PHYSICAL INACTIVITY AND GLYCEMIC CONTROL IN THE ELDERLY

THE INFLUENCE OF REDUCED DAILY AMBULATION ON GLYCEMIC CONTROL, BODY COMPOSITION AND PHYSICAL FUNCTION

IN OLDER ADULTS

By

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the

Requirements for the Degree Master of Science  
  
  
  
  
  
  
  
  
  
McMaster University

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McMaster University MASTER OF SCIENCE (2015) Hamilton, Ontario (Kinesiology)

TITLE: the influence of reduced daily ambulation on glycemic control, skeletal muscle and physical function in older adults

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NUMBER OF PAGES: ix, 71

**LAY ABSTRACT**

Periods of physical inactivity such as hospitalizations decrease daily steps for older adults and this inactivity can cause losses of muscle, strength, and symptoms of diabetes. It was unknown if by simply returning to normal physical activity older persons could ‘reverse’ the consequences of step-reduction so we conducted a study involving two weeks of step-reduction and two weeks of recovery. While there was no change in strength or muscle mass, we found that when older adults reduced their daily steps to fewer than 1000/day, after two weeks they became ‘resistant’ to insulin, a hormone that helps control blood sugar and is connected to the development of type II diabetes. Although these older adults resumed normal step-count levels in the recovery phase, they did not recover their insulin sensitivity such that two weeks of normal daily activity was not sufficient to overcome the consequences of two weeks of inactivity.

**ABSTRACT**

Short-term physical inactivity in older adults has been shown to cause muscular atrophy and impaired glycemic control, however, the ability to recover remains unknown. We aimed to determine the impact of step-reduction (SR) on older adults and if they could recover simply by returning to habitual activity. Ten older adults (6 men, 4 women, 69 ± 3 yr) completed 7d of normal baseline activity (BL), subsequently underwent SR by 86 ± 9% (8568 ± 3741 to 973 ± 76 steps/d; p<0.001) for 14d and then returned to 8383 ± 4513 steps/d for 14d (RC). During an oral glucose tolerance test (OGTT), SR resulted in elevated plasma glucose concentration ([G]) area under the curve (AUC; 325 ± 126 to 375 ± 137, p = 0.13), maximum [G] (10.2 ± 2.4 to 11.9 ± 1.7 mM, p = 0.027) and 2-hr [G] (7.9 ± 1.3 to 9.1 ± 1.1mM, p = 0.085), while all [G] indices returned to BL after RC. However, Matsuda insulin sensitivity index was reduced (3.5 ± 0.3 to 2.7 ± 0.7, p < 0.001) and homeostatic model assessment of insulin resistance was elevated (2.8 ± 0.3 to 3.6 ± 0.7, p = 0.02) with SR, remaining different than BL after RC (p < 0.005). During free-living conditions, 3-hr post-prandial [G] (PPG) AUC and peak PPG increased following SR (p > 0.05), returning to BL with RC. Body composition and physical function remained unchanged with SR. These results show that periods of physical inactivity, characterized by reduced daily stepping, do not present detectable changes in body composition or physical function yet result in reduced glycemic control in older adults. While elevations in blood [G] are abolished with 14d of normal physical activity, our findings suggest that the SR-associated reductions in insulin sensitivity are not normalized as quickly.

**ACKNOWLEDGEMENTS**

I would like to first thank Dr. Phillips for giving me the opportunity to join the lab as an undergrad and get involved with a field I have become very passionate about. It has been an unforgettable experience and what I have learned under your leadership will never be forgotten as I move forward with my career. I also greatly appreciate the guidance of my committee, Dr. MacDonald and Dr. Ljubicic, who have helped me get the most out of this opportunity.

To the members of the Phillips lab, you have been an outstanding group of friends and colleagues over the past three years. Chris, even though I still can’t understand you, you’ve been a great mentor. Todd and Tracy, your lab support always kept our wheels in motion. Tanner, thanks for keeping the study running strong.

Sara and Skelly, I couldn’t be happier to have spent all my time with you, thanks for the fun with friends. Thank you to all of the friends I have made along the way as well, it has been the best part of grad school.

Kristen, you have been here every step of the way and always motivate me to be my best. Thank you for your endless love and support; you will always be my rock.

Mom, Dad, Brian, Katie and Gibson, I wouldn’t be here without you. Thank you for everything you have given me and allowed me to achieve.

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**LIST OF SYMBOLS AND ABBREVIATIONS**

∆ change

30CS 30-second chair stand

ADL activities of daily living

ALM appendicular lean mass

CGMS continuous glucose monitoring system

CVD cardiovascular disease

DEE daily energy expenditure

DXA dual-energy x-ray absorptiometry

FFM fat-free mass

FM fat mass

HOMA-IR homeostatic model assessment of insulin resistance

HR heart rate

IAFM intra-abdominal fat mass

ISI insulin sensitivity index

ISO-MVC isometric maximal voluntary contractions

LM lean tissue mass

MPB muscle protein breakdown

MPS muscle protein synthesis

MRI magnetic resonance imaging

NS non-significant

OGTT oral glucose tolerance test

PA physical activity

PPG post-prandial glucose

RTD rate of torque development

RWT Rockport walk test

SR step-reduction

T2DM type 2 diabetes mellitus

TUG timed up-and-go

VAT visceral adipose tissue

**DECLARATION OF ACADEMIC ACHIEVEMENT**

MT von Allmen and SM Phillips were responsible for study design. MT von Allmen obtained ethical approval. MT von Allmen, C McGlory, T Stokes, and T Rerecich were responsible for participant recruitment. MT von Allmen, C McGlory, T Stokes, T Rerecich and SM Phillips were responsible for data collection. MT von Allmen analyzed and collected research data.

# INTRODUCTION

## AGING AND CO-MORBID DISEASES

Older adults (>65yr) currently comprise slightly more than 14% of the Canadian population, however, they account for approximately 45% of spending on healthcare services1. Current estimates project the world population of adults over the age of 65 will triple, reaching ~1.5 billion people by the year 20502. The impending issues that this demographic shift poses for global healthcare are undeniable. Ultimately, increasingly prevalent chronic illnesses in older adults such as cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), osteoporosis and fractures will demand extensive resources and will come to represent a disproportionate amount of healthcare spending1. All of the aforementioned chronic conditions are exacerbated by many of the major underlying health risks of aging; however, one of the more prominent contributors to increasing chronic disease risk is the age-related loss of muscle mass and strength – sarcopenia.

### Sarcopenia

Sarcopenia was a term coined in 1989 by Irwin Rosenberg to draw attention to the issue of common and, oftentimes, severe muscle atrophy in aging adults3, since which a wealth of research has been devoted to the epidemiology, physiology and clinical relevance of sarcopenic elderly. Sarcopenia encompasses the age-related loss of lean muscle mass, known as myopenia, and muscle function, known as dynapenia. Muscle mass is at its peak in the third decade of life, however, progressive muscle mass losses begin, for most, in the fifth decade of life and proceeds at ~0.6-1.2% annually. Strength, in contrast drops more rapidly at ~1.5-3.5% per year in adults up to 75 years of age 4–6. It is important to understand that sarcopenia is a non-pathological condition and quite prevalent in otherwise healthy, community-dwelling older adults7–10; however, beyond the functional role of muscle, the metabolic roles mean a lower muscle mass is a predisposing factor for chronic disease. Current estimates project that anywhere from 20-45% of elderly adults are sarcopenic based on a definition developed by Baumgartner et al.7, which is having a relative appendicular muscle mass divided by height less than two standard deviations below sex-matched younger counterparts. Nearly half of all adults over the age of 80 are considered sarcopenic7,8,10.

### Functional impairments

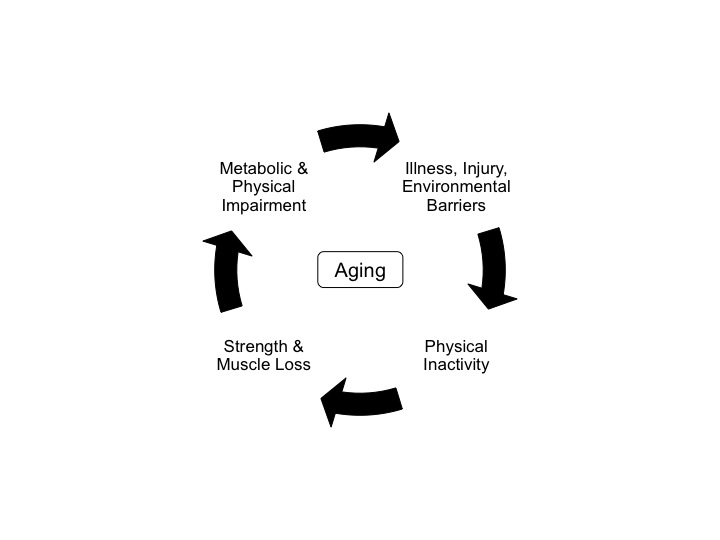
Functional impairments of elderly adults with sarcopenia have a considerable impact on their health and quality of life. The basic function of skeletal muscle is to produce force, which under defined conditions is proportional to muscle cross-sectional area11. Thus, sarcopenic adults who have experienced significant declines in muscle mass while aging lose strength as well6,12. However, muscle strength is lost at an even more precipitous rate compared to mass 4,6, implicating and age-related decline in muscle quality (force per muscle area) as a process in aging. In this regard, results from a large cross-sectional study showed that amongst 4505 older adults those deemed to be moderately and severely sarcopenic, by lower levels of relative muscle mass, more often reported difficulty crouching or kneeling, balancing or lifting and carrying 10 pounds, independent of age and race13. Despite the results of specific tests in this study13, a relationship between functional impairment and disability was only found for those considered to be severely sarcopenic (having 30% lower muscle mass compared to normal younger reference populations); however, once muscle loss progressed this far, 7% of older men and 10% of older women who were severely sarcopenic had a 2-3-fold greater likelihood of experiencing functional declines and disability. Therefore, a significant number of sarcopenic older adults often suffer from disability and impairments in completion of activities of daily living (ADL) 7,10,13–15. This physical inability translates into poor mobility and manifests as impairments in basic skills important for everyday activities. The result is an inability to, for example, use the bathroom or shop independently, and thus a diminishing quality of life for older adults.

Dynapenia can ultimately result in a loss of independence, mobility and quality of life for older adults, however, it has also been identified as an independent risk factor for mortality whereas reductions in relative muscle mass alone have not16. Newman et al.16 examined the mortality rates of 2292 older adults over six years and found robust correlations between grip and quadriceps strength and risk of mortality. Longitudinal studies following aging adults and assessing the sarcopenic elderly have now begun monitoring cut-points of functional capacity based on strength variation across cohorts of older adults16–19. Although there is currently no evidence to suggest threshold levels of skeletal muscle strength in the elderly that would predict functional decline, consequences such as functional impairment, inability to complete ADL, mobility dependence and mortality all emphasize the importance of understanding sarcopenia. In order to create clinically relevant interventions for age-related muscle loss, the underlying causes of sarcopenia and declining strength warrant investigation.

### Metabolic impairments

It is well understood that maintaining muscle mass with good oxidative capacity is critical to preserve metabolic skeletal muscle function as a site for glucose disposal20, and lipid oxidation21 as well as the tissue as a whole being a major contributor to basal metabolic rate22. As a result, maintaining sufficient muscle mass with aging is of primary concern as declines in muscle mass are often associated with chronic diseases such as obesity, T2DM, osteoporosis, CVD and cancer23–25. Despite this theoretical link between aging, sarcopenia and chronic disease, studies of sarcopenic populations provide conflicting evidence for age-related losses in lean mass and the prevalence of chronic illness. For example, reductions in resting metabolic rate were seen with aging in institutionalized elderly adults26. Nonetheless, an 8-year longitudinal study of older adults found no link between sarcopenia and the development of cardiovascular disease27. A growing body of work provides contrasting evidence for a relationship between declining muscle mass with age and mortality risk16,28–30. Although the aetiology of sarcopenia has been the focus of intense research, the role of muscle mass losses in the development of co-morbidities of aging and mortality remains unclear.

Aging is a primary risk factor for the development of insulin resistance and T2DM31. Estimates are that ~22% of men and 15% of women over 65 years of age in Canada are diabetic32. Older adults exhibit reduced whole-body insulin sensitivity compared with younger adults 33. The physiological mechanisms underpinning this state are likely an impairment in peripheral (i.e., skeletal muscle) glucose uptake and oxidation related to inflammation status, visceral adipose tissue accumulation, mitochondrial dysfunction and a reduction in skeletal muscle mass34. Sarcopenia is also a contributing factor to the morbidity risk of T2DM, 10,13 and the consequences of sarcopenia are augmented with the additive effects of obesity. This scenario of so-called ‘sarcopenic obesity’ estimated to affect 2-10% of older adults 35,36. A contributing factor to the development of T2DM in older adults is also a reduction in habitual physical activity (PA)37. There are likely causes of reduced PA and/or increased sedentary time in older adults that are not present in younger persons. For example, older people are more susceptible to reduced PA during convalescence from illness, chronic disease (osteoarthritis and rheumatoid arthritis), or mobility rehabilitation after injury, and that resultant increase in risk is also likely greater for older adults. In addition, as opposed to younger adults, the PA levels of the elderly are likely affected more by environmental factors such as cold and icy conditions during winter months or hot and humid conditions during the summer months, which could limit time spent walking outdoors and engaging in PA. These influences on the prevalence of inactivity for older adults can lead to a vicious cycle of metabolic impairment, disease 24, disability, functional inability17 and falls38 (Figure 1).

****

**Figure 1.** Vicious cycle of inactivity commonly occurring with advancing age.

## AETIOLOGY OF SARCOPENIA

Sarcopenia is unavoidable39, however, it does seem that there is a plasticity of skeletal muscle such that the rate of loss of muscle mass could be altered even in older (i.e., >75 years) adulthood. This means there is a hypothetical means to attenuate the trajectory of sarcopenia, with increased PA and loading (resistance) exercise potentially attenuating the rate of loss and inactivity, while the lack of these factors (i.e., unloading, physical inactivity) would accelerate muscle loss. Current approximations of annual declines in muscle mass (~0.6-1.2% annually) and muscle strength (~1.5-3.5% annually) with advancing age are based on population estimates, but such estimates would encompass a range of individual rates. We now know, for example, that the loss of skeletal muscle mass and function with age is not always linear but can be transiently accelerated with periods of muscular disuse for some older adults (Figure 2)40. Evidence suggests that physical inactivity/unloading of skeletal muscle is a cause of reductions in skeletal muscle mass with aging. Periods of inactivity and unloading transiently reduce muscle protein synthesis (MPS)41,42 resulting in a rapid decline in muscle mass. Thus, in an aging person who frequently undergoes periods of inactivity, sarcopenic muscle loss is punctuated by transient accelerated loss of muscle mass and function (Figure 2: disuse-accompanied).

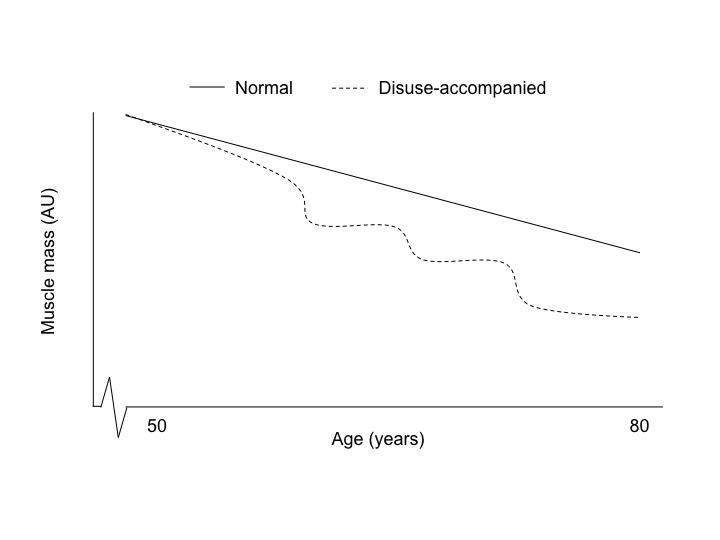


Figure 2. Theoretical loss of muscle mass in arbitrary units based on population estimates compared to accelerated sarcopenia punctuated by periods of inactivity due primarily to illness and injury.

The risks of inactivity with aging are clear. Bortz43 was the first to propose the idea that disuse is a modifiable factor accelerating the aging process, citing the phenotypic change exhibited by his own leg after casting and showcasing the similarities between disuse and traditional aging. For example, three weeks of bed rest results in equivalent decrements in V̇O2peak as 40 years of aging44. This type of disuse period, due to hospitalization, is more common with aging and as such these disuse events punctuate the lives of elderly individuals.

Asher45 first recognized the iatrogenic effects of physical inactivity prescription by healthcare providers in hospitals, which he saw as a prevalent occurrence in older adults with increasing disability46. Acute illnesses such as influenza often leave older adults convalescing in hospital for more than eight days47, while laparoscopic surgeries, procedures touted for their more rapid return to activity, commonly require (in older persons) 3-5 days of hospitalization with an average of one additional week of convalescence at home before returning to normal activity48. Of utmost concern though are the consequences of such periods of inactivity on the risk for falls amongst older adults. In Canada, between 20-30% of older adults report falling each year, making it the most common reason for hospitalization amongst the elderly. A fall often requires a hospital stay of 10-12 days49 and such periods of muscle unloading with bed rest or limb casting in older adults can have almost irreversible consequences.

Despite the inherent risks of inactivity, which pose a greater risk for the elderly compared to younger adults, little research has actually been conducted on disuse with older adults (reviewed by Wall et al.50). Over 28 days, bed rest results in muscle mass loss at a rate of 0.2%/day51; however, this loss primarily occurs early during the unloading period since the rate of loss of muscle with 7-10 days of bed rest is 0.5-0.6% per day52–54. Thus, it seems evident that the majority of muscular atrophy occurs within an the early stages of disuse, possibly the first 10 days50. It has been also been shown that leg strength losses with 10-14 days of bed rest are 2-3x more precipitous than the resultant muscular atrophy for older adults52,54–56. Such losses of muscle mass and strength, seen in only a 10-day period of muscle unloading, are equivalent to those seen annually with normal aging and so an appropriate formula would be that 10 days of bed rest is equivalent to 1 year of ‘normal’ age-related muscle loss. This observation demonstrates how muscular disuse in the elderly can markedly accelerate sarcopenia, or at least act as a compounding factor in the decline in muscle mass and strength. Therefore, focusing research efforts on the mechanisms underpinning the loss in muscle mass and strength over such short-term periods of disuse would yield relevant data and insight into sarcopenic mechanisms in aging persons.

What likely underpins the muscular atrophy seen with muscle disuse is a blunted sensitivity to the anabolic properties of dietary protein, termed ‘anabolic resistance’. Glover et al.57 found that amino acid infusion stimulated a greater rise in MPS in a normally active limb (control leg) compared to an immobilized leg. Older adults, whose skeletal muscle already possesses an inherent anabolic resistance58, also exhibit a blunted feeding-induced MPS response with muscle disuse anywhere from 21-40%51–54; however, an unresolved question is whether or not sexual dimorphism exists in this response. While most research examining short-term unloading in younger adults39,57,59,60 suggests roughly equal atrophy between males and females61, there are no studies looking at disuse atrophy in older women. Older women may, in fact, be more susceptible the consequences of unloading due to anabolic resistance60,62 beyond that which is observed with unloading57 and normally as a consequence of aging63. A number of studies51–56,64,65 have reported data from models of complete immobilization of a limb or bed-rest and as such are models of *complete* inactivity and recapitulate recovery from fracture and illness with no ambulation. Thus, as models bed rest and limb immobilization do not represent what I view as a more common form of muscle disuse/inactivity in older adults that would occur during illness that allows ambulation, albeit at a reduced level. I propose that an accurate model to capture the more frequent nature of immobility intermittently present in the years after onset of sarcopenia would simply be a period of reduced daily steps.

## STEP-REDUCTION

Relative reductions in daily ambulation result in seemingly benign periods of inactivity. In the elderly, reductions in ambulation can be the result of anything from inclement weather (e.g. winter in Canada) to a brief period of hospitalization. Hospital-associated deconditioning is common for older adults as they are oftentimes told to remain in bed for periods of time during hospitalization, which is often a prescription without reason66. Thus a number of hospitalized older adults remain immobile during a hospital stay despite possessing the capacity to remain ambulatory 66,67. Studies of hospitalized older patients indicate averages of 45-60 minutes of standing or walking per day67,68 resulting in approximately 500-700 steps per day69–72. Periods of limited ambulation such as these are, much like bed-rest and aging, associated with functional declines in ADL and increased mortality even after controlling for comorbidies66,73,74.

Despite being a moderate, and seemingly benign form of inactivity, step-reduction (SR) results in a potent metabolic disturbance for both young and older adults. As a result, the SR model is now being employed in experimental research as a means of mimicking periods of reduced PA (Table 1). Studies looking at SR generally do so with the purpose of investigating the consequences associated with walking less than what is recommended in public health guidelines, reflecting general physical inactivity75–81, or to reflect the periods of reduced mobility with acute disability such as might be seen in hospitalized older persons82. The implications of SR outside of the hospital setting are profound, with physically inactive older adults twice as likely to be sarcopenic compared to those who are even moderately active13. A recent clinical trial investigating mobility in older men found that those who exhibited the lowest frequency and extent of mobility had a non-cancer mortality rate of ~41% compared to a rate of ~3% in the most mobile group over four years of follow-up83. It is now a widely accepted guideline that adults of all ages should be trying to achieve 10000 steps daily84, however, it is estimated that the average American walks ~5000 steps per day85 and as a consequence they are at a greater risk for developing chronic metabolic disease86. A consensus opinion is that taking less than 5000 steps a day is a threshold level for classification of someone as having a sedentary lifestyle index that may be associated with several physiologic consequences76. Implementation of models of reduced PA (fewer than 5000 steps per day) in younger men have ranged in duration from 377 to 587 to 7 days75 and have resulted in impaired glucose control, insulin resistance and altered adipose tissue gene expression in75,77. With a further reduction in steps to less than 1500 per day for two weeks, near the lower extreme of daily steps for many adults86, younger adults experienced: a reduction in peripheral (muscle) insulin sensitivity, a 7% reduction in V̇O2peak, a 7% increase in visceral fat mass and a 2.8% loss of leg lean mass78–80. This SR intervention has been repeated in elderly men and despite evidence suggesting that older adults atrophy slower than younger individuals, as a result of muscle unloading56, older men lose a comparable amount of muscle mass compared to younger men with SR78,82. In fact, there is evidence to suggest that older men are more prone to losses in strength with muscle offloading than younger men64,88, and over an acute period of ≤1500 SR elderly subjects lost approximately 7% of knee extensor strength89. In addition, older men and women exhibited a 21% increase in fasted and 43% increase in fed insulin resistance after a two weeks of acute SR82.

The spectrum of consequences associated with acute periods of physical inactivity still has not been fully characterized. Evidence suggests that transient reductions in ambulation may cause deleterious effects on vascular structure/function and microvascular function90–92, which are implicated in the development of CVD and T2DM. The impact of SR on the ability to perform ADL has not been determined, although it may be an important factor in the development of disability and loss of independence for older adults. It may be that during these transient periods of inactivity there can be significant age-related losses of muscle mass and function and possibly the development of T2DM in older adults. Of even greater concern is that older adults have greater difficulty recovering from overt muscle disuse in comparison to younger adults56.

## RECOVERY FROM MUSCLE DISUSE IN OLDER ADULTS

The recovery of atrophic muscle loss following disuse takes much longer than the time taken to lose muscle regardless of age56. In older persons, evidence regarding the potential for recovery, not just of lost muscle mass and strength, but also of the decline in muscle metabolic health, following muscular disuse, is scant. Nonetheless, it seems as though younger adults recover their atrophy-induced losses of muscle mass more rapidly than older adults56,79. Clinical evidence shows that 25-50% of hospitalized elderly develop functional impairments in ADL over short-term (~6-8 days) hospitalizations74,93. One year later, nearly a third of these patients have not fully recovered their functional abilities94,95 and it may take up to 30 months after hospital discharge to completely recover, if there is recovery at all95. In addition, 15% of those elderly who are discharged with no functional changes show reduced physical function at follow-up one year later94,95. Suetta et al.56 immobilized the limbs of younger and older men for 14 days and showed that older men only regained 63% of their lost muscle mass despite undertaking four weeks of intensive resistance exercise (i.e., not standard rehabilitation) following the immobilization56. Older subjects from the same study were also shown not to have fully recovered maximal strength nor muscle rate of force development after the resistance exercise recovery protocol64. Extending some of the findings from overt immobilization in older adults, showing incomplete recovery not only of muscle mass56 but also of strength56, it is possible that similar results may be seen in recovery from a period of reduced steps.

**Table 1.** Summary of available studies involving step-reduction protocols.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Study | Age (mean age) | Sex (M/W) | Steps  (per day; % ↓) | Duration (days) | % ∆ FFM | % ∆ strength | Glycemic control | Other |
| Olsen et al. (2008)1 | Young  (27) | M | <1500  (78) | 7 | - | - | ↑ 53% insulin AUC (OGTT) | - |
| 14 | - | - | ↑ 61% insulin AUC (OGTT) | - |
| 22 | - | - | ↑ 79% insulin AUC (OGTT) | - |
| Olsen et al. (2008)2 | Young  (24) | M | <1500  (87) | 14 | ↓ 2.2 (body) | - | ↑ 57% insulin AUC (OGTT) | ↑ 7% IAFM  ↑ 21% plasma TG (OFTT) |
| Krogh-Madsen et al. (2010) | Young  (24) | M | <1500  (>85) | 14 | ↓ 2.8 (leg) | - | ↓ 17% GIR (H-E clamp) | ↓ 7% rel. V̇O2max |
| Mikus et al. (2011) | Young  (29) | M/W | <5000  (67) | 3 | - | - | ↑30-50% ∆PPG, ↑ 26% peak PPG (CGMS)  ↓30.5% ISI, ↑ 49.4% HOMA-IR (OGTT) | - |
| Knudsen et al. (2012)\* | Young  (24) | M | <1500  (85) | 14 | ↓ 0.5 (body, *NS*) | - | ↓ 29% ISI (NS), ↑ 37% insulin AUC (OGTT)  ↓ 44% GIR, ↓ 28% glucose Rd (H-E clamp) | ↑ 50% VAT (cm2)  ↓ 3% rel. V̇O2peak |
| Walhin et al. (2013)\* | Young  (25) | M | <4000  (71) | 7 | ↑ 4.5 (body) | - | ↓ 48% ISI, ↑ 95% HOMA-IR (OGTT) | - |
| Krogh-Madsen et al. (2014)\* | Young  (22) | M | <1500  (84) | 14 | ND | - | ↑ 10% [G]24h, ↑ 9% [G]max  (CGMS) | ↑ 30% VAT (cm3)  ↓ 6% rel. V̇O2peak |
| Reynolds et al. (2014) | Young  (24) | M | <5000  (>50) | 5 | - | - | ↓ 29% ISI (OGTT)  ↑ 9% peak PPG (CGMS) | - |
|  |  |  |  |  |  |  |  |  |
| Breen et al. (2013) | Elderly  (72) | M/W | <1500  (76) | 14 | ↓ 3.7 (leg) | ↑ 1.5 (*NS)* | ↑ 9% glucose AUC, ↑ 12% insulin AUC,  ↓ 43% ISI, ↑ 12% HOMA-IR (OGTT) | ↓ 26% MPS (%.h-1)  ↑ 3% trunk FM% |
| Devries et al. (unpublished)† | Elderly  (70) | M | <1500  (80) | 14 | ↓ 1.4 (leg) | ↓ 7.0 | - | - |

All values based on presented averages from published studies; no difference (ND), post-prandial glucose (PPG), area under curve (AUC), oral glucose tolerance test (OGTT), continuous glucose monitor (CGMS), oral fat tolerance test (OFTT), insulin sensitivity index (ISI), homeostatic model assessment of insulin resistance (HOMA-IR), intra-abdominal fat mas (IAFM), visceral adipose tissue (VAT), fat mass (FM), muscle protein synthesis (MPS); 1 sub-study 1, 2 sub-study 2, \* study involved hypercaloric diet, † values taken from unpublished, grouped raw data

It is possible to translate the knowledge on reduced steps to recapitulate a likely clinical scenario for an older person. Oftentimes older adults contract influenza that is serious enough to result in hospitalization for up to 8 days96, which would involve bed rest with minimal ambulation. On discharge from the hospital, patients often suffer from post-influenza asthenia characterized by lassitude and malaise97. This general lack of energy can possibly linger for weeks, meaning the days spent recuperating involve minimal PA and daily walking. Thus the flu-induced reduction in ambulation and subsequent muscular deconditioning can have a profound effect on metabolic health and could also result in muscle atrophy that is difficult, if not impossible, to recover. Importantly, illnesses, such as the flu, are not typically followed up with any form of musculoskeletal rehabilitation and as such, bouts of influenza may beget impaired functional status for older adults even months later98. An important message stemming from even short-term hospitalization in older adults is that the consequences may reach far beyond simply the treatment of the reason for hospitalization, but also lead to atrophy and deconditioning, which may well be permanent in older persons. Remarkably, the likelihood of early hospital readmission after acute illness in older adults is inversely related to the number of steps taken daily in hospital and upon dismissal99. Specific ambulatory ‘recovery’ protocols do not currently exist for inpatients or outpatients recovery despite evidence of the importance of mobility while convalescing from acute illness or injury. A majority of patients are discharged to their home without any additional support prescribed to them 67 while experiencing reductions in performance in functional ability tasks at levels well below normal73,94. This means rehabilitation of skeletal muscle mass and function often incomplete with potentially permanent losses of muscle mass and strength.

## SIGNIFICANCE

Periods of physical inactivity contribute to a deleterious cycle resulting in accelerated age-related loss of muscle mass and strength. Decreased PA and daily steps accompanying sarcopenia100 have implications for development of disease24, changes in functional ability, and even mortality101. In particular, the association between strength and falls in older adults 102, typically considered a watershed moment for loss of independent mobility103, play a clear role in this cycle (Figure 2). I propose that repeated cycles of disuse and loss in older adults increases the risk of a loss of independence and mobility and lowers their quality of life. Conservative estimates of the expenditures associated with sarcopenic disability for the American healthcare system were $18.5 billion annually and these estimates would be expected to be increased with the aging of the US population104. Falls already account for the majority of hospitalizations in older Canadians (65 years and above) and nearly 30% of fall-related injuries occurring in Canadians involve people between the ages of 65-6949. By characterizing factors that compromise the mass and function of skeletal muscle, an organ with an important role in metabolism and physical function, we can generate information that may allow for strategies to promote successful aging and more efficient healthcare provision.

## STATEMENT OF RESEARCH QUESTION AND HYPOTHESIS

I propose that short periods of disuse arising from an acute reduction in daily steps, common in older adults experiencing increasingly frequent hospitalization with age, will transiently accelerate muscle loss at a rate greater than average sarcopenic muscle loss (~0.6-1.2% annually). In addition, periods of reduced steps will result, in older persons, in an inability to recover metabolic function. Currently, there is also a lack of information on the potentially important differences between older men and women that may occur with aging and as a result of inactivity. Therefore, a further aim of this study was to investigate the effects of imposed inactivity and a subsequent return to normal activity in both older men and women. Specifically, I studied the impact of two-weeks of, and recovery from, reduced daily ambulation (fewer than 1000 steps per day) on insulin sensitivity, muscle mass and physical function in older men and women. I hypothesized that older men and women would experience declines in muscle mass, strength and physical function, as well as impairments in glycemic control and insulin sensitivity as a result of two weeks of SR. Furthermore, I hypothesized that older adults will not be able to fully recover from acute physical inactivity with two weeks of resumption of normal activity patterns.

# METHODS

### Participants

Ten healthy, older adults (6 men, 4 women, 69 ± 3 yrs, 1.67 ± 0.08 m, 75.8 ± 13.3 kg; means±SD) were recruited from Hamilton and the surrounding area through advertisements in local newspapers. All participants were non-smokers between the ages of 64 and 75 years old. Exclusion criteria were: presence of chronic health conditions such as heart disease or diabetes, regularly taking a statin or non-steroidal anti-inflammatory medication, use of assistive walking devices, and a minimum habitual step count of 3000 steps per day. Habitual daily steps were determined through the use of hip-placed pedometer data (Piezo SC-StepX Health System, StepsCount, Deep River, ON, Canada) collected over a seven-day period at least one week prior to the intervention (Figure 3). Sample and data collection was completed over the fall, winter and spring seasons (November 2014 – April 2015).

### Study protocol

In a repeated measures design, participants were involved in a five-week protocol with three distinct phases differing in levels of daily steps (Figure 3). During a baseline (BL) period, participants were provided with a pedometer seven days prior to the SR period and instructed to record their daily steps while completing habitual activity steps, PA and exercise for one week. Pedometers were placed on the waistband of clothing at the hip, vertically above the dominant knee. This seven-day period also included the subjects’ wearing of a SenseWear armband accelerometer (BodyMedia, Pittsburgh, PA, USA) placed on the upper arm on the non-dominant *triceps brachii*. The SenseWear armband was used to internally validate (the data were not available to the research participants) steps taken, and to objectively estimate caloric expenditure as well as duration of activity.

During the SR phase of the research, participants were instructed to reduce their daily step-count to less than 1000 steps per day, and were encouraged to remain as inactive as possible within their step-count limit. This reduction in daily ambulatory activity lasted for 14 days and was monitored and reinforced by frequent contact with the participant, as well as the use of the pedometer, and SenseWear armband. For the subsequent 14 days participants entered into a recovery phase (RC) during which they were instructed to return to their habitual step-count and levels of PA.

Participants reported to the Exercise Metabolism Research Group at McMaster University on 11 occasions. On the first occasion, participants were screened for eligibility and, if deemed eligible, provided written informed consent to proceed as a research participant. The research protocol was approved by the Hamilton Integrated Research Ethics Board (REB #11-267) and complied with all standards as set out in the Canadian tri-council policy on human research (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS\_2\_FINAL\_Web.pdf). The trial was registered as clinical trial NCT02347137. The second and third visits occurred the day prior to and on the first day of the study protocol. These visits involved repeated familiarization to study measures: isometric maximal voluntary contractions (ISO-MVC), the 30-second chair stand task (30CS) and the timed up-and-go task (TUG). The remainder of the visits were for the performance of study measures and separated into three days (Figure 4). Participants each reported to McMaster University for insertion of the continuous glucose monitor system (CGMS; visits 3, 6 and 9) at least 36 hours prior to the oral glucose tolerance test (OGTT). The OGTT was performed midway through BL, the day prior to the end of SR and two days prior to the end of RC (visits 4, 7 and 10). The dual energy x-ray absorptiometry (DXA), strength and function measures were performed on the day following the OGTT (visits 5, 8 and 11).

### Dual-energy x-ray absorptiometry (DXA)

Participants underwent DXA scans (Lunar iDXA, GE Medical Systems Lunar, Madison, WI, USA) on a unit that was calibrated daily with a 3-compartment Universal Whole Body DXA Phantom (Oscar, Jr; Orthometrix, Naples, FL). Body scans were used to determine body fat percentage, total body fat mass (FM), leg FM, total body lean mass (LM), arm LM and leg LM (Lunar enCORE version 14.1, GE Medical Systems Lunar, Madison WI, USA). VAT was determined using the GE Healthcare CoreScan™ software. Appendicular lean mass (ALM) was determined from the equation7:   
 ALM = ((total arm FFM + total leg FFM)/1000)/height2)

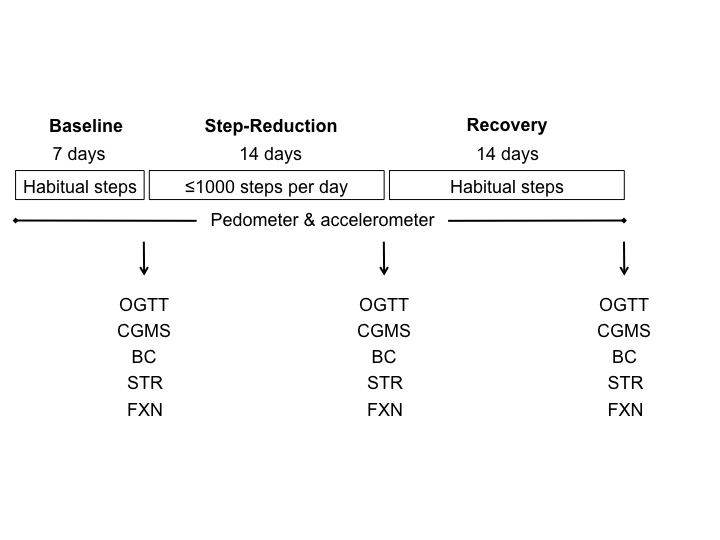


Figure 3. Research schematic depicting the five-week study protocol and approximate timing of data collection. Oral glucose tolerance test (OGTT), continuous glucose monitoring system (CGMS), body composition (BC), strength (STR), function (FXN).

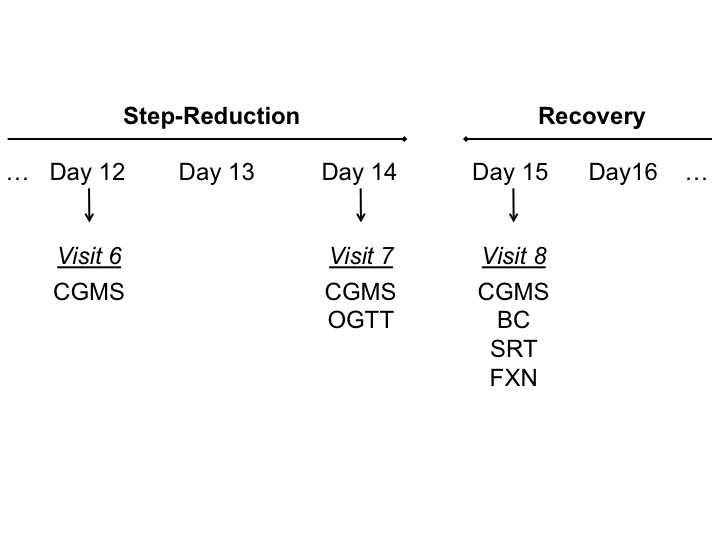
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Figure 4. Research schematic depicting specifics of data collection timing at the end of the step-reduction and beginning of the recovery phase. Oral glucose tolerance test (OGTT), continuous glucose monitoring system (CGMS), body composition (BC), strength (STR), function (FXN).

### Isometric maximal voluntary contraction (ISO-MVC)

Study participants underwent strength testing to determine peak torque production of the knee extensor muscles during ISO-MVCs. Using a dynamometer (Biodex System 3, Shirley, NY, USA) participants were instructed to extend their knee (i.e. contract their quadriceps to produce a kicking motion) as rapidly as possible with as much force as possible and hold that contraction with maximal effort for five seconds. Each unilateral test consisted of three ISO-MVCs of the knee extensors at an angle of 60˚ from the neutral 90˚ resting position and trials were separated by 120 seconds of rest. In order to test peak knee extension torque, participants were familiarized to the nature of the dynamometer testing protocol twice prior to BL testing, and re-familiarized with at least one maximal contraction before proceeding with the test during each testing session. Peak ISO-MVC values were recorded through PowerLab (26T, AD Instruments, CO, USA) and LabChart 7 Pro (v7.3.5, AD Instruments, CO, USA). Sampling rate for torque was 1000 Hz with a low-pass digital filter. The highest recorded torque production from each leg was taken as the ISO-MVCpeak. ISO-MVCmean was defined as the peak torque averaged across all three trial contractions during each testing phase. All settings were recorded to replicate conditions on all testing occasions. Relative ISO-MVC was defined as the peak ISO-MVC per kg body mass.

### Rate of torque development (RTD)

RTD was determined from the tangential slope of the torque production trace from the trial with the highest torque production. Peak RTD (RTDpeak) was defined as the maximal slope of the torque-time curve. RTD was partitioned into epochs of 0-50 ms (RTD50), 50-100 ms (RTD100). 100-150 ms (RTD150) and 150-200 ms (RTD200) after the time at which 3% of peak torque was produced, and the mean RTD from these intervals was calculated. Torque production was also partitioned and averaged by quartiles of force production (0-25% RTD25%, 25-75%, RTD75% and 75-100%, RTD100%). Both absolute and relative (RTD/ISO-MVCpeak) were considered in the analysis.

### Physical function

A battery of tests for the performance of ADL were conducted after each study phase. In each trial of the 30CS task, a participant was instructed to rise from a chair as many times as possible in 30 seconds, without using their arms for assistance105. The 30CS consisted of three trials separated by a two-minute break. The TUG task involved participants rising from a chair without using their arms, walking at a self-selected pace into a designated zone three metres in front of them, turning around and returning to sit down in the chair at the original start position106. Participants performed a six-minute walk test (6MWT) by walking at a self-selected pace around a 200 m track for six minutes107. The total distance walked in six minutes was measured with use of a rolling distance measuring wheel. Participants performed the Rockport walk test (RWT) by walking at a self-selected pace on a 200 m track to complete 8 full laps (1 mile or 1.6 km)108. Participants were timed until completion and heart rate (HR) was measured using digital HR monitors (H7 heart rate sensor, Polar, Lachine, QC, Canada). The score associated with this test represents the estimated V̇O2peak of each participant and was calculated as follows:  
Estimated V̇O2peak (ml·kg-1·min-1) = 132.853 – 0.0769(mass) – 0.3877(age) + 6.315(sex) – 3.2649(time in min) – 0.1565(HR)

### Continuous glucose monitoring system (CGMS)

Free-living glucose concentration was measured through use of CGMS over two days, one ‘free-living’ 24-hour period and a 24-hour period that included an OGTT. CGMS sensors (Enlite, Medtronic, California, USA) were inserted percutaneously at the abdomen (periumbilical). CGMS devices (iPro 2, Medtronic, California, USA) were secured to the abdomen on the day prior to data collection and remained for the next two days. As per manufacturer’s instructions, participants recorded four blood glucose measurements per day for calibration of the CGMS device through point-of-care capillary blood glucose devices (OneTouch UltraMini, LifeScan, BC, Canada) upon waking, before lunch, before dinner and before bed. Participants were provided with an individualized two-day isocaloric diet of pre-packaged food containing energy based on the Harris-Benedict equation26 with an associated activity factor (determined through the subject’s responses to the International Physical Activity Questionnaire). The diet was consumed for two CGMS testing days. Diets provided 1.0 ± 0.1 g • kg-1 body weight of protein and 40 ± 3% of all caloric intake and 53 ± 6% of all protein intake at dinner, with near equal distribution of calories and protein at the breakfast and lunch meals (Table 2). Participants recorded meal consumption in a detailed log and repeated the timing of each meal during each subsequent phase of the study. When CGMS devices were removed all measurements of interstitial glucose concentration were converted to blood glucose concentration during data download using the provided CGMS Carelink™ software (Medtronic).

Recordings from the CGMS devices provided blood glucose measurements every five minutes. Analysis of CGMS allowed for characterization of three-hour post-prandial glucose AUC (PPGAUC), peak post-prandial glucose (∆PPGpeak = max blood glucose – premeal blood glucose) and time spent above target PPG (time >7.9 mM)109. PPG analysis began at the time prior to self-reported meal consumption (no more than five minutes before) and continued over the subsequent three hours. No differences were detected between meals, therefore PPG analysis was pooled for breakfast, lunch and dinner. Maximum blood glucose concentration ([G]max), minimum blood glucose concentration ([G]min) and 24-hour blood glucose concentration mean ([G]24h) were determined from 24-hour free-living CGMS as well. During the OGTT, blood glucose concentration AUC ([G]AUC), maximum blood glucose concentration ([G]max), time spent in hyperglycemia (time >10 mM) and blood glucose concentration two hours post-OGTT ([G]120) were determined via CGMS as well.

Table 2. Summary of diet characteristics provided on the free-living day of CGMS measurements

|  |  |  |
| --- | --- | --- |
|  | Energy (kcal) | Protein (g • kg-1) |
| Breakfast | 659 ± 106 | 0.25 ± 0.02 |
| Lunch | 659 ±106 | 0.22 ± 0.02 |
| Dinner | 878 ± 142 | 0.53 ± 0.04 |
| Carbohydrates (% of daily diet) | 56 ± 3 | |
| Fat (% of daily diet) | 30 ± 3 | |
| Protein (% of daily diet) | 14 ± 1 | |
| Protein intake (g • kg-1 • d-1) | 1.0 ± 0.1 | |

Values are means±SD, n=10 (6 men, 4 women)

### Oral glucose tolerance test (OGTT)

On the day after the ‘free-living’ day and an overnight fast, participants began a two-hour OGTT with blood glucose concentrations simultaneously obtained through finger prick capillary blood testing and subsequently from venous blood plasma samples. Two fasting blood samples and glucose finger prick tests were taken (-15 min, 0 min) after which participants consumed a beverage containing 75 g of glucose (Trutol, Thermo Scientific, Pittsburgh, PA, USA). Blood sampling and capillary blood glucose tests were performed every 15 minutes for the first 90 minutes with final samples taken at 120 minutes. Plasma glucose concentrations were determined through automated glucose analysis (YSI 2300 Stat Plus, Yellow Springs, OH). Blood glucose concentration AUC ([G]AUC), maximum blood glucose concentration ([G]max) and blood glucose concentration two hours post-OGTT ([G]120) were determined by collected samples.

Plasma insulin was determined through solid-phase two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). Homeostatic model assessment of insulin resistance (HOMA-IR)110 calculations were made using the average of fasting samples of plasma glucose and insulin (ie. -15 and 0) and Matsuda insulin-sensitivity index (ISI)111 calculations used the plasma glucose concentrations from samples from 30, 60, 90 and 120 minutes after consumption of the OGTT beverage.

### Statistics

Statistical analyses were performed using SPSS (Version 22.0, IBM, Chicago, IL, USA). AUC calculations were made using Prism 6 (GraphPad, CA, USA). Statistical significance was set at p < 0.05 and tested with Pearson correlations and separate one-way repeated measures analysis of variance (ANOVA). Simple planned contrasts were used to further examine the effects from the omnibus ANOVAs where appropriate. Power calculations were made with G\*Power (v 3.1.9.2, University of Düsseldorf, Germany; alpha, 0.05; power, 0.8; two tailed comparison of means) for the purposes of post-hoc analysis. Values are expressed as means±SD.

# 

# RESULTS

### Daily steps

From BL to SR there was a drop of 86 ± 9% in daily step count estimated using the pedometer located on the hip (8568 ± 3741 to 973 ± 76 steps per day; p < 0.001; Figure 5). The estimation of daily steps as measured by the SenseWear armband accelerometer showed daily steps were reduced by 81 ± 13% with SR (p = 0.002). PA level also changed in concert with step-count, with the number of active minutes per day (at or above three METs) being reduced by 79 ± 17% (p = 0.02) and daily energy expenditure (DEE) reduced by 20 ± 11% (p = 0.01). All measures of PA were significantly greater at RC than SR (p < 0.05) with no difference between BL and RC (p > 0.05; Table 3).  
  


Figure 5. Individual participant’s daily step-count means during each study phase as measured by pedometer. Participants are each denoted by a unique symbol.   
\* Significantly different from BL, p < 0.001. n=10 (6 men and 4 women).

**Table 3.** Summary of participant characteristics

|  |  |  |  |
| --- | --- | --- | --- |
|  | BL | SR | RC |
| Age (years) | 69 ± 3 | | |
| Height (m) | 1.67 ± 0.08x | | |
| Body mass (kg) | 75.8 ± 13.3 | 75.8 ± 13.6 | 75.8 ± 13.6 |
| BMI (kg • m-2) | 27.2 ± 4.7 | 27.4 ± 4.7 | 27.4 ± 4.7 |
| Pedometer Steps (steps • d-1) | 8568 ± 3741 | 973 ± 76\* | 8383 ± 4513 |
| Armband Steps (steps • d-1) | 7860 ± 4054 | 1188 ± 608\* | 6918 ± 4562 |
| DEE (kcal • d-1) | 2455 ± 693 | 1908 ± 298\* | 2256 ± 484 |
| PA > 3MET (min • d-1) | 121 ± 105 | 22 ± 21\* | 97 ± 89 |

\* significantly different from baseline, p < 0.018; BMI, body mass index; PA, physical activity; MET, metabolic equivalent. Values are means±SD; n=10 (6 men and 4 women).

### 

### Body composition

There were no significant changes in measures of body fat or LM from BL to SR to RC (Table 4). Total FM and leg FM both increased with SR and decreased with RC (NS). VAT increased by 3.1 ± 8.2% after SR (p = 0.454) and did not return to BL levels after RC. Leg LM was reduced by an average of ~73 g or 0.8 ± 0.2% (p = 0.139) and increased after RC (Figure 6).

Table 4. Summary of body composition characteristics as measured by DXA

|  |  |  |  |
| --- | --- | --- | --- |
|  | BL | SR | RC |
| Body Fat (%) | 32.0 ± 12.7 | 32.0 ± 12.9 | 31.8 ± 12.6 |
| Total FM (kg) | 24.3 ± 12.1 | 24.4 ± 12.2 | 24.2 ± 12.0 |
| Arm FM (g) | 2696 ± 1337 | 2649 ± 1287 | 2649 ± 1287 |
| Leg FM (g) | 7075 ± 3842 | 7162 ± 3909 | 7097 ± 3902 |
| VAT (cm3) | 1261 ± 832 | 1392 ± 925 | 1365 ± 789 |
| Total LM (kg) | 48.7 ± 7.8 | 48.6 ± 8.0 | 45.5 ± 16.2 |
| Arm LM (g) | 5617 ± 1213 | 5544 ± 1147 | 5579 ± 1154 |
| Leg LM (g) | 16 286 ± 3103 | 16 174 ± 3160 | 16 410 ± 3239 |
| ALM (kg • m-2) | 7.8 ± 1.0 | 7.8 ± 1.0 | 7.9 ± 1.0 |

FM, fat mass; VAT, visceral adipose tissue; LM, lean tissue mass; ALM, appendicular lean mass. Values are means±SD; n=10 (6 men and 4 women).



Figure 6. Leg lean mass as measured by DXA. Boxes represent 25-75th quartiles, whiskers represent minimum and maximum, horizontal line represents median, cross represents mean. n=10 (6 men and 4 women).

### Strength

There were no significant changes in peak (Figure 7) or mean absolute or relative torque production for ISO-MVC from BL to SR or SR to RC (Table 5). Relative RTD exhibited no change between study phases for RTD25%, RTD75%, RTD100%, RTD50ms, RTD100ms, RTD150ms (p=0.19) or RTD200ms (Table 5). No differences were detected between study phases for absolute RTD, regardless of the measurement partition (ie. torque percentage or epochs; Table 5).



Figure 7. Knee extensor peak torque production during ISO-MVC measured by Biodex dynamometer. Boxes represent 25-75th quartiles, whiskers represent minimum and maximum, horizontal line represents median, cross represents mean. n=10 (6 men and 4 women).

### Physical function

There was no difference in max 30CS, mean 30CS or mean TUG performance from BL to SR (Table 5), however performance was slightly enhanced in the RC phase compared to BL and SR (NS). The time necessary to complete the TUG was increased by 0.4 seconds and was 0.2 seconds faster in RC than BL (NS, p=0.21). No differences from BL to SR to RC were seen for 6MWT, however a trend was evident for an increase in distance travelled at RC compared to BL and SR (p=0.06, Table 5). Estimated V̇O2peak was decreased by 4.7 ± 10.5% from BL to SR and returned to near BL values with a 3.8% increase from BL to RC (p = 0.12, Table 5).

**Table 5.** Summary of physical performance measures

|  |  |  |  |
| --- | --- | --- | --- |
|  | BL | SR | RC |
| Torque |  |  |  |
| ISO-MVCpeak (Nm) | 147 ± 29 | 147 ± 32 | 144 ± 31 |
| ISO-MVCmean (Nm) | 142 ± 26 | 140 ± 33 | 138 ± 30 |
| Rel. ISO-MVCpeak (Nm • kg-1) | 2.0 ± 0.3 | 2.0 ± 0.4 | 1.9 ± 0.4 |
| Rel. ISO-MVCmean (Nm • kg-1) | 1.9 ± 0.3 | 1.9 ± 0.3 | 1.9 ± 0.4 |
| RTD/ISO-MVC (NM • s-1 • Nm-1) |  |  |  |
| RTDpeak | 10.6 ± 5.8 | 11.1 ± 4.6 | 10.7 ± 7.3 |
| RTD25% | 6.8 ± 3.8 | 7.1 ± 3.4 | 6.0 ± 4.3 |
| RTD75% | 2.7 ± 0.9 | 2.2 ± 1.1 | 2.5 ± 1.3 |
| RTD100% | 0.3 ± 0.2 | 0.4 ± 0.3 | 0.4 ± 0.6 |
| RTD50 | 5.6 ± 1.6 | 5.5 ± 1.6 | 5.1 ± 2.9 |
| RTD100 | 3.5 ± 0.7 | 3.1 ± 0.9 | 3.6 ± 1.7 |
| RTD150 | 2.2 ± 0.9 | 2.2 ± 1.1 | 1.6 ± 1.3 |
| RTD200 | 2.2 ± 0.5 | 2.0 ± 0.7 | 2.1 ± 0.9 |
| RTD (Nm• s-1) |  |  |  |
| RTDpeak | 1529 ± 865 | 1605 ± 686 | 1439 ± 796 |
| RTD25% | 621 ± 1102 | 844 ± 883 | 514 ± 912 |
| RTD75% | 223 ± 370 | 226 ± 298 | 196 ± 323 |
| RTD100% | 30 ± 50 | 43 ± 76 | 54 ± 96 |
| RTD50 | 819 ± 303 | 823 ± 343 | 695 ± 344 |
| RTD100 | 509 ± 84 | 456 ± 172 | 509 ± 263 |
| RTD150 | 320 ± 134 | 308 ± 159 | 224 ± 205 |
| RTD200 | 324 ± 99 | 312 ± 148 | 318 ± 167 |
| 30CS (sit-stand cycles) |  |  |  |
| Peak | 17 ± 4 | 17 ± 3 | 18 ± 4 |
| Mean | 16 ± 4 | 16 ± 3 | 17 ± 4 |
| TUG (seconds) |  |  |  |  |
| Minimum | 7.0 ± 1.4 | 7.4 ± 1.5 | 6.8 ± 1.2 |
| Mean | 7.8 ± 2.0 | 7.8 ± 1.5 | 7.3 ± 1.4 |
| 6MWT Distance (m) | 575 ± 105 | 577 ± 112 | 600 ± 112 |
| RWT estimated V̇O2peak (mL • kg-1 • min-1) | 28.8 ± 12.2 | 27.3 ± 11.5 | 29.7 ± 12.1 |

ISO-MVC, isometric maximal voluntary contraction; RTD, rate of torque development; 30CS, 30 second chair stand; TUG, timed up-and-go; 6MWT, six minute walk test; RWT, Rockport walk test. Values are means±SD, n=10 (6 men and 4 women).

### OGTT

In response to SR [G]AUC, assessed by capillary blood glucose measurements increased 19 ± 26% (p = 0.02) and decreased in RC by 22 ± 14% (p = 0.001; Figure 8A and B, Table 6). A similar increase (BL to SR) and subsequent decrease (SR to RC) in [G]AUC was detected by both CGMS (Figure 8C and D, Table 6) and plasma glucose analysis (Figure 8E and F, Table 6), however these changes did not reach statistical significance. Capillary blood [G]120 was increased by 23 ± 22% with SR (p = 0.01) and decreased 14 ± 9% with RC (p = 0.002). Similar but not statistically significant changes were seen in [G]120 with CGMS (p = 0.25) and plasma glucose (p = 0.09; Table 6). [G]max increased significantly with SR for capillary blood glucose (p = 0.04) and plasma glucose (p = 0.01) and recovered with a return to habitual activity (Table 6). Albeit non-significant, time spent at >10 mM during OGTT increased with inactivity and only partially decreased upon resuming habitual activity (Table 6).

In response to SR, the insulin AUC during OGTT was increased by 15 ± 17% (p = 0.07) and decreased only 7 ± 13% with RC (Figure 9A and B, Table 6). Matsuda ISI was significantly decreased with SR by 22 ± 9% (p < 0.001) and increased by only 8 ± 13% with RC, remaining significantly decreased compared to BL (p < 0.001, Figure 9C, Table 6). HOMA-IR was significantly increased with SR by 29 ± 33% (p = 0.02) with no mean decrease after RC, remaining significantly elevated compared to BL (p = 0.01, Figure 9D, Table 6).



Figure 8. Glucose concentration and glucose area under the curve during 2-hour OGTT obtained by various measures. Boxes represent 25-75th quartiles, whiskers represent minimum and maximum, horizontal line represents median, cross represents mean. n=10 (6 men and 4 women). A) Time course of glucose concentration via capillary blood sampling. B) Capillary blood glucose concentration area under the curve, \* significantly different from BL, p = 0.023. C) Time course of glucose concentration via CGMS. D) CGMS glucose concentration area under the curve. E) Time course of glucose concentration via venous blood plasma sampling. F) Plasma glucose concentration area under the curve



Figure 9. Venous blood plasma insulin variables determined from 2-hour OGTT. Boxes represent 25-75th quartiles, whiskers represent minimum and maximum, horizontal line represents median, cross represents mean. \* denotes significantly different from BL value. n=10 (6 men and 4 women). A) Time course changes of plasma insulin concentration. B) Plasma insulin area under the curve.   
C) Calculated Matsuda insulin sensitivity index value, \* p < 0.001. D) Calculated homeostatic model of insulin resistance value, \* p < 0.02

### Free-living glycemic control

Two weeks of SR did not result in any significant differences in glycemic control over a 24-hour free-living period. SR did not affect [G]24h, however it was elevated 4 ± 8% from SR to RC (Table 6). [G]min and [G]max were reduced and elevated, respectively, with SR compared to BL and were both elevated with RC (Table 6). In the three hours after standardized meals PPGAUC (Figure 10A and B), ∆PPGpeak and time with blood glucose concentration >7.9 mM were all increased with SR and further increased with RC, however, none of these elevated levels reached statistical significance (Table 6).



Figure 10. Post-prandial blood glucose variables measured by CGMS in five-minute intervals. n=10 (6 men and 4 women). A) Time course of post-prandial blood glucose concentration spanning the three hours post meal-consumption. B) Post-prandial glucose area under the curve.

Table 6. Summary of glycemic control during OGTT and free-living conditions measured via capillary blood finger prick testing and CGMS

|  |  |  |  |
| --- | --- | --- | --- |
|  | BL | SR | RC |
| OGTT |  |  |  |
| Capillary Blood Glucose |  |  |  |
| [G]AUC (AU) | 430 ± 111 | 503 ± 118\* | 394 ± 128 |
| [G]120 (mM) | 7.3 ± 1.5 | 9.1 ± 1.4\* | 7.5 ± 1.7 |
| [G]max (mM) | 10.8 ± 1.9 | 11.6 ± 1.6\* | 10.8 ± 2.1 |
| CGMS |  |  |  |
| [G]AUC (AU) | 331 ± 154 | 387 ± 144 | 367 ± 206 |
| [G]120 (mM) | 7.3 ± 1.4 | 8.3 ± 1.7 | 6.9 ± 3.2 |
| [G]max (mM) | 9.9 ± 2.5 | 10.5 ± 2.1 | 9.3 ± 3.9 |
| Time >10 mM (min) | 24 ± 51 | 46 ± 39 | 34 ± 48 |
| Plasma |  |  |  |
| [G]AUC (AU) | 325 ± 126 | 375 ± 137 | 331 ± 148 |
| [G]120 (mM) | 7.9 ± 1.3 | 9.1 ± 1.1 | 8.8 ± 1.8 |
| [G]max (mM) | 10.8 ± 2.4 | 11.9 ± 1.7\* | 11.1 ± 1.9 |
| Insulin AUC (AU) | 3188 ± 1254 | 3808 ± 1502 | 3548 ± 1460 |
| Matsuda ISI | 3.5 ± 0.3 | 2.7 ± 0.3\* | 2.9 ± 0.4\* |
| HOMA-IR | 2.8 ± 0.3 | 3.6 ± 0.7\* | 3.5 ± 0.7\* |
| Free-living |  |  |  |
| [G]24h (mM) | 5.7 ± 0.7 | 5.6 ± 0.7 | 6.0 ± 0.7 |
| [G]min (mM) | 4.3 ± 0.6 | 4.0 ± 1.1 | 4.6 ± 0.7 |
| [G]max (mM) | 8.0 ± 1.2 | 8.5 ± 1.1 | 8.2 ± 1.7 |
| PPGAUC (AU) | 149 ± 90 | 174 ± 94 | 196 ± 129 |
| ∆PPGpeak (mM) | 1.6 ± 1.0 | 2.0 ± 1.1 | 2.2 ± 1.3 |
| PPG time >7.9 mM (min) | 11 ± 26 | 13 ± 26 | 24 ± 40 |

All free-living glucose measures obtained from CGMS; OGTT, oral glucose tolerance test; CGMS, continuous glucose monitor system; [G], concentration of blood glucose; AUC, area under the curve; ISI, insulin sensitivity index; HOMA-IR, homeostatic model assessment of insulin resistance; \* significantly different than BL, p < 0.05. Values are means±SD , n=10 (6 men and 4 women).

### Correlations

The self-reported reduction in daily steps from BL to SR measured by pedometer were correlated with measures of PA measured from the armband accelerometer including estimated daily step count (R2 = 0.90), DEE   
(R2= 0.67) and time spent at or above 3 METs (R2 = 0.65). The only end-point measure associated with the reduction in daily steps was [G]120 measured by capillary blood during OGTT, which was best correlated with absolute changes in daily ambulation (p = 0.01, R2 = 0.62; Figure 11).



Figure 11. Correlation between the absolute change from BL to SR in daily steps and the absolute change in capillary blood glucose concentration at the final time point of OGTT. p = 0.01, R2 = 0.62; n=10 (6 men and 4 women).

### Influence of sex on outcomes

Women on average completed ~3600 fewer steps per day at BL compared to men (6394 ± 2303 and 10018 ± 3963 steps • d-1, respectively) resulting in an 82 ± 12% reduction in daily steps for women and an 89 ± 5% reduction for men with the SR intervention (Figure 12A). There were no statistical differences between sexes for steps in any study phase. Sex differences were assessed for all primary study outcomes: leg LM, ISO-MVCpeak, [G]AUC (Figure 12B), ISI (Figure 12C) and HOMA-IR (Figure 12D). No statistically significant differences were found between men and women for these key measures (data not shown).



**Figure 12.** Comparison of men and women’s percentage changes from BL to SR. Men represented by closed bars, women represented by open bars. Individual participants represented by individual bars and remain in the same column between figures. n=10 (6 men and 4 women). A**)** Percentage change in daily steps. **B)** Percentage change in leg LM. **C)** Percentage change in VAT. **D)** Percentage change in glucose area under the curve during OGTT as measured by capillary blood. **E)** Percentage change in Matsuda ISI, *NB*: n = 9.  **F)** Percentage change in HOMA-IR.

# DISCUSSION

The primary purpose of this investigation was to determine the extent to which glycemic control, body composition and muscle function were altered following two weeks of SR in older men and women. The secondary aim was to determine whether or not any observed impairments in these variables could be recovered two weeks after returning to normal activity patterns (RC). This study provides novel data showing that two weeks of reduced daily ambulation caused no detectable changes in body composition, leg strength or physical function. However, SR did result in impaired glycemic control in both a free-living and laboratory setting. Indeed, markers of PPG control were negatively impacted with SR and failed to fully recover even with a return to normal daily steps. Additionally, the [G]AUC, [G]120 and [G]max during an OGTT were significantly increased after SR and decreased with RC. Despite the recovery of blood glucose control with RC, the decreased Matsuda ISI and increased HOMA-IR seen after SR were still different from BL after RC. Thus, future work that characterises the time course change in Matsuda ISI and HOMA-IR following two weeks of SR are now needed to establish the long-term impact of reduced PA on glucose control.

An original aim of this work was also to characterize the effects of the protocol in men and women with the a priori hypothesis that the glycemic regulatory variables of the older women would be adversely affected to a greater degree than seen in men. Regrettably, due to time constraints, I was unable to collect enough data from older women to make a sex-based comparison worthwhile. Thus, the data in this thesis should be considered a smaller-scale study with a partial dataset of the larger study, which will be completed in the future.

### OGTT

Our results provide strong evidence to suggest that SR induces impaired glycemic control, which returns to pre-intervention levels with the resumption of habitual daily steps, however insulin sensitivity remains impaired. Measured with three separate techniques, the blood glucose concentration AUC during a two-hour OGTT was increased after SR. Furthermore, despite the return of glucose AUC to BL levels, the elevated insulin AUC, insulin resistance and depressed insulin sensitivity, which persisted after the RC phase, indicate that blood glucose homeostasis is recovered at the expense of a reduction in insulin sensitivity. Our results are in line with those of Breen et al.82, we observed greater increases in plasma glucose AUC (15% vs. 9%), plasma insulin AUC (15% vs. 12%) and HOMA-IR (29% vs. 12%) after SR in comparison to that previous report. These differences between studies may be due to greater relative reduction in steps with the current study compared to the study of Breen et al.82 (86% vs. 76%). Although the reduction in Matsuda ISI was nearly two-fold greater after SR in the former study (22% vs. 43%), insulin sensitivity reported by Breen et al.82 was lower at study-entry and the absolute change in sensitivity after SR was nearly four-fold greater in the current study. The difference in pre-intervention insulin sensitivity may account for differing results between Breen et al.82 and our current findings.

Compared to younger men, the degree of impairment in insulin sensitivity we observed with SR seems to be of a lesser magnitude. For example, after two weeks of SR below 1500 steps per day, a very similar model of inactivity as ours, insulin AUC in younger men was increased by 57-61%80. Furthermore, Matsuda ISI and HOMA-IR showed greater impairments in younger adults compared to our older adults (ISI: ~30%; HOMA-IR: ~40%) after only 3-5 days of fewer than 5000 steps per day77,87. A possible reason for this attenuated response is the fact that older participants in the current study presented with substantially impaired values of insulin sensitivity and resistance compared to younger adults at study-entry and prior to SR. The average Matsuda ISI and HOMA-IR in this study were 3.5 and 2.8 respectively, both of which fall beyond the suggested cut-points for whole body insulin resistance (ISI: 5.0; HOMA-IR: 2.3)112. Comparatively, prior to any SR intervention in younger adults, Matsuda ISI and HOMA-IR values were approximately 14.3 and 0.8 respectively77. Even after SR in younger participants, their Matsuda ISI and HOMA IR values were not beyond insulin resistance cut-point values112. While the absolute and relative metabolic response to SR in older adults may be diminished, in a population already exhibiting impairments in glycemic control, the changes in insulin sensitivity we observed could have relevant clinical consequences.

The novel finding of this study was the fact that insulin sensitivity (Matsuda ISI) and insulin resistance (HOMA-IR) failed to recover to BL levels with RC. Insulin resistance precedes progression to overt T2DM113 and is proposed to be an initiating factor in a number of chronic diseases including CVD, hypertension, as well as cancer114, which highlights the significance of our finding in an elderly population. To our knowledge, only two other studies have looked at the recovery of glycemic control with a return to habitual activity. In younger men, one day of PA was insufficient to recover insulin sensitivity after five days of SR87, however two weeks of habitual ambulation returned all measures of glycemic control back to study-entry levels in another study79. Our study is, to our knowledge, the first evidence that older adults are not able to recover from the consequences of SR, from the perspective of glucose control. While this finding supports our hypothesis, it is still conceptually surprising when you consider observations that a single bout of moderate intensity exercise can increase insulin sensitivity,115 minimal PA is associated with improved glycemic control37,116 and moderate intensity exercise can acutely enhance glucose homeostasis in impaired glucose tolerant older adults117. Thus, despite returning to their normal activity patterns, the recovery period (14d) for older adults after inactivity was insufficient to recover insulin sensitivity. The recovery of glycemic control and insulin sensitivity, at least in older persons by comparison younger persons, is protracted and may last for an unknown period of time. Further research elucidating the time course of recovery of glycemic control and insulin sensitivity in older adults for periods of inactivity would be instrumental in understanding how long the effects of reduced PA due to, as an example, influenza or inclement weather may persist.

Krogh-Madsen et al.78 found that the metabolic consequences of SR manifests prior to changes in body composition, a finding that strongly agrees with the current study. This evidence suggests that muscle atrophy and increased adipose tissue may be limited in their role as a potential mechanisms for SR-mediated insulin resistance. In fact, with as little as three days of fewer than 5000 daily steps, younger men and women exhibited a 31% decrease in Matsuda ISI and 49% increase in HOMA-IR77 while significant impairments can manifest after only 24 hours of sitting and lying down with significantly reduced PA118. Despite the indexes of insulin sensitivity/resistance used in this study being estimations of whole body insulin sensitivity, the use of the clamp technique in similar models of SR in younger adults suggest that the insulin resistance exhibited with SR is peripheral78. Therefore potential mechanisms for peripheral insulin action are of interest, as are the underlying causes of metabolic disruption with SR.

An OGTT by itself is not considered the ‘gold standard’ procedure for determining insulin sensitivity, but when compared to insulinemic-euglycemic clamp, both ISI and HOMA-IR independently show a strong association119. It should also be noted here that there was a lack of statistical agreement between measurement techniques used for blood glucose concentration analysis in the current study. In particular, statistically significant increases in glucose AUC, [G]max ­­and [G]120 were seen with capillary blood measurements using finger prick-point-of-care glucose monitors while only [G]max was significantly increased according to plasma-measured glucose concentration. Interestingly, we observed no significant differences with CGMS. Numerically, however, all three methods reported increases in measures of blood glucose concentration with SR. Glucose meters are subject to many sources of error and it is suggested that only 63% of readings from the best available meter fall within 5% of true blood glucose concentrations120. Venous plasma samples are considered the most accurate measure of blood glucose and by comparison capillary whole blood glucose concentrations are 10-15% lower on average121 and elevated by 8% compared to venous samples after an oral glucose load122. Interstitial fluid glucose concentrations are used to estimate blood glucose concentrations with CGMS, yet there is a reported lag between venous glucose and interstitial fluid which can range from 0123 to 30 minutes124. CGMS measurements vary ±12-13% from venous measurements125 and have a coefficient of variation of ~3%126. While not all measures agree, they each offer unique advantages related to practicality (capillary), frequency of measures (CGMS) and accuracy (venous plasma). It is likely that the current sample size was too small and thus we were underpowered to detect statistically significant changes with all methods.

### Free-living glycemic control (CGMS)

Measures of blood glucose homeostasis in settings outside of the laboratory provide unique insight into metabolic function in free-living persons. Of particular importance are the changes in PPG and whether this is reaching hyperglycemic concentrations, which have been shown to precede T2DM127 and CVD128. Although it did not reach statistical significance in this study, after SR both older men and women exhibited elevated PPGAUC, ∆PPGpeak and time spent above the threshold for non-insulin resistant postprandial hyperglycemia109. These elevated excursions in blood glucose after mixed meals remained above BL levels despite a return to habitual activity, in line with the metabolic impairments reported by OGTT. After 3-5 days of SR below 5000 daily steps in younger adults, CGMS reported increases in max PPG ranging between 9%87 and 26% and ∆PPGpeak from 30% to 50%77. Population, SR protocol and meal composition differences between previous studies and the current investigation limit comparisons, however, collectively the findings indicate SR negatively affects PPG. The study by Mikus et al.77 did not show the same consequences of SR in younger adults, revealing impairments in PPG glycemic control via CGMS but no increase in OGTT glucose AUC. Comparatively, our study indicated both an increase in OGTT glucose AUC and free-living PPG, potentially strengthening the concept that SR is more consequential to older adults. Reynolds et al.87 found that one day of PA failed to return PPGpeak and PPG AUC (NS) to pre-intervention levels in younger men, a finding analogous to our finding, but after two weeks in older adults. Measures of free-living glycemic control therefore provide continued evidence that the impaired glycemic control seen after acute periods of physical inactivity in older adults are pronounced and protracted despite a return to normal daily ambulation.

### Body composition.

Unexpectedly, all measures of body composition remained statistically unchanged with two weeks of SR. The ~3% increase in VAT reported in this study is the first reported increase of visceral fat mass with acute physical inactivity in older adults, yet is less than the ~7% increases in IAFM seen in younger men after SR80. Furthermore, we observed only moderate declines in leg LM after SR. These findings are in agreement with previous studies employing the two-week SR model in both young and older adults (Table 1), however, the losses in leg LM we report here (~0.8%) are attenuated by comparison. Disuse-induced muscle atrophy is generally more pronounced in younger adults than older adults56, however, previous studies using the less aggressive SR model report equivocal losses of LM, regardless of age78,80. Nonetheless, recent reports suggest that two weeks of SR in older adults can induce reductions in leg LM of 1.4%89 to 3.7%82. It is therefore surprising that in the present study decrements in LM in older participants were not observed, or exhibited to a lesser extent. One explanation for the discrepancy between our data and previous studies is that we employed DXA as a means to assess changes in body composition rather than using MRI, which is a more robust measurement tool for body composition analysis. Although DXA has been well correlated with MRI derived characterization of body composition129, the validity of the measure remains highly influenced by factors such as hydration status130,131, where it is suggested a 5% change in FFM hydration could influence FM estimates up to 2.5%132. While attempts were made to control the hydration status of our participants, it is important to acknowledge that DXA also has an intrinsic measurement error. This error (albeit measured in children) accounts for up to ~5% variation in FM and up to ~3% variation in FFM133, a variation (~0.4-1.4 kg) that is further amplified for FFM measurements in obese and for FM measurements in leaner humans133,134. Nearly all of the changes in leg LM from the current study fall well within the lower limit of this measurement error and might provide an explanation as to why no clear muscular atrophy was seen with SR (Figure 13). This intrinsic measurement error would also question the accuracy of muscle loss measurements seen with other SR studies that have used DXA for body composition analysis75,78,79,82. Unfortunately, the use of MRI to assess changes in body composition in this study was financially prohibitive, but as this study shows, the use of MRI should be considered critical for the accurate determination of changes in body composition in future studies using the SR model. Of note are some recent unpublished findings (Dr. D.R. Moore, personal communication) from a study in older people undergoing SR, conducted by our group89, in which participants had a 1.4% reduction in leg LM. This reduction was accompanied by a reduction of mean muscle fibre CSA by 19% (4634+/- 276 vs. 3824 ± 160 µm2; p<0.05), but in a fibre-type specific manner with a difference of 25% (4510 ± 422 vs. 3346 ± 181 µm2; p<0.05) in type-II muscle fibre CSA. Thus, we propose that our changes, while small when only DXA is concerned, are manifested at the fibre level as well.

Another potential explanation for the lack of change in body composition in this study was a lack of adherence to the SR protocol. However, armband-worn accelerometer measures (not visible to the subject) provided an unbiased estimation of daily steps and PA that shows participants did in fact adhere to the protocol and reduced their steps and PA. These findings should be sufficient to determine that compliance was not an issue, yet findings of other studies have shown just how small the muscular stimulus needs to be to offset muscular unloading (bed rest) or complete disuse (limb immobilization). In young men as little as 140 high-load muscle contractions attenuated muscle atrophy associated with knee immobilization135, while daily isometric contractions prevented muscle atrophy during 20 days of bed rest136. Furthermore, Devries et al.89 found that low-load resistance exercise thrice weekly was able to ablate two weeks of SR-induced muscle atrophy. In the current study participants were explicitly asked to refrain from any form of exercise while in the SR phase and were frequently contacted to ensure compliance, but participants remained out of the laboratory environment for at least 10 consecutive days during SR and inadvertent isometric contractions or PA (not recorded by the armband accelerometers) could not be accounted for. It is unlikely that the SR protocol undergone by participants was not a sufficient degree of disuse, but the 973 ± 76 steps per day completed during SR are at least ~25% greater than the ~600-750 steps per day completed by hospitalized patients71,72 and thus findings of muscular atrophy seen here would likely be of even greater relevance in hospitalized patients with compounding pathologies.



Figure 13. The change in leg LM from BL to SR with DXA measurement error. Each participant represented by an individual bar, ranked by absolute change. Dotted line represents lower limit (± 400 g) and hashed line represents upper limit (± 1300 g) of FFM DXA measurement error. n=10 (6 men and 4 women).

### ISO-MVC

The impact of SR on skeletal muscle function remains contentious. For example, Breen et al.82 reported no change in peak torque production using ISO-MVC, findings that are in-line with our data. Conversely, Devries et al.89 reported a ~7% loss of strength in older men taking fewer than 1500 steps per day for two weeks. It is possible that the SR protocol did not provide sufficient musculoskeletal unloading to result in a decrement of strength. Fibre type changes during disuse in the elderly are not well characterized with some reports indicating a decrease in type II fibres56,137 and others supporting a shift towards type II instead137. Preservation of type II fibres may further support an ability to activate muscles and preserve the ability to produce maximal forces despite disuse137,138, potentially as a protective mechanism for impaired older adults during disuse, but further histochemical analysis is required to support these claims.

Losses of strength are consistently exhibited in older adults during bed-rest51,52,54,55 and immobilization56,64,65, however, the understanding of the effects of SR (i.e., incomplete disuse) are underdeveloped. It is possible that similar factors for which we could not account nor control (i.e., performance of the occasional isometric contraction136) could have offset any strength losses seen with SR. Devries et al.89 reported that low-load resistance exercise was sufficient to counter the declines in ISO-MVCpeak resulting from SR in older men, and therefore muscular activation during the sedentary SR phase may have impacted the expected strength loss in this study. While the lack of change in voluntary force production are congruent with what was reported by Breen et al.82, such a discrepancy between this study and Devries et al.89 could be related to the variation in ISO-MVCpeak measurement. Peak torque production did not differ significantly between the first and second familiarizations or BL in the current study; however, BL ISO-MVCpeak was 3.6% lower (NS) than the average of both familiarizations. Furthermore, 70% of participants did not match the peak torque produced during familiarizations when producing ISO-MVCs during BL testing. Older adults may require more familiarization to limit variation when performing maximal tests of strength and some have suggested more than 7 sessions139. Isometric knee extension torque measured using a standard dynamometer can vary by ±19 Nm due to factors unrelated to research interventions in older adults140. This degree of variation is approximately ±13% of the measured torque production in the current study and much greater than any anticipated change in strength with a two-week SR protocol. Even with a familiarization session there is still a systematic bias for knee extension ISO-MVCpeak to increase ~8 Nm with repeated testing140. We attempted to limit the variability in ISO-MVC by recording all apparatus settings for testing, providing a reasonable number of familiarization sessions (two), and giving verbal encouragement to participants141. Nonetheless, the use of visual feedback141 and/or superimposed twitch interpolation142 would be other employable options to achieve true maximal contraction during voluntary contractions. Therefore, the dynamometer used to assess ISO-MVCpeak may simply not be sensitive enough to measure the strength loss associated with SR.

### RTD

Impairment in the rate of force production has been found after disuse and is seemingly more pronounced in older adults64,65,88,137. Previous work has shown RTD was decreased nearly two-fold in older men compared to younger men after two weeks of knee immobilization, suggesting the elderly may be more susceptible to the neuromuscular consequences of muscular disuse56,64. Such disuse-induced reductions in RTD have been associated with an impaired ability to recruit skeletal muscle through reduced central neural drive in the elderly after disuse56, particularly in the recruitment of motor neurons innervating type II muscle fibres, which could exacerbate the natural decline of neural activation with aging88. While robust reductions in RTD are evident with complete disuse56,64,65, the results of this study indicate there is no effect of SR on the RTD during ISO-MVC in older adults. There is in fact previous work to support that older adults, despite disuse, are able to preserve or even increase the shortening velocity of their skeletal muscle fibres and thus are better protected against disuse-induced declines in RTD137,138. Furthermore, with no reductions in ISO-MVCpeak between BL and SR, measures of muscle activation during isokinetic maximal contractions could potentially offer further insight into the effects of SR on neuromuscular recruitment in older adults88 and provide a different perspective for human skeletal muscle dynamic strength52,64.

### Physical function

We did not find any impairment, after two weeks of SR, in the ability of our subjects to perform ADL as tested by 30CS, TUG and 6MWT, which are traditional clinical measures of physical function. In fact, the increased level of performance seen for the 30CS and 6MWT after RC compared to BL suggest that a ‘practice effect’ may have occurred for these tasks, despite repeated familiarization sessions. Given the opportunity to reduce the effects of repeated testing in this battery of physical function tests, it is possible that differences resulting from SR may have been elucidated. The only test that may suggest an effect of SR was the TUG which increased by 0.4 seconds to reach an average of 7.4 ± 1.5 seconds, an increase that is not clinically relevant106.

Decrements in ADL performance are frequently reported within a shorter time period than measured in this study. For example, even ~30% of ‘highly mobile’ older patients were discharged with declines in ADL after acute hospitalizations with an average 7.5 days spent in hospital74. Of course, the impact of the illness that the patient had while in the hospital could have affected this outcome. While an SR-induced impairment in physical function seemed likely, due to evidence from hospitalization of the elderly66,73,74,94,95 as well as an established relationship between knee extensor ISO-MVCpeak and ability to complete ADL17, without the concomitant losses of muscle mass and strength this association would not be expected. Our results agree with the findings of Breen et al.82 who showed no effect of two weeks of SR on Short Physical Performance Battery tasks in older adults.

The RWT was included as an estimate of V̇O2peak because of the important role cardiorespiratory fitness plays in maintaining functional ability143, mobility144 and ultimately independence in older adults145. Although the decline in estimated V̇O2peak in the current study did not reach statistical significance, a post-hoc power analysis suggests that we were underpowered to detect any effect of SR on V̇O2peak as measured by the RWT. We would have required a sample size of 34 participants to show that the SR was sufficient to reduce V̇O­2peak. There was also complete numerical recovery of estimated V̇O2peak with a return to habitual PA suggesting these impairments in cardiorespiratory fitness were, if they were present, transient.

### Inter-individual variability

What is most evident is the large range of daily steps taken, and it is therefore of interest to question how different habitual ambulation might affect the degree of change in various variables induced with SR. Our study, in agreement with previous work78, showed that there is no relationship between the reduction in habitual daily steps and changes in primary endpoint measures such as LM or insulin sensitivity. Although, the [G]120 after OGTT, which is a strong predictor of mortality independent of age146,147, was significantly correlated with the reduction in daily steps. This is only one measure, however, and in general it does not seem that increased habitual levels of daily steps were in any way protective against the effects of SR, nor does higher BL daily stepping predispose older adults to greater consequences of SR.

While the current study was underpowered to detect any differences between men and women, we attempted to see if there were patterns or associations between multiple measures independent of sex. For example, during an OGTT the time spent in a toxic hyperglycemic state (>10 mM), associated with cardiovascular consequences128,148,149, was not affected in all participants. However, some participants spent anywhere from 20 to 95 more minutes in hyperglycemia, regardless of their individual increase in OGTT glucose AUC. What becomes abundantly clear from the investigation of individuals (Figure 14) is that some older adults responded with greater impairments in glycemic regulation as a result of the SR intervention, while others showed a limited response. It seems that all older adults are subject to some degree of impairment in insulin sensitivity after SR, which suggests that Matsuda ISI may be an important end-point outcome to target health after physical inactivity, rather than LM or strength. Furthermore, while many of the results from this study did not reach statistical significance due to the small sample size, it does not mean the effects of SR on individuals are not clinically relevant. Hypothetically, if at least one in ten participants from this current study experienced severe declines in glycemic control, strength, cardiovascular fitness or LM, when extrapolated to the Canadian population of older adults this could mean that ~500 000 Canadians1 are in some way negatively affected by acute periods of SR that approximate what we experimentally induced here. While underlying factors that make some older adults more vulnerable to SR than others are not yet understood, nor is a population-wide effect well established, SR may have underappreciated consequences for an individual.



Figure 14. Individual change responses of participants (each with unique symbol) from BL to SR, organized from left to right by increasing reduction in daily steps during SR. n=10 (6 men and 4 women). A) Individual percentage change in leg LM mass. B) Individual percentage change in maximal knee extensor ISO-MVC. C) Individual percentage change in estimated V̇O2peak from RWT. D) Individual percentage change in capillary blood glucose AUC during OGTT. E) Individual percentage change in Matsuda ISI. F) Individual percentage change in HOMA-IR.

### Conclusions

In summary we report that after two weeks of SR of fewer than 1000 steps per day, older adults experienced impairments in glycemic control measured with OGTT and free-living CGMS. However we did not detect significant declines in muscle mass, strength and physical function. Furthermore, despite a return to habitual levels of ambulation and exercise, older adults failed to recover their insulin sensitivity, which was significantly reduced after SR. This is the first study to report an inability for older adults to recover from a seemingly relatively brief period of physical inactivity such as a reduction in daily steps. When compared to the recovery potential of younger adults56,79, the findings of the current study highlight the increased vulnerability of older adults to muscular disuse. No therapeutic guidelines currently exist for ambulation in hospitals66, and as such, the results of SR studies direct attention towards care that would limit the iatrogenic effects of reduced ambulation during acute illness and hospitalization.

### Future directions

In order to address some of the limitations of the current study, subsequent research should place primary importance on using well controlled measures or ‘gold standard’ methodologies for measuring outcomes such as muscle mass and strength. Using a larger sample size for increased statistical power would provide a better opportunity to investigate differences between men and women in response to physical inactivity, as well as understand any relationships between habitual PA and susceptibility to the consequences of SR. While the current model of SR and RC attempted to mimic a non-pathologic inactivity and recovery period in older adults, clinical investigations of SR may show augmented consequences of SR due to concomitant pathologies. This study suggests that some older adults may be able to defend against two weeks of SR, however, more protracted periods of SR (eg. illness/injury and convalescence lasting 3-4 weeks) may surpass this threshold and have greater adverse health consequences. Future studies altering the length or degree of SR may in fact find these changes impact the consequences of SR for older adults, while longer post-SR periods may help characterize the time course of recovery for the elderly.

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