14</td>
<td>0.08</td>
</tr>
<tr>
<td>Exercise intensity (% HRmax)</td>
<td>EX+SIT</td>
<td>72±8</td>
<td>70±8</td>
<td>0.17</td>
</tr>
<tr>
<td>Exercise intensity (% HRmax)</td>
<td>EX+BR</td>
<td>72±7</td>
<td>70±9</td>
<td>0.19</td>
</tr>
<tr>
<td>Walking breaks intensity (% HRmax)</td>
<td>EX+BR</td>
<td>63±7</td>
<td>60±10</td>
<td>0.16</td>
</tr>
<tr>
<td>Exercise RPE</td>
<td>EX+SIT</td>
<td>12±2</td>
<td>11±2</td>
<td>0.41</td>
</tr>
<tr>
<td>Exercise RPE</td>
<td>EX+BR</td>
<td>11±2</td>
<td>11±2</td>
<td>0.70</td>
</tr>
<tr>
<td>Walking breaks RPE</td>
<td>EX+BR</td>
<td>9±2</td>
<td>9±2</td>
<td>0.65</td>
</tr>
</tbody>
</table>

% HRmax, percent of maximum heart rate (220-age); RPE, ratings of perceive exertion. Heart rate (Polar Electro, Kempele, Finland) and RPE were taken at five-minute intervals during exercise during the final minute of each walking break. Heart rate was taken as the average of three measurements taken over one minute. RPE scale runs between 6-20; light intensity is 9-11; moderate-intensity is 12-15. Data are unadjusted means ±SD compared by independent samples t-test.
Table S4. Effect of baseline blood pressure on net 8-hour blood pressure.

<table>
<thead>
<tr>
<th>Output from mixed model</th>
<th>SIT</th>
<th>EX+SIT</th>
<th>EX+BR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coefficient for baseline systolic BP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female net systolic BP (mmHg∙h)*</td>
<td>-215 [-309 to -122] §</td>
<td>-114 [-208 to -20] †</td>
<td>-148 [-242 to -54] ‡</td>
</tr>
<tr>
<td>Male net systolic BP (mmHg∙h)*</td>
<td>-119 [-219 to -19] †</td>
<td>-120 [-222 to -18] †</td>
<td>-181 [-281 to -81] §</td>
</tr>
<tr>
<td><strong>P (female vs male)</strong></td>
<td>P=0.17</td>
<td>P=0.93</td>
<td>P=0.64</td>
</tr>
</tbody>
</table>

| **Coefficient for baseline diastolic BP** |        |        |         |
| Female net diastolic BP (mmHg∙h)* | -55 [-151 to 40] | -186 [-283 to -89] § | -46 [-142 to 50] |
| Male net diastolic BP (mmHg∙h)* | -27 [-103 to 49] | -101 [-177 to -24] ‡ | -156 [-233 to -80] § |
| **P (female vs male)** | P=0.65 | P=0.17 | P=0.08 |

BP; blood pressure. Net systolic and diastolic blood pressure, calculated as total incremental area below the curve subtracting the area below baseline BP from that above. Regression coefficients calculated from a mixed effects linear regression model (condition by sex by baseline blood pressure interaction). Regression coefficients represent the change in net 8-hour blood pressure associated with a one unit increase in baseline blood pressure. *Net systolic BP examines the effect of baseline systolic BP on systolic BP net area under the curve, net diastolic BP examines the effect of baseline diastolic BP on diastolic BP net area under the curve. Models were adjusted for age, waist circumference, treatment order and testing site, significant coefficients within the model are denoted by †P<0.05; ‡P<0.01; §P<0.001. Data are marginal means and 95% CI.
4.3 Summary

The results of the study presented in Section 4.2 suggest that for post-menopausal females, exercise-induced reductions in blood pressure can be amplified by subsequent breaks in sitting. For males, the results highlight the importance of a morning bout of exercise for maintaining optimal blood pressure during the day. Exercise prior to prolonged sitting reduced average 8-hour blood pressure by 2.8 mmHg for females, but 4.1 mm Hg for males. However, when exercise was followed by breaks in sitting, average 8-hour blood pressure was reduced by 6.0 mmHg for females, but 4.2 mmHg for males.

For comparison, a recent meta-analysis of randomised controlled trials compared 194 trials investigating antihypertensive medication versus 197 trials evaluating the antihypertensive effect of exercise.73 The results demonstrated that medication reduced resting systolic by -8.8 mm Hg and exercise reduced resting systolic by -4.84 mm Hg.73 However, participants included in trials of antihypertensive medication typically have higher blood pressure. Further analysis revealed that when comparing those with systolic >140 mmHg, exercise or medication lowered average systolic to the same degree, approximately 9 mm Hg.

Future studies should seek to extend these findings to a longer-term setting. This is especially important given the central role that blood pressure plays in both cardiometabolic and cognitive health. Future research should be directed towards understanding whether the sustained effects of interventions that target both exercise and sedentary behaviour translate to a reduced incidence of T2D, cardiovascular disease, stroke or dementia; all diseases for which hypertension is a risk factor.70,71,74

The complex and integrated nature of the vasculature in terms of physiological function explains why the cardiovascular benefits of exercise extend to many disparate disease outcomes.74 Stroke is an example of a disease directly linking cardiovascular function to cognitive health. Exercise is associated with a reduced incidence of stroke,75,76 and it also plays an important role in rehabilitation following stroke.77 However, the mechanisms remain unclear. The findings presented in this chapter have suggested that the cardiovascular effects of exercise can be modified by also considering sedentary behaviour. For the final empirical paper in this thesis, the combined effects of these behaviours on a cardiovascular mechanism with direct implications for brain health will be investigated; brain blood flow (Chapter 5).
CHAPTER 5
The effects of exercise with and without breaks in sitting on cerebral blood flow

5.1 Introduction

Chapters 3 and 4 demonstrated that the metabolic and cardiovascular benefits of exercise can be modified by also considering sedentary behaviour. While these results have implications for cardiometabolic and cognitive health over the long term, cerebral blood flow has more immediate effects on cognition as well as long term implications for brain health. Thus, the effects of exercise on cerebral blood flow represent a salient example of how exercise may influence cognitive health through a cardiovascular mechanism.

Exercise may provide some protection against the cognitive decline that occurs with age due to its ability to improve cerebrovascular function. Chapter 1 identified the importance of cerebral blood flow in maintaining optimal brain health and suggested that it may play an important role in mediating the effects of sedentary behaviour in this regard. Given the immediate effect of acute exercise on cerebral blood flow, measuring its response to exercise, with or without breaks in sitting, provides an opportunity to better understand the influence of sedentary behaviour on the link between exercise and brain health.

However, to date no study has determined whether the effects of exercise on cerebral blood flow are influenced by subsequent prolonged sitting or breaks in prolonged sitting. Section 5.2 reports on the final empirical paper of this thesis, which aimed to examine the combined effect of exercise plus prolonged sitting, or breaks in prolonged sitting, on cerebral blood flow.

This peer-reviewed paper was published in the Journal of Applied Physiology in 2019 and won the American Physiological Society’s “Select” award for distinction in scholarship. A copy of this paper is provided in Section 5.2, with further discussion in Section 5.3
RESEARCH ARTICLE

Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older adults

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Wheeler MJ, Dunstan DW, Smith B, Smith KJ, Scheer A, Lewis J, Naylor LH, Heikonen I, Ellis KA, Cerin E, Ainslie PN, Green DJ. Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older adults. J Appl Physiol 126: 1049–1055, 2019. First published February 7, 2019; doi:10.1152/japplphysiol.00001.2019.—Preventing declines in cerebral blood flow is important for maintaining optimal brain health with aging. We compared the effects of a morning bout of moderate-intensity exercise, with and without subsequent light-intensity walking breaks from sitting, on cerebral blood velocity over 8 h in older adults. In a randomized crossover trial, overweight/obese older adults (n = 12; 70 ± 7 yr; 30.4 ± 4.3 kg/m2), completed three acute conditions (6-day washout); SIT: prolonged sitting (8 h, control); EX+SIT: sitting (1 h), moderate-intensity walking (30 min), followed by uninterrupted sitting (6.5 h) and EX + BR: sitting (1 h), moderate-intensity walking (30 min), followed by sitting (6.5 h) interrupted with 3 min of light-intensity walking every 30 min. Bilateral middle cerebral artery velocities (MCAVs) were determined using transcranial Doppler at 13 time points across the day. The temporal pattern and average MCAV over 8 h was determined. The pattern of MCAV over 8 h was a negative linear trend in SIT (P < 0.001), but a positive quadratic trend in EX + SIT (P < 0.001) and EX + BR (P < 0.01). Afternoon time points in SIT were lower than baseline within condition (P ≤ 0.001 for all). A morning dip in MCAV was observed in EX + SIT and EX + BR (P < 0.05 relative to baseline), but afternoon time points were not significantly lower than baseline. The average MCAV over 8 h was higher in EX + SIT than SIT (P = 0.007) or EX + BR (P = 0.024). Uninterrupted sitting should be avoided, and moderate-intensity exercise should be encouraged for the daily maintenance of cerebral blood flow in older adults. The clinical implications of maintaining adequate cerebral blood flow include the delivery of vital oxygen and nutrients to the brain.

NEW & NOTEWORTHY This is the first study to measure the combined effects of an exercise bout with breaks in sitting on cerebral blood velocity in older adults. Using frequent recordings over an 8-h period, we have performed a novel analysis of the pattern of cerebral blood velocity, adjusting for concurrent measures of mean arterial pressure and other potential confounders in a linear mixed effects regression.

acute exercise; brain health; older adults; sedentary behavior; transcranial Doppler

INTRODUCTION

The prevalence of stroke and dementia is increasing due to population aging (14). Aging is also associated with an increased prevalence of cardiovascular risk factors for cerebrovascular disease such as physical inactivity, obesity, hypertension, hyperlipidemia, and dysglycemia (43). Therefore, strategies to maintain cerebrovascular health among older adults with cardiovascular risk factors are a public health priority. Evidence demonstrates that exercise in particular is associated with a reduced incidence of stroke (24, 39) and may also delay the progression of dementia (19, 25). However, the mechanisms underlying these benefits in humans remain unclear. While exercise may exert some of its cardiovascular effects by modifying traditional risk factors (18, 22), there are also direct benefits of exercise on arterial function and health (17, 37). In addition, regular exercise can mitigate the decline in cerebral blood flow associated with aging (1). Insight from animal studies demonstrates the importance of exercise-induced increases in cerebral blood flow for neurogenesis, cerebral angiogenesis, and related growth factors (3, 28, 30, 35). To understand cerebrovascular regulation in response to exercise in humans, many studies focus on cerebral blood flow during or immediately after exercise (27). However, few experiments have characterized the cerebral blood flow response to different patterns of physical activity over the whole day, an imperative for the design of optimal exercise interventions.

Over a whole waking day, older adults spend ~5% of time engaged in exercise of moderate-to-vigorous intensity but spend a majority of time in sedentary behavior, which carries an increased risk for all-cause mortality (15, 20, 21). Recent evidence suggests that sedentary behaviors such as prolonged...
sitting may be negatively associated with aspects of brain health such as cognitive function and medial temporal lobe thickness (13, 33). In addition, laboratory studies that have investigated reducing and breaking up sitting with intermittent physical activity have reported beneficial impacts on multiple systems relevant to brain health, including carbohydrate and lipid metabolism (4, 16), blood pressure (5, 10), sympathetic function (10), and vascular function (7, 32, 36). In response to accumulating evidence, some government guidelines now recommend reducing sitting in addition to engaging in moderate-to-vigorous intensity exercise (2, 11). In the United States, the scientific report that informed the 2018 Physical Activity Guidelines for Americans highlighted a need for future studies to investigate different patterns of physical activity and sedentary behavior on brain health outcomes (29). However, it is currently unknown whether engaging in moderate-to-vigorous intensity exercise would mitigate any potential decline in cerebral blood flow during a subsequent period of prolonged sitting. It is also unknown whether combining a bout of moderate-to-vigorous exercise with subsequent breaks in sitting would further enhance the cerebral blood flow response.

The aim of this study was to assess the impact of a moderate-intensity exercise bout, with or without subsequent breaks in sitting, on middle cerebral artery velocity (MCAv) in older adults. We hypothesized that an acute bout of exercise would enhance cerebrovascular responses over an 8-h period, relative to prolonged uninterrupted sitting. In addition, we hypothesized that cerebrovascular responses following acute exercise would be further enhanced by subsequent exposure to breaks in sitting.

MATERIALS AND METHODS

This experiment is a substudy of a larger randomized crossover trial (ACTRN12614000737639), and the detailed methods have been published independently (12).

Participants. Men and postmenopausal women (n = 10 men and 2 women, age ≥55 to ≤80 yr; body mass index ≥25 to <45 kg/m²; English-speaking) were recruited from the local community via advertisement in Perth, WA, Australia. Full participant characteristics are found in Table 1. This study was approved by the Human Research Ethics Committee of The University of Western Australia (RA/4/1/6990). Participants provided written informed consent before testing. All participants were screened for cardiovascular risk and previous cardiovascular events. Exclusion criteria included self-reported sitting <5 h/day, self-reported engagement in moderate-intensity exercise ≥150 min/wk for >3 mo, probable dementia (Telephone Interview of Cognitive Status score of <19), cognitive impairment (Mini Mental State Exam <24), depressive symptoms of clinical relevance (Geriatric Depression Score >6 or Hospital Anxiety and Depression Scale Score: depression score >8), diagnosed diabetes, use of glucose/lipid-lowering medication, antidepressant medications, β-blockers, antianxiety medication, excessive alcohol consumption (>8 points on the Alcohol Use Disorders Identification Test), abnormal ECG (determined by study doctor), high resting blood pressure (office systolic >160 mmHg or diastolic >100 mmHg), or major illness/physical problems (acute or chronic) that would limit ability to perform moderate-intensity exercise.

Study design. Participants were randomized to participate in three laboratory sessions, separated by a minimum of 6 days (Fig. 1). The order of conditions was block randomized and stratified by sex by an independent third party using a computer-generated random sequence and stored in sealed envelopes as previously outlined (12). Researchers were blinded to the order of conditions until familiarization was complete, and participants were blinded to the conditions until each day of testing. Experimental conditions included the following: sitting (SIT): uninterrupted sitting (8 h, control); exercise + sitting (EX + SIT): sitting (1 h), moderate-intensity walking (30 min, denoted by walking figure) followed by uninterrupted sitting (6.5 h); and exercise + breaks (EX + BR): sitting (1 h), moderate-intensity walking (30 min) followed by sitting (6.5 h) interrupted every 30 min with 3 min of light-intensity walking. Walking breaks are denoted by vertical lines in the EX + BR condition. During each condition, participants consumed a standardized breakfast and lunch meal and transcranial Doppler, mean arterial pressure, and heart rate were recorded simultaneously across the day.

Fig. 1. Experimental design. Participants completed 3 conditions in a random order separated by a minimum of 6 days. Conditions were as follows: sitting (SIT); uninterrupted sitting (8 h, control); exercise + sitting (EX + SIT): sitting (1 h), moderate-intensity walking (30 min, denoted by walking figure) followed by uninterrupted sitting (6.5 h); and exercise + breaks (EX + BR): sitting (1 h), moderate-intensity walking (30 min) followed by sitting (6.5 h) interrupted every 30 min with 3 min of light-intensity walking. Walking breaks are denoted by vertical lines in the EX + BR condition. During each condition, participants consumed a standardized breakfast and lunch meal and transcranial Doppler, mean arterial pressure, and heart rate were recorded simultaneously across the day.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
</tr>
<tr>
<td>Sex (women/men)</td>
<td>2:10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.4 ± 4.3</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>103.4 ± 11.0</td>
</tr>
<tr>
<td>Office SBP,* mmHg</td>
<td>128 ± 13</td>
</tr>
<tr>
<td>Office DBP,* mmHg</td>
<td>76 ± 13</td>
</tr>
<tr>
<td>Fasting glucose,† mmol/l</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>Fasting insulin,† pmol/l</td>
<td>30 ± 24.7</td>
</tr>
<tr>
<td>Fasting cholesterol,† mmol/l</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>Fasting triglycerides,† mmol/l</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Fasting HDL,† mmol/l</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Fasting LDL,† mmol/l</td>
<td>3.3 ± 0.6</td>
</tr>
</tbody>
</table>

Data are means ± SD; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. *Measured during familiarization visit. †Measured during first experimental visit.
EXERCISE IMPROVES THE PATTERN OF CEREBRAL BLOOD FLOW

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tailed for each participant during the familiarization session to induce a heart rate indicative of moderate-intensity, defined as 65–75% of age predicted maximum heart rate. Each 3-min light-intensity walking break performed during EX + BR was completed on a treadmill with 0% incline at a speed of 3.2 km/h, which was a walking speed for all participants. Heart rate (Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE; scale 6–20; light intensity 9–11 RPE; moderate-intensity 12–15 RPE) were collected at 5-min intervals during the 30-min bout of exercise and at the end of each 3-min walking break.

Experimental day protocol. Participants reported to the laboratory at ~7 AM following an overnight fast (>10 h). Participants remained seated while equipment was set up and the bilateral middle cerebral arteries were located as detailed below, before the start of the experiment at ~8 AM (0 h). The experiment began with baseline recordings of MCAv, blood pressure, and heart rate, which were obtained before the administration of a standardized breakfast meal. Breakfast and lunch were administered at 40 and 280 min into the experiment and were consumed over a 20-min period. All meals were standardized according to randomization and participants were instructed to remain seated apart from leaving the chair to void or to perform predetermined treadmill walking in the EX + SIT and EX + BR conditions. Study outcomes were measured at multiple time points across the day (Fig. 1). All measures of MCAv were taken during steady-state sitting periods, such that in the EX + BR condition measures were collected at least 25 min after the most recent activity break.

Cerebrovascular function. Cerebral blood flow was indexed using transcranial Doppler (TCD; Spencer Technologies, Seattle, WA). Bilateral measures of MCAv were determined with a 2-MHz probe transfixed to the posterior aspect of the temporal window of the skull using the Mark 600 headframe (Spencer Technologies, Seattle, WA). The headframe was secured in place to negate movement effects on the insonation site, and participants remained instrumented for the entire experiment to avoid relocating the MCA. The location of the middle cerebral artery was determined by locating the trifurcation of the circle of Willis (~45–65 mm) in the anterior circulation of the brain, as previously outlined (42). The MCAv was continuously sampled for 5 min at baseline and for 30 s during subsequent time points, at 1,000 Hz via an analogue-to-digital converter (PowerLab; 16/30 AD Instruments, Colorado Springs, CO). Data were analyzed offline using a specialized analytical software package (LabChart 8; AD Instruments). The sum of bilateral velocities was calculated for statistical analyses. The sum of bilateral velocities represents a surrogate measure of the total amount of blood being delivered to the brain. Summing the bilateral velocities also accounts for expected anatomical differences between the left and right MCA, the detection of which would be diminished by averaging the bilateral velocities.

Assessment of hemodynamic variables. Resting blood pressure and heart rate were measured in a seated position. A photoplethysmographic method was used for serial BP assessment (Finometer Pro; Finapres Medical Systems, Amsterdam, The Netherlands), and this was calibrated against automated brachial oscillimetry (HEM-907; Omron, Kyoto, Japan). In all conditions, blood pressure and heart rate were measured contemporaneously with MCAv and at a time consistent with the period immediately preceding the 3-min walking break during the EX + BR condition.

Statistical analysis. Based on previous evidence, we estimated the effect size (Cohen’s d for repeated measures) of exposure to intermittent light-intensity walking breaks relative to uninterrupted sitting to be ~1.1 for MCAv (6). With the assumption of a within participant correlation of 0.6, the effective sample size to detect this difference with a power of 0.80 and a two-tailed probability of 0.05, is 9 participants. The order of conditions was block randomized and stratified by sex by an independent third party using a computer-generated random sequence and stored in sealed envelopes as previously outlined (12). Analysis was performed by technicians blinded to the study conditions. Following recent recommendations on data analysis of crossover trials (23), linear mixed models with random intercepts were used to evaluate the differential effects of the experimental conditions on the selected outcomes. Mixed models are appropriate for correlated data (repeated measures) with various distributional assumptions and can easily accommodate missing data (31). A treatment-by-time interaction term was included in regression models to examine between condition differences in temporal patterns of MCAv across the day. Marginal means (i.e., adjusted mean of the dependent variable when fixed effects are held at their mean) were calculated for individual time points and within condition comparisons, relative to baseline, and were performed for the sum of bilateral MCAv. Between condition comparisons of individual time points were performed on heart rate and mean arterial pressure variables. All models were adjusted for baseline, age, sex, waist circumference, and treatment order. Models with MCAv as the dependent variable were additionally adjusted for mean arterial pressure, which was recorded simultaneously with MCAv. Due to the large number of comparisons in the within and between condition analysis of individual time points, adjustment for multiple comparisons using a Sidak correction was performed. A probability level of 0.05 was adopted. Statistical analyses were performed using Stata 15 for Windows (StataCorp).

RESULTS

Exercise response. The initial 30-min exercise bout induced similar (P > 0.05) heart rate and RPE responses (means ± SD) under each condition (EX + SIT: 104 ± 10 beats/min, 69 ± 7% HRmax, 11 ± 3 RPE; EX + BR: 108 ± 15 beats/min, 72 ± 11% HRmax, 11 ± 3 RPE). Average HR and RPE across all 12 walking breaks was 93 ± 14 beats/min, 62 ± 10% HRmax and 8 ± 2 RPE.

Temporal variation: 8-h pattern of cerebral blood velocity. Recording the MCAv across an 8-h time period enabled the assessment of the pattern of cerebral blood velocity across the day. Observation of the response across time revealed a persistent decline in SIT (Fig. 2A). In the EX + SIT and EX + BR conditions, the initial decline of MCAv was followed by an afternoon recovery (Fig. 2, B and C). In support of these observations, a significant main effect of time was found for the sum of bilateral velocities (P < 0.001). Post hoc analysis revealed a negative linear trend in SIT (P < 0.001) but a positive quadratic trend for both EX + SIT (P < 0.001) and EX + BR (P < 0.01). A positive quadratic trend identifies the response as a convex curvilinear pattern. A significant main effect of time was also observed for left MCAv (P < 0.001) and right MCAv (P = 0.04). Left MCAv followed a negative linear trend in SIT (P < 0.001) but a positive quadratic trend in EX + SIT (P < 0.001) and EX + BR (P < .001). Right MCAv followed a negative linear trend for SIT (P < 0.001), a positive quadratic trend in EX + SIT (P = 0.02), and no significant trend for EX + BR (P > 0.05). Within condition analysis of the time course data in the SIT condition revealed a significant decline in the sum of bilateral MCAv during the morning period relative to baseline, which was sustained until the end of the condition (Fig. 2A). However, an initial decline in MCAv relative to baseline was followed by a recovery, which was sustained for the final 2.5 h of the EX + SIT condition and final 4 h of the EX + BR condition (Fig. 2, B and C).

Average cerebral blood velocity across the day. The sum of bilateral velocities (cm/s), averaged across the day (Fig. 3A),
was higher in the EX + SIT condition 87 [95% confidence interval 79–96] relative to SIT 85 [76–93, \( P = 0.005 \)] or EX + BR 85 [77–93, \( P = 0.02 \)]. These between condition differences in MCAv (cm/s) were largely driven by the left MCA, which was higher in EX + SIT 44 [42–46] compared with SIT 43 [41–45, \( P = 0.009 \)] or EX + BR 42 [40–44, \( P < 0.001 \)] (Fig. 3B). However, no significant differences were observed between conditions in the average right MCAv (cm/s); SIT 45 [40–51], EX + SIT 45 [40–50], and EX + BR 46 [41–51] (Fig. 3C).

Comparison between conditions in hemodynamic data. Heart rate, when averaged across the day, displayed a pattern of increase with increasing activity; SIT 68 [64–71], EX + SIT 72 [69–75, \( P < 0.001 \) vs. SIT], and EX + BR 73 [70–77, \( P < 0.001 \) vs. SIT]. This was predominantly due to increased heart rate following the 30-min bout of moderate-intensity exercise. In EX + SIT and EX + BR, heart rate remained elevated for ~2 h following the moderate-intensity exercise bout, relative to SIT (Fig. 4A). Despite a higher heart rate, mean arterial pressure was lower for ~2 h following the moderate-intensity exercise bout in EX + SIT and EX + BR, relative to SIT, although no significant differences between conditions were observed during this time. There was a small increase in the mean arterial pressure (mm Hg) averaged across the day in EX + BR 102 [96–107], compared with SIT 98 [92–104, \( P = 0.02 \)], but no difference compared with EX + SIT 99 [93–105].

DISCUSSION

We observed that the pattern of MCAv during prolonged uninterrupted sitting was that of negative linear trend, with significant declines relative to baseline during the final 3.5 h of the experiment. In contrast, the pattern of MCAv following a morning bout of exercise with or without breaks in sitting, was that of a convex curvilinear response characterized by an initial decline followed by a subsequent recovery. Interestingly, the recovery of MCAv after the initial decline began earlier in the EX + BR condition, compared with EX + SIT, which may represent a benefit of intermittent walking on the temporal pattern of MCAv. The clinical implications of such a pattern of MCAv may be in avoiding sharp declines in the delivery of oxygen and nutrients to the brain (34). A decline in the delivery of glucose to the brain, for example, risks exposing the brain to hypoglycaemia, which can increase the risk of developing dementia (41). Previously, we hypothesized that fluctuations in glucose availability, more specifically than absolute concentrations, pose a risk to brain health and breaks in sitting may help
mitigate this risk by maintaining a more stable supply of glucose to the brain (40). While the current data suggest that MCAv was most stable in the EX + BR condition, we did not measure glucose availability to the brain. Future studies to determine the effect of breaks in sitting on central glucose concentrations and oxygen delivery would be highly informative. To our knowledge, this is the first study to examine the 8-h pattern of MCAv in this way. This type of analysis involving frequent transcranial Doppler assessment offers unique insights into the temporal regulation of cerebral blood flow and may have implications for understanding cerebrovascular health.

We also observed that a morning bout of exercise sustained a higher average MCAv across a subsequent period of prolonged sitting. However, the finding that adding regular activity breaks to a morning bout of exercise abolished the increase in average MCAv was somewhat unexpected. There are some possible explanations worth exploring. First, day-to-day differences in the probe angle and location used when establishing the MCAv signal may have introduced measurement error into the between condition comparisons of average MCAv. Our within condition analysis of the pattern of MCAv helps mitigate this potential source of error as participants remained instrumented for the entire experiment to avoid relocating the MCA. Second, subtle changes in MCA diameter, undetectable by TCD, may have altered MCAv during intermittent walking. With the use of magnetic resonance imaging (MRI), changes in MCA diameter would translate to a decrease in velocity and vice versa for a decreased diameter, assuming constant flow.

The effects of intermittent walking on MCAv have been documented in one previous study of lean healthy “desk workers” (6). The authors demonstrated an increase in MCAv pre to post a 4-h period involving breaks in sitting, compared with prolonged uninterrupted sitting (6). Although we observed an attenuation in average MCAv following intermittent walking, this was after an antecedent bout of morning exercise in a population of older overweight and obese adults (mean age 70 yr), compared with walking breaks alone in a younger healthy population (mean age 36 yr) in the study by Carter et al. (6). These differences between studies likely represented a different stimulus to a range of mechanisms responsible for regulating cerebral blood flow.

While our study was not designed to address the mechanisms responsible for effects on MCAv, several possibilities may exist. Brain blood flow is controlled by multiple redundant and integrative mechanisms and is highly protected by local and reflex pathways. Although differences existed between conditions in blood pressure responses (Fig. 4), MCAv data were statistically adjusted for contemporaneous blood pressure in regression analyses, and it is therefore unlikely that our cerebrovascular findings are primarily related to underlying changes in driving pressures. This type of correction avoids the need to meet the stringent assumptions required for ratio normalization, where one number is divided by another (9). A further mechanism that controls cerebral blood flow is the partial pressure of carbon dioxide in the circulating blood, and it is possible that the exercise bouts induced differences in this parameter. However, the major differences we observed between conditions occurred more than 4 h after the morning exercise bout and all of our MCAv data were obtained under quiet resting conditions. Furthermore, an impact of active breaks on carbon dioxide at the time of measurement seems unlikely, since there was ~25 min between these brief periods of walking and the subsequent resting measure of MCAv.

A strength of this study is the well-controlled randomized crossover design, which controls for person-specific factors.
and affords smaller sample sizes. Trial conditions were also standardized for potential confounders such as diet, physical activity, medications, and baseline values. There are also some limitations to our study. The experiment was designed as a superiority trial, and we did not include a fourth condition involving walking breaks alone. This was due to the general acceptance of the health benefits associated with continuous exercise bouts; we considered this the minimum standard for prescription. Our aim in the present study was to determine whether additive benefit was possible beyond that obtained from a morning bout of exercise. Our measure of cerebrovascular function, based on TCD ultrasound, is widely used, provides sensitive time course information, and has been shown to be a useful surrogate measure of cerebral blood flow between individuals (26). However, direct measures of intracranial diameters are not currently possible using ultrasound and velocity is therefore relied on as a surrogate measure of flow. This is less of an issue for within subject experimental designs because blood flow changes are heavily dependent on velocity change. However, we cannot rule out distinct effects on artery diameter responses that went undetected. Future experiments utilizing electroencephalography or near-infrared spectroscopy may help to better understand complementary and temporal patterns of change in cerebrovascular function in the future. Furthermore, positron emission tomography and MRI would provide information on spatial distribution of brain blood flow. In addition, it is unknown whether the changes observed simply represent a local effect on the brain vessels per se or an impact on cerebral activation that subsequently affected brain blood vessels. Future studies, perhaps including functional MRI, may be utilized to test how brain networks are affected by the combination of exercise and breaks in sitting. This is relevant since metabolic activity in the brain is known to also affect regional cerebral blood flow. Finally, given expected regional differences in cerebral blood flow, our findings are not generalizable to the posterior circulation.

Conclusion. We have demonstrated in older overweight to obese adults, that the pattern of cerebral blood velocity over 8 h is improved following a morning bout of moderate-intensity exercise with or without subsequent breaks in sitting. In addition, a morning bout of exercise sustained a higher average MCAv during a period of subsequent sitting. Interestingly, adding intermittent walking breaks to a morning bout of exercise abolished the increase in average MCAv, which was unexpected. Future studies should seek to replicate these findings with more direct measures of cerebral blood flow using positron emission tomography or MRI. In addition, future studies using TCD should take advantage of the high temporal resolution this measure offers and collect frequent recordings to analyze the temporal pattern of cerebral blood velocity. Collecting and analyzing data in this way can also take advantage of current statistical techniques such as linear mixed effects modeling, which are particularly suited to repeated measures analysis and within subject study designs. In conclusion, our findings suggest that uninterrupted sitting should be avoided, and moderate-intensity exercise should be encouraged for the daily maintenance of cerebral blood flow.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


GRANTS

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Downloaded from www.physiology.org/journal/jappl at Australian Catholic Univ (203.010.044.060) on May 26, 2019.
5.1 Summary

The findings presented in Section 5.1 represent a novel contribution to the literature in which frequent sampling enabled analysis of the pattern of middle cerebral artery velocity (MCAv). This analysis revealed that during prolonged sitting, bilateral MCAv declined by ~20%; an effect which was sustained over 8-hours. However, when morning exercise was performed, this initial decline of ~20% was followed by a recovery in the afternoon, hours after exercise cessation, such that afternoon time-points were not significantly different to baseline. When exercise was followed by prolonged sitting, this recovery occurred 4.5 hours into the experiment. However, when exercise was followed by breaks in sitting, this recovery occurred earlier, at 3 hours into the experiment. This may represent a benefit of intermittent walking on the pattern of cerebral blood velocity.

These results should be considered in light of the hypothesis outlined in Chapter 1; that a stable fuel supply to the brain is an essential component of brain health, and that sedentary behaviour may contribute to a decline in that fuel supply. The results of the current study support the hypothesis that prolonged sitting can impair brain blood flow and suggest that exercise should form part of a daily routine in order to preserve the brain’s fuel supply.

In addition to fuel supply, cerebral blood flow may impact brain health through modifying shear stress within cerebral blood vessels. The ‘vascular niche’ hypothesis suggests that adult neurogenesis occurs in highly vascularised regions of the brain and involves co-depndant signalling between neuronal cell precursors and endothelial cell precursors. Indeed, endothelial cells secrete growth factors such as BDNF and vascular endothelial growth factor, known to support neurogenesis and angiogenesis. Dynamic co-culture experiments have revealed that shear stress is an important signal for endothelial cells, promoting cross-talk with neighbouring neural stem cells to increase neuronal differentiation. Animal models also suggest an in vivo casual interaction between angiogenesis and neurogenesis. For example, in exercise-trained mice, increased dentate gyrus blood volume post training was correlated with increased neurogenesis measured post-mortem. In the same study, exercise-trained humans also increased dentate gyrus blood volume which was correlated with increased aerobic fitness and improved cognition. In the context of this research, the current results suggest that prolonged sitting should be avoided and moderate-intensity exercise encouraged for the daily maintenance of brain health. The wider implications of the work presented in this thesis, along with future directions, are considered in Chapter 6.
CHAPTER 6

Discussion

This final thesis chapter synthesises the experimental findings presented in Chapters 1-5 and incorporates these findings into context with the prevailing literature. It is divided into subheadings which introduce various discussion points. While each chapter (above) includes a manuscript incorporating discussion specific to the aims of that paper, the goal of Chapter 6 is to integrate those findings in a way that builds a ‘Gestalt’ picture, particularly relating to the influence that physical activity and sedentary behaviour can have on cardiometabolic and cognitive health. Since no individual experiment is ever completely definitive, nor perfect in design or execution; a section is also included on limitations. This will address lessons learned through the candidature journey and new questions that have arisen as a result of the work presented in this thesis, with proposals for future areas of research.

6.1 Conceptual framework and fundamental questions posed by this thesis

In Chapter 1, the public health perspective on physical activity and sedentary behaviour was introduced. This was presented to give the reader some perspective on the translational focus of the thesis. An important theme of this chapter was that while physical activity and sedentary behaviour may be behaviourally distinct with unique determinants, they nonetheless coexist in real world settings on a daily basis. An individual can be compliant to physical activity guidelines, but also indulge in prolonged periods of uninterrupted sitting. Alternatively, one can be ‘fidgety’ and regularly interrupt sedentary behaviour with light-intensity activity breaks, but without satisfying the extant guidelines on achieving sufficient physical activity to sustain health benefit. Between these extremes lies a spectrum of inter-related behaviours that are impacted by work, leisure, transport, environmental and lifestyle factors of contemporary society.

Recently, some guidelines have started to recognise the complexity and implications of this spectrum, but recommendations remain general, especially with respect to sedentary behaviour.\textsuperscript{24,25} Indeed, it has recently been suggested that the evidence base around sedentary behaviour still needs to mature in order to support quantitative guidelines for behaviour change.\textsuperscript{89} Studies such as those reported in this thesis may help by adding to this evolving evidence base. The experimental work presented in Chapters 2-5 was...
designed to be ecologically valid, but laboratory based, in order to better understand, in a controlled setting, the physiological implications of real-word patterns of behaviour.

Another core idea presented in Chapter 1 relates to the links between physical activity, cardiometabolic function and cognition, recognising the interconnectedness of these determinants of brain function and health in humans, and the evolutionary foundations of these connections. From an evolutionary perspective, if our ancestors were required to both outrun and outplan their peers in the hunt for food, and efficiently use those gained resources in order to survive and reproduce, this would create environmental pressure favouring physiology that promotes a virtuous cycle between physical activity, cardiometabolic function and cognition. A benefit in one component would generate a benefit to all. Despite this evolutionary context, modern society is characterised by an environment that encourages prolonged sitting as the default behaviour, with physical activity as a deliberate and planned endeavour. Time is ‘set aside’ for structured exercise, as opposed to it being incidental and the historical nexus between exercise and food procurement has been fundamentally disrupted.

The overarching aim of this thesis was to understand the physiological consequences of contemporary behaviours as they typically manifest in various modern scenarios. For example, the sedentary office worker or retiree who predominantly sits over the course of a normal 8-hour day; versus the ‘active couch potato’ who achieves the recommended daily amount of exercise but who sits for prolonged periods outside of their planned exercise; or the ‘active commuter’ who exercises in the morning and may also break up their sitting throughout the working day. This understanding was sought through a synthesis of literature in Chapter 1, which introduced the conceptual framework that informed subsequent experimental investigation. An overview of the findings from Chapters 2-5 is presented below (Figure 10). Through these chapters, the combined effects of exercise and sedentary behaviour on cognitive, metabolic, cardiovascular and cerebrovascular function were systematically studied to address the overarching and fundamental questions posed in this thesis:
1) What are the consequences of prolonged sitting over the course of 8-hours for cognitive, metabolic, cardiovascular and cerebrovascular function and health?

2) Is it sufficient to achieve the guideline-recommended daily amount of exercise – 30 minutes of moderate intensity – in order to warrant protection from the aforementioned physiological consequences of prolonged sitting?

3) Can the physiological benefits of a 30-minute bout of moderate-intensity exercise be enhanced by subsequently interrupting a period of sitting with brief, regular light-activity breaks?
Figure 10. Overview of thesis main findings. This figure depicts the main findings of this thesis as they relate to different patterns of physical activity and sedentary behaviour. Each ring represents a typical 8-hour day. The health impacts of each day, according to the findings of this thesis, are displayed within each ring. The table gives a more detailed overview of the effect of each condition on specific outcomes. Plus signs denote a positive health impact; minus signs, negative health impact; tilde, unclear health impact. BDNF: brain-derived neurotrophic growth factor. SIT; sitting condition. EX+SIT, exercise plus sitting condition. EX+BR; exercise plus breaks condition.
6.2 Implications of improved executive function and working memory

Chapter 2 contained the first empirical paper of this thesis. It explored the acute effects of exercise, with or without subsequent breaks in prolonged sitting, on cognitive performance and BDNF. The results demonstrated that prolonged sitting over the course of 8-hours impaired cognitive test scores, and resulted in decreased levels of BDNF. Conversely, when participants performed a morning bout of moderate-intensity exercise, there were sustained increases in cognitive performance and BDNF levels. However, a novel finding was that the acute effects of exercise on cognition were influenced by whether-or-not subsequent sitting is interrupted. Relative to prolonged sitting alone, a morning exercise bout improved executive function during a subsequent period of prolonged sitting. However, when exercise was followed by breaks in sitting, working memory, but not executive function, was improved relative to prolonged sitting. In addition, BDNF was increased following exercise, with or without breaks in prolonged sitting.

Multiple aspects of cognition were assessed; psychomotor function, attention, visual learning executive function and working memory. However, significant effects were only observed for executive function and working memory. Previous studies in older adults investigating the effects of acute and chronic exercise on cognition demonstrate the largest effect sizes for executive function.\textsuperscript{55,90,91} This is consistent with the current finding that exercise improved executive function, and this improvement was evident during a subsequent period of prolonged sitting. It was surprising that combining exercise with breaks in sitting, which increased the overall amount of physical activity, did not further improve executive function. However, as outlined in Chapter 2, executive function is an umbrella-term that involves multiple cognitive processes, of which working memory is one. From this perspective, the two different exercise conditions could have affected different subcomponents of executive function. That different behavioural strategies were associated with distinct impacts on varying components of cognition is, in itself, an important and novel contribution.

It can be difficult to compare acute exercise studies which have measured the higher-order, prefrontal lobe-dependant domain of cognition known as “executive function”.\textsuperscript{92} Apart from differing intensity, duration and timing of exercise relative to cognitive testing, as well as studies being performed in different populations, executive function itself is a complex psychological construct. Indeed, there is no standardised or agreed set
of subcomponents that collectively reflect executive function. Some suggest it comprises working memory, reasoning, task flexibility, problem-solving and planning. Others suggest it involves volition, planning, purposeful behaviour and effective performance, or novelty, planning and acting on appropriate strategies for conducting performance. In addition, there is a plethora of different tests, and ways in which they are administrated, that are used to assess executive function. Despite this complexity, there is a general notion in the scientific literature that executive functions tend not to be critical for the execution of routine, well-learned behaviours, but rather are specifically required in novel and unfamiliar circumstances.

Executive functions, therefore, might be described as the ability to ‘think on your feet’. Improving this capability can have important practical advantages, for example in work contexts that encourage prolonged sitting but require competent decision making. One study analysed 1,112 judicial decisions which were collected over 50 days from 8 judges. The authors found that sitting judges deliberated longer over rulings at the start of the day, of which ~ 65% were favourable to the defendant. As judges continued to sit, deliberation time along with the percentage of favourable decisions gradually declined to ~0% until the judge had a break, after which deliberation time went up and decisions became favourable again. This pattern was found to repeat across the day. This suggests that judges can become mentally fatigued the longer they sit and work without a break, which can make them more likely to reject requests and make unfavourable rulings. On the basis of the findings of this thesis, it could reasonably be speculated that if judges were to structure their day by performing a morning exercise bout followed by regular light-activity breaks from sitting, this may help them maintain consistent decision-making ability across the working day.

A similar pattern emerges in the industrial setting, where there seems to be a linear relationship between time-on-task and injury risk. One study assessed all accidents that occurred at a car assembly plant in the UK over a three year period, finding that the relative risk of accidents was double after 90-119 minutes of continuous work, compared to 0-29 minutes. The authors suggest that breaking up the time-on-task would be an effective risk reduction strategy. This strategy aligns well with the regular activity breaks investigated in the current study, as a break from sitting can also be a break from time-on-task. However, the effectiveness of such a strategy to improve cognitive performance is likely to be task or context-specific.
Driving is another context in which sustained benefits in cognitive performance would be beneficial, especially for older adults. The proportion of fatal multi-vehicle collisions at intersections may increase exponentially past a certain age. One study demonstrated that such crashes are 2.26 and 10.62 more likely in those aged 65-69 and 85+, respectively, compared to those aged 40-49.\textsuperscript{99} Moreover, a review of studies on factors associated with safe driving and crash risk in older adults concluded that not all older drivers are impaired in this regard, but those with impaired executive function tend to be less safe on the road.\textsuperscript{100} These sources of evidence clearly point to the practical advantages of sustained improvements in cognition, particularly in the setting of prolonged inactivity. However, the cognitive benefits observed in the current study may also affect longer-term health-related behaviour.

One theory, coined ‘the effort hypothesis’, posits a reciprocal relationship between executive function and adherence to exercise.\textsuperscript{101,102} According to this theory, practicing effortful exercise requires planning and inhibition (i.e. the ability to focus on one task while inhibiting distractions). Therefore, practicing regular exercise may be synonymous with practicing concentration and willpower, qualities that facilitate adherence to healthy behaviours. This theory is associated with two perspectives: 1) that regular exercise improves executive function, an idea supported by much empirical evidence,\textsuperscript{103} and 2) that those with good executive function are better equipped to plan and adhere to healthy behaviours, including exercise. Although this is a less studied theory,\textsuperscript{101} In support of this second point, one study found that among 125 women aged 65-75, those who made greater improvements in executive function during an exercise training intervention exhibited increased levels of self-reported physical activity during a one year follow up period.\textsuperscript{104} Interestingly, improvements in executive function during the exercise intervention were not related to adherence during the exercise intervention, only to adherence after the intervention had ended. In another study of 64 undergraduate students (19.0±2.5 years), those who scored higher on a Go/No-Go response inhibition task demonstrated greater correspondence between the amount of physical activity they intended to do during the upcoming week, and the amount they self-reported doing at the end of that week.\textsuperscript{105}

Aside from exercise, executive function has also been shown to predict adherence to medication in older adults.\textsuperscript{106} While taking medication may be a routine task, this
association may be explained by those times when routine was interrupted and executive function was required to remember to take the medication in an unplanned situation. In this light, the improvements in executive function and working memory observed in the current study could represent the beginning of a virtuous cycle leading to improved cognitive function in the long term; where initial improvements in cognition may lead to an increased adherence to health-enhancing behaviours (Figure 11).

![Figure 11](image)

**Figure 11.** Cycles of physical (in)activity, executive function and adherence. This figure depicts two hypothetical scenarios. In scenario A, a bout of exercise improves executive function, which leads to an improved capacity to adhere to a plan involving health-enhancing behaviours, including exercise. Scenario A is conducive to the building of habits that may benefit health, productivity and quality of life over the long term. In scenario B, prolonged sitting leads to poorer executive function, which may reduce a person’s capacity to adhere to a plan involving health-enhancing behaviour, including exercise. Scenario B is less conducive to the building of healthy habits, and may be detrimental to productivity, quality of life and health over the long term.
Potential mechanisms underpinning the link between physical activity and cognition

A greater appreciation for the intimate link between physical activity and cognition may be gained by looking through the lens of evolutionary biology. While this thesis seeks to establish how important this link is in modern humans, it helps to appreciate that this relationship has likely been conserved from the earliest forms of life. In his book “I of the Vortex: From Neurons to Self” neuroscientist Rodolfo Llinás describes insights from evolutionary biology and mathematical modelling of neuron-to-neuron communication, which support his theory that animals evolved nervous systems in order to internalise the properties of the outside world, and to translate those internalisations into movement patterns to increase the chance of obtaining food for survival. One key example used is that of the sea squirt, which develop as a free-swimming tadpole-like larva with a primitive eye. The larva swims until it finds a surface to attach to and will then digest its own nervous system, eye and other parts that classify it as a chordate, the same phylum as fish, birds, reptiles and mammals. The germane observation is that, at the point when the sea squirt no longer needs to move or make decisions, it digests its own nervous system. Hence neuronal activity and movement are intimately linked, as Llinás states; “That which we call thinking, is the evolutionary internalisation of movement”.

Of particular relevance to this thesis are the mechanisms related to physical activity and sedentary behaviour that may influence the trajectory of age-associated cognitive decline. Recently, The Lancet commission on dementia concluded that up to 35% of all cases of dementia may be attributable to nine potentially modifiable risk factors, including physical inactivity, hypertension, obesity, smoking, hearing loss, social isolation, education, depression and diabetes. Other estimations have also suggested that this list of risk factors, with the exceptions of hearing loss and social isolation, contribute to approximately 50% of all cases of Alzheimer’s disease, the most common cause of dementia. Physical inactivity may be the key risk factor among this list due to its influence on multiple aspects of health. Current evidence suggests that prescribing exercise can play an important role in the treatment of up to 26 different diseases and conditions by improving multiple risk factors including blood pressure, ischaemia (lower limb, cardiac and cerebrovascular), cardiorespiratory fitness, abdominal obesity, insulin resistance, inflammation and depression.

There are multiple biomarkers and mechanisms which may potentially explain the effects of both acute and chronic exercise on cognition and brain health. These include cerebral
blood flow,\textsuperscript{78} catecholamines,\textsuperscript{111} inflammatory markers,\textsuperscript{112} reactive oxygen species,\textsuperscript{113} glucose/insulin metabolism,\textsuperscript{114} and growth factors responsible for neurogenesis and angiogenesis.\textsuperscript{115} As outlined in Chapter 2, increased BDNF was observed over an 8-hour period following exercise with or without breaks in sitting. This has potential implications for longer term brain health, since BDNF is involved in the growth and maintenance of neurons, particularly in the dentate gyrus region of the hippocampus, a part of the brain involved in memory consolidation.\textsuperscript{115,116} Important insights into the role of BDNF in brain health have come from Mendelian randomisation studies where participants with the Val66Met mutation in the BDNF gene exhibit decreased secretion of BDNF, decreased brain volume, impaired episodic memory and increased anxiety and depression.\textsuperscript{117,118} However, BDNF levels are modifiable, as aerobic exercise training has been demonstrated to increase hippocampal BDNF, with greater increases in BDNF being associated with increased hippocampal size and improved spatial memory.\textsuperscript{119} The lack of correlation between BDNF and cognitive performance outcomes described in Chapter 2 may be related to the fact that peripheral, rather than central, BDNF was measured. Nonetheless, the observed increases in BDNF reinforce the idea that exercise is important for stimulating BDNF secretion. However, the results do not demonstrate additive increases in BDNF when combining exercise with regular interruptions to prolonged sitting. This is despite an additive effect of breaks on postprandial insulin, which, in itself, may have implications for brain health.

6.3 Implications of central and peripheral insulin action
Chapter 1 presented a hypothesis describing how sedentary behaviour may exert a negative influence on brain health by facilitating glycaemic excursions. However, when investigated in Chapter 3, exercise with or without breaks in sitting did not lower postprandial glucose, relative to prolonged sitting. Indeed, small increases were observed which equate to an approximate 0.2 mmol\textperiodcentered L\textsuperscript{-1} increase in average glucose over 8-hours. Such increases are unlikely to have adverse clinical consequences, and may be important for muscle glycogen re-synthesis after exercise.\textsuperscript{120} However, large decreases in 8-hour insulin concentrations of 25\% and 18\%, and insulin:glucose ratio of 28\% and 21\%, were observed following exercise with or without breaks in sitting, respectively. In light of Chapter 1, it is worth exploring the potential implications of this for brain health.

The brain is highly insulin-sensitive. Contrary to the periphery however, the role of central insulin seems not to be the transport of glucose, since the predominant glucose
transporters in the brain are the insulin insensitive GLUT-1 and GLUT-3. Nonetheless, insulin receptors are abundant throughout the brain of both rodents and humans. Insulin action in the brain appears to have a beneficial effect on a number of different processes including cognition, hunger/satiety and peripheral glucose metabolism. The following synthesis of evidence may appear to represent a paradox in that central insulin action is described as beneficial, but the current study observed reductions in peripheral insulin that are likely beneficial for brain health. However, chronic exposure to elevated insulin may induce ‘brain insulin resistance’ whereby the beneficial effects of central signalling are diminished despite elevated peripheral insulin.

Central insulin signalling has multiple benefits for cognitive function. For example, one study examined the impact of 8-weeks of intranasal insulin administration on memory. Before and after insulin administration, a word list was verbally presented to participants which had to be recalled immediately and again one week later. Results demonstrated that intranasal insulin improved the delayed recall, but not the immediate recall of that word list. The effect of insulin on memory may be related to its ability to induce growth factors in the brain, important for growth and development of the central nervous system. Indeed, central insulin signalling has been shown to regulate synaptic plasticity and synaptic density in vivo. However, intranasal insulin administration has also been shown to acutely improve other aspects of memory, such as verbal working memory, visuospatial memory and olfactory memory.

In addition to impacts on cognition, central insulin signalling may also influence food intake and peripheral metabolism, further highlighting the link between metabolic and cognitive function. Higher postprandial increases in insulin correlate with decreased activation of appetite regions of the brain, along with lower subjective appetite ratings to food cues. Similarly, intranasal insulin administration has been shown to reduce ad libitum calorie intake compared to placebo. In addition, knockout of neuronal insulin receptors has been demonstrated to induce hyperphasia, obesity, fasting hyperinsulinemia and hypertriglyceridemia in mice. This suggests a role of central insulin in energy intake that may be important in order to counteract overeating.

In humans, intranasal insulin administration, compared to placebo, has been demonstrated to increase the thermogenic response to food while reducing the postprandial insulin and c-peptide response by approximately 35% and 20%, respectively, despite similar glucose
Findings from other studies suggest that central insulin signalling reduces hepatic glucose production and improves whole body insulin sensitivity.\textsuperscript{133–135} Interestingly, one study found that intranasal insulin administration reduced the glucose infusion rate during a hyperinsulinemic-euglycemic clamp in lean participants versus a placebo spray, suggesting that central insulin signalling improves whole body insulin sensitivity.\textsuperscript{135} However, the same study found intranasal insulin had no effect on the glucose infusion rate in obese participants, suggesting brain insulin resistance in this group relative to lean participants.\textsuperscript{135} Indeed, in another study, obese adults demonstrated reduced cortical activity in response to peripheral insulin administration compared to lean counterparts.\textsuperscript{136}

These findings raise the question of what might cause insulin resistance in the brain. While not fully understood, one mechanism may be reduced transport of insulin across the blood brain barrier. Interestingly, in rats, prolonged exposure to peripheral hyperinsulinemia can decrease insulin receptors at the blood brain barrier, resulting in decreased transport of insulin into the brain.\textsuperscript{137} In insulin resistant humans, it has been established that peripheral insulin levels are high, whereas cerebrospinal fluid insulin levels are decreased.\textsuperscript{138,139} Therefore, exposure to hyperinsulinemia may reduce the transport of insulin across the blood brain barrier, and thus reduce the beneficial effects of central insulin signalling described above.

Taken together, these results suggest that central insulin signalling may have multiple benefits for cognitive and metabolic function in humans. This evidence is relevant to the current findings as well as being informative to the broader context in which the current study was conducted. Specifically, this evidence suggests that perhaps the prolonged sitting and concomitant hyperinsulinemia observed in the current study should be avoided in order to preserve blood to brain insulin transport and maintain beneficial central insulin signalling in the long term. From a broader perspective, the role of central insulin signalling on cognition and food seeking behaviour underscores the connection between metabolic and cognitive function. When considering this evidence, it is reasonable to speculate that the memory enhancing effect of insulin may be related to evolutionary pressures to consolidate information that would lead to obtaining food. Therefore, insulin not only regulates the availability of substrates for cellular respiration in the periphery, but it may regulate behaviour and other cognitive processes involved in the ability of the organism to obtain food in the first place. This is relevant to the findings reported in
Chapters 2 and 3 that different patterns of physical activity and sedentary behaviour affect cognitive function and insulin levels in the short term. Taken together, it suggests that these acute effects could also have longer-term implications for central insulin signalling; affecting cognition, food seeking behaviour and postprandial metabolism.

**Cardiometabolic implications of exercise and sedentary behaviour**

It has been proposed that more people in the world now die from over-nutrition than under-nutrition. Part of the problem is the widespread availability of cheap, high glycaemic index, energy dense foods making for unhealthy options. Indeed, the postprandial state is a common feature of modern life. Fuel partitioning in response to food is both a strong indicator, and key driver of, metabolic function in health and disease. The ‘carbohydrate-insulin model of obesity’ posits, for example, that hyperinsulinemia in response to high glycaemic foods promotes unhealthy fat and glycogen deposition which ultimately contribute to the development of obesity and insulin resistance. However, it is also acknowledged that there is considerable interpersonal variability in the glycaemic response to identical meals, suggesting limited efficacy for universal dietary recommendations aimed at reducing postprandial responses.

Physical activity undertaken during the postprandial period is an approach that can be used to lower postprandial markers of metabolic risk. The large reductions in 8-hour insulin reported in Chapter 3 suggest that the postprandial insulin response is sensitive to the daily pattern of physical activity and sedentary behaviour. Such findings may have implications for the development of cardiovascular disease and T2D over the longer term. For example, a chronic pattern of prolonged sitting and postprandial hyperinsulinemia would indicate an elevated demand on insulin secretion. This, in turn, could progress the development of insulin resistance. In addition, Chapter 3 also reported a 6% reduction in the 8-hour triglyceride response during EX+BR, which was significantly lower compared to both SIT and EX+SIT. The dual reduction of postprandial insulin and triglycerides during EX+BR may not be surprising, considering the bi-directional relationship between these two biomarkers. On one hand, a reduced postprandial triglyceride response could be viewed as secondary to the large reduction in postprandial insulin that occurred in this condition. Insulin is a rapid and potent inhibitor of lipolysis and stimulator of glucose uptake and free fatty acid esterification, mediated by attenuation of adrenergic signaling of adipose tissue lipases, including lipoprotein lipase. Conversely, intrahepatic triglyceride accumulation is a strong predictor of
insulin resistance in liver, muscle and adipose tissue. \textsuperscript{145} Taken together, these results potentially indicate broad implications of combining exercise with breaks in sitting for multiple physiological processes related to longer-term metabolic health.

### 6.4 Vascular function: At the crossroads of physical activity, metabolism and cognition

The aim of this discussion has been to integrate the findings from each chapter of the thesis. One variable linking much of what has already been discussed is vascular function. The final two empirical papers of this thesis reporting on blood pressure (Chapter 4) and cerebral blood velocity (Chapter 5) deal with something that connects peripheral metabolic health to cognitive health; the vasculature. The metabolic perturbations previously discussed that affect cognitive health, are mediated by, and have direct impact on, artery function and health. For example, a bi-directional relationship is observed between hyperinsulinemia and large artery stiffness. \textsuperscript{146} This may be related to hyperinsulinemia-induced sodium retention, \textsuperscript{147} and increased activity of the renin-angiotensin-aldosterone system; \textsuperscript{148} which can ultimately lead to fibrosis. \textsuperscript{149} Conversely, stiff arteries may transmit increased pulsatility to the microvasculature, impairing insulin-mediated glucose disposal and contributing to insulin resistance. \textsuperscript{150} Therefore, a negative feedback loop may become established whereby hypertension accentuates the severity of T2D, and vice versa. \textsuperscript{151,152} The findings of the current study suggest that combining exercise with breaks in sitting may be an effective strategy that mitigates such a negative feedback loop, given the additive reductions of combining these behaviours on both insulin and systolic blood pressure over 8-hours.

There are also implications of reduced blood pressure for cognitive health. Recent findings from The Whitehall II study suggest that systolic blood pressure >130mmHg in midlife increases the risk of dementia in later life. \textsuperscript{153} In this analysis, systolic and diastolic blood pressure was measured in 8,639 men and women in 1985, 1991, 1997 and 2003; and the incidence of dementia was followed until 2017. The results demonstrated that exposure to systolic blood pressure >130 mmHg at age 50 increases the risk of developing dementia by between 11\%-70\%. Interestingly, only the development of increased blood pressure in mid-life (50 years) but not later life (60 or 70 years) was associated with increased dementia incidence. One explanation for this may be that those with hypertension at aged 50 are exposed to the detrimental effects of high blood pressure for longer. This is directly relevant to the findings in Chapter 4, which demonstrated reduced
blood pressure following exercise in men and women, and additional reductions when
exercise was followed by breaks in sitting for women only. It is possible that these acute
effects may have implications for brain health over the long term.

A potential mechanism linking hypertension and dementia relates to the impact of
increased blood pressure on function and/or structure of cerebral microvessels. According
to this hypothesis, there is a ‘sweet spot’ for cerebral blood flow between 50 to
150 mmHg. Pressures lower than this may result in hypoperfusion and impaired
cerebral endothelial function, while higher pressures can also result in impaired
endothelial function and microbleeds or transient ischaemic attacks. An associated idea,
known as the ‘biomechanical hypothesis’ of brain damage in ageing, suggests that age-
related arterial stiffening can lead to increased pulse pressure and pulse wave velocity,
inducing ‘wear and tear’ on cerebral blood vessels and ultimately accelerating cognitive
decline. Indeed, the brain may be particularly susceptible to the mechanical impacts
of increased pulse wave velocity due to a lack of elastic lamina within intra-cranial
arteries leading to a reduced capacity to buffer pulse pressure. As a consequence,
the pulse pressure wave may propagate further along the vascular tree, causing damage
within the cerebrovascular microcirculation. Indeed, a cross-sectional analysis in
elderly individuals has revealed an association between structural abnormalities in white
matter and middle cerebral artery pulsatility. Increased arterial stiffness, pulse pressure
and carotid artery pulse wave velocity have been prospectively associated with brain
atrophy and cognitive decline. Notably, an analysis from the Whitehall II study
measured carotid artery pulse wave velocity, which is a direct measure of the intensity of
pulse waves travelling to the brain. This analysis included 3,191 participants who had
measures of carotid artery pulse wave intensity measured between 2003-2005 and
longitudinal measures of cognitive function assessed between 2002-2004 and 2015-
2016. The results demonstrated that higher pulse wave velocity at baseline was
associated with accelerated cognitive decline during follow-up. Moreover, this
association was driven by those in the 25% of highest pulse wave velocity at baseline,
who were ~50% more likely to develop rapid cognitive decline (top 15% most rapid
cognitive function during follow-up) than those in the lowest 25% of pulse
wave velocity, after adjustment for other well-established cardiometabolic risk factors.

These results are interesting in the context of Chapter 5 where an increased MCAv
following exercise was presumed to be beneficial for brain health. It suggests a paradox;
i.e. that exercise-induced increases in pulse wave velocity are beneficial to brain health,
but increased resting pulse wave velocity is detrimental. However, as we will discuss, the physiological environment underlying these increases are distinctly different, and have contrasting influences on brain health.

**Endothelium as the gatekeeper between haemodynamic forces and brain health**

The mechanisms underpinning the ‘biomechanical hypothesis’ of brain damage in ageing are not fully understood, but are likely to be influenced by endothelial function, given the sensitivity of the endothelium to mechanical forces. Indeed, the endothelium possesses a complex system of mechanosensors capable of transducing forces of shear stress, hydrostatic pressure and circumferential strain into chemical signals that lead to the activation of intracellular cascades. Depending on the pattern of haemodynamic forces, the endothelium can secrete an array of molecules that regulate vascular tone and redox balance which can ultimately affect the functional and structural properties of blood vessels with implications for brain health. It is worth clarifying the difference between the haemodynamic forces associated with ‘wear and tear’ of the cerebral blood vessels and those associated with exercise, especially in the context of the ‘biomechanical hypothesis’ of brain damage in ageing. According to this hypothesis, the beating heart transmits force (shear stress and circumferential strain) along the vasculature and into the brain, damaging cerebral microvessels and ultimately accelerating cognitive decline. Indeed, the negative linear association between resting heart rate and cognitive decline may be indirect evidence of this. However, the increased haemodynamic forces associated with ‘wear and tear’ occur in a different environment to the increased haemodynamic forces during exercise, which dictate whether these forces impair or enhance brain health.

The vascular environment associated with an acute bout of prolonged sitting is one of impaired glucose and insulin dynamics, reduced shear stress, vascular resistance and increased blood pressure. These factors converge on the wall of the blood vessel inducing endothelial dysfunction, which may ultimately result in arterial stiffening and loss of buffering capacity of large arteries. While arterial stiffening can increase pulse wave velocity, it is typically associated with a net reduction in global cerebral blood flow, which, in turn, is associated with cognitive decline. Reduced brain blood flow may result from an increased pulse wave velocity and subsequent early pulse wave reflection, augmenting systolic pressure and reducing diastolic flow over time. A reduction in diastolic flow indicates that blood flow would occur mostly during systole and reflects an oscillatory pattern. Such an oscillatory pattern of blood flow and shear
stress promotes further endothelial dysfunction and endothelial cell senescence, generation of reactive oxygen species (ROS), and a pro-inflammatory environment; as opposed to laminar flow which improves redox balance and promotes an antioxidant environment.\textsuperscript{164,171–173} Conversely, during acute exercise, there are large increases in blood flow and shear stress, which along with increases in core body temperature, result in improved endothelial function and vasodilation.\textsuperscript{174–176} In response to repeated exposure to such stimuli, vascular remodelling occurs whereby the size of the lumen increases to normalise the shear stress stimulus.\textsuperscript{164,177} This, coupled with the exercised-induced secretion of angiogenic factors which increase cerebral capillary density and brain perfusion,\textsuperscript{81,178–182} could improve cerebral blood flow and ‘unload’ the capillaries by spreading pulse wave velocity across a denser network of cerebral microvessels. A denser network of capillaries in the presence of exercise, may also synergistically support adult neurogenesis via enhanced delivery of circulating growth factors such as BDNF, vascular endothelial growth factor and insulin like growth factor-1, which increase in response to exercise and are known to support the development of neural progenitor cells into functional neurons (Figure 12).\textsuperscript{115,179} Indeed, the ‘vascular niche’ hypothesis describes evidence that neurogenesis and angiogenesis are intimately linked, as neural and endothelial precursor cells proliferate together in tight clusters around capillaries, supported by circulating growth factors.\textsuperscript{94} Indeed, the increased BDNF observed in Chapter 2, and increased cerebral blood flow observed in Chapter 5, support the idea that exercise acts synergistically on mechanisms that support neurogenesis.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure12}
\caption{The relationship between angiogenesis and neurogenesis. Increases in blood flow and shear stress in response to exercise upregulate growth factors responsible for the growth and development of capillaries and neurons. Increased capillary density and brain perfusion, in turn, facilitates the delivery of growth factors to developing neurons. BDNF; brain derived neurotrophic factor. IGF-1; insulin like growth factor 1. VEGF; vascular endothelial growth factor. NO; nitric oxide.}
\end{figure}

The endothelium of cerebral microvessels also acts as a gatekeeper due to its strategic location at the blood brain barrier. Damaged endothelium within the microcirculation can allow toxic substances into the interstitial space which can directly damage neurons and
glial cells. For example, microhemorrhage as a result of increased pulse pressure/pulse wave velocity may introduce haemoglobin into the interstitial space which upregulates amyloid beta (Aβ), resulting in the accumulation of Aβ plaques at areas of microhemorrhages. In an experiment to test this idea, Purushothuman and colleagues made small needlestick legions into the brains of adult rats. The results demonstrated elevated depositions of haem, Aβ and phosphorylated tau along the needle track and in nearby neurons and astrocytes, which was similar to the features of human senile plaques also investigated in this study for comparison. Interestingly, it has been suggested that the binding of Aβ to haemoglobin protects against the immediate toxic effects of haemoglobin, but over the long term promotes the oligomersation of Aβ which develop into plaques that are eventually toxic to neurons. This suggests it is possible the pathology of Alzheimer’s disease, as described with an accumulation of senile plaques, could result from pulse-induced microhaemorrhage.

Additionally, increased pulse pressure/pulse wave velocity can promote endothelial production of reactive oxygen species (ROS), which may inhibit adult neurogenesis (although some ROS production is necessary for neurogenesis). Additional ROS production in the context of arterial stiffening may tip the balance in favour of neural apoptosis. Moreover, ROS can impair endothelial function and increase blood brain barrier permeability. This ultimately affects the capacity of the brain to match blood flow (and thus oxygen and nutrients) to metabolic demand (known as neurovascular coupling), resulting in energetic stress which can impair the function of astrocytes and neurons. Increased blood brain barrier permeability can also facilitate the infiltration of activated immune cells and inflammatory cytokines into the brain. Indeed, increased systemic markers of inflammation have been prospectively associated with structural abnormalities in the brain and more rapid cognitive decline. Once they have infiltrated the blood brain barrier, inflammatory cytokines can induce neuronal apoptosis and inhibit neurogenesis. Moreover, activated immune cells (in the process of producing inflammatory cytokines) consume high amounts of energy and may compete with the brain in this regard, resulting in further energetic stress. This highlights the important barrier function played by the endothelium within the cerebral microcirculation, in addition to its role in regulating redox balance, angiogenesis and neurogenesis; all important factors in brain function and health.
Taken together, these data highlight the integral role the vasculature plays in supporting brain function and health (Figure 13). This has direct relevance for the findings of the current study. Specifically, as outlined in Chapter 5, sustained reductions in middle cerebral artery velocity (MCAv) were observed during the prolonged sitting condition, but MCAv increased following morning exercise, with or without breaks in sitting. This exercise-induced increase in MCAv, if repeated on multiple occasions, would be expected to improve endothelial function, promote vascular remodelling and improve blood supply to the brain. This may be important in assisting with prevention of the age-associated decline in cerebral blood flow and attendant decline in cognition. Moreover, sustained decreases in cerebral blood flow may be detrimental to cerebral artery endothelial function, thereby increasing the susceptibility of the cerebral circulation to damage from high blood pressure or alterations in pulse wave velocity, ultimately impairing cognitive function. The endothelium may also be sensitive to the subtle differences observed in MCAv between EX+SIT and EX+BR, where a more stable pattern was observed in the latter condition. This may represent a benefit of intermittent walking on cerebral blood flow, as a more stable supply of blood to the brain is likely conducive to steady laminar flow and shear stress, which represent a beneficial stimulus for the endothelium. Future studies assessing the impact of exercise, with or without breaks in sitting, on cerebrovascular endothelial function would be highly informative in this regard.
Figure 13. A vascular hypothesis of physical (in)activity and brain health. This figure depicts the relationship between vascular and cognitive health in the context of sedentary behaviour or physical activity. An acute bout of prolonged sitting impacts both metabolic and vascular function. A repeated pattern of prolonged sitting, in the context of a vicious cycle, can lead to a cascade of adaptations that may ultimately accelerate cognitive decline. Conversely, exercise has acute benefits to metabolic and vascular function, that when repeated, cultivates an environment for optimal cognitive function. Aβ; amyloid beta. BBB; blood brain barrier. BDNF; brain derived neurotrophic growth factor. ET-1; endothelin-1. IGF-1; insulin like growth factor-1. NO; nitric oxide. VEGF; vascular endothelial growth factor.
6.5 Limitations and opportunities for future research

Every study has limitations. In the broader context, such limitations can constructively guide and inform future research. The following discussion points specifically address the limitations of this thesis and aspects of the findings that would be fruitful areas for future research.

**Acute study design and lack of condition investigating breaks in sitting alone**

There are pros and cons to the design of both acute and chronic exercise interventions. Exercise training interventions offer insight into adaptations that occur when a bout of exercise is repeated on multiple occasions over a period of weeks, months or years. However, they do not necessarily offer insight into the immediate effects of a single exercise bout, and results can be confounded by the other behaviours outside of the prescribed exercise *per se*. Conversely, acute exercise studies offer insight pertaining to immediate effects. The ideal scenario might be to draw on evidence from acute studies that offer insight on how a response may be optimised, then put this strategy to the test in a longer-term intervention. While future studies should consider the longer-term implications of exercise plus prolonged sitting versus breaks in sitting, more acute studies are still necessary. Specifically, studies in the acute setting which test the effects of breaks in sitting alone. We omitted such a condition from the current study as we considered the existing evidence on the acute benefits of 30-minutes of moderate intensity exercise to be strong. Our aim was not to test the physiological consequences of stepping down from this guideline-enshrined minimum prescription; rather we wanted to better understand whether the established benefits of this target could be further enhanced by the addition of short intermittent breaks in sitting.

**Findings are only generalizable to morning exercise**

The current study tested the effects of a morning bout of exercise combined with subsequent breaks in prolonged sitting. Without comparison to timings of exercise at other timepoints, this does not offer insight into the best time of day to undertake exercise. This is relevant since exercise can regulate the internal body clock, and the best time to exercise might be dictated by the presence of disruptions in circadian rhythm. Such disruptions are pervasive in modern 24-hour society (jetlag, social jet jag), and shift work is a common demand for many professionals; e.g. doctors, nurses, paramedics, commercial airline pilots, military personnel and information technology (IT) specialists. Moreover, circadian misalignment has been associated with increased risk of T2D,201,202
cardiovascular events, and dementia. Exercise at various times of the day may be one effective countermeasure for circadian misalignment. One study demonstrated that exercise had the largest effect on circadian rhythm at 7AM or between 1PM-4PM, when it advances circadian rhythm to an earlier time, and between 7PM-10PM, when it delays circadian rhythm to a later time. Phase advancing has the effect of inducing an earlier wake and sleep time and can help re-align circadian rhythm following eastward air travel or coming off a rotation of night shift, and vice versa for phase advancing. The current study investigated a morning bout of exercise, however, this may not be the optimal time to exercise following westward travel or if trying to adjust to a night shift schedule. Moreover, ageing is associated with circadian misalignment and some older adults can suffer from being phase advanced, and waking up too early. In this scenario, evening exercise might be preferable in order to induce phase delaying effects.

Other factors under circadian control may influence what the optimal time of day to exercise is. For instance, blood pressure is lowest during sleep (10-20% less than daytime mean, known as “the cardiovascular holiday”), but surges in the morning and displays small postprandial decreases. Indeed, the systolic blood pressure results in Chapter 4 followed a diurnal pattern with the highest values observed in the morning (~8AM), with postprandial dips following breakfast and lunch. Chapter 4 also described post-exercise hypotension following a 30-minute bout of exercise performed at ~9AM. However, if this exercise bout had been undertaken in the evening, the hypotensive effect may have been greater. Previous evidence demonstrates that post-exercise hypotension is diminished or even reversed when exercise is performed in the morning between 4AM-10AM, compared to exercise performed between 6PM-8PM, which results in the greatest decreases in blood pressure. In contrast to continuous exercise, intermittent exercise performed in the morning does not seem to result in diminished post-exercise hypotension. In light of this, future studies should specifically test whether breaks in sitting followed by an evening bout of exercise results in lower average blood pressure than morning exercise followed by breaks in sitting.

Investigate strategies in populations not considered high-risk
The current study investigated the effects of exercise plus breaks in sitting in a group at increased risk of cognitive decline (overweight/obese older adults). However, the findings do not offer insight on other groups who may have less immediate risk, such as a younger, leaner cohort. The optimal daily balance between continuous MVPA, intermittent light-
activity and sedentary behaviour may be different among different populations and in different contexts. For example, among older adults, sedentary behaviour involving cognitive stimulation such as computer use may have beneficial cognitive effects compared to television viewing.210 Future studies may improve the generalisability of results by aligning intervention goals to practical outcomes, for example, by investigating for how long a bout of exercise might improve cognition as assessed by driving performance. In addition, the effect of physical activity on cognition and academic performance in children is of widespread practical interest from both a public health and economic perspective. Indeed, physical activity in children aged 5-13 is associated with improved cognitive function, although effects on academic performance are mixed.211 More research is required to better model in a controlled laboratory setting, the patterns of physical activity that might be realistic for children in the school or home environment. This will likely involve the combined effects of multiple behaviours across the physical activity spectrum. Investigating whether such strategies benefit those at less risk is an important step in bridging the gap between improving individual health versus population health. Indeed, the evidence provided in this thesis may inform interventions targeted at high-risk individuals. However, a public health approach that only targets high-risk individuals has its disadvantages.212 For example, the disease processes leading to cardiovascular disease, T2D and Alzheimer’s disease can take decades to manifest.213–215 The pathophysiological processes of disease can continue unabated for years before an individual may be considered high-risk. Even among high-risk individuals, it is very difficult to predict who will develop disease.216–218 Therefore, it is important to test strategies aimed at primary prevention of disease in individuals at low initial risk. Moreover, such strategies should seek to incorporate novel measures that may improve the predictive value of disease progression beyond traditional risk factors.

More detailed clinically relevant measures: energy expenditure and substrate utilization

As energy expenditure or substrate utilization was not measured in the current study, it is unknown whether these factors explain some of the findings. However, there is reason to suspect they may have had some influence. For example, increased energy expenditure can reduce circulating triglycerides in order to replenish intra-muscular triglycerides. Glycogen depletion has been shown to be an important moderator of this effect, hence activity of longer duration (>1 hour) appears to be associated increased utilization of intra-muscular triglycerides.219 In a recent whole room calorimetry study of sedentary
overweight/obese adults, breaking up sitting increased reliance on carbohydrate as a fuel compared to a single energy matched continuous bout of postprandial exercise, which increased reliance on fat.\textsuperscript{220} It was suggested that the continuous bout of moderate-intensity exercise may have reduced glycogen stores more than the regular interruptions to sitting time. This is relevant because a reduction in hepatic glycogen can facilitate fat oxidation, especially in participants with obesity who are impaired in this regard.\textsuperscript{221} It is possible that the morning exercise bout undertaken in the current study ‘primed’ the subsequent breaks in sitting to increase fat oxidation by an initial reduction in hepatic glycogen, and the effect on triglycerides may not have been observed without this. Future studies to determine the combined effects of exercise and prolonged sitting versus breaks in sitting on energy expenditure and substrate utilization would be highly informative in light of the current results.

\textbf{More detailed clinically relevant measures: a nuanced assessment of lipoproteins}

As lipids are hydrophobic, in order to be transported in the blood they must be packaged into lipoprotein particles. Lipoproteins are potentially atherogenic, especially in the case of small lipoprotein remnants which can cross into the sub-endothelial space and become oxidized.\textsuperscript{222,223} Indeed, higher postprandial triglyceride levels have also been prospectively associated with increased risk for myocardial infarction, ischaemic heart disease and all-cause mortality.\textsuperscript{224} While triglyceride-lowering pharmacological trials to prevent cardiovascular events can be effective,\textsuperscript{225} others show mixed results.\textsuperscript{226} This may be partly explained by the fact that plasma measures of triglycerides indicate the total mass of triglycerides, rather than the total number of lipoprotein particles within which they are carried. In a recent analysis of data from 654,783 older men and women (mean age, 62.7 years) including 91,129 cases of coronary heart disease; a 0.56 mmol/L decrease in fasting triglycerides was associated with an 18\% decrease in coronary heart disease risk.\textsuperscript{227} However, this association was no longer evident after adjusting for differences in apolipoprotein B, a surface protein indicative of the number of lipoprotein particles. Indeed, individual lipoproteins can become trapped in the arterial wall and contribute to atherosclerosis.\textsuperscript{222,223} This suggests that reductions in triglyceride levels are associated with reductions in coronary heart disease risk proportional to their reduction in particle number, rather than total triglyceride concentration \textit{per se}. Future studies investigating the combined effects of exercise and prolonged sitting versus breaks in sitting which measure changes in the number of apolipoprotein B particles would be highly informative in this context.
More detailed clinically relevant measures: sex differences in blood flow & fatty acid uptake

The observed sex differences in the blood pressure response to exercise and breaks in sitting are fascinating and warrant further investigation. While males exhibited no additional reduction in blood pressure when exercise was followed by breaks in sitting, there may have been beneficial effects on more subtle measures of regional blood flow. For example, we have previously demonstrated that regular, physically active interruptions to prolonged sitting can increase average femoral artery blood flow over 5 hours by +46% [+0.3% to +92%], relative to prolonged sitting (Appendix C).228

Interestingly, when stratifying the results from this study by sex, only males demonstrated significant increases in average femoral artery blood flow when sitting was interrupted with light-activity breaks +55% [+11% to +99%, p=0.01]. Blood flow increases were non-significant in post-menopausal females +34% [-5% to +118%, p=0.43]. A strategy which can increase lower-limb blood flow may have implications for lower-limb uptake of fatty acids. This may be germane to males who are impaired in this regard relative to females. Indeed, males demonstrate decreased lower-limb adipose tissue blood flow, and an increased ratio of abdominal-to-lower limb fatty acid uptake in response to a meal, compared to premenopausal women.229 In addition, abdominal obesity is more prevalent in men than premenopausal women. It is interesting to speculate that this increased prevalence of central adiposity in males may be related to an increased ratio of abdominal-to-lower limb fatty acid uptake. If this is the case, it would be interesting to test whether intermittent physical activity, which can increase postprandial femoral artery blood flow, can also increase lower-limb adipose tissue blood flow and reduce the ratio of abdominal-to-lower limb fatty acid uptake in a sex specific manner. Indeed, intermittent light-activity breaks from sitting have been shown to regulate the expression of up to 36 genes within subcutaneous adipose tissue (to a fold change ≥0.5 in either direction).230 However, the systemic physiological drivers of this effect are unknown and functional assessment of adipose tissue blood flow would be highly informative in this context.

Incorporate multiple techniques in the assessment of cerebrovascular function

Given the central role the vasculature plays in mediating the effects of exercise on metabolic and cognitive health, future studies should take advantage of technology that can better characterise vascular function. Chapter 5 reported on the effects of exercise and breaks in sitting on MCAv, measured via transcranial Doppler ultrasound. While this technique has good temporal resolution, it is a surrogate measure of blood flow since it
cannot measure blood vessel diameter. This is less of an issue for within-subject experimental designs because blood flow changes are heavily dependent on velocity change. However, constant diameter of cerebral blood vessels during exercise cannot be assumed. Exercise has been shown to increase the diameter of extra-cranial arteries measured via Duplex ultrasound. Conversely, measurement of the intracranial middle cerebral artery diameter via high-resolution 7-tesla magnetic resonance imaging (MRI), reveals constriction during exercise. While techniques such as MRI, positron emission tomography (PET) and computerized tomography (CT) can measure global or regional cerebral blood flow, a disadvantage of these techniques is that they cannot measure the dynamic response to a physiological stressor. In addition, CT and PET require the injection of a contrast agent or radioactive tracer, respectively, and the participant is exposed to some ionizing radiation. Therefore, this technique may not be suitable for repeated assessments or in certain populations, i.e. pregnant mothers, small children or those who might be allergic to iodine. Conversely, techniques such as transcranial Doppler and near-infrared spectroscopy do not expose the participant to additional risk and can be applied to collect continuous measures. Nonetheless, they represent proxy measures of cerebral blood flow. In addition, the measurement of local cerebral blood volume and oxygenation using near-infrared spectroscopy may be contaminated by scalp blood flow. It has been suggested that one way to overcome the limitation of continuous measures that are proxies of cerebral blood flow is to concurrently measure global cerebral blood flow via Duplex ultrasound by combining flow in the extra-cranial internal carotid and vertebral arteries. Therefore, studies in the future would likely benefit from combining techniques that can complement each other in the assessment of cerebrovascular function.

**Explore whether distinct improvements in cognition have implications for exercise adherence**

The idea that improved cognition may increase the capacity to adhere to exercise is of great interest and potential relevance. Extending this concept, a question that arises is whether exercise plus breaks in sitting would be superior to exercise plus prolonged sitting, in terms of longer-term benefits? Future studies should specifically compare these interventions to test whether there are improvements in distinct aspects of cognition and whether different aspects of cognition at baseline predict adherence to physical activity strategies. In addition, such studies could also attempt to tease out what mechanisms may
be involved in supporting a virtuous circle between physical activity and cognitive function.

6.6 Conclusions
The findings presented in this thesis help clarify a number of points relevant to the public health agenda and may inform targeted interventions to improve clinical outcomes in future.

1) Prolonged sitting should be avoided in order to maintain optimal cardiometabolic and cognitive health. From this perspective, it may be beneficial to avoid or modify environments that encourage prolonged sitting, at home, at work or during (in)activity of daily living.

2) A bout of exercise performed in the morning can have a sustained 8-hour benefit to a broad spectrum of cardiometabolic and cognitive health markers, relative to prolonged sitting. Given that this timeframe spans an 8-hour working day, the results may inform how people structure their day.

3) For some cardiometabolic outcomes, achieving the daily-recommended level of exercise (30 minutes of moderate intensity) may not be sufficient to mitigate the impact of subsequent prolonged sitting (i.e. postprandial insulin, triglycerides and blood pressure). This finding may inform the design of future recommendations on physical activity and sedentary behaviour.

4) Relatively simple changes to the pattern of exercise and breaks in sitting throughout the day may be targeted to benefit specific outcomes (i.e. cognitive performance) or to benefit specific populations (i.e. blood pressure reduction in male versus females).

While public health policies are beginning to incorporate advice regarding reduction and breaking up sitting in addition to regular exercise, there has been a lack of experimental evidence testing the acute effects of breaking up sitting in addition to a bout of exercise. The findings of this thesis highlight the idea that no behaviour occurs in isolation and studying the combined effects of behaviours may lead to a more nuanced understanding of their health impacts. Specifically, the results of this thesis emphasise that studies performed in the acute setting are important testing grounds for understanding how to better optimise long term behaviour to improve clinical outcomes.

In conclusion, the results of this work highlight the importance of engaging in exercise and avoiding prolonged sitting in order to maintain optimal cognitive, metabolic,
cardiovascular and cognitive health. By seeking to understand the relationships between sedentary behavior, physical activity, cerebral blood flow, metabolism and cognition, this thesis has developed a more nuanced understanding of the combined implications of physical activity and sedentary behaviour for health. Studying the combined effects has also informed some practical questions about the physiological consequences of realistic patterns of behaviour for many older adults. The findings have addressed these questions which are fundamental to this thesis:

1) **What are the consequences of prolonged sitting over the course of 8-hours for cognitive, metabolic, cardiovascular and cerebrovascular function and health?**

It appears that, across many of the outcomes, prolonged sitting resulted in the least favourable results, including impaired cognition, reduced levels of BDNF, elevated postprandial insulin and triglycerides, elevated blood pressure and a sustained decline in brain blood flow.

2) **Is it sufficient to achieve the guideline-recommended daily amount of exercise – 30 minutes of moderate intensity – in order to warrant protection from the aforementioned physiological consequences of prolonged sitting?**

According to the findings of this thesis, the answer to this question depends on which outcome is considered. The results suggest that for outcomes directly related to brain health, including overall cognitive performance, BDNF and brain blood flow, a 30-minute bout of exercise in the morning was sufficient to mitigate the detrimental effects of prolonged sitting for the remainder of the 8-hour condition. However, for metabolic outcomes such as postprandial insulin, triglycerides and blood pressure, the results suggest that 30-minutes of exercise may not be enough to mitigate the detrimental effects of subsequent prolonged sitting, and this may have implications for brain health over the long-term.

3) **Can the physiological benefits of a 30-minute bout of moderate-intensity exercise be enhanced by subsequently interrupting a period of sitting with brief, regular light-activity breaks?**
There was evidence of additive effects of exercise plus breaks in sitting for postprandial insulin and triglycerides. In addition, females demonstrated additive effects of exercise plus breaks in sitting on lowering blood pressure. However, there was also the suggestion of additive effects when looking at the working memory results in isolation. In addition, combining exercise with breaks in sitting resulted in an earlier recovery of afternoon cerebral blood velocity, compared to exercise plus prolonged sitting which may represent a benefit of intermittent walking on brain blood flow.

Finally, by attempting to answer questions about the relationship between physical activity, sedentary behaviour and metabolic, cardiovascular, cerebrovascular and cognitive health; many more questions have arisen that will hopefully be fruitful areas for future investigation.


48. Sparling PB, Howard BJ, Dunstan DW, Owen N. Recommendations for physical activity in older adults. BMJ. 2015;350:h100-h100. doi:10.1136/bmj.h100


85. Li Q, Ford MC, Lavik EB, Madri JA. Modeling the neurovascular niche: VEGF-


Appendices

Appendix A: Methods paper for The Brain Breaks Study published in Mental Health and Physical Activity

Appendix B: Supplemental material from The Brain Breaks Study

Appendix C: Co-first authored paper on the effects of breaking up sitting on flow mediated dilation published in the *Journal of Applied Physiology*

Appendix D: Wider impact of the research in this thesis
Appendix A

Methods paper for The Brain Breaks Study published in

*Mental Health and Physical Activity.*

Interacting effects of exercise with breaks in sitting time on cognitive and metabolic function in older adults: Rationale and design of a randomised crossover trial

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ABSTRACT

Background: A single bout of moderate-to-vigorous intensity exercise improves both metabolic and cognitive function. In addition to exercise, emerging evidence suggests that reducing sitting time may be another strategy for improving metabolic function. However, the combined effects of acute exercise with reductions in sitting time on cognitive and metabolic function are largely unknown.

Methods/design: This is a dual-site randomised crossover trial involving three acute experimental conditions separated by a minimum six-day washout period. This trial included physically inactive and sedentary older adults (55–80 years) who were overweight to obese (body mass index 25–45 kg/m²). Participants were recruited to complete the following eight hour laboratory-based conditions in a random order; 1) Sitting: uninterrupted sitting (8hrs, control); 2) Exercise: sitting (1hr), moderate intensity walking (30mins) followed by uninterrupted sitting (6.5hrs); 3) Exercise + Breaks: sitting (1hr), moderate intensity walking (30mins) followed by sitting (6.5hrs) interrupted every 30 minutes with three minutes of light intensity walking. The primary outcome will be cognitive function, assessed using a battery of memory tests (Cogstate). Secondary outcome measures will include postprandial glucose, insulin, triglycerides, brain derived neurotrophic factor (BDNF), catecholamines, brain blood flow, brachial artery flow mediated dilation (FMD) and blood pressure.

Discussion: This evidence will inform practical preventive strategies aimed at optimising daily cognitive, vascular and metabolic function among the large number of older adults who are behaviourally exposed to prolonged uninterrupted sitting.

1. Introduction

As a consequence of population ageing, the total number of people living with dementia globally is estimated to increase from 35.6 million in 2010, to 115.4 million by 2050 (Prince et al., 2013). In the absence of effective pharmacotherapy, lifestyle modification remains the most effective strategy for reducing the risk of Alzheimer’s disease (AD) and other causes of dementia (Winblad et al., 2016). For example, regular moderate-to-vigorous physical activity (MVPA) reduces many of the metabolic risk factors for cognitive decline such as obesity, hypertension and altered glucose metabolism (Baumgart et al., 2015). Indeed, several prospective studies support the idea that regular MVPA is associated with a reduced risk of cognitive decline, AD and other dementias (Buchman et al., 2012; Rovio et al., 2005; Xu et al., 2017). However, the evidence is mixed and not all prospective studies agree (Sabia et al., 2017).

Intervention studies aimed at improving cognition by increasing physical activity levels over a period of weeks or months also show mixed results. Some studies demonstrate improvements in cognitive function (Zheng, Xia, Zhou, Tao, & Chen, 2016; van Uffelen, Chin A Paw, Hopman-Rock, & van Mechelen, 2008), but some more recent studies show no improvement in cognitive function following exercise intervention (Sink et al., 2015; Snowden et al., 2011; Young, Angevaren, Rusted, & Tabet, 2015). These null findings contrast with...
experimental evidence from laboratory studies demonstrating that an acute bout of exercise can improve cognitive function (with the largest effect size seen for executive function tasks) (Chang, Labban, Gapin, & Etnier, 2012). Acute exercise is also a potent stimulus for the induction of growth factors such as brain derived neurotrophic factor (BDNF) (Piepmeier & Etnier, 2015), neurogenesis and angiogenesis which are beneficial to brain health and cognition (Cotman, Berchtold, & Christie, 2007; Ratey & Loehr, 2011; Tarumi & Zhang, 2015). Taken together, it appears difficult to translate the cognitive benefits of acute exercise seen in laboratory settings into exercise interventions which seek to maintain improvements over a longer period of time.

Such discrepancies may be due to between-study differences in methodology, including varying degrees of effort to account for potential confounding factors. For example, an intervention which increases MVPA may result in a compensatory increase in sedentary behaviour which may be a confounding variable. Indeed, many exercise intervention studies focus on achieving the recommend level of MVPA; 150 minutes per week, accumulated in bouts of > 10 minutes (Garber et al., 2011). However, outside of this time spent exercising, prolonged sitting may have a negative impact on health. Observational evidence indicates that increased sedentary time is associated with a higher risk of multiple chronic diseases such as type 2 diabetes (T2D), cancer and cardiovascular disease, after adjusting for MVPA (Biswas et al., 2015). Conversely, breaking up sitting with intermittent activity improves markers of disease risk, most notably, postprandial carbohydrate and lipid metabolism (Benatti & Ried-Larsen, 2015; Grace et al., 2017), blood pressure (Bhammar, Sawyer, Tucker, & Gaesser, 2017; Dempsey, Sacre, et al., 2016) and vascular function (Restaino, Holwerda, Credeur, Fadel, & Padiila, 2015; Thosar, Bielko, Mather, Johnston, & Wallace, 2015). There is rationale to suspect that prolonged sitting may have a negative impact on cognitive function, due to its effects on risk factors for cognitive decline (Wheeler et al., 2017).

Indeed, some observational evidence indicates that higher levels of sitting time are associated with lower cognitive function (Falek, Davis, & Lio-Ambrose, 2016). In an experimental setting, it has been demonstrated that reducing and breaking up sitting with intermittent light intensity activity may improve cognitive function over a six-hour period (Mullane, Homan, Zeigler, Crespo, & Gaesser, 2016). However, not all studies support this idea (Wennerg et al., 2016). Differences between studies may be due to the total volume of accumulated activity.

While acute exercise and breaking up sitting may both exert a positive influence on cognition, the combined effects of both activities on cognitive function have not been studied in a controlled, experimental setting.

1.1. Aims and hypotheses

This study will aim to compare the acute effects of prolonged uninterrupted sitting to a single bout of moderate-intensity exercise, with or without subsequent light-intensity breaks in sitting time, on (i) cognitive function, (ii) metabolic function and (iii) cerebrovascular function in older, overweight adults during the course of an eight hour experimental condition.

Hypothesis 1: A continuous (30 minute) bout of moderate-intensity exercise will improve cognitive function compared with uninterrupted sitting.

Hypothesis 2: The magnitude of the improvement in cognitive function resulting from a moderate-intensity exercise bout will be greater when combined with a series of brief intermittent light-intensity breaks from prolonged sitting, relative to uninterrupted sitting.

2. Methods

2.1. Participants

Participants were recruited from the local community at two sites: 1) Physical Activity Laboratory, Baker Heart and Diabetes, Melbourne, Australia; and 2) Human Cardiovascular Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia (UWA), Perth, Australia. Recruitment was complete by the time of manuscript submission. This study has been approved by the Alfred Human Research Ethics Committee and Human Research Ethics Committee of The University of Western Australia. All participants provided written, informed consent prior to testing.

Inclusion criteria included: Age ≥ 55 to ≤ 80 years; body mass index ≥ 25 kg/m² to < 45 kg/m²; English-speaking. Exclusion criteria include: Pregnancy; self-reported sitting ≤ 5 hours per day; self-reported engagement in moderate-intensity exercise ≥ 150 min/week for ≥ three months; probable dementia (Telephone Interview of Cognitive Status score of ≥ 19); cognitive impairment (Mini Mental State Exam (MMSE) < 24 or Montreal Cognitive Assessment (MoCA) ≥ 26 when MMSE is between 24 and 28); depressive symptoms of clinical relevance (Geriatric Depression Score > 6 or Hospital Anxiety and Depression Scale score > 8); diagnosed diabetes; use of glucose/lipid lowering medication; antidepressant medications; beta blockers; anti-anxiety medication; peri-menopausal or menopausal women (must be post-menopausal); excessive alcohol consumption (> 8 points on the Alcohol Use Disorders Identification Test); abnormal ECG (determined by study doctor); high resting blood pressure (measured systolic > 160 mmHg or diastolic > 100 mmHg); major illness/physical problems (acute or chronic) that may limit ability to perform moderate intensity exercise.

2.2. Screening

Participants were initially telephone screened for eligibility during which time the following was administered: Telephone Interview of Cognitive Status (Welsh, Breitner, & Magruder-Habib, 1993); and the Geriatric Depression Scale assessment (Lyness et al., 1997). During the phone screening the study was verbally explained to the participant. After passing the telephone screening, participants were invited to attend a fasted blood test (after an overnight fast ≥ 10 h) at a local pathology centre to screen for diabetes and determine general health status. Following the blood test participants were booked in for a familiarisation visit.

2.3. Familiarisation

Participants were instructed to avoid caffeine, alcohol and MVPA for 48 hours prior to familiarisation. During familiarisation the following information was obtained: Written informed consent; completion of the MMSE (Folstein, Folstein, & McHugh, 1975), where participant’s score was between 24 and 28 on the MMSE the MoCA was also be administered (Nasreddine et al., 2005); Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983); Physical Activity Readiness Questionnaire + (Bredin, Gledhill, Jammik, & Warburton, 2013); Dutch Eating Behaviour Questionnaire (van Strien, Frijters, Bergers, & Defares, 1986); resting blood pressure; resting 12-lead ECG and anthropomorphic measurements. Participants were familiarised with cognitive testing on a commercially available computerized test battery (Cogstate Ltd, Melbourne, Australia). Following familiarisation of cognitive testing, participants were given the opportunity to walk on a motorised treadmill to allow for the determination of the incline that equates to a relative moderate-intensity (65–75% of age predicted maximal heart rate). To determine this incline, participants walked for a total of 30 min at a speed of 3.2 km/h⁻¹. Ratings of perceived exertion and heart rate (Polar Electro, Kempele, Finland) were tracked every three minutes and the incline was adjusted by between ± 0.5–2% until the participant reached the required heart rate zone. The final incline was then applied during the experimental conditions with a speed of 3.2 km/h⁻¹.
2.4. Randomisation and blinding

The order of experimental conditions was randomised by a third party using a computer generated sequence of numbers and sealed envelopes. Block randomisation was performed with balanced blocks, stratified by sex. Study personnel were blinded to the order of conditions until the night before the first experimental visit. Participants were blinded to the order of conditions until the morning of the second experimental visit. Blood sample analysis and data analysis will be performed by technicians blinded to the study conditions.

2.5. Standardisation of dietary intake and physical activity

Participants were instructed to avoid caffeine, alcohol and moderate to vigorous intensity physical activity for 48 hours prior to each experimental condition. To assess physical activity levels, participants were fitted with two objective physical activity monitors – an accelerometer (Actigraph model GT3X+) worn on the hip and an inclinometer (actiPAL3) worn on the thigh, in combination with an activity diary. Activity monitors were worn during the time between visits, starting from the familiarisation session. Fully charged devices replaced battery depleted devices during each experimental visit. Participants were instructed to keep a weighed food diary for the visits, starting from the familiarisation session. Food records will be assessed using Australian-specific dietary analysis software (Foodworks: Xyris Software, Highgate Hill, AUS). Food intake was controlled from the evening before each experimental condition with a meal pack provided for consumption at home between 7pm and 9pm in place of any other food, with no food allowed after that time. The evening meal packs were individualised using the Schofield equation (Schofield, 1985) to meet 33% of estimated daily energy requirements and using dietary analysis software, were designed to have an energy macronutrient profile of 55–59% carbohydrate, 29–31% fat and 12–15% protein, as has been used previously in similar research (Dempsey, Larsen, et al., 2016). During the experimental conditions, breakfast and lunch meals were provided which were individualised in the same manner as the evening meal, and participants consumed the same breakfast and lunch during each experimental condition.

2.6. Experimental day protocol

Participants reported to the laboratory at ~7am following an overnight fast (> 10 h) (Fig. 1). Blood pressure and brachial artery flow mediated dilation (FMD) were measured prior to a “steady-state” sitting period. Baseline measures of cognitive function and cerebrovascular function at the UWA site only were taken during the steady state sitting period. A fasting blood sample was obtained prior to the breakfast meal. Following steady state sitting, conditions become unique as per their experimental protocol and blood draws, blood pressure, subjective appetite, cognitive, and cerebrovascular function tests (at the UWA site only) occurred at multiple time points across the day. At the completion of the experimental condition brachial artery FMD was measured again.

3. Outcomes

3.1. Cognitive function

Cognitive function is the primary outcome, measured using a computerized test battery (Cogstate - www.cogstate.com.au) that has been specifically developed for brief repeated testing of cognitive performance in clinical and research trials, with good acceptability, efficiency and stability, and minimisation of practice effects (Collie, Maruff, Darby, & McStephen, 2003; Falleti, Maruff, Collie, & Darby, 2006; Fredrickson et al., 2010). Previous work has demonstrated that executive function tasks are the most sensitive to improvement following acute exercise (Chang et al., 2012). The test battery covers multiple cognitive domains using the following tasks: (1) The Groton Maze Learning Test to measure executive function; (2) Detection Test to measure psychomotor function and speed of processing; (3) Identification Test to measure attention and (4) One Card Learning Test to measure visual learning and (5) One Back Test and Two Back Test to measure working memory. The total administration time is approximately 25 minutes.

3.2. Blood sampling

Venous blood samples were collected using an indwelling cannula inserted in an antecubital vein. Coded samples were sent to an independent National Association of Testing Authorities/The Royal College of Pathologists of Australasia-accredited laboratory on the day of testing for the determination of glucose, triglycerides and full blood evaluation. Blood was collected into fluoride/oxalate tubes for analysis of plasma glucose using the hexokinase method. Blood was collected into lithium heparin tubes for analysis of triglycerides using a COBAS Integra 400 + analyser (Roche Diagnostics, Indianapolis, IN). Plasma and serum samples were also collected at each time point for storage at –80 °C and later analysis. Plasma samples were collected and centrifuged immediately prior to –80 °C storage and serum samples were allowed to clot for 1 h at room temperature (22–24 °C) prior to centrifuging. Plasma and serum samples were spun at 2000 rpm for 15 min at 4 °C at room temperature (22–24 °C) prior to subsequent storage at –80 °C.

3.3. Brachial artery function

This test was performed at the beginning and end of each eight hour condition, according to accepted guidelines (Thijssen et al., 2011). Participants remained supine for 20 minutes in a dimly lit, temperature controlled room (22–24 °C) prior to initial recording. Participants were measured again at the conclusion of the eight hour testing condition. Brachial artery FMD was measured with a high-resolution ultrasound machine (Terason 1320™, Teratech, Burlington, MA, USA) in conjunction with a 10 MHz multifrequency linear array probe and insonation angle of 60°. A rapid inflatable cuff (SCI12D™, D.E. Hokanson Inc., Bellevue, Washington) was placed around the forearm, distal to the cubital fossa. Once an optimal image of the artery was obtained, a one minute recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated for five minutes (220 mmHg). After five minutes of inflation, the cuff was released to induce reactive hyperemia. A further three minutes of continuous duplex ultrasound recording was undertaken to observe the post deflation diameter profile and peak response.

3.4. Cerebrovascular function

Cerebral blood flow was measured with transcranial Doppler only in participants recruited to the UWA site. Bilateral measures of middle cerebral artery flow velocities were determined with a 2 MHz probe transfixed to the posterior aspect of the temporal window of the skull using the Mark 600 headframe (Spencer technologies, Seattle, USA). The location of the middle cerebral artery within the brain was achieved by locating the trifurcation of the circle of Willis (~45–65 mm) in the anterior circulation of the brain, as previously outlined (Willie et al., 2011).

3.5. Blood pressure

Resting blood pressure and heart rate were measured in a seated position on the contralateral arm to the cannula; taken as the average of three serial measurements using an automated oscillatory method (HEM-907, Omron, Kyoto, Japan). During the exercise plus break condition, blood pressure and heart rate were measured prior to the
Subjective appetite was assessed as there is a body of literature suggesting that walking can acutely reduce sugary food cravings (Ledochowski, Ruedl, Taylor, & Kopp, 2015; Oh & Taylor, 2013) and ad libitum snacking in a simulated workplace environment (Oh & Taylor, 2012). This evidence represents an indirect mechanism by which walking may ultimately affect glucose levels. Appetite variables were assessed using visual analogue scales, where a mark is placed on a 100 mm line between two opposite states (i.e. "not hungry at all" to "as hungry as I have ever felt"). Subjective states assessed include, hunger, fullness, desire to eat, prospective food consumption and food preference, as previously described (Flint, Raben, Blundell, & Astrup, 2000). Non-appetite sensations of calmness and drowsiness were also assessed by a visual analogue scale (VAS) as they have previously been shown to correlate with food intake (Parker et al., 2004). Subjective fatigue was assessed using an 18 item VAS, previously validated against the Stanford Sleepiness Scale and the Profile of Mood States (Lee, Hicks, & Nino-Murcia, 1991), and demonstrated to be sensitive to change from intermittent light intensity walking (Wennberg et al., 2016).

4. Statistical methods

4.1. Sample size calculation

Power calculations have been made in relation to the primary outcome measure of cognitive function and reflect the cross-over design of the study. Based on recent evidence (Mullane et al., 2016), we estimate the effect size (Cohen's d for repeated measures; ES) of additional exposure to light-intensity breaks (as compared to sole exposure to a moderate-intensity exercise bout) would be ~0.40 for executive function tasks. From a clinical perspective, this moderate effect size is similar in magnitude to the difference in cognitive decline over 36 months between older adults with high brain levels of amyloid beta versus low levels of amyloid beta (Lim et al., 2015). Sample size calculations indicate that 52 participants would be needed to detect an ES of 0.40 based on one baseline and one post assessment per experimental condition. However, this trial will include three post assessments per experimental condition, which gives 3 x participant data points per condition. Assuming a within-participant, within-condition correlation between assessments of 0.6, the effective sample size per condition is [3 x participants]/[1+(3−1)*0.6] = [3 x participants]/2.2. To achieve an effective sample size of 52, we need 52*(2.2/3) ~ 40 participants. Thus, the sample size needed to detect an ES of 0.40 with a power of 0.80 and a two-tailed probability of 0.05 is 40 (with three post-assessments per person and experimental condition). In a recent trial adopting a similar design, we achieved a 15% attrition rate. However, as a safeguard, assuming a conservative attrition rate of ~20%, the required sample size is 48 participants.

4.2. Statistical analyses

Following recent recommendations on data analysis of cross-over trials (Kenward & Roger, 2010), generalized linear mixed models with random intercepts will be used to evaluate the differential effects of the experimental conditions on the selected outcomes. All models will include a binary variable indicating the experimental condition (intervention vs. control), adjusted for relevant confounders. Mixed models are appropriate for correlated data (repeated measures) with various distributional assumptions and can easily accommodate missing data (Rabe-Hesketh, Yang, & Pickles, 2001). A probability level of 0.05 will be adopted. Statistical analyses will be performed using Stata 15 for...
5. Discussion

Maintaining optimal cognitive, vascular and metabolic function are essential for health and quality of life, especially for older adults. Regular exercise is currently the most effective known strategy to prevent cognitive decline, but whether such benefits could be enhanced by avoiding prolonged periods of uninterrupted sitting during the ‘non-exercise’ periods of the day is currently unknown. This controlled acute experimental trial will provide evidence in the context of an eight hour experimental model, in a population at heightened risk of cognitive decline – overweight older adults. The study will contribute new knowledge pertaining to the effects of a morning bout of exercise (at an intensity and duration consistent with current public-health guidelines) on cognitive function across the day, and further, will examine the additive effects of subsequent breaks from prolonged sitting. With the prevalence of dementia and associated metabolic and vascular pathologies on the rise across ageing populations, there is the need to identify practical preventive strategies that can optimise daily cognitive, vascular and metabolic function among the large number of older adults who are at risk.

Funding

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The format of this protocol paper has been guided by the Standard Protocol Items: Recommendations for Interventional Trials 2013 guidelines. The authors declare no conflicts of interest.

Trial registration

ACTRN12614000736939

References

D.W. Dunstan et al.

10.1016/j.socscimed.2012.11.007.


Appendix B

Supplemental material from The Brain Breaks Study

Provides supplementary material on case report form, participant informed consent, information and recruitment flyers and ethical approval.
## PRE TRIAL CHECKLIST

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is the room booked?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Participant booked into VIP?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Ingredients for breakfast and lunch bought &amp; prepared?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Envelope prepared with zip lock bags - for posting back of handbook, activity monitors, sleep questionnaire?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Correct CRF and path form prepared/printed?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>*If final visit, has an ATO “Statement by a supplier” form been prepared/printed?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>If final visit has a requisition for payment (RFP) form been prepared/printed?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Has 3 VAS-F been printed? Sleep Questionnaire &amp; VAMS?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Have labels for the tubes &amp; aliquots been prepared/printed?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Do we have enough Flur Ox, Serum, GSH/EGTA, and EDTA tubes?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Consumables for blood taking ready? Cups for ice?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Plenty of ICE with SPARE in Freezer?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>Scales, BP cuffs? HR time correct?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>17</td>
<td>What BP cuff size, arm and HR strap does PP use??</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>18</td>
<td>Accelerometer &amp; Inclinometer initialised and ready - numbers recorded below?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>21</td>
<td>Participant comfort - blankets, water cup, eating bench?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>Ultrasound equipment set up? On?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>23</td>
<td>Participant Diary Filled in with ID and details of meal pack and next visit</td>
<td>Yes</td>
<td>No</td>
</tr>
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</table>
## 1. PRELIMINARY CHECKLIST

<table>
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<tr>
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<th>No</th>
<th>TIME</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Has the participant fasted this morning?</td>
<td>Yes</td>
<td>No</td>
<td></td>
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<tr>
<td>1.2</td>
<td>*Did the participant complete a 2 day food record?</td>
<td>Yes</td>
<td>No</td>
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<td></td>
<td>Notes:</td>
<td></td>
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<td>TIME</td>
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<tr>
<td>1.3</td>
<td>Did the participant consume last night’s meal pack?</td>
<td>Yes</td>
<td>No</td>
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<td>1.4</td>
<td>Has the participant avoided caffeine for 48hrs?</td>
<td>Yes</td>
<td>No</td>
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<td>1.5</td>
<td>Has the participant avoided alcohol for 48hrs?</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>1.6</td>
<td>Has the participant avoided MVPA for 48hrs?</td>
<td>Yes</td>
<td>No</td>
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<td>1.7</td>
<td>Participant wearing <em>accelerometer</em>? (if needed)</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>1.8</td>
<td>Participant wearing <em>inclinometer</em>? (if needed)</td>
<td>Yes</td>
<td>No</td>
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</table>

**NOTE:** Participant water consumption log = bottom of page 23
Experimental Condition: Sitting □ Exercise □ Ex + Breaks □

Control LOG:

This log is to be used to note any environmental / timing issues that need to be kept consistent across conditions □ Check if NONE

<table>
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<tr>
<th>Item No.</th>
<th>Comment</th>
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</tbody>
</table>
2. BASELINE MEASURES & PREPARATION: -1:00 → 0:00HR

MEASUREMENTS A

2.1. Participant completed Sleep Questionnaire? Yes  No

2.2. Weight (1): . kg

PREPARATION FOR EXPERIMENTAL CONDITION

2.3. Heart rate monitor fitted? Time: : HR Timer Start: :

2.4. Accelerometer fitted (if need)? Time: : Accel No:

2.5. Inclinometer fitted (if need)? Time: : Inclin No:

2.6. IV. catheter inserted? Time: : Arm used: L R

2.7 Blinds Closed Yes  No

3.2 Height of adjustable table set Yes  No cm

MEASUREMENTS B (0)

2.7. Seated blood pressure (1 min between): Arm used: L R Cuff size: S L  

(a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

(b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

(c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

AVERAGE (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

HR rest

2.8 PRE Flow Mediated Dilation Complete (1) Yes  No
3. STEADY STATE PHASE: 0:00 → 1:00HR

CONDITION BEGINS – START ALL TIMERS timer start (real time) : am

Seated blood pressure (00:00HR) (1 min between): Arm used: L R Cuff size: S L

(a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

AVERAGE (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

3.1 VAS-F Questionnaire Complete Yes No

OBSERVATIONS (0:00HR)
3.2 Participant comfortable and understands must remain seated for the next hour (participant can read, knit answer emails but NO television, DVDs etc.? Yes No (mention standard toilet break at midday)

3.3 Participant instructed to call if necessary (e.g. toilet visit – check if free 1st)? Yes No

3.4 Prepare breakfast meal

3. Cognitive Test Battery 1 (0:05 HR)

Cognitive Test [1] (0:05HR)

3.4 Head set fitted and adjusted: Yes No

3.3. VAMS complete: Yes No

Actual Time of Cognitive Test 1 (start): :

Actual Time of Cognitive Test 1(end): : Length of Test (GML) (minutes)

Actual Time of Cognitive Test 2(start): :
Brain Breaks Study – HREC 181/14

Experimental Condition: Sitting  Exercise  Ex + Breaks

Actual Time of Cognitive Test 2(end): : Length of Test (Card Over)(minutes)
Actual Time of Cognitive Test 3(start): :
Actual Time of Cognitive Test 3(end): : Length of Test (Red)(minutes)
Actual Time of Cognitive Test 4(start): :
Actual Time of Cognitive Test 4(end): : Length of Test (Seen before)(minutes)
Actual Time of Cognitive Test 5 (start): :
Actual Time of Cognitive Test 5(end): : Length of Test (N-back)(minutes)

3.4 Cognitive Test Complete (1) Yes  No

3.5 Total Length of Test Battery(minutes)

1st BLOOD COLLECTION (0:35HR)

3.4. Actual time of blood collection: :

1st  5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)
2nd  6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)
3rd  4.5 mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for lipid profile)
4th  3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)
5th  3mL (EDTA lilac 3mL tube) (deliver to pathology for full blood evaluation)
6th  2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

Satiety VAS (0:39HR)

Satiety VAS (1) Complete Yes  No

5. BREAKFAST (3min) 0:40 HR

BREAKFAST MEAL (0:40 HR)

3.6 Give Breakfast Meal

Actual time breakfast meal started : , and finished : [Goal = 20 mins]

3.5. Dominant activity for the past hour:________________________________________________________
4. **EXERCISE: 1:00HR**

Actual time exercise started: 

Target Heart Rate (bpm): 

Working Speed km/h: 

Working Incline %: 

4.2. **Average Heart Rate(1)**

<table>
<thead>
<tr>
<th>Time</th>
<th>HR</th>
<th></th>
<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 5 mins</th>
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<tr>
<td>4 mins</td>
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<td>4:30 mins</td>
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<tr>
<td>5 mins</td>
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4.3. **Average Heart Rate(2)**

<table>
<thead>
<tr>
<th>Time</th>
<th>HR</th>
<th></th>
<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 10 mins</th>
</tr>
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<tr>
<td>9 mins</td>
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<tr>
<td>9.30 mins</td>
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<tr>
<td>10 mins</td>
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4.3. **Average Heart Rate(3)**

<table>
<thead>
<tr>
<th>Time</th>
<th>HR</th>
<th></th>
<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 15 mins</th>
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<td>14 mins</td>
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<td>14.30 mins</td>
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<td>15 mins</td>
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4.3. **Average Heart Rate(4)**

<table>
<thead>
<tr>
<th>Time</th>
<th>HR</th>
<th></th>
<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 20 mins</th>
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<tr>
<td>19 mins</td>
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<td>19.30 mins</td>
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<td>20 mins</td>
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</table>

4.3. **Average Heart Rate(5)**

<table>
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<tr>
<th>Time</th>
<th>HR</th>
<th></th>
<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 25 mins</th>
</tr>
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<td>24 mins</td>
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<td>24.30</td>
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</tr>
<tr>
<td>25 mins</td>
<td></td>
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4.3. **Average Heart Rate(6)**

<table>
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<tr>
<th>Time</th>
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<th></th>
<th>AVERAGE HR</th>
<th>RPE @ 30 mins</th>
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<tr>
<td>29 mins</td>
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<td>29.30 mins</td>
<td></td>
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<td></td>
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<tr>
<td>30 mins</td>
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</table>

**2nd BLOOD COLLECTION (1:30HR)**

5.1. Actual time of blood collection: 

1st: 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)

2nd: 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)

3rd: 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

4th: 2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)
**Experimental Condition:** Sitting [ ] Exercise [ ] Ex + Breaks [ ]

**BLOOD Pressure (1:35HR)**

Actual time of blood pressure collection: :

5.2 Seated blood pressure (1 min between): Arm used: L R

(a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

(b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

(c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

**AVERAGE** (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

**3rd BLOOD COLLECTION (1:55HR)**

3.4. Actual time of blood collection: :

1st 4mL (red serum 6mL tube) (rest 30 minutes, spin, aliquot, freeze)

2nd 2.5 mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for triglycerides)

3rd 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

4th 2mL (fluoride oxalate grey 2mL tube)(deliver to pathology for glucose)

(1:58) VAS-F Questionnaire Complete Yes No

**5. Walking Break 1 (3min) 2:00 HR**

Actual Time of Break (start): :

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed? Yes No</td>
</tr>
<tr>
<td>Dominant activity past hour (reading ect):</td>
<td>b) RPE final (6-20)?</td>
</tr>
<tr>
<td></td>
<td>c) HR final</td>
</tr>
</tbody>
</table>

**BLOOD Pressure (2:20HR)**

Actual time of blood pressure collection: :

5.2 Seated blood pressure (1 min between): Arm used: L R
Experimental Condition: Sitting [ ] Exercise [ ] Ex + Breaks [ ]

(a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

AVERAGE (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

4th BLOOD COLLECTION (2:25HR)

3.4. Actual time of blood collection: :

1st 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)
2nd 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)
3rd 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)
4th 2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

5. Walking Break 2 (3min) 2:30 HR

Actual Time of Break (start): :

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed? Yes No</td>
</tr>
<tr>
<td></td>
<td>b) RPE final (6-20)? c) HR final</td>
</tr>
</tbody>
</table>

5. Cognitive Test 2 (2:35 HR)

Cognitive Test [2] (2:35HR)

3.3. VAMS complete: Yes No

Actual Time of Cognitive Test 1(start): :
Actual Time of Cognitive Test 1(end): Length of Test 1 (minutes)
Actual Time of Cognitive Test 2(start): :
Brain Breaks Study – HREC 181/14

Experimental Condition:  Sitting  Exercise  Ex + Breaks

Actual Time of Cognitive Test 2 (end):  :  Length of Test 2 (minutes)
Actual Time of Cognitive Test 3 (start):  :
Actual Time of Cognitive Test 3 (end):  :  Length of Test 3 (minutes)
Actual Time of Cognitive Test 4 (start):  :
Actual Time of Cognitive Test 4 (end):  :  Length of Test 4 (minutes)
Actual Time of Cognitive Test 5 (start):  :
Actual Time of Cognitive Test 5 (end):  :  Length of Test 5 (minutes)

Cognitive Test Complete  Yes  No

5.2. **EXERCISE BREAK 3** (3:00HR)

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<tr>
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<th>Active Condition (with 3 min breaks)</th>
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<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed? Yes No</td>
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<tr>
<td>Dominant activity past hour (reading ect):</td>
<td>b) RPE final (6-20)?</td>
</tr>
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<td></td>
<td>c) HR final</td>
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</table>

5. **Walking Break 3 (3min) 3:00 HR**

Actual Time of Break (start):  :

5.1. **5th BLOOD COLLECTION** (2:58HR)

Actual time of blood collection:  :

1st  **4mL** (red serum 6mL tube) (rest 30 minutes, spin, aliquot, freeze)
2nd  **2.5 mL** (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for triglycerides)
3rd  **3mL** (EDTA lilac 3mL tube) (spin, aliquot, freeze)
4th  **2mL** (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)
### Blood Pressure (3:24 HR)

**5.3. Seated blood pressure (1 min between): Arm used:** L R

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<tr>
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<th>L</th>
<th>R</th>
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<tbody>
<tr>
<td>a) Systolic (mmHg):</td>
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<td>Diastolic (mmHg):</td>
<td></td>
<td></td>
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<tr>
<td>HR(60sec):</td>
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<td>b) Systolic (mmHg):</td>
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<td>Diastolic (mmHg):</td>
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<tr>
<td>HR(60sec):</td>
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<tr>
<td>c) Systolic (mmHg):</td>
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<tr>
<td>Diastolic (mmHg):</td>
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<tr>
<td>HR(60sec):</td>
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**AVERAGE**

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<td>Diastolic (mmHg):</td>
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<tr>
<td>HR(60sec):</td>
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### 6th BLOOD COLLECTION (3:27HR)

Actual time of blood collection:

1st: 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)

2nd: 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

4th: 2mL (fluoride oxalate grey 2mL tube)(deliver to pathology for glucose)

### 5. Walking Break 4 (3min) 3:30 HR

Actual Time of Break (start):

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
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<tr>
<td>No. of accumulated breaks so far:</td>
<td>a) Break completed? Yes No</td>
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<tr>
<td></td>
<td>b) RPE final (6-20)?</td>
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### TURN ON OVEN

### Toilet Break (3:40 HR)

Actual Time of Break (start): | Actual Time of Break (end): |  |
VA-S F Questionaire 3:55 HR

VAS-F Questionaire Complete Yes No

7th BLOOD COLLECTION (3:57HR)

Actual time of blood collection: : 
1st 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)
2nd 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)
3rd 2.5mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for triglycerides)
4th 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)
5th 2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

5. Walking Break 5 (3min) 4:00 HR

Actual Time of Break (start): :

Extra walking breaks (toilet ect)
No. of accumulated walking breaks so far:
Dominant activity past hour (reading ect):

Active Condition (with 3 min breaks)
a) Break completed? Yes No
b) RPE final (6-20)?
c) HR final

5. Cognitive Test 3 (4:04 HR)

Cognitive Test (3) (4:04HR)

3.3. VAMS complete: Yes No

Actual Time of Cognitive Test 1 (start): :
Experimental Condition: Sitting Exercise Ex + Breaks

Actual Time of Cognitive Test 1(end): : Length of Test 1 (minutes)
Actual Time of Cognitive Test 2(start): :
Actual Time of Cognitive Test 2(end): : Length of Test 2 (minutes)
Actual Time of Cognitive Test 3(start): :
Actual Time of Cognitive Test 3(end): : Length of Test 3 (minutes)
Actual Time of Cognitive Test 4(start): :
Actual Time of Cognitive Test 4(end): : Length of Test 4 (minutes)
Actual Time of Cognitive Test 5 (start): :
Actual Time of Cognitive Test 5(end): : Length of Test 5 (minutes)

Cognitive Test Complete [3] Yes No

Blood Pressure (4:25HR)
6.3. Seated blood pressure (1 min between): Arm used: L R
   (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
   (b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
   (c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
AVERAGE (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

5. Walking Break 6 (3min) 4:30 HR

Actual Time of Break (start): :

Extra walking breaks (toilet etc)
No. of accumulated walking breaks so far:

Active Condition (with 3 min breaks)
   a) Break completed? Yes No
   b) RPE final (6-20)?
   c) HR final
**Satiety Measure** (4:35HR)

Actual time of VAS measures: 

Satiety VAS measures complete: YES NO

**8th BLOOD COLLECTION** (4:37HR)

5.5. Actual time of blood collection: 

1st 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)

2nd 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)

3rd 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

4th 2mL (fluoride oxalate grey 2mL tube)(deliver to pathology for glucose)

---

**5. LUNCH 6 (3min) 4:40 HR**

**Lunch MEAL** (4:40 HR)

3.6 Give Lunch Meal

Actual time lunch meal started , and finished [Goal = 20 mins]

---

**5. Walking Break 7 (3min) 5:00 HR**

Actual Time of Break (start): 

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
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</thead>
<tbody>
<tr>
<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed? Yes No</td>
</tr>
<tr>
<td>Dominant activity past hour (reading ect):</td>
<td>b) RPE final (6-20)?</td>
</tr>
<tr>
<td></td>
<td>c) HR final</td>
</tr>
</tbody>
</table>
Brain Breaks Study – HREC 181/14

Experimental Condition:  Sitting  Exercise  Ex + Breaks

Blood Pressure (5:23HR)

6.3. Seated blood pressure (1 min between): Arm used:  L  R

   (a) Systolic (mmHg):  Diastolic (mmHg):  HR(60sec):
   (b) Systolic (mmHg):  Diastolic (mmHg):  HR(60sec):
   (c) Systolic (mmHg):  Diastolic (mmHg):  HR(60sec):

AVERAGE  (a) Systolic (mmHg):  Diastolic (mmHg):  HR(60sec):  HR rest

9th BLOOD COLLECTION (5:27HR)

5.5. Actual time of blood collection:  

   1st  5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)
   2nd  6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)
   3rd  2.5 mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for triglycerides)
   4th  3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)
   5th  2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

5. Walking Break 8 (3min) 5:30 HR

   Actual Time of Break (start):  

   Extra walking breaks (toilet ect)  Active Condition (with 3 min breaks)

   No. of accumulated walking breaks so far: a) Break completed?  Yes  No
   b) RPE final (6-20)?  c) HR final

Satiety Measure (5:35HR)

   Actual time of VAS measures:  

   VAS measures complete:  YES  NO
Experimental Condition: Sitting [ ] Exercise [ ] Ex + Breaks [ ]

(5:55) **VAS-F Questionnaire Complete**

Yes No

---

**10th BLOOD COLLECTION (5:58HR)**

5.5. Actual time of blood collection: :

1st 4mL (red serum 6mL tube) (rest 30 minutes, spin, aliquot, freeze)

2nd 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

3rd 2mL (fluoride oxalate grey 2mL tube)(deliver to pathology for glucose)

---

**5. Walking Break 9 (3min) 6:00 HR**

Actual Time of Break (start): :

---

<table>
<thead>
<tr>
<th>Sedentary Condition</th>
<th>Active Condition (with 3 min breaks)</th>
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</thead>
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<tr>
<td>No. of walking breaks:</td>
<td>a) Break completed? Yes No</td>
</tr>
<tr>
<td>Dominant activity past hour (reading ect):</td>
<td>b) RPE final (6-20)?</td>
</tr>
<tr>
<td></td>
<td>c) HR final</td>
</tr>
</tbody>
</table>

---

**Satiety Measure (6:05HR)**

Actual time of VAS measures: :

VAS measures complete: YES NO

---

**Blood Pressure (6:23HR)**

6.3. Seated blood pressure (1 min between): Arm used: L R

<table>
<thead>
<tr>
<th>(a) Systolic (mmHg):</th>
<th>Diastolic (mmHg):</th>
<th>HR(60sec):</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) Systolic (mmHg):</td>
<td>Diastolic (mmHg):</td>
<td>HR(60sec):</td>
</tr>
<tr>
<td>(c) Systolic (mmHg):</td>
<td>Diastolic (mmHg):</td>
<td>HR(60sec):</td>
</tr>
</tbody>
</table>

**AVERAGE** (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
**Experimental Condition:** Sitting  [ ] Exercise [ ] Ex + Breaks [ ]

**11th BLOOD COLLECTION (6:28HR)**

5.5. Actual time of blood collection:

1st 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)

2nd 4mL (red serum 6mL tube) (rest 30 minutes, spin, aliquot, freeze)

3rd 2.5 mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for triglycerides)

4th 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

5th 2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

---

**5. Walking Break 10 (3min) 6:30 HR**

Actual Time of Break (start):

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
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</thead>
<tbody>
<tr>
<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed?     Yes No</td>
</tr>
<tr>
<td></td>
<td>b) RPE final (6-20)?      c) HR final</td>
</tr>
</tbody>
</table>

---

**Satiety Measure (6:35HR)**

Actual time of VAS measures:

VAS measures complete: YES NO

---

**12th BLOOD COLLECTION (6:55HR)**

5.5. Actual time of blood collection:

1st 5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)

2nd 6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)

3rd 3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)
5. Walking Break 11 (3min) 7:00 HR

Actual Time of Break (start): 

<table>
<thead>
<tr>
<th>Extra walking breaks (toilet ect)</th>
<th>Active Condition (with 3 min breaks)</th>
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</thead>
<tbody>
<tr>
<td>No. of accumulated walking breaks so far:</td>
<td>a) Break completed? Yes No</td>
</tr>
<tr>
<td>Dominant activity past hour (reading ect):</td>
<td>b) RPE final (6-20)?</td>
</tr>
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<td></td>
<td>c) HR final</td>
</tr>
</tbody>
</table>

Satiety Measure [7:05HR]

Actual time of VAS measures: 

VAS measures complete: YES NO

Blood Pressure [7:25HR]

6.3. Seated blood pressure (1 min between): Arm used: L R

(a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(b) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
(c) Systolic (mmHg): Diastolic (mmHg): HR(60sec):

AVERAGE (a) Systolic (mmHg): Diastolic (mmHg): HR(60sec):
Brain Breaks Study – HREC 181/14

Experimental Condition:  Sitting  Exercise  Ex + Breaks

5. Walking Break 12 (3min) 7:30 HR

**Extra walking breaks (toilet etc)**

| No. of walking breaks: |

**Active Condition (with 3 min breaks)**

| a) Break completed? | Yes | No |
| b) RPE final (6-20)? |   |   |
| c) HR final |

5. Cognitive Test 4 (7:35 HR)

**Cognitive Test [4] (7:35HR)**

| 3.3. VAMS complete:  Yes | No |

| Actual Time of Cognitive Test 1(start): | : |
| Actual Time of Cognitive Test 1(end): | : Length of Test 1 (minutes) |
| Actual Time of Cognitive Test 2(start): | : |
| Actual Time of Cognitive Test 2(end): | : Length of Test 2(minutes) |
| Actual Time of Cognitive Test 3(start): | : |
| Actual Time of Cognitive Test 3(end): | : Length of Test 3 (minutes) |
| Actual Time of Cognitive Test 4(start): | : |
| Actual Time of Cognitive Test 4(end): | : Length of Test 4 (minutes) |
| Actual Time of Cognitive Test 5 (start): | : |
| Actual Time of Cognitive Test 5(end): | : Length of Test 5 (minutes) |

| Cognitive Test Complete [4] | Yes | No |
Experimental Condition:  
- Sitting
- Exercise
- Ex + Breaks

---

**VAS-F Questionnaire** *(7:57HR)*

VAS-F Questionnaire Complete:  
- Yes
- No

**Satiety Measure** *(7:58HR)*

Actual time of VAS measures:  

VAS measures complete:  
- YES
- NO

---

**13th BLOOD COLLECTION** *(8:00HR)*

5.5. Actual time of blood collection:  

1st  
5.5mL (transfer from syringe to 15 mL falcon tube with added EGTA/GSH) (spin, aliquot, freeze)

2nd  
6mL (red serum 10mL tube) (rest 30 minutes, spin, aliquot, freeze)

3rd  
4.5 mL (lithium heparin 4.5 mL tube) (spin, aliquot, freeze) (deliver to pathology for lipid profile)

4th  
3mL (EDTA lilac 3mL tube) (spin, aliquot, freeze)

5th  
3mL (EDTA lilac 3mL tube) (deliver to pathology for full blood evaluation)

6th  
2mL (fluoride oxalate grey 2mL tube) (deliver to pathology for glucose)

---

**Testing End** Dominant activity past hour (reading ect): ________________

---

**Blood Pressure** *(8:05HR)* Pre FMD 2

6.3. Seated blood pressure *(1 min between)*:  
Arm used:  
- L
- R

(a) Systolic (mmHg):  
(b) Systolic (mmHg):  
(c) Systolic (mmHg):

Diastolic (mmHg):  
Diastolic (mmHg):  
Diastolic (mmHg):

HR(60sec):
HR(60sec):
HR(60sec):

AVERAGE (a) Systolic (mmHg):  
Diastolic (mmHg):  
HR(60sec):

HR rest

2.9 POST Flow Mediated Dilation Complete:  
- (2) Yes
- No
Experimental Condition: Sitting [ ] Exercise [ ] Ex + Breaks [ ]

13.2. *IV. catheter* removed? Time: 

13.3. *Heart rate monitor STOPPED* and removed? Time: (don’t forget HR belt!!)

**TRIAL OFFICIALLY OVER**

See next page for final checklists before participant departs

---

### PARTICIPANT WATER CONSUMPTION LOG

1. **Number** of 500 mL cups consumed (mark off over trial):

\[
\text{500mL x Number} = \text{mL} - \text{amount in final 500mL cup} (\ldots\text{mL})
\]

Total volume of water participant consumed over trial period: mL

---

### 14. FINAL CHECKLIST

| 12.1 | Check participants current Activity/Diet Handbook? | Yes | No |
| 12.3 | Participant received postage envelope and protective wrapping for sleep questionnaire? | Yes | No |
| 12.4 | Participant received parking ticket? | Yes | No |
| 12.5 | If final visit, has participant completed ATO “Statement by a supplier” form? | Yes | No |
| 12.6 | If final visit, participant completed requisition for payment (RFP) form? | Yes | No |
| 12.10 | Accelerometer & inclinometer mailed back/collection and data uploaded/recorded? | Yes | No |

- Document parking ticket number
- Scan all completed trial visit docs in this order: CRF, Path CRF, Sleep questionnaire, VAS-F and VAMS.
- Remind PP to: Fast, Avoid MVPA/caff/alcohol, Bring pack with diary, Remind PP to bring back SCALES, hydrate!

Scan Handbook and Pathology hard copy separately.
Experimental Condition:  
- Sitting  
- Exercise  
- Ex + Breaks  

COMMENT LOG:

This log is to be used for clarification of CRF data or entry of significant comments by investigator or study co-ordinator. Please initial and date any comments made.

☐ Check if NONE

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Comment</th>
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Participant Information Sheet/Consent Form

Non-Interventional Study - Adult providing own consent

Baker Heart and Diabetes Institute

<table>
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<th>Title</th>
<th>Taking a break for brain health: Interacting effects of exercise bouts with breaks in sitting time on cognitive and cerebrovascular function in overweight adults</th>
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<tbody>
<tr>
<td>Short Title</td>
<td>Brain Breaks</td>
</tr>
<tr>
<td>Project Number</td>
<td>181/14</td>
</tr>
<tr>
<td>Project Sponsor</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>Coordinating Principal Investigator/ Principal Investigator</td>
<td>Prof David Dunstan</td>
</tr>
<tr>
<td>Associate Investigator(s)</td>
<td>Prof Daniel Green, Dr Kathryn Ellis, Prof Ester Cerin, Prof Bronwyn Kingwell, Prof Neville Owen, Prof Gavin Lambert, Dr Robyn Larsen, Dr Louise Naylor, Prof Nicola Lautenschlager, Dr Patrik Wennberg, Dr Carl-Johan Olsson, Dr Ilkka Heinonen, Mr Michael Wheeler, Mr Paddy Dempsey</td>
</tr>
<tr>
<td>Location</td>
<td>Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.</td>
</tr>
</tbody>
</table>

1 Introduction

You are invited to take part in this research project. This is because you are between 55 and 80 years of age, and you are in a weight range classed as overweight to obese. This places you in an important target group for measuring strategies to manage or prevent a number of diseases. In recent years, studies have shown that adults who are physically active are more likely to perform better on several tests of memory, problem solving, and decision making (cognitive function). Furthermore, recent studies conducted in this laboratory suggest that reducing sedentary behaviour (prolonged sitting time) leads to improvements in control of blood sugar, blood pressure, and blood flow – all of which have been shown to affect cognitive function. The present study aims to investigate whether breaking up sitting time, with and without a single bout of exercise, improves, blood flow to the brain (cerebrovascular function), cognitive function, and how cells in the body perform their functions (metabolic function).
This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don’t understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don’t wish to take part, you don’t have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

It is well known that being physically active is important for maintaining good health. However, new evidence has emerged which shows that being sedentary (sitting for prolonged periods) is linked to signs of poor health, such as elevated blood sugars, blood fats, and blood pressure. Interestingly, too much sitting may even be a stand-alone risk factor above and beyond doing too little exercise.

In recent years, studies have also shown that adults who are physically active are more likely to perform better on several tests of cognitive function compared to those who are inactive. Regular exercise in mid-life is also associated with a significantly slower rate of cognitive decline and reduced risk of developing dementia later in life. Furthermore, recent studies conducted in this laboratory have shown that reducing prolonged sitting time results in metabolic improvements in blood sugar control, blood pressure, and blood flow – all of which have been shown to affect cognitive function. While suggestive of a link, no studies to date have investigated direct associations between prolonged sitting time, metabolic function, and cognitive health.

Therefore, in this study we will examine whether, compared to uninterrupted sitting, there are cognitive benefits achieved throughout the day following a single 30-minute bout of moderate-intensity exercise. We will also examine whether breaking up sitting time throughout the day with regular bouts of walking, in addition to the 30-minute exercise bout, will provide further improvement in cognitive function compared to exercise alone. Furthermore, we will also examine the associations between breaking up prolonged sitting, improvements in metabolic markers (from blood tests), and improved cognitive function.

This research has the potential to offer at risk adults advice to modify their lifestyle and, in doing so, reduce their risk of cognitive decline and dementia, particularly given that there are currently no medications available to offer similar protection.

This research has been initiated by Professor David Dunstan (Head of Physical Activity Laboratory, Baker Heart and Diabetes Institute) and has been funded by the National Health and Medical Research council (NHMRC).
3 What does participation in this research involve?

The first step will be a telephone screening. This will be used to ensure you meet the inclusion criteria. After passing this, you will be referred to attend your local Melbourne Pathology, in a fasted state (overnight fast), to have a free health screening blood test. This blood test will provide information on your general health as baseline information for comparison with samples we plan to take during the experimental days of the study. After passing this blood test, you will be booked in for your 4 visits to Baker.

Participation in this project will involve 4 visits in total to the Physical Activity Laboratory at Baker Heart and Diabetes Institute, spread over a minimum period of 4 weeks. The first visit will be a familiarisation visit where we will provide instructions on our requirements of you, and familiarise you with the testing procedures and measurement devices we plan to use. We will require you to undergo a number of tests to ensure your ability to participate in the study and to provide baseline data. The tests include cognitive function tests, a resting ECG and a moderate-intensity exercise test on a motorised treadmill to determine your target heart rate range for the experimental conditions.

During visits 2, 3 and 4 (separated by a minimum of 6-days) you will be required to complete each of the three experimental conditions described below in a random order. For visits 2, 3 and 4 you will be required to fast (not consume any food or drinks – except water) from 10pm the night before your appointment. For an overall diagram of the study schedule just described, please see page 5.

Consent/Screening/Familiarisation

Screening blood test (21mL) (equivalent of ~1.4 tablespoons)

During the phone screening the study was verbally explained to you. After passing the telephone screening, you were asked to give verbal consent to attend your local Melbourne Pathology. The PICF was then sent out to you (which you are reading now) with a cover letter explaining that you have consented to having a screening blood test and outlining the next steps that need to be taken. After this you will be asked to attend your local Melbourne Pathology for a free health screening blood test. You will be required to attend this blood test in a fasted state, after an overnight fast.

From this blood, a number of tests will be performed to give an indication of general health, and also to provide baseline measurements in which we will look for changes to indicate possible benefits for cognitive function. Preparing for the blood test is quick and relatively straightforward. To ensure the accuracy of the other blood tests, you should avoid alcohol, caffeine or any other stimulants 12 hours before this visit. A small needle will be inserted into a vein in your arm to enable the collection of a venous blood sample. We will be in touch a few days after this to let you know your results and having passed this screening we can then book you in for your first visit to the BakerInstitute.

Visit 1: Familiarisation – (approximately 2 hours).

You will be asked to attend an initial visit at the Physical Activity Laboratory, Baker Heart & Diabetes Institute. You are asked to avoid caffeine, alcohol and vigorous exercise 48 hours before this visit. At this stage you will be given the opportunity to ask questions before providing your consent to participate. The research staff will then collect information about your education and medical history. Your height, weight, blood pressure, and hip and waist measurements will then be taken.

During this visit you will also complete:

i) Cognitive function testing
A small number of computer-based and paper-based tests commonly used to assess, cognitive ability and signs of anxiety and depression.
ii) A medical examination
Both the study coordinator and our study doctor will conduct a medical examination to ensure that it is safe for you to take part in this study. This will involve an overall evaluation of general health, vital sign checks, a resting ECG (electrocardiogram, this is a device which can measure the electrical activity of your heart).

During this first visit you will be asked to perform a moderate-intensity exercise test. The test will require you to walk on the treadmill at a progressively steeper incline until you reach moderate intensity. Once all the inclusion and exclusion criteria have been met and you have been deemed suitable to take part in the study, appointments will be made for the remaining three visits. The three experimental conditions will be assigned to you in a random order. During this familiarisation visit you will get the opportunity to practice and become acquainted with the treadmill walking at the required intensity levels, and to become familiar with the cognitive testing tasks.

You will also be fitted with two small lightweight devices which measure and store information regarding the frequency and duration of times spent sitting, standing and walking. One instrument, known as an accelerometer, will be worn with a belt at hip height. The other instrument, known as an ActivPAL inclinometer, will be fixed to the front of your upper thigh with Micropore tape, a gentle, breathable paper tape. Micropore is hypoallergenic and latex free and can be applied straight onto your skin without needing to shave. Both instruments can be easily removed and refitted after sleeping or engaging in water activities (e.g. shower or swimming). You will be required to wear both devices everyday (during waking hours) starting from your familiarisation visit to the completion of the 4th and final experimental condition – a maximum of 21-22 days. We will ask you to keep a log of your physical activity to help us gather information about non-walking physical activities during that period (e.g. weight lifting, yoga, cycling).

At the completion of this visit, you will receive a standardised meal pack, to be eaten on the evening prior to the first experimental condition (Similar packs will be provided prior to each of the remaining visits). We will request that you eat food only from this pack and not other foods. This will help to minimise possible variability in results caused by diet. It is anticipated that this first visit will take approximately two hours.

Experimental procedures
Visits 2, 3 and 4: Experimental condition – (8 hours). Total blood collected/visit 216.5mL (equivalent of ~14.6 tablespoons)

Preparation for each experimental condition:
In the 48 hour period prior to each experimental condition, we request that you avoid all alcohol and caffeine (e.g. tea, coffee, caffeinated soft drinks) and do not engage in any moderate (e.g. gentle swimming, social tennis, golf) and/or vigorous (e.g., running, aerobics) physical activity. We will ask you to keep a record of your food and drink intake in the provided diary during this 48-hour period. On the night before and after each experimental condition, we ask that you eat only the foods provided for you in your ‘meal pack’ and no other foods.

Visits 2, 3 and 4: After having fasted (no food/drink – except water) from 10pm, you will attend the Physical Activity Laboratory, Baker Heart and Diabetes Institute between 7:15 and 7:30 am on each treatment day. Upon arrival, you will have your weight, height, and waist circumference measured, and your blood pressure taken.

Next a small continuous glucose sensor will be inserted into the subcutaneous (just under the skin) fat tissue of your lower back, where it will remain until 7:30 am the next morning. A protective plastic film is then placed over the inserted sensor. This sensor captures blood glucose patterns and will require you to take blood samples with a finger prick device so that the sensor can be calibrated. The sensor will require re-calibration one hour after it is connected to the glucose meter, 3 hours after connection, just prior to your evening meal and before going to sleep the night of this visit and again the morning after, just before removing the sensor. To remove the sensor first start by
carefully removing the plastic film that has been covering the sensor. Once this has been removed, carefully pull the sensor away from your body. All this will be demonstrated by the study coordinator at the familiarisation visit.

A cannula (small plastic tube) will be inserted into a vein in your arm to enable the collection of blood samples. Cannulation involves inserting the small, soft, indwelling plastic tube into a vein in your arm via needle, where it will stay until all blood samples have been collected.

A test for artery health, called flow mediated dilation (FMD), will be performed next using a small ultrasound device about the size of a mobile phone, which will rest against your arm as you lie down. We use a special hypoallergenic water soluble gel with this device to be able to see an image of your artery. The test involves looking at your artery at rest for 1 minute. A cuff will be wrapped around the lower part of your arm, like a blood pressure cuff, which will be inflated after one minute, and will stay inflated for 5 minutes, after which the cuff will deflated. We will continue to look at your artery for a further 3 minutes. Doing this allows us to view your artery under conditions of altered blood flow to assess artery health.

On each experimental day, you will sit quietly in a chair for an initial 1-hour “steady-state” period. During this time, baseline measures of cognitive function will be obtained via series of computerised tests. You will then be provided a standardised breakfast meal, commencing the experimental condition thereafter. Lunch will also be provided around midday.

On each experimental day blood samples will be drawn from the cannula at regular intervals for the analysis of BDNF (a protein which helps maintain healthy brain cells), glucose, triglycerides and serum insulin. You will be asked to provide small volumes of blood (approximately 6-20 mL, equivalent of ~ 0.4 – 1.3 tablespoons) on 13 occasions. These 13 blood draws will total 216.5mL (equivalent of ~ 14.6 tablespoons) per day. This volume is relatively small (about one quarter of the volume typical of a blood bank donation) and is quickly replenished naturally by the body.

Cognitive function will be measured on 4 occasions throughout each condition using a number of computerised tests. These will include tests of visual attention, ability to concentrate, working memory, spatial problem solving, and visual learning and memory.

**Experimental condition A: Uninterrupted sitting**
The experimental condition will commence following the baseline measurements. During the 8-hour period, you will be asked to sit quietly in a comfortable lounge chair. You will be able to read or work quietly whilst you are seated.

**Experimental condition B: Continuous exercise + uninterrupted sitting**
Following the 1-hour steady-state sitting period, you will complete 30 minutes of continuous exercise at moderate-intensity. You will then sit quietly, as in condition 1, for a remaining 6 1/2 hours.

**Experimental conditions C: Continuous exercise + interrupted sitting**
This experimental condition will begin identically to condition B, however following the moderate-intensity exercise bout you will be asked to sit quietly for the next 30 minutes before completing 3 minutes of light-intensity walking on motorised treadmill at 3.2km/hr. You will then be asked to sit quietly again. Your sitting time over the remaining 6 ½ hour period will be interrupted every 30 minutes by repeated 3-minute bouts of light-intensity walking (total walking time 36 minutes).

You will be able to have a toilet break whenever you need regardless of the experimental condition.

Randomisation (i.e., “coin flipping”) will decide if you will follow experimental condition A, B, or C at visits 2, 3 and 4. For example, if it is decided that you will follow experimental condition B at visit 3, you will follow experimental conditions A or C at visits 3 and 4.
Study Schedule

<table>
<thead>
<tr>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>Familiarsation</td>
<td>2-4 days</td>
<td>Experimental Condition A, B or C</td>
<td>1 week</td>
</tr>
</tbody>
</table>

Reimbursement

You will receive reimbursement to offset the cost of car parking and time associated with participating in this study. This will include $18 for the familiarisation visit and $125 per day for each of the experimental days. You will only be reimbursed for days you attend therefore, if during screening, you are deemed ineligible to participate or if you withdraw from the study you will receive a partial payment according to the number of visits you attended. This will be paid to you on completion of all study visits.

4 What do I have to do?

In order to participate in the study, you must have time and be willing to attend all scheduled visits and have the relevant tests and procedures done at each visit.

You will be asked to continue your normal routine and not to begin any strenuous new physical activities while participating in the study.

You will be asked to record all food intake during the 48-hour pre-experimental period through food diaries that we will give you. You will be asked to not drink any caffeine drinks (coffee, tea, caffeinated soft drinks, etc.) and alcohol during 48 hours preceding the three experimental visits. The day prior to each visit you will be asked to consume food only from the meal packs given to you either at Visit 1.

You will be asked to avoid any moderate and/or vigorous intensity activity for at least 48 hours prior to each visit. During this period, we will ask to wear the small devices on your hip and thigh to measure your physical activity. You can shower with them and take them out for water-based activities if needed.

Summary of requirements

<table>
<thead>
<tr>
<th>Visit</th>
<th>Melbourne Pathology</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrain from alcohol and caffeine for 48 hours prior to this visit</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Refrain from vigorous exercise for 48 hours prior to this visit</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Record all food items consumed for 48 hours prior to this visit</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consume food from meal pack only in the evening prior to this visit</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Wear the devices to measure physical activity 48 hours prior to this visit

Fast from 10pm the night before this visit

There are a number of factors that would restrict you from participating in this study, these include:

- You are outside the age range of 55-80 years of age
- You are outside the BMI range 25 – 45 kg/m²
- You are non English speaking
- You are pregnant
- You are in a peri-menopausal or menopausal stage of life
- You have a semi-active to active occupation i.e., less than 5 hours sitting per day
- You currently watch less than 3 hours of television per day
- You have been regularly undertaking moderate-to-vigorous physical activity i.e., 150 minutes per week for longer than three months
- You are scheduled or plan to schedule an elective surgery procedure during your participation in the study
- You have Type 1 or 2 diabetes
- You have heart disease, high blood pressure or an abnormal ECG.
- You are taking any medication to control your body weight, blood sugar, cholesterol, anxiety and/or depression, anxiety or cardiovascular disease
- You have a major illness and/or physical problems (acute or chronic) that limit your ability to perform exercise
- You have a condition that, in the opinion of the investigator, may confound the evaluation and interpretation of the data.
- You drink excessive amounts of alcohol

5 Other relevant information about the research project

A total of 69 male and female individuals who are overweight and sedentary will participate in this study at the Physical Activity laboratory, at Baker Heart & Diabetes Institute. This study involves a collaboration of researchers from the Baker Heart and Diabetes Institute in Melbourne and from The University of Western Australia who will also be recruiting 69 participants to their study.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part you don’t have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you decide to take part in this study, you will be given this Participant Information Sheet/Consent Form to sign and given a copy to keep.

Your decision whether to take part or not to take part in this study, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Baker Heart & Diabetes Institute and the Alfred Hospital.

7 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include individualised health profile, knowledge and understanding of your baseline metabolic health and what happens to your metabolic indicators of health when you are sedentary and/or actively breaking up your sitting over the course of a day. We expect that the findings from this study will make an important contribution to the design, implementation and evaluation of intervention strategies in the community to reduce the risk for diabetes and cardiovascular disease through the adoption of healthier behaviours and lifestyles.
What are the possible risks and disadvantages of taking part?

This research involves a number of procedures in which you may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with the study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms.

Many side effects go away following the completion of a procedure. However, sometimes side effects can be serious, long lasting or permanent. Your study doctor should discuss the best way of managing any side effects with you.

The risks associated with the individual procedures involved in the study are as follows:

**Continuous glucose sensor placement and finger prick tests:**
Placement of the sensor is spring loaded and fast, but may cause some discomfort, much like the finger prick test. Once the small needle is removed, you may still feel the sensor’s presence. Bleeding, swelling, irritation and/or infection at the insertion site are possible risks.
The finger prick calibration will require you to use a spring loaded lancet to prick your finger to allow a droplet of blood to form. You will then use a glucose sensing strip to measure your glucose level as demonstrated by the research team. In total 5 finger prick calibrations will be required over a 24 hour period.

**Cannula placement and blood sampling:**
Cannula placement and blood sampling may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which blood is taken could become inflamed. Rarely, there could be a minor infection or bleeding. If this happens, it can be easily detected and treated by our trained nurse or study doctor. It is also possible that you may feel faint during the catheter placement and/or blood tests. To minimise your risk, only our trained nurse or doctor will perform the cannulation procedure during which time you will be lying down.

**ECG:**
For some people, a skin reaction may occur due to the electrode patches that are attached to the chest for the ECG, but any skin irritation usually disappears when the patches are removed. Because an ECG simply monitors the electrical activity of the heart and does not emit electricity, there is no risk of shock.

**Flow mediated dilation (FMD):**
A slight feeling of tightness on your arm and cold/tingling sensation in your hand might be felt during the time the arm cuff will be inflated.

**Moderate-intensity exercise:**
On two occasions during this study you will be asked to perform 30 minutes of moderate-intensity exercise. The intensity of the exercise you will perform on trial days will be determined during the familiarisation visit. You will also be able to practice this activity during the familiarisation visit to ensure you will comfortably be able to perform it on the experimental days. To help minimise your risk, this experimental sessions will be supervised by trained staff and will be tailored to your ability. There is a small possibility that you will experience minor and temporary muscle soreness initially following the exercise sessions. There may be additional unforeseen or unknown risks and participation in this study can be suspended or terminated if a medical issue or distress occurs.

**Walking activity breaks:**
As part of this study, on one occasion following a moderate-intensity exercise bout, you will be asked to walk on a treadmill at a low/moderate pace for 3 minutes, repeated every 30 minutes.
for 6 hours. To help minimise your risk, trained staff will supervise all experimental sessions. There is a small possibility that you will experience minor and transient muscle soreness initially following the exercise sessions. There may be additional unforeseen or unknown risks and participation in this study can be suspended or terminated if a medical issue or distress occurs.

Screening of participants:
Being deemed ineligible to participate may be concerning for participants. Particularly if attributed to failure of the cognitive function testing. If this were the case, appropriately qualified staff (principle investigators associated with this study) would explain the results and implications to you and refer you to relevant and independent specialists for counselling and/or further investigation.

9 What will happen to my test samples?

On the experimental days, a fasting blood sample (21mL equivalent of ~ 1.4 tablespoons) will be collected from a vein in your arm via a cannula. Further blood samples will be collected at regular intervals for 13 occasions in total. The blood collected will be analysed for BDNF (a protein that helps maintain healthy brain cells), blood glucose, triglycerides, and insulin. A total of 216.5mL (equivalent of ~ 14.6 tablespoons) of blood will be collected over the course of each assessment day.

At each blood collection, 7.5mL (equivalent of ~ 0.5 tablespoons) of blood will be drawn and stored for future analysis. We would like your permission to store these extra samples for future research into cognitive function, diabetes, and heart disease. These samples will be stored indefinitely at -80°C locked freezer at Baker, Alfred Centre, 99 Commercial Rd, Melbourne. Only study investigators will have access to your samples. By checking the appropriate box and signing the Consent Form for tissue sample storage and use, you consent to this request. The storage of any excess sample is optional. Your blood samples will not undergo genetic analysis. Once you have completed the study a summary of your individual results will be sent in the mail. A section on the Baker website (www.baker.edu.au) accessible to all participants will report findings of the evaluation of group data. The results will be published in a peer reviewed scientific journal.

10 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

11 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are
involved in the research project. With your permission, the study doctor will consult with your GP should this need arise.

12 What if I withdraw from this research project?

If you decide to withdraw from this research project, please notify a member of the research team before you withdraw. A member of the research team will inform you if there are any special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them when you withdraw from the research project.

13 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons including unacceptable side effects, but this is unlikely.

14 What happens when the research project ends?

At the completion of the study, you will be provided with an individual report of your results. If requested, you can also receive a report of the main findings of the study and copies of any subsequent publications.

15 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research study that can identify you will remain confidential and will only be used for the purpose of this research study. It will only be disclosed with your permission, or in compliance with the law.

To access the information collected on your CGM sensor (the Medtronic iPro2 Elite blood glucose system) we will need to upload your glucose measurements onto the Medtronic database. The data uploaded onto the database will be stored at the Medtronic offices in Heerlen, Netherlands. Medtronic has issued their own privacy statement and consent form to explain that your glucose data will be stored with them for quality assurance purposes. You will be asked to sign their form before being fit with the CGM. We will take steps to maintain participant privacy when handling CGM data. When uploading your glucose data, we will not submit your personal information to Medtronic’s database. We will enter it in a re-identifiable format (which means it will be coded so that only study staff will be able to identify who the data belong to, eg. X309). The signed privacy statement and consent form issued by Medtronic will be stored securely in your participant file at The Baker Heart & Diabetes Institute. Although unlikely, a Medtronic representative may need to view the consent form to ensure that you have properly entered their data sharing agreement (where they may use your uploaded information for the purpose of advancing or improving its products, therapies or services by analysing, studying, conducting education, and/or monitoring the data stored on the Medtronic CareLink® iPro servers, usually in aggregate form). If this is the case, your identity may be disclosed to Medtronic.
Study data will be stored on password-protected computers, belonging to the researchers involved in the study. Hard copy data will be stored in a locked filing cabinet in the Study Coordinator’s office at the Baker, Alfred Centre, 99 Commercial Rd, Melbourne. Your data will be stored in a re-identifiable (coded) format with your personal details stored in a separate file. Data will be stored indefinitely as per Alfred Hospital Study policy. At the completion of the study, electronic and hard copy data will be archived securely off site.

Your contact details, pathology (blood) results in your name and medical history will be kept in a locked filing cabinet in the Study Coordinator’s office at the Physical Activity laboratory, at Baker Heart & Diabetes Institute. Identifying information will not be entered into the study research forms, the study database or appear in any data reports. Your information will only be used for the purpose of this research study, however if you give permission for any remaining samples to be used for related research (by signing the second consent form), your coded data will be used for further analysis. Your information will only be disclosed with your permission, or in compliance with the law.

This research study involves the establishment of a databank. When you sign the attached consent form, you are consenting your information being used for this specific study and, if you give consent, to future related research.

If you have a regular local doctor, it is desirable that they be advised of your decision to participate in this research study. If you do have a local doctor, by signing this consent form, you are agreeing to inform him/her of your participation in the study. With your permission, we will write to your local doctor to inform them of your participation in this study.

It is anticipated that the results of this research study will be published and/or presented in a variety of forums, which may include publication in scientific journals, presentations at scientific conferences and clinical trial registries such as www.clinicaltrials.gov. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Only group data will be published and presented.

In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

16 Complaints and compensation

If you suffer any injuries or complications as a result of this research study, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

17 Who is organising and funding the research?

This research study is being conducted and funded by the Baker Heart and Diabetes Institute (National Health and Medical Research Council grant).

You will not benefit financially from your involvement in this research study even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to the Baker Heart and Diabetes Institute. In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

The Baker Heart and Diabetes Institute will receive payment for the direct research costs for this study from National Health and Medical Research Council. No member of the research team
will receive a personal financial benefit from your involvement in this research study (other than their ordinary wages).

18 Who has reviewed the research study?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research study have been approved by the HREC of The Alfred Hospital, Melbourne.

This study will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

19 Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this study or if you have any medical problems which may be related to your involvement in the study (for example, any side effects), you can contact the Study Coordinator Mr Ian Mullis (03 8532 1931) at any time or any of the following people:

Michael Wheeler
Telephone: 03 8532 1898

Professor David Dunstan (Principal Investigator)
Telephone: 03 8532 1873

Complaints contact person

If you have any complaints about any respect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Position: Complaints Officer, Office of Ethics & Research Governance, Alfred Health
Telephone: 03 9076 3619
Email: research@alfred.org.au

Please quote the following Alfred Health project number: 181/14.
Title
Taking a break for brain health: Interacting effects of exercise bouts with breaks in sitting time on cognitive and cerebrovascular function in overweight adults

Short Title
Brain Breaks

Project Number
181/14

Project Sponsor
National Health and Medical Research Council

Coordinating Principal Investigator/ Principal Investigator
Prof David Dunstan

Associate Investigator(s)
Prof Daniel Green, Dr Kathryn Ellis, Prof Ester Cerin, Prof Bronwyn Kingwell, Prof Neville Owen, Prof Gavin Lambert, Dr Robyn Larsen, Dr Louise Naylor, Prof Nicola Lautenschlager, Dr Patrik Wennberg, Dr Carl-Johan Olsson, Dr Ilkka Heinonen, Mr Michael Wheeler, Mr Paddy Dempsey

Location
Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.

Declaration by Participant
I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project, as describe in the Participant Information Sheet.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to The Alfred Hospital concerning my condition and treatment for the purposes of this project. I understand that such information will remain confidential.

Name of Participant (please print)

Signature ________________________ Date __________________________

Name of Witness* to Participant’s Signature (please print)

Signature ________________________ Date __________________________

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.
Declaration by Study Doctor/Senior Researcher†
I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher† (please print) ________________________________

Signature ________________________________ Date ________________________________

† A senior member of the research team must provide the explanation of, and information concerning, the research project. Note: All parties signing the consent section must date their own signature.
Form for Withdrawal of Participation - Adult providing own consent

<table>
<thead>
<tr>
<th>Title</th>
<th>Taking a break for brain health: Interacting effects of exercise bouts with breaks in sitting time on cognitive and cerebrovascular function in overweight adults</th>
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</tr>
<tr>
<td>Location</td>
<td>Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC.</td>
</tr>
</tbody>
</table>

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with The Alfred Hospital.

Name of Participant (please print)  
Signature ______________________ Date ____________________

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher† (please print)  
Signature ______________________ Date ____________________

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.
CONSENT FORM FOR THE STORAGE AND USE OF BLOOD AND TISSUES

Taking a break for brain health: Interacting effects of exercise bouts with breaks in sitting time on cognitive and cerebrovascular function in overweight adults

Researchers: A/Prof David Dunstan, Prof Daniel Green, Dr Kathryn Ellis, Prof Ester Cerin, Prof Bronwyn Kingwell, Prof Neville Owen, Prof Gavin Lambert, Dr Robyn Larsen, Dr Louise Naylor, Prof Nicola Lautenschlager, Dr Patrik Wennberg, Dr Carl-Johan Olsson, Dr Ilkka Heinonen, Mr Michael Wheeler, Mr Paddy Dempsey

Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne VIC.

I have read the Participant Information Form and give consent to the storage and use of blood and tissue samples taken from me for use in:

☐ this specific research project
☐ other research that is closely related to this research project (i.e. for future research into diabetes and cardiovascular disease approved by the Alfred Human Ethics Committee).

as described in Section 9 of this document by Professor David Dunstan.

Participant’s name (printed) ……………………………………………………………………………………………………………………………

Signature:………………………………………………………………………… Date:………………………………

Name of witness to participant’s signature (printed) ………………………………………………………………………………………

Signature:………………………………………………………………………… Date:………………………………

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher’s name (printed) ……………………………………………………………………………………………………………………………

Signature:………………………………………………………………………… Date:………………………………
What is Brain Breaks?
The Brain Breaks study is a randomised control trial, which will look at how exercise and breaking up sitting time could benefit cognitive function.

Why exercise and breaking up sitting?
Previous studies have indicated that exercise is important for health, as well as for cognitive function. Recent research has also indicated that reducing and breaking up sitting time is important for health. However it is unknown whether breaking up sitting will benefit cognitive function. It is also unknown if breaking up sitting plus exercise is better for cognitive function than exercise alone.

Why is this research important?
Optimal cognitive function is important for productivity and quality of life. This is especially relevant for Australia’s ageing population, where the number of people with dementia is increasing. This research has the potential to offer lifestyle advice to reduce the risk of cognitive decline and dementia. This protective effect of lifestyle modification is essential to brain health, as there are currently no medications available to offer similar protection against cognitive decline and dementia.

Who are we?
The Brain Breaks team includes researchers affiliated with Baker IDI in Melbourne, Deakin University, Melbourne University, The University of Western Australia and Umeå University in Sweden. This multidisciplinary team includes experts in exercise science, neuroscience, psychology and medicine, who will work together to find better ways to improve our brain health.

What’s involved?
Participants will undergo tests for blood pressure, memory function and blood tests. The study will compare how these tests might be affected by sitting, exercise and breaks in sitting time. Each participant will perform 3 different conditions, in a random order, each lasting a total of 8 hours.

These Conditions are:
1. Uninterrupted Sitting
2. Exercise (30min) followed by uninterrupted sitting
3. Exercise (30 min) followed by interrupted sitting (3 min walk every half hour).

Who can participate?
Researchers are looking for healthy people aged between 55-80 with a BMI between 25 and 45 (kg/m²). Main exclusion criteria are smoking, regularly active (defined as more than 150 minutes of moderate intensity exercise per week), diagnosed diabetes, dementia, cancer (in the last 5 years), peri menopause or menopause. Eligibility criteria will be determined via a phone screening questionnaire.

Further Information
For further information please contact Michael Wheeler, Ian Mullis or Kym Rickards.
Michael- Tel: (03) 8532 1898 or michael.wheeler@bakeridi.edu.au
Ian - Tel:(03) 8532 1932 or ian.mullis@baker.edu.au
Kym- Tel: (03) 8532 1864 or kym.rickards@bakeridi.edu.au
Ever wondered if too much sitting affects your brain?

Studies suggest that exercise can improve cognitive function. Researchers from Baker IDI seek to determine if breaking up sitting time, in addition to exercise, is better for improving cognitive function than exercise alone.

Men and Women are eligible for this study, if they are:

- 55-80 years
- Overweight/obese but otherwise healthy
- A non smoker
- Post– menopausal
- Not regularly physically active
- Working in a sedentary job.
- Not diagnosed with depression, dementia, cancer (within the last 5 years) or diabetes.

The study involves 4 visits to Baker IDI
- 1 familiarisation visit (2 hours)
- 3 experimental conditions (8 hours each, breakfast and lunch provided)

Participants will undergo:

- Free blood tests
- Free memory tests
- Free blood pressure tests

Participants will be reimbursed for their time and parking will be provided for all visits.

For further information please contact Michael Wheeler or Ian Mullis.

Michael - Tel: (03) 8532 1898 or email: michael.wheeler@bakeridi.edu.au
Ian - Tel: (03) 8532 1100 or email: ian.mullis@bakeridi.edu.au
Our Ref: RA/4/1/6990 30 July 2014

Winthrop Professor Daniel Green
School of Sport Science, Exercise & Health
MBDP: M408

Dear Professor Green

HUMAN RESEARCH ETHICS APPROVAL - THE UNIVERSITY OF WESTERN AUSTRALIA
Taking a Break for Brain Health Study Interacting Effects of Exercise Bouts with Breaks in Sitting Time on Cognitive & Cerebrovascular Function in Overweight Adults

Student(s):

Ethics approval for the above project has been granted in accordance with the requirements of the National Statement on Ethical Conduct in Human Research (National Statement) and the policies and procedures of The University of Western Australia. Please note that the period of ethics approval for this project is five (5) years from the date of this notification. However, ethics approval is conditional upon the submission of satisfactory progress reports by the designated renewal date. Therefore initial approval has been granted from 30 July 2014 to 01 August 2015.

You are reminded of the following requirements:

1. The application and all supporting documentation form the basis of the ethics approval and you must not depart from the research protocol that has been approved.
2. The Human Research Ethics Office must be approached for approval in advance for any requested amendments to the approved research protocol.
3. The Chief Investigator is required to report immediately to the Human Research Ethics Office any adverse or unexpected event or any other event that may impact on the ethics approval for the project.
4. The Chief Investigator must submit a final report upon project completion, even if a research project is discontinued before the anticipated date of completion.

Any conditions of ethics approval that have been imposed are listed below:

Special Conditions

None specified

The University of Western Australia is bound by the National Statement to monitor the progress of all approved projects until completion to ensure continued compliance with ethical standards and requirements.

The Human Research Ethics Office will forward a request for a Progress Report approximately 30 days before the due date.

If your progress report is not received by the due date for renewal of ethics approval, your ethics approval will expire, requiring that all research activities involving human participants cease immediately.

If you have any queries please contact the HREO at hreo-research@uwa.edu.au.

Please ensure that you quote the file reference – RA/4/1/6990 – and the associated project title in all future correspondence.

Yours sincerely
Dr Mark Dixon
Associate Director, Research Integrity
This is to certify that

Project No: 181/14

Project Title: Taking a break for brain health: Interacting effects of exercise bouts with breaks in sitting time on cognitive and cerebrovascular function in overweight adults

Principal Researcher: A/Professor David Dunstan

Project Proposal Version 1 dated: 7-May-2014

Participant Information and Consent Form Version 3 dated: 22-May-2014

was considered by the Ethics Committee on 29-May-2014, meets the requirements of the National Statement on Ethical Conduct in Human Research (2007) and was APPROVED on 4-Jun-2014

It is the Principal Researcher’s responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or progress report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- Any expiry of the insurance coverage provided with respect to sponsored clinical trials and proof of re-insurance;
- A delay of more than 12 months in the commencement of the project, and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Progress Report on the anniversary of approval and on completion of the project (forms to be provided);

The Ethics Committee may conduct an audit at any time.

All research subject to the Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Hospital Ethics Committee is a properly constituted Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (2007).

SPECIAL CONDITIONS

None

Please quote project number and title in all correspondence
Appendix C

Co-first authored paper on the effects of breaking up sitting on flow mediated dilation published in the *Journal of Applied Physiology*.

The following publication resulted from my involvement in another research project in the lab during my candidature. My primary involvement here was in statistical analysis, preparing the tables and figures and writing the results section. I also helped with study design, data collection and critical revision of the manuscript as it was being prepared for publication.

RESEARCH ARTICLE

Simple intermittent resistance activity mitigates the detrimental effect of prolonged unbroken sitting on arterial function in overweight and obese adults

Rachel E. Climie,1,2* Michael J. Wheeler,1,3* Megan Grace,1 Elisabeth A. Lambert,1,4 Neale Cohen,1 Neville Owen,1,4 Bronwyn A. Kingwell,1,5 David W. Dunstan,1,3,6,7,8,9 and Daniel J. Green3

1Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia; 2Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia; 3School of Human Sciences (Exercise and Sport Science), The University of Western Australia, Perth, Western Australia, Australia; 4Swinburne University of Technology, Melbourne, Victoria, Australia; 5Central Clinical School and Department of Physiology, School of Medicine, Nursing and Health Services, Monash University, Melbourne, Victoria, Australia; 6School of Public Health, University of Queensland, Brisbane, Queensland, Australia; 7Mary MacKillop Institute of Health Research, Australian Catholic University, Melbourne, Victoria, Australia; 8School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; and 9School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia

Submitted 18 June 2018; accepted in final form 5 September 2018

This evidence beyond acute observations to better understand the potential longer-term vascular-related consequences of prolonged sitting.

arteries; blood flow; blood pressure; obesity; sedentary lifestyle

INTRODUCTION

Arterial dysfunction, particularly that related to the inner (endothelial) lining, represents one of the earliest detectable stages of atherosclerotic disease (2, 24). Atherosclerotic lesions are not uniformly distributed, developing primarily in the coronary and carotid arteries as well as in the lower limb (21), which suggests that local factors, such as abnormal arterial shear stress, may play a role. Shear stress is modulated by physical (in)activity and sedentary behaviors (i.e., prolonged sitting (25, 32, 38, 39)), making such activities key contributors to arterial (dys)function and atherosclerosis. Excessive time spent sitting is now ubiquitous in modern-day society, with the average adult spending 9 h a day sitting (17). Moreover, prolonged sitting (defined as ≥30 min of uninterrupted sitting) accounts for 4 h per day (3). Importantly, high volumes of sitting are associated with elevated risk of cardiovascular disease (CVD) (31) along with other adverse health consequences (5), and it is possible that sitting-induced decrements in arterial function contribute to increased risk of CVD.

Previous research has shown that prolonged sitting leads to impairment in lower limb arterial function and dilation, effects that may be negated or reversed by light-intensity activity (25, 32, 38). These studies have been restricted to young, healthy populations, and it is unknown whether prolonged sitting may affect arterial function in those already at a heightened risk of CVD (such as overweight/obese adults). Moreover, mechanisms relating to the effect of prolonged sitting on vascular function remain unexplored. Candidate mechanisms include nitric oxide (NO), a potent vasodilator released from endothelial cells in response to shear stress (10). Furthermore, high insulin concentration (such as in insulin-resistant type 2 diabetes or overweight/obese populations) is associated with en-

* R. E. Climie and M. J. Wheeler are co-first authors of this work.
Address for reprint requests and other correspondence: R. E. Climie, PhD, Baker Heart and Diabetes Institute, 99 Commercial Rd., Melbourne, Victoria, 3181 AUSTRALIA (e-mail: rachel.climie@baker.edu.au).

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dothelin-1 (ET-1; a vasoconstrictor) upregulation (6, 29, 33) and expression of pro-atherogenic molecules [intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] (26). It is conceivable that the combination of CVD risk factors and reduced shear stress in the lower limb associated with prolonged sitting promotes an exaggerated pro-atherogenic environment. However, this hypothesis has not been directly addressed in those at increased risk of CVD.

Most earlier work has examined the effect of interrupting prolonged sitting with intermittent walking activity, but it has been suggested that a more pragmatic option for working adults could be to interrupt sitting without having to move away from their workstation, for example by performing simple resistance activities (SRA) in a static position using their own body weight (11). Indeed, a recent study in patients with type 2 diabetes demonstrated that interrupting prolonged sitting with brief bouts of SRA was as effective as light walking for reducing the impact of a day of uninterrupted sitting on postprandial glucose and insulin (11). However, it is unclear whether benefits in arterial function could be gained via SRA during periods of prolonged sitting. The aim of this study was to examine the effects on arterial function of prolonged uninterrupted sitting, relative to regular physically-active interruptions (SRAs) to sitting time, in adults at increased CVD risk.

MATERIALS AND METHODS

Subjects

Sedentary overweight/obese [body mass index (BMI) ≥25–40 kg/m²] adults were recruited via local advertisements. The exclusion criteria included pregnancy, self-reported sitting less than 5 h per day, self-reported regular engagement in moderate- to vigorous-intensity physical activity (≥150 min/wk), diagnosed diabetes, use of glucose/lipid lowering medications, being a current smoker, or having any major acute or chronic illness that might limit their ability to perform the SRA. Premenopausal women were excluded, as assessed via self-report. One woman who reported being perimenopausal completed both study conditions; sensitivity analysis (data not shown) revealed that statistical significance and interpretation of the experiment were unaltered by inclusion/exclusion of this participant’s data.

Study Overview and Randomization

This study was a randomized crossover trial (ACTRN12-316000578404), undertaken at the Baker Heart and Diabetes Institute research clinic. Potential participants were initially screened via a telephone questionnaire to determine their eligibility and were asked about their general health and medical history. Eligible participants were requested to undergo a fasted screening blood test at a local pathology clinic (Melbourne Pathology; Sonic Healthcare Ltd.) for glycated hemoglobin, glucose, and lipid profile. Participants attended the laboratory on three separate occasions: a familiarization visit and two repeat attendances. Timing of medication (if applicable) was standardized to occur with the breakfast meal before the 0 h time point. Postprandial blood samples were collected, blood pressure (BP) was measured, femoral and brachial artery flow-mediated dilation (FMD) were recorded, and participants were then given 15 min to consume a standardized breakfast meal before the 0 h time point. Postprandial blood samples were collected at 30 min, 1 h, and then hourly up to the end of the 5-h condition. Options for the breakfast meal consisted of bran-based cereal, fruit salad, ham-and-cheese croissant, and juice (200 ml). Lunch was provided after 5 h (at the end of the condition and after the final FMD measurement) and options included a salad and meat bread roll and juice. A note was made regarding each individual’s meal choice, and these were replicated on the repeat attendance. Timing of medication (if applicable) was standardized to occur with the breakfast meal during each condition.

Experimental Conditions

On the trial days, participants arrived at the laboratory between 0730 and 0800 in a fasted state (>10 h). Weight was remeasured, and BMI was calculated. An indwelling venous catheter was inserted in the antecubital vein for blood sampling. Each condition began with a 1-h “steady-state” seated period. During the steady-state period, blood samples were collected, blood pressure (BP) was measured, femoral and brachial artery flow-mediated dilation (FMD) were recorded, and participants were then given 15 min to consume a standardized breakfast meal before the 0 h time point. Postprandial blood samples were collected at 30 min, 1 h, and then hourly up to the end of the 5-h condition. Options for the breakfast meal consisted of bran-based cereal, fruit salad, ham-and-cheese croissant, and juice (200 ml). Lunch was provided after 5 h (at the end of the condition and after the final FMD measurement) and options included a salad and meat bread roll and juice. A note was made regarding each individual’s meal choice, and these were replicated on the repeat attendance. Timing of medication (if applicable) was standardized to occur with the breakfast meal during each condition.

Participants were instructed to sit upright in a comfortable lounge chair for the duration of the condition and were asked to minimize...
excessive movement. In the SIT condition, participants sat uninterrupted for 5 h, only rising from the chair to void. The SRA condition was similar, but sitting was interrupted every 30 min for 3 min of SRA. The SRA were light-intensity, body weight-resisted exercises undertaken in a standing posture, including half squats, calf raises, and single knee raises with gluteal contractions. These lower-body activities were selected because they are considered to be safe for most individuals; can be implemented easily using one’s own body weight without having to move away from the desk (unlike moving to a wall to perform wall pushups for the upper body, for example); involve large muscle groups (gluteal and quadriceps), thereby maximizing the effect of muscle-mediated glucose uptake and reducing postprandial glucose concentrations; and reduce the likelihood of dislodging the cannula (which may occur during upper body exercise). Each exercise was performed for 20 s at a tempo of 1 repetition every 2 s, 3 times, for a total of 3 min. To ensure appropriate movement standardization, tempo, and correct form, participants mimicked a video recording. Half squats and knee raises were tailored to the range of motion of each participant, where knee/hip angle was between 45° and 90° for half squats/knee raises, assessed during the familiarization session. The alternate trial condition was completed after a minimum of 6 days washout period.

Measurements

Arterial function. Participants were seated for 20 min in a dimly lit, temperature-controlled room (22°C –24°C) before the steady-state recording of FMD. Brachial and superficial femoral artery function (i.e., FMD) were assessed in the seated position using a high-resolution ultrasound machine (Terason t3200, Teratech, Burlington, MA) in conjunction with a 10 MHz multifrequency linear array probe and insonation angle of 60° according to current guidelines (36). Femoral artery FMD was measured in the right leg with the foot placed flat on the floor. A rapid inflatable cuff (SC-12-D, D.E. Hokanson Inc., Bellevue, WA) was placed around the thigh at the distal end of the femur. Once an optimal image of the artery was obtained, a 1-min recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). Resting shear rate was calculated as $4 \times$ velocity in cm per second/diameter in cm, using the average velocity and diameter during the 1-min recording before cuff inflation. The cuff was then inflated for 5 min (≥200 mmHg). After 5 min of inflation, the cuff was released to induce reactive hyperemia. A further 3 min of continuous duplex ultrasound recording was then undertaken to observe the post-deflation diameter profile and peak response. The FMD response is presented as the percentage change from preceding resting diameter to peak dilation. Femoral artery FMD was measured at the start of the condition during the steady-state period (0 h), 30 min, 1 h, 2 h, and 5 h. Brachial FMD, resting blood flow, and resting shear rate were measured before the femoral measurements at the start of each condition and again at 5 h. The arm was extended and supported by a pillow at the level of the heart. The inflatable cuff was placed around the forearm, distal to the cubital fossa, and the artery was imaged following a similar protocol as that described for the femoral FMD measurement. FMD measurements were taken in the same limbs for all trial conditions. Impor-
tantly, all FMD measures occurred before the SRA to avoid measuring any transient effects of the SRA that might have influenced the measurement. We have previously published a reproducibility study utilizing the software and analytical approaches adopted in this experiment (42). Our coefficient of variation for intra-observer reproducibility when repeated analysis was undertaken on the same images by a single observer was 6.7%. Between-visit reproducibility, when repeat scans were collected from the same operator and analyzed in a blinded manner, was 14.7% (this includes day-to-day biological variability).

Analyses of artery diameter and blood velocity were performed offline using automated edge detection and wall tracking software (42) by one scanner, who was blinded to the condition. Resting diameter and peak diameter post cuff release was used to calculate FMD percentage change. Shear rate (s⁻¹), calculated from blood velocity and diameter, was used as an estimate of shear stress on the artery wall. The shear stimulus was calculated as the shear rate area under the curve (AUC) from time of cuff release to peak dilation, using the sum of trapezoids method.

Resting BP. Hourly resting brachial BP was measured in triplicate at 1 min intervals using an automated oscillometric BP monitor (Dinamap Vital Signs Monitor 184465X, HEM-907, Omron, Kyoto, Japan) and an appropriately sized cuff, as per recommended guidelines (8). All measurements were taken in the same arm for both conditions by trained research staff. The first measurement was discarded, and the average of the second two was used in the analysis.

Biochemical analysis. Whole blood was collected into EDTA tubes and centrifuged within 5 min of collection (2,000 revolutions/min for 15 min at 4°C), and the plasma fraction was separated and stored at −80°C. Samples for ET-1, VCAM-1, and ICAM-1 were analyzed by sandwich immunoassay technique using kits from R&D systems (Minneapolis, MN) according to the manufacturer’s instructions. The final product of the ELISA was quantified using a Benchmark Plus Microplate spectrophotometer and standard curve (Bio-Rad Laboratories, Hercules, CA) at 450 nm (14). Plasma nitrate and nitrite were measured as an indirect measurement of total NO using a commercial colorimetric kit from Cayman Chemical Company (Ann Arbor, MI).

Statistical Analysis

The total AUC across the 5-h protocol on each day was calculated for ET-1, nitrates plus nitrates, ICAM-1, and VCAM-1 using the trapezoidal method. We examined within- and between-condition effects using generalized linear mixed models with random intercepts in Stata 14.2 (StataCorp LP). Outcome variables were adjusted for potential covariates to reduce the total variance of the model. All models were adjusted for age, sex, BMI, values at 0 h, and condition order. A condition by time interaction with post hoc comparisons was used to compare individual time points between conditions and within condition relative to 0 h. Post hoc comparisons between time points were adjusted for multiple comparisons using a Šidák correction. Associations between variables were assessed using Spearman’s rank correlation coefficients. Descriptive data are presented as means ± standard deviation (SD), and output from mixed model analyses are presented as marginal means ± standard error where P < 0.05 was considered statistically significant.

RESULTS

Participant Characteristics

Of the 21 participants randomized, 19 completed the study (Fig. 2). The mean ± SD age was 57 ± 12 yr, participants were all overweight or obese (30.6 ± 3.4 kg/m²), and 6 were taking medication for hypertension. The participant characteristics are presented in Table 1.

FMD and Hemodynamics

The hemodynamic and absolute (i.e., unadjusted) FMD data are presented in Table 2. Table 3 displays adjusted data with statistical comparisons. Femoral artery FMD was not significantly different at the 0 h time point between conditions, nor at the 30 min time point, but was significantly lower at 1 and 2 h in the SIT condition compared with SRA (3.3 ± 0.6% vs. 9.3 ± 0.6%, P < 0.001 and 5.4 ± 0.8% vs. 8.9 ± 0.8%, P = 0.007 respectively; Table 3, Fig. 3). Femoral artery FMD at 5 h was not significantly different between conditions (P > 0.05). However, femoral artery FMD averaged across the 5-h day was lower in the SIT condition compared with the SRA condition (5.3 ± 0.6% vs. 8.4 ± 0.5%, respectively, P < 0.001; Fig. 3B). No significant differences between conditions were observed for brachial artery FMD at either the 0 or 5 h time point (P for both > 0.05). Additional adjustment for resting diameter or shear stimulus had no significant impact on the models for femoral or brachial FMD (P > 0.05) and so were not included as covariates.

Mean resting femoral shear rate averaged across 5 h was lower in the SIT condition relative to SRA, although the difference did not reach statistical significance (23.1 ± 9.7/s vs. 45.7 ± 9.6/s, P = 0.052). Mean resting femoral blood flow averaged across 5 h was lower in the SIT condition relative to SRA (1.6 ± 0.4 ml/s vs. 2.3 ± 0.4 ml/s, P = 0.049). No differences in resting systolic or diastolic BP averaged across 5 h were observed between the SIT condition and SRA conditions,117 ± 2 mmHg vs. 115 ± 2 mmHg, P = 0.618 and 69 ± 1 mmHg vs. 71 ± 1 mmHg, P = 0.094, respectively). Mean heart rate averaged over 5 h was significantly lower in the SIT relative to SRA condition (70 ± 2 beats/min vs. 72 ± 2 beats/min, P = 0.003).

Blood Biomarkers

Plasma ET-1 total AUC was 14% higher in the SIT condition relative to SRA (8.1 ± 0.3 pg·hr·ml⁻¹ vs. 7.0 ± 0.3

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Sex, male/female</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Waist circumference, cm</td>
<td>104.3 ± 10.3</td>
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<tr>
<td>Clinic systolic blood pressure, mmHg</td>
<td>121 ± 11</td>
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<tr>
<td>Clinic diastolic blood pressure, mmHg</td>
<td>74 ± 10</td>
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<tr>
<td>Glycated haemoglobin, %</td>
<td>5.4 ± 0.4</td>
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<td>Glycated haemoglobin, mmol/mol</td>
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<td>Fasting glucose, mmol/l</td>
<td>5.2 ± 0.8</td>
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<tr>
<td>Fasting insulin, mmol/l</td>
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<td>HOMA2-IR</td>
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<td>Fasting cholesterol, mmol/l</td>
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<td>Fasting triglycerides, mmol/l</td>
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<td>Fasting HDL cholesterol, mmol/l</td>
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</tr>
<tr>
<td>Fasting LDL cholesterol, mmol/l</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers, n (%)</td>
<td>3 (16)</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitors, n (%)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Calcium channel blockers, n (%)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Diuretic, n (%)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Serotonin reuptake inhibitors, n (%)</td>
<td>2 (11)</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless otherwise stated. HDL, high-density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment index of insulin resistance; LDL, low-density lipoprotein cholesterol.
In addition, a significant but weak negative correlation was observed between ET-1 concentrations at 1, 2, and 5 h were all significantly lower than at 0 h. The plasma nitrate plus nitrite, ICAM-1, and VCAM-1 were not significantly different between conditions, *P < 0.001* within condition vs. 0 h.

**DISCUSSION**

The principal novel finding of this study was that femoral artery function (measured via FMD) was lower in the SIT condition relative to SRA at the 1 and 2 h time points, suggesting that introducing intermittent activity breaks during the first 2 h of prolonged sitting appears to exert the greatest impact on lower limb arterial function. We also found that ET-1 was significantly elevated following 5 h of prolonged sitting, compared with the SRA condition. These findings provide pathophysiological insights into the impact of prolonged uninterrupted sitting on arterial dysfunction and increased CVD risk in overweight/obese adults.

We measured femoral artery FMD at multiple time points across 5 h. The magnitude of the decline in femoral FMD in the SIT condition was greatest after 1 h of uninterrupted sitting. In line with previous work (38, 39), this suggests that the first hour of prolonged sitting could elicit the greatest impact on femoral artery function and corresponds with the immediate diminishing of leg blood flow upon sitting (41). In addition, the relative insulin resistance induced by prolonged sitting after a meal (compared with sitting interrupted by breaks) (9, 13), may be associated with impaired arterial function and blood flow via effects on NO bioavailability and ET-1 (19, 20). It should be noted, however, that our subjects acted as their own controls in this crossover designed study, and they consumed exactly the same standardized breakfast and lunch meal for each condition, as well as evening meal the night before the trial day. Our study was well controlled in this regard, relative to many previous experiments.

We observed a slight rebound effect in femoral FMD at 5 h. However, differences between the SIT and SRA conditions did persist to some extent, even at the 5 h time point (2.6% FMD difference between conditions). There are a number of reasons that may explain this rebound effect, including a gradual increase in venous return to the heart and a reduction in arterial stiffness.

**Table 3. Hemodynamics and adjusted flow-mediated dilation during 5 h of uninterrupted sitting and sitting interrupted with simple resistance activities**

<table>
<thead>
<tr>
<th></th>
<th>Femoral</th>
<th>Brachial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 h</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIT resting blood flow, ml/min</td>
<td>60 ± 8</td>
<td>78 ± 44</td>
</tr>
<tr>
<td>SRA resting blood flow, ml/min</td>
<td>60 ± 8</td>
<td>85 ± 58</td>
</tr>
<tr>
<td>SIT resting shear rate, s⁻¹</td>
<td>15.3 ± 2.6</td>
<td>108.0 ± 84.9</td>
</tr>
<tr>
<td>SRA resting shear rate, s⁻¹</td>
<td>17.2 ± 2.6</td>
<td>105.0 ± 92.6</td>
</tr>
<tr>
<td>SIT resting diameter, mm</td>
<td>6.9 ± 0.02</td>
<td>0.6 ± 0.9</td>
</tr>
<tr>
<td>SRA resting diameter, mm</td>
<td>7.0 ± 0.02</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>SIT FMD, %</td>
<td>7.4 ± 0.7</td>
<td>10.6 ± 4.5</td>
</tr>
<tr>
<td>SRA FMD, %</td>
<td>6.2 ± 0.7</td>
<td>9.9 ± 6.2</td>
</tr>
<tr>
<td><strong>5 h</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIT resting blood flow, ml/min</td>
<td>110 ± 52</td>
<td>99 ± 55</td>
</tr>
<tr>
<td>SRA resting blood flow, ml/min</td>
<td>165 ± 54</td>
<td>216 ± 59</td>
</tr>
<tr>
<td>SIT resting shear rate, s⁻¹</td>
<td>20.8 ± 12.3</td>
<td>29.8 ± 35.9</td>
</tr>
<tr>
<td>SRA resting shear rate, s⁻¹</td>
<td>40.2 ± 8.5</td>
<td>84.8 ± 37.08</td>
</tr>
<tr>
<td>SIT resting diameter, mm</td>
<td>7.1 ± 0.02</td>
<td>7.0 ± 0.02</td>
</tr>
<tr>
<td>SRA resting diameter, mm</td>
<td>7.0 ± 0.02</td>
<td>7.0 ± 0.02</td>
</tr>
<tr>
<td>SIT FMD, %</td>
<td>5.5 ± 0.8**</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>SRA FMD, %</td>
<td>8.9 ± 0.8***</td>
<td>8.3 ± 1.0</td>
</tr>
</tbody>
</table>

Data are marginal mean ± SE. All models adjusted for age, sex, body mass index, treatment order, and multiple comparisons. Time points 30 min, 1 h, 2 h, and 5 h additionally adjusted for value at 0 h. FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA, sitting interrupted with simple resistance activities. **P ≤ 0.01 between conditions, ***P ≤ 0.001 between conditions, †P < 0.05 within condition vs. 0 h.
diminution in terms of shear rate and/or habituation in terms of other mechanisms (oxidative or inflammatory effects). Since we did not assess glyceryl trinitrate (GTN) responses, the possibility also remains that a form of tachyphylaxis may occur in smooth muscle cells to repeated NO exposure.

The reasons for a sitting-induced decline in FMD are not completely understood but may be due to reductions in shear stress and blood flow (41, 42) as well as an increase in blood viscosity (16) in the SIT condition. Although we observed a marked decrease in femoral artery FMD in the SIT condition, we did not observe a concomitant decrease in resting shear rate or blood flow, as observed in some previous studies (14, 32, 38, 39). The participants in these earlier studies were, however, young healthy adults, which is in contrast to the relatively older, overweight to obese, and sedentary population included in the current analysis. It is possible that shear and function relationships differ with age and long-term exposure to CVD risk factors (35).

Femoral FMD was elevated at 1 h and 2 h in the SRA condition relative to the SIT condition. This is similar to previous work in healthy populations, which has demonstrated that interrupting sitting with light-intensity walking (32, 38), moderate-intensity activity breaks (25), or “fidgeting” (27) improves lower limb artery function relative to prolonged uninterrupted sitting. The increase in femoral FMD observed in the current study occurred despite no within-condition increase in resting blood flow or shear rate, which has been reported in previous work (27, 32, 38). This suggests that the improvement in FMD seen with the introduction of intermittent SRA could be due to intrinsic improvement in vessel wall function and not merely an increase in the FMD stimulus. It is pertinent to mention that the FMD measurement occurred before the SRA break in our study, as opposed to immediately following exercise, as in some previous experiments (7, 32). We therefore avoided any transient impact of activity-related blood flow and shear rate changes on FMD. McManus et al. (25) also measured shear rate and FMD before activity breaks and observed no difference in shear rate compared with the sedentary condition. Despite no between-condition differences in resting blood flow at individual time points, we did observe a between-condition difference in the resting blood flow averaged over 5 h. Therefore, we cannot completely rule out the possibility that differences in blood flow and arterial hemodynamics may, in part, explain the differences observed in FMD.

Relative to SRA, ET-1 AUC was elevated across the 5 h of the SIT condition. ET-1 is a potent vasoconstrictor that plays a role in regulating vascular tone and blood flow, especially in older populations (37, 40). There was a weak but significant correlation between ET-1 and resting blood flow and similarly between ET-1 and resting shear rate. Indeed, it has been demonstrated that low levels of shear stress stimulate ET-1 secretion from cultured cells, whereas higher levels of shear stress have an inhibitory effect (22). In addition, sustained increases in shear stress following hand heating have been shown to result in uptake and clearance of arterial ET-1 via endothelin type B (ET_\text{B}) receptors (4). The authors of this study noted that by blocking the ET_\text{B} during hand heating, the decline in arterial ET-1 was prevented and radial artery FMD was reduced, despite sustained increases in shear stress. It is possible that the weak correlation between shear rate and ET-1 and lack of correlation between ET-1 and FMD in the current study may be due to measuring venous rather than arterial concentrations of ET-1. That said, our observation of elevated ET-1 in the SIT condition suggests that in older, sedentary, and at-risk populations, interactions between blood flow, shear stress, and ET-1 may contribute to sitting-induced impairment in arterial function. More work is required to confirm our findings.
In keeping with previous studies (32, 39), we did not observe a significant reduction in brachial FMD in the SRA condition compared with SIT. It should be noted, however, that brachial FMD was only measured at the start and end of each condition, and the possibility remains that transient differences may have occurred throughout the day. This is supported by the time course of effect in the femoral artery FMD, where differences were less apparent at 5 h than they were throughout the intervention period. Furthermore, it is also likely that the SRA had a varied effect on the upper and lower limbs. Alternatively, sitting may differentially impact upper and lower limb artery function, given that in the seated position, the lower limbs are subjected to unique structural and functional milieu (39). Upper body SRA may have been a better stimulus for improvements in brachial FMD; however, given that atherosclerotic lesions develop primarily in the lower limbs (1, 21), the primary focus of this work was the effects of SIT versus SRA on the lower body. Future studies utilizing similar measurement time points for both the brachial and femoral arteries will be necessary to establish or definitively rule out a generalized arterial effect of prolonged sitting (18, 28, 30).

Although we did not observe differences in BP between conditions, an increase in average heart rate across 5 h was evident in the SRA condition relative to SIT. The absence of a BP-lowering impact of activity breaks contrasts with previous evidence demonstrating that regular walking breaks or SRA can lower BP relative to prolonged sitting (12, 23). It should be noted that the resting BP of our study population was relatively low and possibly indicates limited potential for improvement.

**Limitations**

This study was performed in a laboratory setting, and although this environment allows for rigorously controlled trials to be conducted, it does not reveal the impact of prolonged sitting on arterial function in a real-life setting, such as in the workplace or at home. Moreover, we did not assess changes in GTN responses, which limits our findings to changes in arterial function or NO-mediated dilator function, rather than specifically to endothelial function. We adopted a seated “steady-state” period, rather than supine, which may have influenced our data, given the impacts of seated postures on blood flow (32, 41). However, we undertook a resting baseline period in accordance with current guidelines regarding optimal FMD and BP assessment (36) and utilized a seated posture during this phase so as to be consistent with subsequent seated postures throughout the experiment; a change in posture from supine to seated may have equally influenced our data. We only measured brachial FMD at 0 and 5 h, limiting the possibility to examine any transient effects in upper limb FMD across the day. Furthermore, only lower body SRAs were performed. Future work may examine whether similar activities in the upper limb have the same effect on arterial function. Finally, our results cannot be generalized beyond the current study population, and further research is needed to compare the effect of prolonged sitting, compared with breaks in sitting, on arterial function in other high risk groups.

**Conclusion**

This is the first study to show that prolonged, uninterrupted sitting has detrimental effects on arterial function in older, overweight/obese adults at heightened risk of CVD across a 5-h day. We also demonstrate that brief periods of simple resistance exercise effectively mitigates this impairment. Given the ubiquitous high volumes of prolonged sitting in contemporary work and recreational settings, and the associated increased risk of CVD and all-cause mortality (5, 15), short, frequent bouts of light-intensity resistance activities may provide a practical and easily translated approach to maintaining healthy arterial function, particularly within the first 2 h of prolonged sitting. Future work should aim to examine the longer-term impacts of prolonged unbroken sitting, and the impacts of different interventions that interrupt this ubiquitous behavior, on arterial (dys)function in high risk populations.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


Appendix D
Wider impact of the research in this thesis

The wider impact generated by some of the research in this thesis is displayed below. This page gives an overview of the research that featured in news articles, blogs, and on social media. Please click on the DOI to be taken to a summary of this content for each paper. The subsequent pages are some selected media articles (pages 195-206) pertaining to the research and the American Physiological Society “Select Award” certificate for distinction in scholarship, is presented on the final page.


Could too much sitting be bad for our brains?

July 27, 2017 12.10pm AEST

Sitting affects our glucose levels, which affects our brain. Unsplash/Andrew Branch, CC BY-SA
In many aspects of life where we need to use our brain power, we also tend to sit down: at school, at work, sitting exams or concentrating on a crossword. In a new paper, we explore how prolonged sitting may affect the brain’s fuel supply and have a negative impact on brain health.

The brain is a glucose hungry organ. It weighs about 2% of body mass but demands about 20% of our resting energy requirements, which is mostly in the form of glucose, the primary brain fuel. If this energy supply is disrupted it can impair and even damage brain cells. Therefore, the availability of glucose to brain cells may have implications for brain health.

Exposure of the brain to both high glucose levels and low glucose levels can increase the risk of developing dementia. Also, switching between a high and low glucose level, known as glucose variability, is important, as higher glucose variability has been associated with lower cognitive function. This indicates that tight control of glucose is essential for brain health.

**The problem with too much sitting**

Too much sitting can increase the risk of early death. It’s estimated that 60-75 minutes a day of moderate to vigorous intensity exercise is required to offset the increased risk of death associated with more than eight hours a day of sitting.

This is a lot of exercise. At least twice as much as the current minimum recommended amount for adults. So reducing sitting may be an additional health-enhancing strategy.

Multiple studies have demonstrated that reducing and replacing sitting with light intensity walking improves glucose control after food consumption. That means glucose levels that do not spike too high, or dip too low. This may be explained by the way working muscles can use up some of the glucose in our system, helping to keep glucose in the optimal range.
Evidence suggests that when it comes to glucose control, light intensity physical activity spread across the day can be superior to a day in which a single bout of moderate to vigorous exercise is performed in the morning. Even when the total energy expenditure of the light intensity activity is equal to the energy expenditure of the single bout of higher intensity activity.

Improved glucose control may explain some of the health benefits of reducing sitting time, but what about effects on brain function?

High sitting time and brain function

Studies investigating the effects of excessive sitting on brain function have had mixed results. Laboratory studies both support and fail to support the idea that a day of sitting can impair performance on memory related tasks, relative to a day where sitting is interrupted by regular activity breaks.
We need to get into the habit of sitting less anyway. Unsplash/Grovemade, CC BY

Other types of studies that track a large number of people over a number of years suggest an association between higher sitting time and impaired brain function. But drawing conclusions from these studies is made difficult by the multitude of different measurements used. Generally, methods that do not rely on the self-reporting of participants are preferred, because self-reporting isn’t always accurate. This isn’t always practical though.

Apart from directly measuring performance on cognitively demanding tasks, another approach is to measure something that would theoretically support improved brain function. For example, researchers at New Mexico Highlands University demonstrated that foot impact during walking sends pressure waves through blood vessels to increase brain blood flow.

Brain blood flow is involved in regulating supply of glucose to the brain, and this likely has implications for brain health over time. For example, we know decreases in brain blood flow are associated with a more rapid decline in brain function in those with Alzheimer’s disease.

What can we do?

For scientists, the way in which sitting is likely to affect brain function poses a research challenge. Based on the available evidence, it’s more likely that reducing sitting would slow cognitive decline, rather than improve cognitive function.

For everyone else, despite a current lack of conclusive studies linking brain health and sitting, reducing sitting time is already advised to prevent other adverse health outcomes linked to poor glucose control. With improved glucose control in mind, reducing sitting is especially important after meals.
So take a walk after lunch, wash the dishes by hand after dinner and take an active commute to and from work if possible. There is much opportunity to reduce sitting time throughout the day, and therefore much potential to have a positive impact on health.
A morning spurt of exercise can keep the brain sharper for hours afterwards, researchers found. (Mabel Amber/Pixabay)

**BETTER LIFE**

**Morning Exercise Improves Decision-Making in Elderly**

Moderate-intensity exercise has notable impact on cognitive ability, which is good for the elderly and the young

**BY BEL MARRA HEALTH, WWW.BELMARRAHEALTH.COM**
May 13, 2019  Updated: May 13, 2019

A new study out of Australia has found that exercise in the morning can improve cognitive performance such as decision-making across the day compared to prolonged sitting without exercise. “Sedentary behavior is associated with impaired cognition, whereas exercise can acutely improve cognition,” say the authors of the study.

The study titled, ‘Brain Breaks’ led by the Baker Heart and Diabetes Institute and The University of Western Australia shows that there are distinct responses in cognitive performance and physical activity.
The study looked at a little more than 65 males and females aged 55 to 80 years. It examined the effects of light morning exercise on a treadmill with and without brief eight-minute walking breaks during an eight hour day of prolonged sitting. They compared the aspects of cognition and concentration including psychomotor function, visual learning, working memory, attention, and executive function, such as decision-making.

The researchers found that the benefits of exercise on memory and learning comes from a brain-derived neurotrophic growth factor, a protein which plays an essential role in the survival and growth of information-transmitting neurons in the brain. The conclusion was that this protein was elevated for eight hours during both exercise conditions, relative to prolonged sitting.

The study published in the British Journal of Sports Medicine also showed that it wasn't just a little morning exercise that was best for cognition. The best results for short-term memory came with the combination of exercising in the morning along with a brief, but frequent light intensity walking breaks throughout the day.

Physical activity researcher Michael Wheeler says “With an aging population which is looking to live healthier for longer, these studies are critical to people enjoying a productive and satisfying quality of life.”

“This study highlights how relatively simple changes to your daily routine could have a significant benefit to your cognitive health. It also reveals that one day we may be able to do specific types of exercise to enhance specific cognitive skills such as memory or learning.”

Manipulation of Exercise

An interesting note on the study is that Wheeler believes that not all aspects of cognition respond in the same way to a given dose of exercise. He believes it may be possible to manipulate the pattern of exercise throughout the day to optimize specific cognitive outcomes.

The researchers for this study are on a mission to promote ‘brain breaks’ throughout the day as a way to combat the global epidemic of too much sitting. Their ‘brain breaks’ are now being implemented in Australian schools to help refocus and reenergize students throughout the day and help with their decision-making.

This research outlines just how important it is that uninterrupted sitting should be avoided throughout the day. To maintain optimal cognition, moderate-intensity exercise such as a brisk walk is encouraged by doctors for the daily maintenance of brain health.

This article was originally published on Bel Marra Health.
Morning Exercise Helps Keep Blood Flowing to the Brain All Day

April 11, 2019

Rockville, Md. (April 11, 2019)—New research suggests that exercising early in the day protects brain blood flow from some of the negative effects from hours of sitting. The first-of-its-kind study (https://doi.org/10.1152/japplphysiol.00001.2019) is published ahead of print in the Journal of Applied Physiology. The paper was chosen as an APSselect (https://www.physiology.org/journal/apsselect/) article for April.

Previous research has established that prolonged sitting can reduce blood flow throughout the body, including to the brain. Protecting the brain against declines in brain blood flow is important to maintain brain health in aging adults. An international team of researchers studied a group of overweight adults between the ages of 55 and 80 to measure the combined effects of exercise and sedentary behavior session on brain blood flow.

The volunteers participated in three different trials separated by at least six days. The order in which each participant completed each trial was random. The trials were:

- The volunteers sat uninterrupted for eight hours.
- The volunteers sat for one hour, walked on a treadmill at medium intensity for 30 minutes and then sat for 6.5 hours.
- The volunteers sat for one hour, walked at medium intensity for 30 minutes and sat for 6.5 hours, but also completed three-minute, light-intensity walks every 30 minutes.

The research team measured blood pressure, heart rate and blood flow to the middle cerebral artery (MCA) before the participants ate breakfast (before they sat for the initial hour) and other times during long sitting periods. The MCA is one of the main arteries that supplies blood to the largest and uppermost section of the brain (cerebrum).

In all trials, blood flow in the MCA was highest at the beginning of the day. Throughout the morning until lunchtime, blood flow dropped by about 20 percent when the volunteers sat continuously, and it stayed at that reduced rate for the rest of the day. But in both exercise trials, blood flow increased again in the afternoon instead of remaining lower. When the volunteers took frequent exercise breaks, blood flow increased earlier in the day than when they exercised for 30 minutes and then sat for the rest of the day.
“Our results show that the majority of the benefit, in terms of brain blood flow, is coming from the morning bout of exercise. If people can fit in a morning bout of exercise before sitting for the rest of the day, they will be protected to a degree from the effects of prolonged sitting,” wrote Michael Wheeler of The University of Western Australia, and first author of the study.

Read the full article, “Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older adults published ahead of print in the Journal of Applied Physiology. It is highlighted as one of this month’s “best of the best” as part of the American Physiological Society's APSselect program. Read all of this month’s selected research articles.

NOTE TO JOURNALISTS: To schedule an interview with a member of the research team, please contact the APS Communications Office or 301-634-7314. Find more research highlights in the APS Press Room.

Physiology is the study of how molecules, cells, tissues and organs function in health and disease. Established in 1887, the American Physiological Society (APS) was the rst U.S. society in the biomedical sciences eld. The Society represents more than 10,000 members and publishes 15 journals with a worldwide readership.
The American Physiological Society

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For distinction in scholarship in the Journal of Applied Physiology for the article

Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in older adults

DOI: 10.1152/japplphysiol.00001.2019

Linda C. Samuelson, Editor-in-Chief, APSselect

Douglas Eaton, Associate Editor, APSselect