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Arto Pesola

Reduced Muscle Inactivity, Sedentary Time and Cardio-Metabolic Benefits

Effectiveness of a One-Year Family-Based Cluster Randomized Controlled Trial

JYVÄSKYLÄN YLIOPISTO

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UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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A driving hypothesis of the evolving field of sedentary behavior is that frequent muscle activity short-circuits detrimental physiological effects of sedentary time. However, the field relies mostly on observational health associations of proxy measures of muscle inactivity, like television viewing or lack of accelerometer-derived impacts. This study tested effectiveness of a family-based cluster-randomized controlled trial aimed at reducing and breaking up sedentary time at work and leisure time of families on muscle inactivity patterns, sedentary time and cardio-metabolic biomarkers. The intervention consisted of a lecture, face-to-face tailored counseling, two follow-up calls and five emails during the first six months, and six months follow-up without the intervention. Detailed muscle inactivity and activity patterns of sitting, standing, normal daily life and the short-term efficacy of the counseling were quantified by special shorts capable of measuring average rectified EMG signal. The long-term effectiveness of the intervention was studied in 133 sedentary parents by measuring accelerometer-derived sedentary time, anthropometrics and blood-drawn cardio-metabolic biomarkers to report if short-term changes in muscle inactivity patterns translate to long-term behavioral and physiological benefits. The main findings of this study were that while muscles were inactive 90% of sitting time and the average muscle activity amplitude was threefold higher in standing (1.6% of EMG_{MVC}) than sitting (0.5% of EMG_{MVC}), the inter-individual differences were tenfold. However, within two weeks of the counseling muscle inactivity time decreased by 37 min in intervention group compared to controls without affecting high intensity activities suggesting that sedentary-time targeted intervention can change specifically muscle inactivity patterns. At three months the intervention was successful in reducing accelerometer-derived sedentary leisure time by 27 min in the intervention group as compared to controls. At 12 months the control group's sedentary leisure time tended to increase whereas that of the intervention group remained at the baseline level suggesting that acutely effective intervention methods may prevent unfavorable long-term changes. Between baseline and endline, significant intervention effects in weight, total lean mass, leg's lean mass, apoA-1 and apoB/apoA-1 -ratio favored intervention group and remained independent of moderate-to-vigorous -intensity physical activity and energy intake. In conclusion, the family-based tailored counseling was effective in reducing muscle inactivity and sedentary leisure time acutely, and in preventing an increase in sedentary leisure time during one year without reducing work or weekend sedentary time. This resulted also in some modest positive changes in anthropometrics and apolipoproteins independent of moderate-to-vigorous physical activity and energy intake. Reducing muscle inactivity produces causal health benefits and may bear public health potential.

Keywords: muscle inactivity, EMG, textile electrodes, sedentary behavior, cluster-randomized controlled trial, tailored counseling, cardio-metabolic biomarkers

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FOREWORD AND ACKNOWLEDGEMENTS

During my university studies, I encountered a question on an exam that asked what elements make up "physical activity". Due to my lack of knowledge and in part because I was feeling a bit rebellious, I answered indifferently: "Who cares, as long as we participate more in physical activity?" After the exam, I remembered that our textbook stated that the elements of physical activity are: frequency, intensity, volume (time), and type of exercise, but I still was not sure why physical activity needed to be cut up and classified like this. I did not understand why physical activity had to be such structured "exercise".

While I enjoy physical activity and participating in all kinds of sport myself, over the years I have wondered about "physical activity" and the seemingly uncontested idea that participating in exercise is the only way to improve our physical fitness and health. Why do we have to put on our exercise clothes in order to improve our health? Is it possible that only meeting these specified physical activity patterns at high enough intensity bring any results?

In 2008, a former classmate and current colleague Olli Tikkanen sent me Professor Marc Hamilton's review article (Hamilton et al. 2007). This article revolutionized my thoughts by explaining that sitting is actually a health risk. The studies reviewed showed that sitting less improved health and even indicated that sitting less could improve health variables that remained unchanged by participating in regular exercise. I read every single word of that review. With each word, I validated the idea that maybe also those people who are not willing or able to comply with the traditional methods of exercise prescription may enjoy active and healthy lifestyle.

Not long after reading the paper, I received a phone call from Professor Taija Juutinen Finni, who at that time was supervising my master's thesis. Taija had received funding for a new project specifically aiming to elucidate if increasing light everyday physical activities might provide an effective addition to the current exercise prescription. Taija had persuaded two of my colleagues to start their PhD studies in this project, both of who refused to snap up. As her third option, Taija inquired my interests towards the project. After a couple hours facile consideration period I accepted the challenge. Needless to say, I'm more than happy for these fortunate events, which enabled me to make the easy decision.

I want to express my deepest gratitude to Taija, who has guided me through the challenges every budding researcher encounters. Taija is one of the busiest persons I know, but those busies have not reduced her caring abundance and supervision at the highest standards. I have been very creative in slowing down my PhD studies with all kinds of extra ideas and projects, but have never heard any discouraging words from Taija. Instead, she has been able to see through these obstacles and inspired me to build my professionalism beyond merely finalizing the thesis. Taija's enthusiasm and talent towards creating new ideas has been a huge motivation along my journey. I couldn't imagine a better supervisor. I'm also grateful to Docent Arja Sääkslahti and Professor

Sarianna Sipilä, who put up our project and whose advices along the project were invaluable. I also want thank my second supervisor Heikki Kainulainen, who has helped me in specific physiological subjects with an expertise of iron.

Making a PhD is teamwork. I have been privileged to work with wonderful and talented fellow PhD students who brought all the life and joy to the long days. The most important guy has been Arto Laukkanen, with whom we started to work in the same project. In the beginning we spent long hours in a tiny office calling to subjects and writing our articles. Arto's calming attitude has always covered his hard work and diligence - even at the busiest times Arto has been himself. Seemingly without any extra effort he steered our project to the right direction. In April 2015 when defending his thesis, Arto showed me an example of a mature scientist who will have a bright future. I'm also grateful for the other wonderful colleagues in our project. Piia Haakana is "the mother of EMG analysis and cleaning". Piia has made a huge work along the years by having her hands on the practical development of the EMG measurement and analysis methods. In addition, Piia and Kasimir Schildt provided an indispensable help in data collection in the long intervention project, and Tahir Masood was helping in data analysis when we couldn't stretch there ourselves. Marko Havu and Risto Heikkinen provided priceless help in developing the data analysis methods by writing code, which still remain beyond my understanding. I also want to acknowledge my other colleagues Lauri Stenroth, Ying Gao, Mika Silvennoinen and Juha Hulmi among the many others with whom we have spent countless hours drinking coffee and having lunches. From the whole PhD project those moments and the many people are the most important outcome. Moreover, many of them keep sending me funny memes of the ridiculous hooha around the whole sedentary behavior topic, which surely have kept me humble.

When thinking of possible expert reviewer's for my thesis, I made a list of scientists who have contributed the most for my thoughts and research throughout my PhD studies. With humble minds, we started approaching the possible reviewers from the top of my list. However, we didn't get far. I'm extremely grateful for Professor David Dunstan, Professor Marc Hamilton and Professor Genevieve Healy who gave us an immediate positive response. I want to thank all of them for their pioneering and inspiring research, which served as a starting point for my PhD, and for the time they committed for reviewing the thesis at the highest scientific standards. Especially warm acknowledgement belongs to Professor Genevieve Healy who agreed to be the opponent in the public defense of this dissertation. These three pioneers all have made an enormous contribution for this work - and together they formed the top three on my list.

In addition to my research work, I have been privileged to implement and share my ideas with similar minded, forward-looking colleagues at Fibion Inc. Olli Tikkanen, Tommo Reti, Ari Peltoniemi and Marko Havu are colleagues, who promote the implementation of science through their multitalented expertise. This expertise is converted into new tools, which support the work of pro-

fessionals at the field of physical activity and health. In Fibion team, I really can realize my passion. I want to warmly recommend practical work outside of academic world for anyone who wants to get a new perspective. The problems to be solved are completely different, and so is the motivation and expertise gained.

I want to express my deepest gratitude to institutions and foundations that have made my PhD studies possible. Finnish Ministry of Culture and Education, Academy of Finland, Juho Vainio Foundation, Yrjö Jahnsson Foundation, Kuntoutumis- ja liikuntasäätiö Peurunka and my home organization, Department of Biology of Physical Activity at the Faculty of Sport Sciences, have provided the resources and funding for the projects I have been working with. Myontec Ltd is acknowledged for their technical support and encouragement for the research we have been doing. I also want to thank the whole staff of our department, especially Minna Herpola, Katja Pylkkänen, Pirkko Puttonen, Risto Puurtinen, Aila Ollikainen, Markku Ruuskanen and Sirpa Roivas for the hard work they have done every day behind the scenes. They have always been available when needed, and those times are many.

My father Tapio, mother Sirpa and the rest of my family also deserve thanks for their support in this project. I also want to acknowledge my friends at JTC, our own telemark skiing club. With them life is always easy and enjoyable.

While spending countless hours on the computer, no one has had the energy to stay by my side as long as my dear wife Tanja. Besides many long evenings spend staring at the computer screen, Tanja has encouraged and inspired me to keep going with her lovely persistence. Tanja has made sure that we discuss topics other than my PhD and because of Tanja and our dearest daughter Lilja, life has included even more important things than physical activity and writing about it. With her honest eyes and insatiable will to explore the world, Lilja has brought joy and a whole new perspective to my life.

This book is dedicated to the many people who devoted their time by being participants in our research projects.

Jyväskylä 10.8.2016
Arto Pesola

ORIGINAL PUBLICATIONS

The thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I. Pesola AJ, Laukkanen A, Tikkanen O, Finni T. 2016. Heterogeneity of muscle activity during sedentary behavior. Applied Physiology, Nutrition & Metabolism 41(11): 1155-1162.

II. Pesola AJ, Laukkanen A, Tikkanen O, Sipilä S, Kainulainen H, Finni T. 2015. Muscle inactivity is adversely associated with biomarkers in physically active adults. Medicine & Science in Sports & Exercise 47(6): 1188-1196.

III. Pesola AJ, Laukkanen A, Haakana P, Havu M, Sääkslahti A, Sipilä S, Finni T. 2014. Muscle inactivity and activity patterns after sedentary-time targeted randomized controlled trial. Medicine & Science in Sports & Exercise 46(11): 2122-2131.

IV. Pesola AJ, Laukkanen A, Heikkinen, R, Sipilä S, Sääkslahti A, Finni T. Objectively measured sedentary work, leisure time and cardiometabolic biomarkers: effectiveness of a one-year family-based cluster randomized controlled trial. Submitted for publication.

Additionally, some previously unpublished results are included in the thesis.

ABBREVIATIONS

ANOVA	Analysis of variance
ApoA-1	Apolipoprotein A-1
ApoB	Apolipoprotein B
ApoB/A-1	Apolipoprotein B/A-1 –ratio
%B	Basal insulin secretion
BMI	Body mass index
CI	Confidence interval
CRCT	Cluster-randomized controlled trial
EMG	Electromyography
FPG	Fasting plasma glucose
HDL	High density lipoprotein cholesterol
HOMA	Homeostasis Model Assessment
IR	Insulin resistance
MVPA	Moderate-to-vigorous physical activity
r	Correlation coefficient
RCT	Randomized controlled trial
SD	Standard deviation
Sedh	Sedentary hour

CONTENTS

1 INTRODUCTION

"Take a seat, please!" This invitation offers you the chance to take a load off your feet, to rest for a moment and settle into that chair. The politeness of this invitation lays in the active history of man, where activities of daily living have been indispensable for survival, but rest has been a welcomed possibility. The development of mankind has enabled convenient living without the necessity for physical activity. Consequently, a modern lifestyle is characterized by abundance of chairs, which portrays the success of development. The necessity of physical activity has turned into an option for physical activity left upon individual choice. At the face of sedentary lifestyle, physical activity is no longer necessary for survival, yet continually more important to sustain our inherent physiological readiness to lead the active lifestyle of the past.

The current physical activity recommendations address the importance of undertaking half an hour of moderate-to-vigorous activity on five days a week to gain health benefits (Haskell et al. 2007). These activities can include walking to work, jogging for fitness, or gardening, if they elevate heart rate and get one out of breath. Specifically, the recommendations state that activities performed at an intensity corresponding to three times the resting energy expenditure, i.e. moderate-to-vigorous intensity, produce health benefits whose scientific foundation is enormous. Of interest is that the recommendations include a note that also the activities performed at amounts below the specified thresholds may be beneficial. However, so far these benefits have remained to be elucidated.

An individual might be nominated physically active if her daily living includes the recommended dose of physical activity. This is good news for an office worker who might pick up her nomination with a half an hour jog after sitting the entire day at work. For decades the physical activity literature has generally distinguished "sedentary" from "physically active" participants based on not meeting the physical activity recommendations (Bennett et al. 2006). However, this categorization does not make any difference between those who perform zero to <30 minutes of moderate-to-vigorous activity per day, or those who are standing the whole day but do not engage in more intense activities. Consequently, the influence of the baseline activity upon which the physically

active lifestyle is to be built, have remained beyond focus during past decades of research.

The rapid development of modern lifestyle has been followed by a fast development of physical activity research, which both have been noticeably skewed towards the direction of sedentary behavior. Advances in environment and transportation, as well as time pressure in work makes us dash physical activity out of our lives. Driven by the technological development, population level sedentary behavior has been increasing throughout the past decades without signs of slowing down (Church et al. 2011; Ng, & Popkin 2012; Archer et al. 2013). An increasing proportion of population is having an elevated risk for sedentary behavior owing to the increased prevalence of office work and sedentary leisure time habits (Brownson et al. 2005; Church et al. 2011) resulting in 9-11 h of total sitting time per day (Matthews et al. 2008; Hagströmer et al. 2010; Colley et al. 2011). At the same time, the number of publications studying the health effects of sedentary lifestyle has experienced an exponential rise (Bauman et al. 2013). These both trends come across in findings that the recommended amount of physical activity may not cancel out the health hazards caused by sitting too much. Sitting has been found to associate with disease incidence and premature mortality, which were previously acknowledged for lack of physical activity as per recommendations (Owen et al. 2010).

During the past years, sedentary behavior has been conceptualized as its own entity affecting health, with possibly distinct underlying physiological mechanisms as compared to lack of physical exercise. This conceptualization is rooted on the epidemiological findings, where the health hazards of sedentary behavior have remained statistically independent of moderate-to-vigorous activity (Hu et al. 2003). The driving hypothesis supporting the causality of these findings has been that frequent activity in antigravity muscles short-circuits the detrimental physiological processes of sedentary time resulting in better cardiometabolic risk profile (Hamilton et al. 2007). An increasing number of acute laboratory experiments have proved that reducing and breaking up sitting with activities of light intensity bring about benefits that are of similar or even higher magnitude as compared to undertaking a similar volume of activity within a single exercise bout (Peddie et al. 2013; Duvivier et al. 2013; Blankenship et al. 2014). These findings have provided hope that increasing even light intensity activities that break up detrimental muscle inactivity could provide an avenue for healthy lifestyle for people living at the mercy of sedentariness.

Even though the field of sedentary behavior research is rooted on the assumption that lack of engagement of muscles during everyday life is detrimental, many of the findings supporting the concept of sedentary behavior as an individual health hazard are based on observational evidence and short-term interventions conducted mostly in laboratory. The association between muscle inactivity and health outcomes has not been shown with direct measures, and it has been unknown how reducing sedentary behavior actually affects muscle activity. Further, currently the only evidence available from interventions targeting sedentary time as a primary outcome is from studies last-

ing less than three months (Martin et al. 2015) with few exceptions (Aadahl et al. 2014; Healy et al. 2016), which is a major gap in the literature. The help for public health is provided only if reducing sitting time is possible and effective in providing long-term causal health benefits.

This study aimed to address these research gaps by testing the effectiveness of a year-long cluster-randomized controlled trial targeted at reducing and breaking up sedentary time at work and leisure time of families. The muscle inactivity and activity patterns during sitting, standing, everyday life and the changes following the intervention were quantified in order to report how reducing sedentary time affects muscle inactivity patterns. The long-term effectiveness of intervention was studied by measuring accelerometer-derived sedentary time, anthropometrics and blood-drawn cardio-metabolic biomarkers to report if short-term changes in muscle inactivity patterns translate to long-term behavioral and physiological benefits.

2 LITERATURE REVIEW

2.1 The evolving concept of sedentary behavior distinct from physical inactivity

Health effects of physically active lifestyle have been well documented and preventive efforts have been targeted at increasing physical activity at the population level for decades. However, the interpretation of the advice to increase physical activity might be very different depending on what an individual considers as physical activity. Jogging for fitness when one gets out of breath, is easily understood as physical activity. However, standing at work, gardening, or fidgeting while sitting, may or may not be physical activity depending on the underlying definitions.

The prevalence of "physically active" and "physically inactive" people, as well as the associations to health outcomes, vary greatly across studies depending on the definitions used (Bennett et al. 2006). A common misconception in physical activity research has been the distinction between physical inactivity and sedentary behavior. Provided that the majority of physical activity literature concerns the effects of moderate-to-vigorous activity, failing to meet this intensity level has been generally considered as *sedentary*. However, sedentary behavior, which can practically understood as sitting with relatively idle muscles, has been more recently defined as its own entity to make a clearer distinction between sedentary behavior and physical inactivity, referring to not accumulating adequate amounts of moderate-to-vigorous physical activity. The updated definitions have clarified the fact that sedentary behavior and physical inactivity are independent classes and they may or may not coexist. The evolution of the key concepts in physical activity research helps in streamlining the knowledge on what physical activity can reduce sedentary behavior.

2.1.1 Definition of sedentary behavior

Sedentary behavior is defined as any waking behavior in seated/reclined positions with energy expenditure at or below 1.5 metabolic equivalents (Sedentary Behaviour Research Network 2012), such as lying down, sitting, or watching television. Like physical activity, daily sedentary behavior is accumulated in various contexts, like occupation, transportation and leisure time. It can also include a modifiable (e.g. television viewing) and a necessary (e.g. office work) component, which can be used for further classification.

Sedentary. In cultural anthropology, "sedentariness" means living in a one place for a long time (Wikipedia). The term "sedentary" originates from a latin word "sedere", which means "to sit" (Hamilton et al. 2007). Unlike being physically inactive, there are no any specific cut points or criteria available for being sedentary. Sedentary people are often stratified from non-sedentary people based on artificial cut-points or sample statistics like quartiles (Wilmot et al. 2012; Edwardson et al. 2012), making a uniform definition of "being sedentary" difficult. Thus, in current research literate the cut points for "sedentary" are selected based on the sample in question. Moreover, it has been common to categorize subjects as "sedentary" based on other groups who participate in physical activities, without actually even measuring sedentary time (Pate et al. 2008).

Muscle inactivity can be defined as time when the muscle activity amplitude remains below a specified muscle inactivity threshold (Tikkanen et al. 2013). It has been proposed that muscles are inactive during sitting and active during physical activity explaining their different effects on metabolic outcomes, but few studies have elaborated on this hypothesis (Hamilton et al. 2007). For example, muscle inactivity time may vary depending on the threshold condition used (Klein et al. 2010). It is important to note that sedentary behavior and muscle inactivity may not correspond; the possible differences between these concepts are explored in this thesis. Moreover, *muscle* inactivity should not be confused with *physical* inactivity.

2.1.2 Definitions of physical activity and physical inactivity

Physical activity is defined as bodily movement produced by contraction of skeletal muscles, which increases energy expenditure over the resting level (Caspersen et al. 1985). Physical activity can be divided into subcategories based on behavioral, environmental or biological criteria, which may overlap to some extent. A common categorization is based on the practical contexts where physical activity takes place, from which typical examples include occupational, transportation, household or leisure time physical activity. Leisure time physical activity can be further divided into recreational activities, exercise training or competitive sports (U.S. Department of Health and Human Services 1996). Across the categories, physical activity may include a volitional aspect, like planned, structured and repetitive *physical exercise* with an objective to increase

or maintain physical fitness (Caspersen et al. 1985). On the other hand, physical activity can be spontaneous such as fidgeting while standing (Levine et al. 2000), or obligatory such as physical activity necessary to carry out occupational tasks (Church et al. 2011).

Physical exercise is defined as physical activity, with an objective to increase or maintain physical fitness (Caspersen et al. 1985). Because of several mutual elements, it has been common to use physical activity and exercise interchangeably. However, in 1990 official recommendations by American College of Sports Medicine stated that the quantity and quality of exercise to gain health benefits may differ from what is recommended for enhancing fitness (Blair et al. 2004).

Physical activity for health. In the context of physical activity research, health has been defined as "a human condition with physical, social and psychological dimensions, each characterized on a continuum with positive and negative poles" (U.S. Department of Health and Human Services 1996). The physical activity guidelines are reasoned "to promote and maintain health" (Haskell et al. 2007), and the amount of *physical activity for health* is commonly paralleled to the physical activity guidelines. For the past decades of physical activity promotion, the recommendation to increase physical activity has included a threshold, which defines on what intensity level the physical activity should be undertaken to gain the desired health benefits. Conversely, it can be implied from this definition that only physical inactivity, not sedentary behavior, matter in terms of health risks.

Physically active. Distinct from the definition of *physical activity*, *physically active* refers commonly to a degree of physical activity which fulfills the physical activity recommendations. The current recommendations encompass moderate-intensity physical activity for a minimum of 30 min on five days each week or vigorous-intensity physical activity for a minimum of 20 min on three days each week, and activities that maintain or increase muscular strength a minimum of two days each week (Haskell et al. 2007). Consequently, to be classified as physically active, one needs to undertake conscious physical activities at a minimum of moderate intensity. Although current physical activity recommendations include strength training, it is common to exclude the strength training part from the criteria, such that only the aerobic exercise part counts for physically active lifestyle.

Physical inactivity can be defined as an insufficient amount of moderate-to-vigorous physical activity, i.e. not meeting physical activity guidelines (Sedentary Behaviour Research Network 2012).

2.1.3 Continuum from sedentary behavior to physical activity

Energy expenditure is an essential determinant of physical activity, as illustrated by both sedentary and physical activity definitions. Following the definitions, this continuum can be further categorized based on posture, external movement and/or muscle activity (Figure 1). Metabolic equivalent of task (METs) is commonly used to categorize the physical activity continuum into intensity classes (Pate et al. 1995). Sedentary behavior such as sitting and lying down typically expend little energy and their MET value is close to resting level (1.0–1.5 METs). Light-intensity activities such as self-care activities and slow walking increase energy expenditure above the resting level with a MET -rage of 1.6-2.9. Moderate (3-6 METs) and vigorous (>6 METs) activities are more likely exercise-related activities, like brisk walking or jogging (Ainsworth et al. 2000). However, it is important to note that occasionally the energy expenditure of daily activities can exceed the light intensity threshold, without being categorized as exercise in case they do not include a conscious effort towards increasing or maintaining physical fitness.

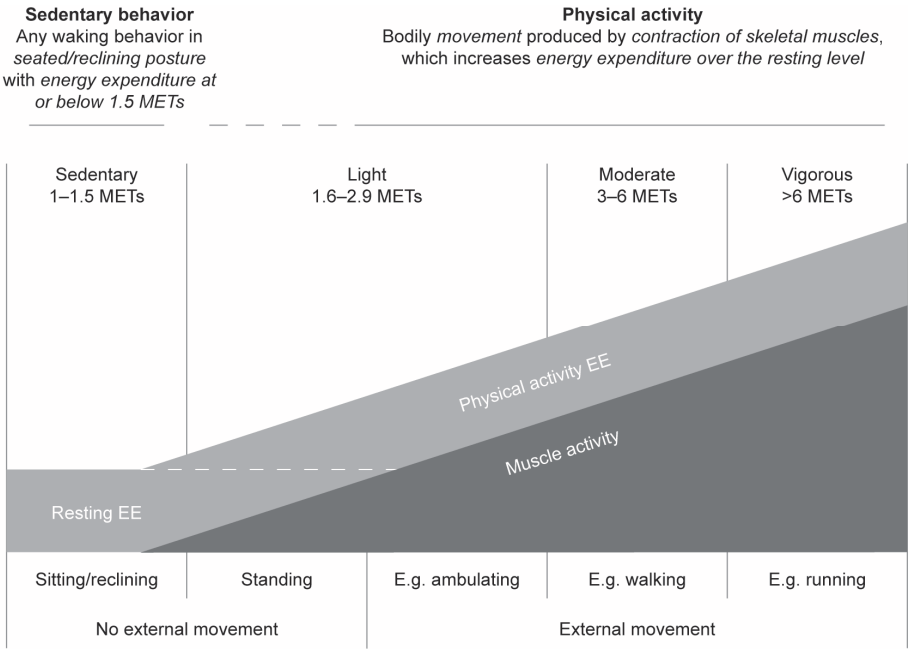

Figure 1 Continuum from sedentary behavior to physical activity is defined based on posture, external movement, energy expenditure and/or muscle activity.

By definition, *physical activity* should include external movement caused by contraction of skeletal muscles. Although standing involves no bodily movement and thus bypasses the conventional definition of *physical activity*, it includes an upright posture with elevated muscle activity and energy expenditure, and is

rather classified as *physical activity* than *sedentary behavior* (Gibbs et al. 2015). Interestingly, standing is distinct from the definition of sedentary behavior based on the measures of posture and energy expenditure, but similar to physical activity based on the measures of muscle activity and energy expenditure. Although energy expenditure is a common distinctive factor, using only energy expenditure in the classification might be problematic because some sitting-based activities might exceed the fixed threshold of 1.5 METs (Mansoubi et al. 2015). Conversely, the energy expenditure of static standing might fall below 1.5 METs especially in overweight people (Mansoubi et al. 2015; Tompuri 2015). Thus it is advantageous to include both energy expenditure and posture in the definitions.

The distinction between sedentary behavior and physical activity definitions would be further clarified if lack of muscle activity was used as an additional determinant of sedentary behavior, and if external movement would not be required for physical activity. Therefore, in this thesis *muscle inactivity* refers to time when the intensity of muscle contraction as measured by EMG remains below the specified inactivity threshold, as described later. This is in line with proposed concept of *inactivity physiology* distinct from *exercise physiology* (Hamilton et al. 2007). Thus, *muscle inactivity* should not be confused with *physical inactivity*.

The total daily sedentary and physical activity behaviors can accumulate in different patterns. The volume of physical activity is accumulated as a multiplication of frequency, intensity, duration and type of activity. A comparable approach has been suggested for sedentary behavior, such that the total pattern of sedentary behavior is a constituent of frequency, interruptions, time and type of sedentary behavior (Tremblay et al. 2010). Figure 2 illustrates that a part of the patterns of sedentary and physical activity behavior can occur independently, but part of the accumulation is conceptually dependent (Figure 2).

The volume of physical activity can be expressed as accumulated time spent at the categories of light, moderate and vigorous intensities. The total volume of physical activity can be further expressed as a time-weighted average of the accumulated METs, e.g. MET-hours/week (METh/wk). This way the time spent at each intensity category can be combined into one variable depicting total energy expenditure (Caspersen et al. 1985; Ainsworth et al. 2000). In respect to sedentary behavior, some authors have suggested that also more than one dimension of sedentary behavior could be depicted in one outcome, such as total number of sedentary bouts divided by their total duration, which indicates the pattern of sedentary time accumulation (Lyden et al. 2012; Chastin et al. 2015c).

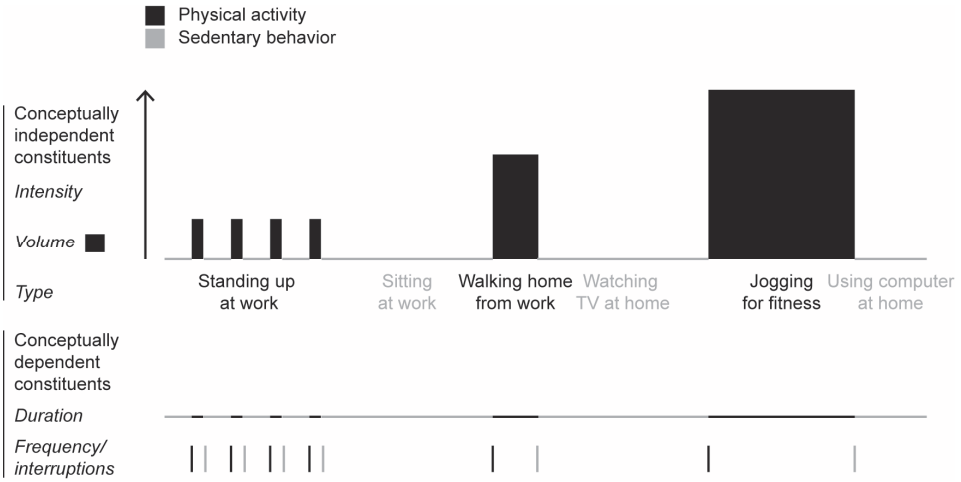

Figure 2 Different types of sedentary and physical activity behaviors are accumulated in bouts of different duration, frequency (dependent), intensity and volume (independent). The degree of dependency between sedentary behavior and physical activity may thus depend on the pattern of an individual.

2.1.4 Behavioral independency of sedentary behavior from physical inactivity

An individual may accumulate her total daily physical activity by meeting several parts of the physical activity continuum. The total volume of physical activity can be obtained by displacing sedentary time with physical activity, and/or by displacing light intensity activities with more intensive ones. Conversely, sedentary time may be accumulated at the expense of light or moderate-to-vigorous activities. From the perspective of sedentary and physical activity patterns, it is important to consider how the reallocation between these behaviors occurs during habitual living. Based on observational evidence, sedentary time and moderate-to-vigorous activity are poorly correlated and physically active people appear to be sitting equal amounts to inactive ones (Craft et al. 2012). This evidence suggests that there is time for both sedentary behavior and moderate-to-vigorous physical activity during the 24 hour day.

One reason for the independency of sedentary behavior from moderate-to-vigorous activity behavior might be that sedentary behavior covers the majority of waking hours, whereas the time spent at moderate-to-vigorous physical activity is short. Studies using objective monitoring have reported that 55% to 70% of waking hours is spent in sedentary behavior (Matthews et al. 2008; Hagströmer et al. 2010; Colley et al. 2011) corresponding to 9-11 hours per day, as compared to less than 30 minutes of moderate-to-vigorous physical activity (Metzger et al. 2008; Colley et al. 2011). Thus, the typical amount of moderate-

to-vigorous physical activity is of insufficient magnitude to displace large amounts of sedentary time. Another possible reason is that the domains where people sit and exercise are very different from each other (Owen et al. 2011). For example, an office worker might have limited options for physical activity during work time resulting in high accumulated sitting time, but he might participate in moderate-to-vigorous activities during leisure time. The behavioral independency suggests that interventions should target both of these behaviors, with likely different methods.

Accumulating sedentary behavior independent of moderate-to-vigorous physical activity is possible due that sedentary behavior occurs in exchange of light intensity physical activity time, but moderate-to-vigorous physical activity displaces randomly sedentary or light activity (Finni et al. 2014; Chastin et al. 2015b). Moreover, it has been suggested that insufficient sleep time may favor sedentary behavior and reduce drive towards physical activities (Tremblay et al. 2007), which is supported by the observational co-dependency between sleep time and sedentary time (Chastin et al. 2015b). Although sedentary time and moderate-to-vigorous physical activity appear to be behaviorally independent, the different activity classes and sleep compose the 24 hours day and are thus inherently linked to each other. If an individual increases or decreases an activity class, it must occur in exchange of other behaviors, and the resulting pattern of sleep, sedentary time and physical activity at different intensities matters more than the modified activity class itself (Chastin et al. 2015b).

2.1.5 Accelerometer -based measurement of sedentary and physical activity patterns

Objective monitoring of physical activity enables the assessment of the total physical activity continuum from sedentary behavior to vigorous activities. Importantly, it serves as a tool to estimate if the proposed constituents of sedentary when measured during habitual living have relevance to health outcomes.

The cut points for the different intensity categories are based on validating the device output, like accelerometer-derived counts, against objectively measured energy expenditure (Matthews 2005). A "count" represents the magnitude of impacts accumulated over a time period, typically over one minute. A typical placement for a device is on waist, where the impacts measured near the center of body mass can be used to estimate total body energy expenditure during movement. The intensity data obtained in a continuous scale is typically categorized into intensity classes following the proposed classifications (Pate et al. 1995). Respective threshold values for waist-worn devices include >100 to 2019 counts/min for light activity and >2020 counts/min for moderate-to-vigorous activity (Troiano et al. 2008). Further, the duration, frequency and total volume of physical activity can be estimated. It is important to note that specifications of different devices influence the output such that e.g. sampling rate (how many samples are collected in a time unit), dynamic range (how high accelerations are collected), and resolution (to how many data points the intensity range is divided) should be considered when analyzing results, and may re-

quire calibration to enable use of same counts between different devices (Matthews 2005). Fortunately for a researcher, some advancement have been made in the development of universal analysis methods which are proposed to give comparable results across different devices (Vähä-Ypyä et al. 2014).

Accelerometers can also be used to estimate time spent sedentary based on lack of movement. Similarly to physical activity intensities, sedentary behavior is typically classified as a period of counts at <100/min (Matthews et al. 2008). However, the ability of a waist-worn device to estimate the actual sitting time is good on a population level, but worse on an individual level (Healy et al. 2011a). Because the definition of sedentary behavior includes also sitting/reclining posture (Sedentary Behaviour Research Network 2012), inclinometers have been applied to measure the actual sitting/standing postures to clarify the difference between sedentary behavior and physical activity beyond lack of movement (Gibbs et al. 2015). Not surprisingly, sensitivity to changes of sitting time has been shown to be higher when the actual sitting posture is measured (Kozey-Keadle et al. 2011; Chastin et al. 2015c). However, the total time spent sedentary or sitting appears to be similarly high (more than 70% of measurement time) with both methods (Kozey-Keadle et al. 2011).

In addition to measuring total sedentary or sitting time, the pattern in which the total time is accumulated is important. For this purpose, a break in sedentary time can be measured as one minute interruption in sedentary time (Healy et al. 2008a) or as an actual change in posture (Judice et al. 2015). That said, a conventional "break in sedentary time" (Healy et al. 2008a) as measured by a waist worn device may actually reflect not only a break in sedentary time, but also a break in activity time because all transitions across the sedentary and physical activity threshold are measured (Kang, & Rowe 2015). A validation study reported that the number of breaks/day as measured with a waist worn device and analyzed based on counts is around 74, which is almost double (39) as compared to inclinometer-derived transitions between sitting and standing postures (Barreira et al. 2015). However, normalizing the number of breaks to actual sedentary time improves the interpretation of breaks as interruptions in sedentary time (Lyden et al. 2012).

Other drawbacks of accelerometers include that a waist worn device may classify static standing completely to sedentary behavior because of lack of movement (van Nassau et al. 2015). Similarly, the energy expenditure of cycling, strength training or fidgeting-like activities during sitting where the device remains stationary, are ignored (Levine et al. 2000; Chen, & Bassett 2005; Marshall, & Merchant 2013). Moreover, both of these methods provide data about absolute intensity of a given activity and are incapable of measuring intensity relative to an individual's physiological capacity. In practice, many of the validation studies are conducted in young, healthy and normal weight individuals whose physiological capacity to perform physical activity is high (Copeland, & Esliger 2009; Ortlieb et al. 2014). An individual with very low fitness level might reach their maximum where the device measures only moderate intensity based on the validations drawn from fit individuals. Similarly, an overweight person

would perform a higher absolute workload at a similar external movement measured because of the extra body weight he needs to carry (Tompuri 2015).

Despite these problems, advancements in objective monitoring of physical activity, posture and sedentary behavior have reduced the inherent problems related to self-reported sitting time. This development has progressed the field tremendously beyond the single self-recalled types of sedentary behavior to assessment of complex patterns where the bouts of sedentary and physical activity time alternate in differing intervals, which are impossible to recall or report.

2.1.6 Measurement of habitual muscle inactivity and activity

Muscle activity measured using electromyography (EMG) reflects the electrical action potential resulting from muscle contraction, which spreads within and across the surface of the muscles fibers. The changes in action potential can be measured from the surface of the skin with EMG electrodes, which capture the summed signal propagated from the active motor units. With increasing force level, the detected electrical activity increases because more muscle fibers are recruited and firing frequency of the fibers increases. Thus, the EMG signal reflects the level of electrical activity that is picked up by using EMG electrodes. In clinical applications surface EMG has been used to study the coordination of movement and the roles different muscles play in specific tasks by detecting the level or lack of their electrical signal. (De Luca 1997).

Because physical activity is defined to be caused by contraction of skeletal muscles (Pate et al. 1995), it is logical to measure the physical activity exposure in this outcome. The anti-gravity muscles, including plantar flexors soleus and gastrocnemius, knee extensors vastus lateralis, vastus medialis and rectus femoris, hip extensors hamstring and gluteal muscles, as well as trunk extensors in erector spinae muscle group among other muscles, keep the body in upright posture during standing and produce movement during more intense physical activities (Panzer et al. 1995; Hof et al. 2002; Tikkanen et al. 2014). Conversely, many of these muscles are inactive when sitting (Panzer et al. 1995; Tikkanen et al. 2013) suggesting that physical activity and lack of it can be measured by EMG.

To date relatively few studies have assessed muscle activity during habitual life, but have been limited mostly to laboratory conditions. Long-term EMG recordings have been mainly utilized in ergonomic applications, where risk factors for muscular constrains in upper extremities have been identified in different occupations (Jensen et al. 1998; Nordander et al. 2000; Mork, & Westgaard 2005; Thorn et al. 2007). In addition, changed EMG activity patterns following a medical condition have been compared to those of healthy controls (Jakobi et al. 2008; Howe, & Rafferty 2009). From ergonomic perspective the gaps in EMG activity are considered to be protective against musculoskeletal constrains caused by long-term low intensity static activation (Nordander et al. 2000). However, from the point of view of sedentary behavior research, the

gaps, or time periods of muscle inactivity, can provide data about the hypothetically hazardous sedentary behavior at the muscular level.

The lower extremity muscles have been shown to be active only for a fraction of day during habitual life. Klein et al. (2010) showed that vastus lateralis muscle was active only 1-3 hours per day at an average intensity below 12% of maximum contractile capacity of the muscle (Klein et al. 2010). By using EMG shorts, Tikkanen et al. (2013) reported that thigh muscles of healthy, physically active individuals, were inactive (at an intensity below that required for standing) for almost 70% of habitual life and the muscle activity intensity averaged only 4% of maximum (Tikkanen et al. 2013). In contrast, although the vastus lateralis and biceps femoris muscles were defined to be inactive at <2% of maximum contractile capacity about half of the eight hour measurement, Harwood et al (2008) showed that the number of bursts where muscle activity amplitude rose above the threshold was more than 11000 (Harwood et al. 2011). This was possible because the duration of a burst was less than two seconds. Similarly, Kern et al. (2001) showed using similar inactivity threshold, that the vastus lateralis muscle was active only for 10% of the time, which however consisted of 6000 bursts (Kern et al. 2001). The average burst duration was less than one second and that the majority of bursts occurred at very low intensity. Of note was that several bursts were recorded also at intensities below the proposed threshold 2% of maximum contractile capacity (Kern et al. 2001). These results imply that the number of muscle inactivity periods can be drastically higher as compared to number of sedentary bouts as measured by accelerometers (Barreira et al. 2015).

Measurement of EMG with large wearable textile electrodes has served a feasible method to assess presence and lack of muscular activity of muscle groups, which is relevant and applicable for the field of sedentary behavior and physical activity. Because the size of the conductive area and the inter-electrode distance of EMG textile electrodes sewn into the inner leg of shorts are larger as compared to traditional bipolar electrodes, they provide data about a global muscle activity data from the entire muscle groups at thigh rather than measuring single muscles (Finni et al. 2007). Finni et al. (2007) showed that the signal, EMG/force -ratio and within-session repeatability recorded with these EMG shorts is in good agreement with that of bipolar electrodes, but the use of EMG shorts might benefit the reproducibility between days because of large electrode area and lack of skin irritation (Finni et al. 2007). Tikkanen et al. (2014) showed that the EMG shorts can be used to estimate energy expenditure and at low loads and at changing terrains they are more accurate than accelerometer and heart rate if individual calibrations are performed (Tikkanen et al. 2014). In another study, Tikkanen et al. (2012) showed that thigh muscles were inactive almost 70% of the measurement time, and that the activity consisted of more than 12 000 quick (1.4 s) bursts obtained at an average intensity (5.8% of EMG during maximum isometric voluntary contraction) below that of walking (Tikkanen et al. 2013). Using similar methods, Finni et al. (2014) showed that the average amount of time muscles were inactive was around 70% of measurement time

within an individual regardless if they participated in exercise for fitness (Finni et al. 2014).

Several physiological and methodological factors need to be considered when interpreting the EMG signal measured during habitual life (Farina et al. 2004). Impedance between the skin and the electrode, and related inter-individual variability of the signal, is typically reduced by normalizing the signal to that measured during maximal voluntary contraction to yield EMG activity in relation to maximum contraction of the muscle (%EMG_{MVC}) (Burden 2010). The selection of inactivity threshold used to separate gaps in the signal from the physiological activity has a considerable influence on the results. Most recent studies have used an inactivity threshold fixed into 2% of EMG_{MVC} (Kern et al. 2001; Harwood et al. 2008; Jakobi et al. 2008; Shirasawa et al. 2009; Harwood et al. 2011; Theou et al. 2013), but also 10% of EMG_{MVC} has been used (Howe, & Rafferty 2009). Klein et al (2010) demonstrated that the selection of higher threshold decreases the muscle activity duration in a curvilinear manner, because the majority of bursts are of low intensity (Klein et al. 2010). The studies utilizing EMG shorts have used a threshold set individually below the muscle activity of standing still (Tikkanen et al. 2013; Finni et al. 2014; Gao et al. 2016). This approach is beneficial because standing is defined as physical activity (Sedentary Behaviour Research Network 2012) and many daily activities are performed at intensities below 2% of EMG_{MVC} (Okada 1972; Panzer et al. 1995).

2.2 Sedentary behavior and health

The research of sedentary behavior and health is aroused from findings about the associations between a robust proxy measure of sedentary behavior, i.e. television viewing, and increased risk of metabolic syndrome, type 2 diabetes, obesity, cardiovascular diseases and early mortality (Kronenberg et al. 2000; Hu et al. 2001; Jakes et al. 2003; Hu et al. 2003; Dunstan et al. 2005; Katzmarzyk et al. 2009; Dunstan et al. 2010; Veerman et al. 2011; Katzmarzyk, & Lee 2012; Matthews et al. 2012; Helajärvi et al. 2014; Helajärvi et al. 2015). Although television viewing is only a single sedentary behavior among the total sedentary behavior pattern, it has been shown to associate with total sedentary time with a reasonable accuracy (Sugiyama et al. 2008). Importantly, the ease of recalling time spent watching television, and the comparably low cost of running the surveys, has enabled expansion of the paradigm around the independent health hazards of sedentary behavior.

Because sedentary behavior might displace moderate-to-vigorous physical activity during daily living, an important concept has been to study whether the health associations of sedentary behavior remain independent of moderate-to-vigorous activity. This is commonly done by statistical adjustment to moderate-to-vigorous, or by stratifying the sample by their moderate-to-vigorous activity levels to study the health associations of sedentary behavior within each subsample of similar moderate-to-vigorous activity behavior. A more recent ap-

proach has been so called isotemporal substitution and compositional models, where the health effects of displacing sedentary behavior with light or moderate-to-vigorous physical activity are studied (Hamer et al. 2014; Buman et al. 2014; Stamatakis et al. 2015; Chastin et al. 2015b). This approach is beneficial because it takes into account the behaviors during the full 24 hours. If one reduces sedentary time, she needs to reallocate it to some other activity - a trade-off which may have an effect on health outcomes beyond the changes of the initially modified behavior itself.

The following paragraphs aim at giving perspective on the observational associations between different patterns and types of sedentary behavior and health outcomes.

2.2.1 Epidemiological findings

During the past years several meta-analyses have been published about the associations between sedentary behavior and a broad range of surrogate and hard end outcomes. In 2011 two individual research groups published separate systematic review articles about the associations between sedentary behavior and prospective health outcomes in adults (Proper et al. 2011; Thorp et al. 2011). The first one included 19 eligible studies, of which 14 were rated to be of high methodological quality (Proper et al. 2011). Based on the authors' evaluation, they found limited evidence for the risk of weight gain, moderate evidence for the risk of type 2 diabetes incidence, insufficient evidence for the relationship of sitting with cardiovascular disease risk factors and endometrial cancer, no evidence for the risk of cancer mortality, but strong evidence for all cause and cardiovascular disease mortality (Proper et al. 2011). The other systematic review included 48 papers (Thorp et al. 2011). Although they did not systematically evaluate the quality of studies, an important improvement was the reporting of physical activity and BMI -adjusted outcomes. They authors found limited evidence for disease incidence, but convincing evidence for longitudinal association between sedentary behavior and all-cause mortality, cardiovascular disease -related mortality and all-other-cause mortality risk independent of BMI and physical activity. They also reported some evidence for a longitudinal relationship between sedentary behavior, weight gain and risk of obesity independent of physical activity in adults, and that these relationships may build up already in childhood and adolescence (Thorp et al. 2011). A more recent meta-analysis included 47 articles, of which 44 applied a prospective design, assessing the associations of sedentary behavior on health outcomes independent of physical activity (Biswas et al. 2015). Sedentary time was associated with mortality from all, cancer and cardiovascular causes, as well as with cardiovascular disease and type 2 diabetes incidence.

The exposure variables of the aforementioned studies have included time watching television, screen time, occupational sedentary time, sedentary leisure time, sedentary traveling time, and self-reported as well as device-measured total sitting or sedentary time. The odds ratios were generally based on comparing participants accumulating low vs. high levels of the exposure

outcome assessed. However, these comparisons do not reveal a synthesis of the absolute sedentary time, which may be of harm. To give insight on the possible dose-response relationship between the hours spent sitting and all-cause mortality, Chau et al. (2013) meta-analyzed studies assessing total sitting time in hours as their exposure (Chau et al. 2013). They found out that at sitting time range of 4-8 hours per day, each hour is associated with 2% increased risk of all-cause mortality. However, those who accumulated more than 8 hours sitting per day had an 8% increased risk of all-cause mortality per hour of sedentary time. These results suggest that a higher sitting time per day is associated with increased mortality risk in a dose-response manner.

2.2.2 Behavioral factors modifying the health associations of sedentary time

Although the reasonable amount of evidence suggests that the relationship between sedentary behavior remains independent of moderate-to-vigorous physical activity, it is incorrect to conclude that this relationship would remain completely unaffected by moderate-to-vigorous physical activity. In a meta-analysis of Chau et al. (2013), adjusting for moderate-to-vigorous physical activity left the relative risk to 2%/hour of siting in those sitting 3-7 hours per day. However, in those sitting more than 7 hours a day the risk increased 5% per hour of sitting time (Chau et al. 2013). Thus, moderate-to-vigorous physical activity appears to be more protective at high sitting time, but the dose-response -relationship remains independent of moderate-to-vigorous physical activity. Similarly, Biswas et al. (2015) reported in their meta-analysis that the hazard ratios associated with sedentary time were more pronounced in those participating in low vs. high levels of physical activity (Biswas et al. 2015). These findings are further supported by studies reporting negative associations between self-reported standing time and all-cause mortality independent of moderate-to-vigorous physical activity (Ploeg et al. 2014), and by the fact that this relationship is stronger in inactive population (Katzmarzyk 2014). Based on these results it appears that light intensity activities like standing can be a healthy alternative to prolonged sitting in addition to participating in adequate amounts of moderate-to-vigorous activity.

In addition to moderate-to-vigorous activity, it is logical to assume that other similar health related behaviors or attributes may mediate the associations between sedentary time and health outcomes. Although sedentary behavior is a health risk independent of BMI, the direction of causality remains unclear bringing up the possibility that obesity may in fact predict sedentary time (Ekelund et al. 2008) and be a confounding factor in analysis with other health outcomes (Petersen et al. 2016). However, other studies have suggested that sedentary time leads to weigh gain (Helajärvi et al. 2014). Another important attribute is cardio-metabolic fitness, which has been shown to lower or remove the risk of sedentary time (Nauman et al. 2015; Shuval et al. 2015).

Although sedentary behavior itself has been cited to be an individual health risk, of interest is that sedentary behavior accumulated in different domains, i.e. at work or leisure, is unequally associated with health risks. Several

studies have associated negative health outcomes to sedentary leisure time (Hu et al. 2003; Grøntved et al. 2011), but to a lesser extent to worksite sedentary time (Hu et al. 2003; Chau et al. 2012; Stamatakis et al. 2013). One reason for the inequality between domains is that leisure sedentary time might coexist with some confounding unhealthy behaviors, like TV viewing and snacking (Heinonen et al. 2013), or with adverse socioeconomic status (Pinto Pereira et al. 2012; Stamatakis et al. 2014; Hadgraft et al. 2015), which add to the risk in an observational setting. Another reason might be the different trade-off between activities at work and leisure time. During leisure time, unlike during worktime routines, the sitting might replace more active leisure time behaviors like moderate-to-vigorous exercise for fitness.

2.2.3 The pattern of sedentary behavior accumulation and health

Recent adoptions of isotemporal and compositional data analysis approaches have found that the activities which replace sedentary behavior modify the magnitude of sedentary behavior -related risk. In a group of healthy participants, a statistical replacement of 10 minutes sedentary time with moderate-to-vigorous physical activity, but not with light activity, showed beneficial associations to cardio-metabolic health markers (Hamer et al. 2014). In another study utilizing similar analysis methods, reallocating 30 minutes of sedentary time to either light activity or sleep was beneficially associated with cardio-metabolic health markers in a comparable magnitude, and moderate-to-vigorous activity provided more sizeable benefits (Buman et al. 2014). Thus, it might be that at least 30 minutes of light activity is required in exchange of sedentary time to show health benefitting associations. A more recent study found that a statistical replacement of one hour self-reported sitting time with both self-reported standing, walking and moderate-to-vigorous activity was associated with a decreased mortality risk, with walking and moderate-to-vigorous activity showing the strongest benefits of similar magnitude (Stamatakis et al. 2015). However, replacing sedentary time with moderate-to-vigorous activity rather than light intensity activity produces a higher gain in total volume of physical activity, which is an important health-enhancing component of healthy physical activity behavior (Ekelund et al. 2005; Ekelund et al. 2007). Thus, it remains unclear from these observational findings if a similar volume accumulated in either light or moderate-to-vigorous intensity would change the interpretations. For example, a study by Wellburn et al. (2016) showed that 50 minutes of light activity is required to produce similar benefits to 10 minutes of moderate-to-vigorous activity supporting the importance of total activity volume which replaces sedentary time (Wellburn et al. 2016).

A further advancement has been so called compositional data analysis framework, which adjusts the health associations of sedentary time not only to moderate-to-vigorous activity, but for the entire composition of relative time budget spent at sleep, light and moderate-to-vigorous activities (Chastin et al. 2015b). Within this composition, both sedentary and light intensity activities showed adverse associations to obesity and cardio-metabolic health markers,

whereas moderate-to-vigorous activity showed a beneficial association. However, replacing moderate-to-vigorous activity with sedentary time rather than light activity time led to stronger negative associations letting the authors conclude that light intensity activity is less harmful, and thus beneficial as compared to sedentary time. Perhaps even more strikingly, the authors were able to show that re-allocating moderate-to-vigorous activity to sedentary behavior was associated with a much stronger harmful effect on obesity and cardio-metabolic health markers, than re-allocating sedentary time to moderate-to-vigorous activity showed benefits (Chastin et al. 2015b). These novel findings suggest that reducing vs. increasing sedentary behavior might be physiologically unequivocal and that preventing an increase in sedentary behavior may be a more effective public health strategy, than efforts to decrease sedentary time.

The pattern in which sedentary time is accumulated may also modify the health risks of total sedentary time. In their highly cited paper utilizing cross-sectional data, Healy et al. (2008) showed that breaks in sedentary time were beneficially associated with waist circumference, BMI, triglycerides and 2-h plasma glucose independent of total sedentary time and moderate-to-vigorous physical activity (Healy et al. 2008a). Later the authors were able to reproduce the finding on waist circumference in a larger dataset (Healy et al. 2008a). As reviewed recently, cross-sectional findings support the association of breaks in sedentary time on obesity metrics (Chastin et al. 2015a; Brocklebank et al. 2015) and on triglycerides independent of moderate-to-vigorous activity or total sedentary time, but the association to triglycerides is driven by adiposity (Brocklebank et al. 2015).

Based on these findings it appears as each part of the sedentary behavior pattern, namely frequency, interruptions, time and type of sedentary behavior, should be considered to have its own unique influence on health outcomes.

2.3 Physiological mechanisms

Although the associative health risks of prolonged unbroken sedentary time seem clear based on epidemiological evidence, the several controversies and the inadequate knowledge of the underlying physiological mechanisms require controlled intervention studies to resolve. The observational findings depict effects at a population level, but do not tell whether the effect is true if an individual changes behavior.

Possible mechanisms underlying the health hazards of sitting include positive energy balance, post-meal glycemic load, oxidative stress, liver and intramuscular lipid accumulation among other factors - many of which contribute to disturbed insulin signaling and thus insulin resistance at the muscle. These factors are well acknowledged contributors to increased cardio-metabolic risk. The next paragraphs aim to clarify the role of sedentary behavior in this orchestra of mechanisms.

2.3.1 Sedentary behavior and positive energy balance

During sitting the activity of large postural muscle is in vain (Tikkanen et al. 2013) and total body energy expenditure drops to a resting level (Levine et al. 2000; Mansoubi et al. 2015). In short-term experimental studies energy intake easily exceeds the low energy expenditure, since there is no compensatory decline in food intake following a large drop in expenditure (Stubbs et al. 2004; Granados et al. 2012). In the long term, low energy expenditure associated with energy surplus promotes positive energy balance, which may be a mediating mechanism underlying the health risks of sedentary time.

Sedentary time and postprandial glycemic load. Every meal is a challenge for our bodies in terms of glucose and lipid load. Post-meal load of nutrients has physiological effects on hemostatic factors, causes oxidative stress, and activates immune system both in people with normal or impaired glucose tolerance. Normally, this response is attenuated following 2-3 hours after a meal. The fluctuations in responses are associated with a degree of glucose intolerance, such that obese, glucose intolerant and type 2 diabetic persons experience a prolonged and strong response (Blaak et al. 2012). Prolonged sedentariness attenuates the normalization of the post-meal nutrient load in both normal weight and obese individuals (Duvivier et al. 2013; Blankenship et al. 2014). Even though the post-meal hyperglycemia and the related lipidemia and insulinemia are normal and necessary physiological reactions, they contribute to the etiology of cardio-metabolic diseases when disturbed (Blaak et al. 2012).

Sedentary time and long-term positive energy balance and systemic lipid overflow lead to ectopic lipid accumulation to other than adipose tissue, e.g. skeletal muscles, causing insulin resistance in the tissue in question (Bergouignan et al. 2011). The resulting increased concentration of free fatty acids may inhibit insulin signaling directly or through accumulated intramuscular lipid metabolites (Yu et al. 2002). Because inactive muscles oxidize intramuscular lipids poorly (Bergouignan et al. 2011), this vicious cycle promotes intramuscular lipid accumulation and inhibition of insulin signaling even more. The flow of dietary fat load is increasingly headed to adipose tissue, liver and other organs promoting local lipid accumulation and insulin insensitivity. As a response to insulin load, the synthesis and storage of lipids exceeds the rate of oxidation in liver, advancing the development of fatty liver. The increased load of dietary fats and the liver lipogenesis together promote production of atherogenic lipids (such as VLDL), feeding the accumulation of visceral and lipid fat stores and the related liver insulin resistance (Bergouignan et al. 2011). This possible chain of events is supported by epidemiological associations between sedentary behavior and fatty liver (Helajärvi et al. 2015; Ryu et al. 2015). Even though this association may be partly driven by visceral adiposity (Helajärvi et al. 2015) or fat mass (Ryu et al. 2015), increasing physical activity has beneficial effects on fatty liver -related enzyme activities without changes in body weight (St George et al. 2009).

Since positive energy balance and obesity can increase insulin resistance by themselves, they may mediate part of the sedentary-behavior -related risk (Figure 3). At the population level, sitting accompanied with low energy expenditure are closely coupled with positive energy balance (Hill 1998; Hill et al. 2003). To develop effective countermeasures for sedentary lifestyle, it is important to understand if sedentary behavior has a harmful role independent of energy balance. At the same time, interventions studying the cardio-metabolic effectiveness of reduced sedentary time should measure and adjust the cardio-metabolic outcomes to energy intake in order to elucidate the effects which are independent of energy balance.

2.3.2 Independent mechanisms of sedentary behavior

If positive energy balance was the only mechanism explaining the harms of sitting, an appropriate countermeasure would be to level the energy balance by eating fewer calories, or by losing more calories with physical exercise, without changes in sitting. However, an inactive muscle can experience insulin resistance through mechanisms which are independent of positive energy balance (Figure 3). In experimental laboratory studies reducing energy intake to match low expenditure during sitting has approximately halved the insulin resistance at the inactive muscle (Stephens et al. 2011). The reason is reduced concentration of circulating dietary fatty acids, glucose and/or amino acids, resulting in less inhibited insulin signaling (Patti 1999; Krebs, & Roden 2004). The other half of the insulin resistance remains to be explained by other factors than energy surplus. Because liver insulin sensitivity and lipid oxidation remain unchanged during sitting, may the sedentary behavior -related insulin resistance be primarily linked to skeletal muscle (Stuart et al. 1988; Bergouignan et al. 2006; Stephens et al. 2011).

Diminished handling of muscular lipids. One of the most acknowledged mechanism underlying the adverse health effects of sitting has been a quick decline of muscle lipoprotein lipase activity following hind limb unloading in rats and mice (Hamilton et al. 1998; Bey, & Hamilton 2003; Hamilton et al. 2004; Zderic, & Hamilton 2006; Hamilton et al. 2007). The loss in muscle lipoprotein lipase activity or content has been shown to associate with decreased plasma triglyceride uptake (Bey, & Hamilton 2003; Bergouignan et al. 2009) and reduced plasma HDL levels (Bey, & Hamilton 2003), as well as metabolic syndrome, systemic oxidative stress (Saiki et al. 2007), systemic insulin resistance and obesity (Wang et al. 2009) and cardiovascular disease (Henderson et al. 1999; Wittrup et al. 1999). Of note is that the quick decline in muscle lipoprotein lipase activity following muscle inactivity does not get worse during days of prolonged muscle inactivity, but the majority of this decline occurs within hours (Bey, & Hamilton 2003). Further, the changes are highly muscle fiber type specific such that changes occur primarily in the unloaded slow twitch muscle fibers through post-translational mechanisms (Bey, & Hamilton 2003).

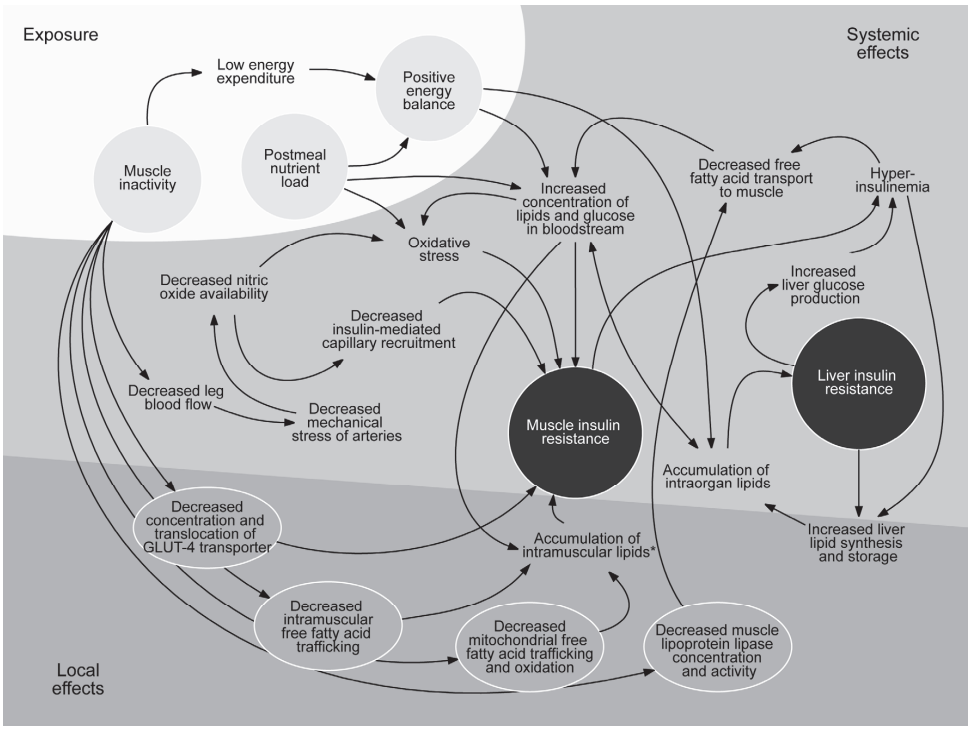

Figure 3 Possible metabolic cascades at local level, like skeletal muscle, and on systemic level following prolonged sitting. The effects of different exposure variables may be mediated partly through the same or independent pathways.
* Cheramides and diacylglycerol are reported to be the key fat metabolites which inhibit insulin signaling within muscle.

Muscle inactivity results in diminished lipid trafficking also within the muscle cell. An experimental physical deconditioning downregulates gene activities which facilitate cytoplasmic transport of fatty acids, as well as mitochondrial transport and oxidation of fatty acids, which disturb early steps in insulin signaling and cause insulin resistance (Lammers et al. 2012; Bergouignan et al. 2013). It is possible that the intramuscular lipid metabolite accumulation during muscle inactivity explains the increased insulin resistance, which is observed as an individual consequence of sedentary behavior. Even though positive energy balance promotes this mechanism, they appear to be partly independent. Prolonged muscle inactivity also directly declines contraction-mediated glucose uptake through reduced concentration of GLUT-4 transporter, which contributes to the development of muscle insulin resistance (Bergouignan et al. 2011). Activation of related pathways has been shown in response to breaking up sitting with light or moderate activity walking (Latouche et al. 2013).

Hemodynamic changes. Reduced arterial shear stress is one central mechanism in the pathology of coronary artery disease (Malek et al. 1999), and can be observed already after half hour of sitting (Padilla et al. 2009). Low arterial shear stress reduces nitric oxide synthase activity leading to reduced nitric ox-

ide availability and oxidative stress (Malek et al. 1999). Already an hour of sitting reduces thigh muscle blood flow contributing to blood pooling to legs, consequently increasing peripheral resistance blood pressure (Thosar et al. 2012). The sitting posture itself physically bends popliteal arteries increasing blood turbulence, which has an effect on formation of plaques and agglomerations and consequently atherosclerosis (Liepsch 2002). Many of these adverse events increase also the risks of varicose veins and deep venous thrombosis, but can be easily prevented by introducing light intensity activity breaks (Howard et al. 2013; Thosar et al. 2015).

The hemodynamic changes during sitting might also relate to insulin-mediated blood flow. Physiological hyperinsulinemia increases skeletal muscle blood flow through nitric oxide -related mechanism leading to increased glucose uptake by the muscle. Muscle inactivity reduces insulin-mediated capillary recruitment, because of the reduced nitric oxide bioavailability in the inactive muscle (Chadderdon et al. 2012). The insulin resistance observed during sedentary behavior could thus be explained also by the reduced insulin-mediated blood flow and capillary recruitment. Because also the increased glucose load, insulin resistance and low mechanical loading of the muscles and blood vessels themselves impair endothelial function, sedentary behavior causes a vicious cycle whose many steps aggregate the development of muscle insulin resistance. Even though acute physical activity increase arterial stress and enhances endothelial function (Tinken et al. 2010), these effects are short lived and periodical. For example, physical exercise was unable to recover the reduced blood flow following prolonged sitting (Younger et al. 2016), which may be partly due the independent mechanistic pathways that control the endothelial functions during sitting vs. physical exercise (Zderic, & Hamilton 2012). Already a frequently undertaken light intensity activity may maintain endothelial functions, because it prevents the adverse events occurring during prolonged sitting (Zderic, & Hamilton 2012).

2.3.3 Sedentary behavior ≠ physical inactivity

From prevention point of view it is important to know if one can reduce the health hazards of prolonged sitting by participating in recommended levels of moderate-to-vigorous activity. Based on the epidemiological findings reviewed before, it is evident that exercise for fitness does not fully remove the statistical association between sitting and adverse health outcomes. In addition to behavioral independency, sitting appears to have distinct effects to cellular events as compared to moderate-to-vigorous physical activity. Especially the changes lipoprotein lipase activity (Bey, & Hamilton 2003) and expression of genes controlling oxidative phosphorylation (Lammers et al. 2012) are order of magnitude bigger following increased sitting than increased moderate-to-vigorous activity.

All of the adverse events of prolonged sitting cannot be reversed by undertaking more physical exercise, because exercising does not influence the ex-

pression of all of the genes which are modified during sitting. Nine days of bed rest changed expression of 4500 genes in skeletal muscle, of which the most influenced where those related to oxidative phosphorylation, endothelial functions and glucose metabolism (Alibegovic et al. 2010). Even though the setting included a period of physical exercise after the four weeks of bed rest, 17% of those genes modified by bed rest remained on the same level regardless of exercise. Of special interest was that the gene PGC-1α was slightly methylated during the bed rest period and all of the gene activities being regulated by it did not recover during the exercise. PGC-1α is a well acknowledged mediator of exercise training adaptations, like oxidative phosphorylation and capillarisation. The authors discussed that prolonged sitting may have epigenetic effects on central signaling pathways regulating insulin sensitivity and an adequate level of everyday activity may be necessary to maintain the activity of these pathways (Alibegovic et al. 2010).

Sitting and physical exercise may have distinct metabolic effects also within the range of physical activity undertaken during habitual living. In an acute intervention, increasing physical exercise in previously inactive participants did not induce benefits on fasting or postprandial insulin concentrations (Bergouignan et al. 2013). Instead, reducing the same volume of total physical activity through increased sedentary behavior, previously active participants experienced worsening of their fasting and postprandial insulin concentrations although being in a strict energy balance. Reducing total physical activity increased the concentration of circulating dietary lipids because of reduced peripheral uptake by the inactive muscles. The decreased lipid oxidation was strongly associated with increased insulin production supporting the important role of muscle lipid oxidation in insulin signaling. Because these effects were evident only in those participants who reduced their total physical activity level, the authors ended up emphasizing the important role of reduced daily activity in contrast to increased physical exercise in respect of these changes (Bergouignan et al. 2013). The findings about different effects of reducing vs. increasing sedentary behavior are in line with observational data as reviewed before (Chastin et al. 2015b).

To conclude, reducing daily sitting time may be beneficial for active, but especially for inactive people. The effects of daily physical activities can be additive to those received from physical exercise through the increased total volume of physical activity, but also through independent mechanisms especially at the muscle level. Maintaining a proper level of everyday activity may be of importance for intramuscular lipid trafficking and oxidation, and consequently for maintaining the activity of insulin signaling pathways within the muscle.

2.3.4 Short-term efficacy of reducing and breaking up sedentary time

Standing up from a chair is a strong stimulus for body. In addition to a mild increase in energy expenditure (13-20%), thigh muscle activity is several fold higher during standing than sitting (Tikkanen et al. 2013). The sympathetic nervous activity increases to adapt cardiovascular system for the requirements

of upright posture (Supiano et al. 1990). During the past years several acute experimental studies have explored the efficacy of different activity patterns on metabolic markers of cardio-metabolic risk using prolonged sitting as their reference condition.

Prolonged standing (Buckley et al. 2014) or alternating between sitting and standing (Thorp et al. 2014) were effective in decreasing postprandial glucose load in mostly overweight office workers. However, the same effect for standing breaks was not seen in normal weight young men, but walking was required to decrease postprandial glucose and triglyceride load as compared to prolonged sitting (Miyashita et al. 2013; Bailey, & Locke 2014). The benefits can be at least partly attributed to increased energy expenditure volume during the effective activity conditions, because energy balance was not controlled for. Interestingly, when the energy expenditure volume of light intensity breaks was matched to that of single exercise bouts, the frequent light intensity activity breaks affected more beneficially glycemic fluctuation (Blankenship et al. 2014) and postprandial triglyceride, non-HDL cholesterol (Duvivier et al. 2013), insulin (Peddie et al. 2013; Duvivier et al. 2013) and glucose concentrations (Peddie et al. 2013) as compared to the single exercise bout. However, Kim et al. (2014) showed in non-obese young men that physical exercise more effectively attenuated triglyceride response to fat tolerance test as compared to breaking up sitting with light activity breaks when the energy expenditure volume was the same, but both were beneficial to sitting (Kim et al. 2014). These findings emphasize the importance of total activity volume, but suggest that accumulating this volume in short frequent, than a single bout, is more beneficial at least for glucose metabolism.

In contrast to these findings, Dunstan et al. (2012) showed that breaking up sitting with both light and moderate intensity breaks was as beneficial for postprandial glucose and insulin concentrations in obese subjects, despite the different activity volume of these conditions (Dunstan et al. 2012). Similarly, Henson et al. (2015) showed that breaking up sitting with either standing or walking was equally beneficial for postprandial glucose and insulin responses in obese subjects, and the beneficial effects reached to the following day (Henson et al. 2015). A hypothesis might be put forward that the muscle activity required at upright posture is higher in obese than normal weight people thus contributing to their greater benefits seen during light intensity activity and standing.

2.4 Behavioral interventions to decrease and break up sedentary time

Although the same time of increased moderate-to-vigorous activity than reduced sedentary time is more effective in decreasing health risks, from behavioral point of view it is important to comprehend that only those preventive

efforts that people are able or willing to undertake are effective. Because levels of moderate-to-vigorous activity continue to be low (Troiano et al. 2008) and sedentary behavior poses an independent thread for health (Hamilton et al. 2008), growing number of interventions have tested the possibility if targeting reduced sedentary time among everyday life would provide an accessible way to healthy activity routines (Marshall, & Ramirez 2011). Of importance is the notion that the domains and determinants of sedentary behavior are different to those modulating participation to moderate-to-vigorous activity (Owen et al. 2011). Consequently, interventions targeting increased moderate-to-vigorous activity have been ineffective at decreasing self-reported sitting time suggesting that these behaviors can coexist and should be targeted with different methods (Chau et al. 2010). In addition, long-term interventions targeting decreased sedentary time in ecological setting are required to prove the health effectiveness of reduced sedentary time.

2.4.1 Domains and determinants of total sedentary time to inform intervention targeting

Modern technology-driven environment which limits the need for manual work in everyday activities is often attributed to be the main determinant of low physical activity and high sitting time at the population level. For example, an individual might be employed in office work with limited options for physical activity during work time. Built indoor environments typically include only chairs to settle in, and social norms discourage standing in a meeting, in classes and while doing business, since the option to sit is always available. Thus, unlike determinants leading to planned and structured exercise behavior, it appears as the determinants of high sitting time are linked to physical and social contexts which are rooted in the modern lifestyle, and are partly beyond the conscious everyday decisions. An ecological approach, which assumes that both individual, social, organizational, environmental and policy influence the sedentary behavior, has been proposed as a framework to study the determinants of sedentary behavior within these various contexts (Owen et al. 2011). Namely, sedentary behavior accumulates in different domains of daily life, within which different behavioral, social or environmental attributes may be associated with the behavior of an individual. Further, individual-level attributes, like preferences, enjoyment or barriers, finally determine how the individual behaves in the given situation, ultimately leading to the individual sedentary behavior pattern.

Sitting takes place in various domains during the daily living. In developed countries working adults spend a large proportion of their workday sitting, even across different occupations (Miller, & Brown 2004; Mummery et al. 2005; Jans et al. 2007). In office work, people are sedentary approximately 80% of their work time, equaling seven hours per day, or nearly half the total sitting exposure over a whole week (Toomingas et al. 2012; Thorp et al. 2012; Parry, & Straker 2013). Physical activity during commuting has decreased over the past decades (Husu et al. 2011), which has been likely replaced with sedentary forms

of commute, like driving a car owing to the increased car ownership (Sala et al. 2005; Levine et al. 2006; McKenzie, & Rapino 2011). In Finland, almost 70% of commuting is done by car, although 58% of commutes are shorter than 5 km (Sala et al. 2005). In U.S and Australia, car is the main form of transport to work for more than 80% of adults (Australian Bureau of Statistics 2009; McKenzie, & Rapino 2011). Watching television is the most prevalent leisure activity occupying more than half of leisure time, even based on recent time-use surveys (Bureau of Labor Statistics 2015). TV viewing is likely accompanied with other screen-based activities, since computer use at leisure time follows TV viewing at its reported frequency and total duration (Wijndaele et al. 2014a; Kim, & Welk 2015). On average, sedentary time accounts for almost 70% of leisure time on workdays and non-work days, and contributes to more than half of the total sedentary time of the week (Parry, & Straker 2013).

The sitting time at and between different domains might be determined by different behavioral, social or environmental attributes, which likely vary across the different domains. Higher occupational sitting time is associated with higher income and professional/managerial occupation, whereas low occupational sitting time is related with being older, having a blue collar occupation and a technical/vocational education (Hadgraft et al. 2015). However, although lower education is associated with less sitting on work days, it relates to more sitting outside of working hours (Proper et al. 2007; Kim, & Welk 2015). Interestingly, low occupational physical activity was associated with more leisure-time physical activity in one study (Hadgraft et al. 2015), whereas other studies have found no differences in leisure-time physical activities between people having an active or sedentary jobs (Tigbe et al. 2011), or even shown an inverse relationship between total physical activity and sitting time on weekdays (Proper et al. 2007). People accumulating high sitting time at work do not compensate by sitting less outside of working hours (Jans et al. 2007). Instead, the most sedentary individuals at work appear to be the most sedentary ones also during leisure time, suggesting that inter-individual differences account for sedentary behavior risk in addition to common environmental determinants (Clemes et al. 2014). Screen-based behaviors at leisure time are most prevalent among males, young, obese, individuals with low education, those not meeting physical activity guidelines, as well as single, divorced and widowed people (Clemes et al. 2015).

Whereas the knowledge of domains and attributes linked to high sedentary time can be used to target interventions, the modifiable intrapersonal and social-cognitive variables associated with sedentary time should be concerned when trying to change the behavior of an individual. For example, the enjoyment towards watching television and modeling of the partner are longitudinally associated with increased TV sitting time (Busschaert et al. 2016). Also, the pleasure and enjoyment are found to be associated with computer use in young and highly-educated individuals (Rhodes et al. 2012). Thus, both intrapersonal and social-cognitive variables might modify the attitudes and social influences towards sedentary behavior. The interventions promoting light-intensity physi-

cal activity should serve similar attributes, like enjoyment and socializing, to be an attainable behavioral substitute for sedentary time. The interventions may benefit from targeting several sectors of the proposed ecological model, like perceived environment, social climate, or physical environment among many other possible factors, to modify the sedentary behavior within the complex network of sedentary behavior determinants (Owen et al. 2011).

2.4.2 Behavioral effectiveness

Two recent meta-analyses have studied the combined effectiveness of interventions targeting sedentary behavior as their primary outcomes (Prince et al. 2014; Martin et al. 2015). The first one of these included three non-randomized trials and five RCTs reporting that a reduction of 1,5 hours of sedentary time per day can be expected from interventions targeting solely sedentary time, mostly at work (Prince et al. 2014). The latter one included two RCTs, which reported a mean decrease of 41 minutes in sedentary time also with methods implemented at worksite (Martin et al. 2015). Both of the meta-analyses extracted data also from studies targeting sedentary behavior as their secondary outcomes, which resulted in more modest changes in sedentary time. These findings led to conclusions that focusing sedentary time as a primary outcome is required to generate clinically meaningful changes. An important limitation of the meta-analyzed studies was that all of them were lasting less than three months, suggesting that the sustainability of the acute changes remains unknown.

After the data extraction of these meta-analyses at least two longer RCTs targeting sedentary behavior have been published. Aadahl et al. (2014) reported that a community-based motivational counseling was able to reduce intervention group's sitting time by half an hour per day at six months follow-up, albeit not statistically significantly (Aadahl et al. 2014). This change translated to increased standing time, but not to increased stepping or breaks in sitting time. Healy et al. (2016) found, that a work-site delivered intervention including organizational, physical environment –related and individual behavior change techniques was able to reduce workplace sitting time by 99 minutes at three months, and by 45 minutes at 12 months (Healy et al. 2016). The reduced sitting time was reallocated to increased standing but not stepping, and the numbers of prolonged and usual sitting bouts were reduced.

The published studies suggest that the strategies implemented to reduce sedentary time result in highly context-specific results. Generally, the effect size of a given intervention is bigger when multiple domains and contexts are intervened and when social, cultural and environmental aspects are considered (Owen et al. 2011). A typical example is a workplace intervention, in which changes in physical environment and targeting the whole workplace community (Healy et al. 2013), instead of intervening merely an individual (Evans et al. 2012), have been found to be beneficial. In addition, the effectiveness has been found to be highly specific to the message delivered (Kerr et al. 2016). However, there remain large gaps in the literature regarding the sustainability of acute changes especially outside of workplace. So far it is largely unclear whether in-

tervention methods targeted at other domains outside of work are effective at short and long term. This is especially important provided that the health risks of sitting during leisure time are more pronounced than those associated to work site sitting.

2.4.3 Cardio-metabolic effectiveness

Few interventions have assessed cardio-metabolic effectiveness of reducing sedentary time outside of laboratory based efficacy studies. Despite work site sitting was reduced more than two hours per day during a four-week intervention, Healy et al. (2013) observed only weak evidence for improved non-fasting glucose (-0.43 mM within intervention group), while effects for triglycerides, fat mass, and diastolic blood pressure were potentially adverse (Healy et al. 2013). However, their study was inadequately powered to detect these changes due to its pilot nature. In contrast, Danquah et al. (2016) found that as compared to control group, 48 minutes/workday reduced sitting time and 43 minutes increased standing time resulted in 0.61 percentage points lower body fat percentage during three-months in 317 overweight office workers (Danquah et al. 2016). In a three-month quasi-experimental study, Alkhajah et al. (2012) were able to demonstrate 0.26 mmol/l increase in HDL cholesterol (as compared to control group) after normal weight office workers reduced their sitting time 97 minutes per day (Alkhajah et al. 2012). In a study by Graves et al. (2015), a worksite-delivered RCT reduced sitting time by 80 minutes, increased standing time by 73 minutes, and resulted in 0.40 mmol/l decrease in total cholesterol during eight weeks in 26 intervention as compared to 21 control participants, who were normal weight office workers (Graves et al. 2015).

To the best of our knowledge, only one study has investigated cardio-metabolic effects of a longer than three months sedentary-time targeted intervention. Aadahl et al. (2014) found that the 30 minutes increased standing time which replaced sitting during their half-year intervention reduced waist circumference (-1.42 cm as compared to control group), fasting serum insulin (-5.9 pmol/l as compared to control group) and homeostatis model –assessed insulin resistance (-0.28 as compared to control group) in overweight participants who were sitting more than nine hours per day at baseline (Aadahl et al. 2014). However, the authors did not report where the increased standing occurred (e.g. at work or leisure).

These results suggest that an adequate sample size and long follow-up facilitate cardio-metabolic benefits attributed to reduced sedentary time outside of laboratory. However, there is limited evidence if reductions of sitting time outside of workplace benefit health. In addition, none of the studies have reported if possible changes in moderate-to-vigorous activity or diet mediate the intervention effectiveness.

3 AIMS OF THE STUDY

The field of sedentary behavior research has progressed rapidly throughout the past years driven by the observational associations between self-reported and accelerometer-derived sedentary time and adverse cardio-metabolic outcomes. Another advancement was made in 2012 by a published definition of "sedentary behavior", which explicitly streamlined the assessment of sedentary behavior to sitting or reclining posture accompanied with low energy expenditure during waking hours (Sedentary Behaviour Research Network 2012). In addition, several acute laboratory interventions have provided preliminary evidence about causal benefits of breaking up prolonged sedentary time supporting the observational evidence.

Distinct from the definition of sedentary behavior, one of the driving hypotheses of the sedentary behavior research is that lack of frequent engagement of the antigravity muscles, particularly the large muscles of the lower limbs, results in detrimental physiological processes and adverse cardio-metabolic profile. However, so far the association between muscle inactivity time and cardio-metabolic outcomes has not been shown with direct measures. In addition, currently the only evidence available from interventions targeting sedentary time as a primary outcome is from studies lasting less than 3 months (Martin et al. 2015) with few exceptions (Aadahl et al. 2014; Healy et al. 2016), which is a major gap in the literature. This study aimed to address these gaps by providing data about the directly measured muscle inactivity and activity patterns at the low end of physical activity spectrum, as well as to measure the causal long-term effects of reduced muscle inactivity time on cardio-metabolic outcomes.

Therefore, the specific aims of this study were:

1) To quantify muscle inactivity and activity patterns during sitting, standing, walking and habitual life (Cross-sectional EMG data, I and II).
2) To study the associations between habitual muscle inactivity and activity patterns and cardio-metabolic outcomes (Cross-sectional EMG data, II).

3) To measure how a tailored counseling targeted at reducing and breaking up sedentary time changes muscle inactivity and activity patterns acutely after a family-based behavioral counseling (Short-term effectiveness of counseling with EMG, III).
4) To test the effectiveness of a family-based behavioral counseling on sedentary time, light activity time and breaks in sedentary time at work and leisure time (primary outcomes), and anthropometric measures and cardio-metabolic biomarkers (secondary outcomes) during one year (Long-term effectiveness of counseling with accelerometers and biomarkers, IV). We hypothesized that the intervention is effective in decreasing sedentary time and increasing breaks in sedentary time both during work and leisure time where it is targeted. Because of the resulting muscle activity and peripheral insulin sensitivity, we hypothesize to see decreased fasting insulin concentrations (Aadahl et al. 2014). Moreover, the increased energy expenditure is expected to decrease body fat percentage (Aadahl et al. 2014; Danquah et al. 2016). Even though the hypotheses are based on shorter studies, we expected that these acute changes can be sustained.

The intervention study was a huge effort of the whole research team. The PhD candidate was involved in conceiving and designing the experiments, recruiting the participants, performing the experiments, delivering the intervention, analyzing the data and being the main author when drafting and writing the papers.

4 METHODS

4.1 Setting and participants

This study consists mainly of data measured at project InPACT (ISRCTN28668090), a sedentary-time -targeted cluster-randomized controlled trial including families with sedentary behaviors (Finni et al. 2011), of which the results regarding children have been reported (Laukkanen et al. 2015). The baseline data was utilized for studies I and II, and longitudinal data was analyzed for studies III and IV as illustrated in Figure 4. In addition, the data measured at project EMG24 (Tikkanen 2014), a cross-sectional study to quantify muscle loading during normal daily life, was pooled for study II.

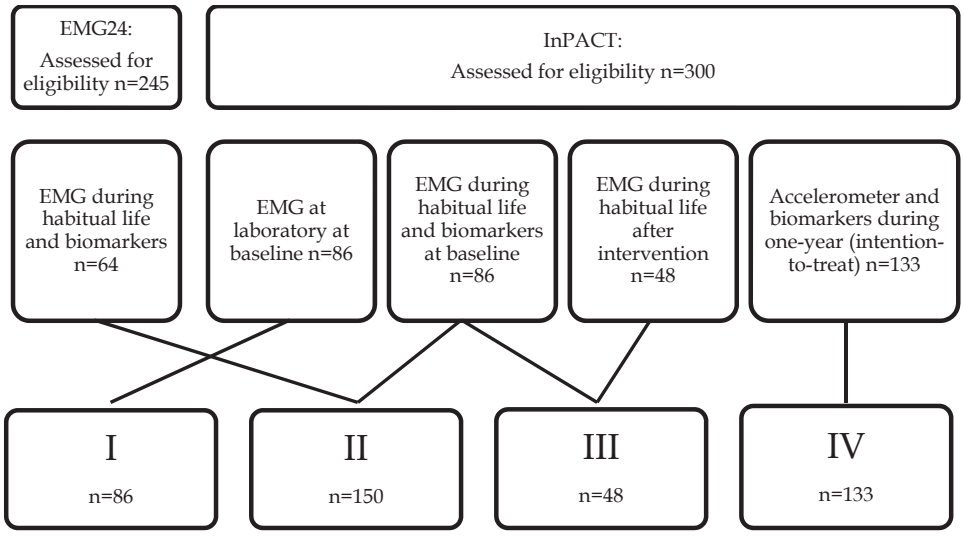

Figure 4 Data used for this study was collected in projects EMG24 and InPACT.

The EMG24 -project was conducted in 2007–2012 and data collection for InPact was done in 2011–2013. Sampling was performed for both studies in the city of Jyväskylä located in central Finland with a population of 133 000. The ethical approval for InPACT was received from the ethics committee of the Central Hospital District of Central Finland, and for EMG24 from the ethics committee of the University of Jyväskylä. The participants were thoroughly informed about the protocol and signed an informed consent prior to the measurements.

4.1.1 EMG sampling for studies I-III

Study I aimed to quantify heterogeneity in muscle inactivity and activity patterns during sitting and standing. EMG was measured from 121 individuals at laboratory, from which 32 were removed because of artifact at any of the four channels and three because of missing data. The final sample for Study I consisted of 86 individuals having artefact-free electromyographic (EMG) signal during laboratory measurements on all four channels.

Study II assessed cross-sectional associations between habitual muscle inactivity and activity patterns and cardio-metabolic outcomes in healthy individuals. Habitual EMG activity was measured from 117 participants on a day which would represent their habitual routines during a weekday at baseline of InPACT. The participants having prolonged (>30 min) artefact (non-physiological signal, caused by e.g. movement of electrodes in relation to skin or close proximity to electronic devices, masking the physiological signal) on all 4 channels (13) or short (<8h) EMG data from self-reportedly typical weekdays (17) were excluded from the analysis. The excluded untypical days included e.g. "having day off, having organized exercise evening at work or staying at home because kids were sick". Of the remaining 87 individuals, one participant had exceptionally high serum triglycerides (8.4 mM) and was excluded giving a final sample of 86 individuals with artefact-free habitual EMG and biomarkers. This data was pooled with the data received from EMG24-project.

Volunteers for the EMG24-project were recruited through general advertisements delivered as posters or emails to local companies and institutions, including hospital of Central Finland, a construction company, a paper manufacturing machinery producer, the University of Jyväskylä and the city of Jyväskylä. A total of 245 persons contacted us to express their interest towards the study. An eligible sample was 109 healthy individuals with no reported chronic diseases and any related medication (diabetes, cardiovascular diseases, cancer, hypertension, rheumatism, osteoporosis) affecting daily ambulatory activity or cardio-metabolic markers.

The eligible participants were measured for EMG on a minimum of one day period and were asked to select a day which would represent their habitual routines during a weekday. Three participants were removed because of short measurement time (<8h). In addition, 40 participants were excluded because of prolonged (>30 min) artefact (non-physiological signal, caused by e.g. movement of electrodes in relation to skin or close proximity to electronic devices, masking the physiological signal) on all 4 channels. Of the remaining 66 indi-

viduals, one participant's fasting plasma glucose (FPG) values were not available and one participant had exceptionally high blood pressure (176/93) and were excluded from the analysis giving a final sample of 64.

The final pooled data for study II was from 150 healthy participants having artefact-free EMG data from a minimum of one self-reportedly typical weekday, and cardio-metabolic biomarker data available.

Study III assessed how tailored counseling targeted at reducing and breaking up sedentary time affects EMG inactivity and activity patterns as measured before and after the counseling session of InPACT. Following criteria resulted in selection of 48 participants: 1) measured days were self-reportedly typical and identical in terms of occupational tasks, workday duration and leisure time activities (31 excluded), 2) both days included artifact-free EMG signal from the same muscles recorded with the same electrodes (34 excluded), 3) length of measurement > 9 hours (10 excluded), and 4) diaries were returned properly filled (3 excluded). In addition, 7 subjects dropped out before the second measurement day. The final Study III sample included 24 subjects in the intervention group and 24 in the control group.

4.1.2 Setting, randomization and recruitment for study IV

Study IV tested effectiveness of the family-based behavioral counseling of InPACT. Primary outcomes included accelerometer-derived sedentary, light and moderate-to-vigorous activity time, as well as on breaks/sedentary hour at work and leisure time during one year. In addition, changes in anthropometric measures and cardio-metabolic biomarkers were studied (secondary outcomes).

The flow diagram of InPACT study is presented in Figure 5. Sampling for InPACT began by identification of homogenous regions around the city. A total of 14 identified regions were divided in seven clusters, such that each cluster included regions homogenous for their socioeconomic status and environmental possibilities for outdoor physical activities within a region. The regions included a total of 8 primary schools and 20 kindergartens (2-5 schools or kindergartens per region), which were then randomized to intervention and control groups within each cluster.

The recruitment began by delivering a total of 1055 recruitment forms to parents via the primary schools and kindergartens in spring 2011, autumn 2011 and spring 2012. In these forms profession, % sitting time at work, health status and contact information were asked. In addition, general information about the study, inclusion and exclusion criteria, and an incentive to get diverse information about personal health, diet and physical activity and motor skills of their children were told (Laukkanen et al. 2015). Inclusion criteria were healthy men and women with children 3–8 years old, parental occupation where they self-reportedly sit more than 50% of their work time, and children in all-day day-care in kindergarten or in the first grade of primary school. Exclusion criteria were severe obesity (BMI > 35 kg/m^2), self-reported chronic, long-term diseases, families with pregnant mother at baseline, children with disorders that

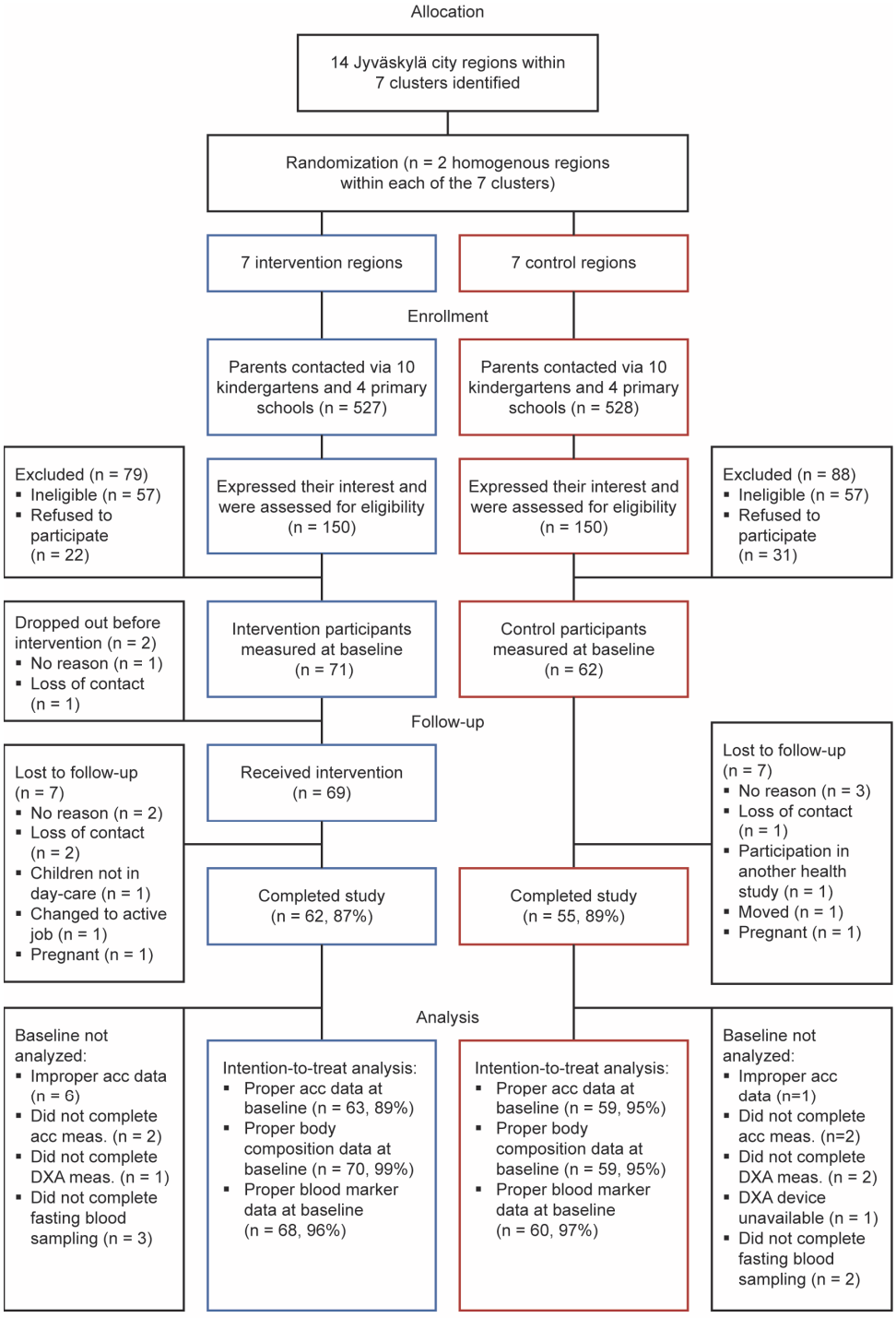

Figure 5 Flow chart of the InPACT -study.

delay motor development, and concurrent participation in another study. No monetary incentive was offered to the participants.

People fulfilling the inclusion criteria were contacted by phone and invited to one of 14 information lectures held in April 2011-April 2012, where the procedures were explained in detail and the first measurement time was scheduled. If people were unable to attend the lecture, details of the project were explained on the phone. Finally, a total of 133 participants were measured at baseline.

4.2 Protocol

The study protocols consisted of laboratory measurements including biochemical, anthropometric and behavioral assessments, as well as structured laboratory test patterns for EMG normalization. The measurements continued by monitoring of habitual life of participants with EMG shorts and accelerometers depending on the study protocols, as depicted in Figure 6 and Figure 7.

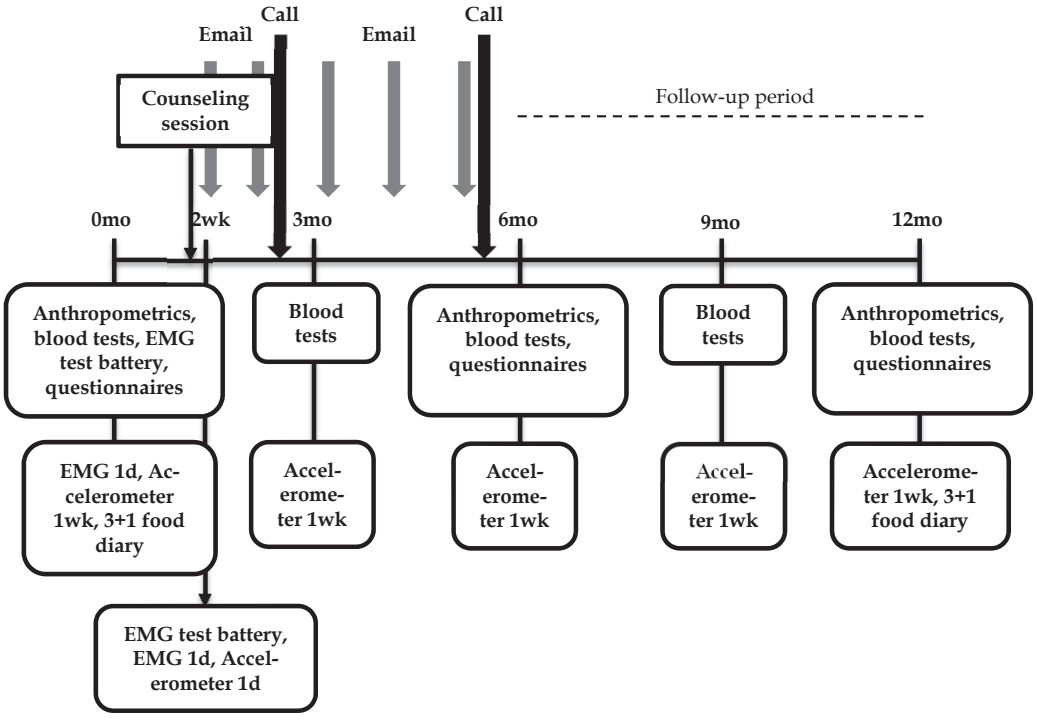

Figure 6 Study protocol for InPACT included several measurements during the 12 month study-period.

In InPACT, the laboratory tests included similar test battery for EMG normalization at the first (0 mo) and second (2 wk) measurements (Figure 6). Blood samples were obtained every three months, and anthropometrics were measured every six months along the study timeline. The measurement of habitual activity was conducted for one week at baseline (EMG one day, accelerometer one week), for one day initially after the intervention (EMG and accelerometer), and for one week at three, six, nine and twelve months (accelerometer). In EMG24, EMG laboratory test battery as well as blood sampling was performed on the first day, and EMG measurements of habitual activity life were conducted on three following separate days if applicable in terms of device availability and a participant's schedule (Figure 7).

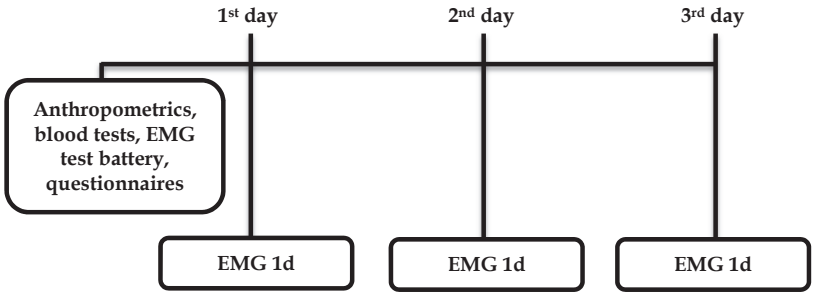

Figure 7 Study protocol for EMG24.

4.2.1 Laboratory protocol

Participants were asked to fast for a minimum of 10 hours and refrain themselves from vigorous exercise the day before coming to the laboratory in the morning for blood tests and anthropometric measurements. After arriving to the laboratory subject's height, weight, triplicate waist circumference and body composition (EMG24: InBody 720, Biospace Ltd, Soul, Korea; InPACT: InBody and DEXA, Lunar Prodigy, GE Healthcare) were measured after asking the participants to visit toilet and to undress for underwear. Next, blood pressure was measured twice in supine position after five minutes resting period. Professional laboratory personnel took and analyzed blood samples. In InPACT, the three and nine-month measurements included only blood tests without anthropometric measurements.

After the fasting assessments participants were offered breakfast while discussing the study protocol briefly and giving instructions for filling in questionnaires and diary. In InPACT, EMG was recorded during sitting at the breakfast table without giving any advice for how to sit. All participants sat on the same lobby chairs with light cushioning (model: Asko Jokke).

4.2.2 EMG test battery

The participants were asked to wear comfortable clothes and shoes that enable light activities, like walking and jogging for a short period. A researcher prepared appropriately sized EMG shorts by putting electrode paste on the electrodes, and helped the participant to dress on the shorts on. Subsequently, the accelerometer was secured to the waist with elastic belt and both devices were set to record simultaneously. The laboratory measurements started with the assessment of EMG level during standing. Standing in different positions was measured for 15 seconds per task, and subjects were asked to stand still as they usually do, except that the weight was first supported by both, and then by one leg at a time (15 s on both legs and 15 s each leg individually). Next, treadmill walking at 5, 6 and 7 $km \cdot h^{-1}$, running at 10 $km \cdot h^{-1}$ (one minute loads in InPACT, three minute loads in EMG24; males in EMG24 were running at 12 $km \cdot h^{-1}$) and stair ascending and descending were measured. In EMG24, additional EMG measurements were performed while pushing strollers, walking in a squatting position, stair ascending and descending with additional loads. The treadmill protocol of EMG24 included also walking uphill (5 $km \cdot h^{-1}$ with 4° ascent) and downhill (5 $km \cdot h^{-1}$ with 4° descent), and a final load where the participants were walking uphill until exhaustion (5 $km \cdot h^{-1}$ with 8° ascending).

Finally, maximal voluntary EMG activity was measured in knee flexion/extension machine (David 220, David Health Solutions Ltd., Helsinki, Finland) with knee angle of 140° in both flexion and extension. This angle was selected because the angles which are close to maximum (110°) are rarely used during daily life. After a warm-up, three 3-5 seconds maximal efforts with strong verbal support were performed with one minute of rest between trials. If the torque improved more than 5%, additional trials were performed.

4.2.3 Habitual EMG and accelerometry

Measurements of habitual physical activity were performed in InPACT directly after the laboratory EMG test battery, and in EMG24 on a separate day from the laboratory EMG test battery to enable proper recovery after the maximal treadmill test performed. The participants were asked to schedule an EMG measurement day, which would represent their typical behavior during a weekday. The data collection always started by a researcher before dispatching the participants for their daily doings. The participants were expected to continue normal life while wearing the EMG shorts and accelerometers and to mark down to a diary any abnormal tasks or behaviors (e.g. abnormal working tasks), exercise for fitness, bedtime and occasions when the devices were not worn. When going to toilet, the participants were instructed to roll the shorts down to move the electrodes minimally, and then to roll the shorts back on. They were instructed to remove the equipment when taking a shower or swimming, and when going to bed at night. In InPACT, the accelerometer measurements continued for full seven days, but in EMG24 the participants were asked to return the devices to the laboratory the next day.

4.3 Description of the intervention

The aim of the intervention delivered in InPACT was to target work time and leisure time sedentary behaviors of families. The intervention program consisted of a lecture, individual and family face-to-face discussion including goal setting, and phone counselling. The behavior change techniques used along the intervention were grounded on previous knowledge of effective interventions and theory of planned behavior (Ajzen 1985; Conn et al. 2011).

Within 2 weeks of the baseline measurements a common 30 min lecture was given for a maximum of six participants at a time. The lecture began with information about the importance of children's physical activities for their healthy growth and development (Laukkanen et al. 2015). The key message was that if parents want to support the healthy growth of their children, it is important to enable natural physical activities for children (e.g. run around and climb trees), to not restrict them unnecessarily, and to act as role models (walking stairs instead of a lift with the child). Next, the health hazards of prolonged sitting and the challenges of the sitting-friendly modern environment from the adults' perspective were described. It was underlined that in this study the aim is at reducing and breaking up sitting time with light intensity activities like light ambulating, because that is the easiest way to overcome the health hazards of prolonged sedentary time. With this statement it was communicated that physical exercise and reducing sitting are different things. The key message was that breaking up sedentary time does not require time, but is easy to carry out during other everyday routines. When breaking up their own sedentary time during leisure time, it is possible to promote physical activities of children and spend more time with family, with healthy outcomes. The lecture was designed to provide information about behavior-health link, to provide information on consequences, to provide information about others' approval, to provide instructions and general encouragement, as well as to prompt identification as a role model for children (Abraham, & Michie 2008)

The lecture was followed by face-to-face discussions with 1) each participant when discussing work time behavior and 2) parents together when discussing leisure time behaviors, if both parents were participants. The participants were first asked about their feelings about the lecture and its relevance to their life. Following a checklist, they were asked to describe their normal daily routines during work time and weekday and weekend leisure time. Next, the frequency of these routines was asked and written down to the checklist by the researcher. The participants were encouraged to think of feasible ways to decrease and break up sitting time during these routines and to increase family-based physical activities, which were then formulated to small step graded goals specific for each domain. The goals and their frequency were written down into an agreement document that was signed by the participant and the researcher. The agreement was copied and given to the participant. The researcher transcribed the goals to a paper with nice background and delivered

these documents to the participant afterwards. An example from the contract of one participant is as follows:

My goals to decrease sitting time and to increase non- exercise physical activity during working time are:

- I stand up from my chair every half an hour;
- When answering the phone, I stand up from the chair;
- Instead of calling, I walk to my colleague's room;
- I take the stairs instead of the elevator; and
- I walk for lunch and once a week choose a restaurant that is farther away.

Mine and my family's goals to decrease sitting time and to increase physical activity during leisure time are:

- At least once a day, we go out as a family in order to replace family sitting activity;
- We cycle to work whenever the weather permits us to do so;
- Instead of taking the car, we walk or bicycle to the grocery shop more often as a family;
- We organize family dancing sessions; and;
- We will work hard with snow removal, using child labour together with us ☺.

The underlying theoretical frameworks were motivational interviewing, to provide general encouragement and instructions, to prompt intention formation and specific goal setting and to agree on behavioral contract (Abraham, & Michie 2008). The lecture and individual discussions were led by researchers (AL, AP, TF) who all had underwent an orientation to good practices in PA counseling.

Five emails containing tips and videos about how to promote physical activity in families were sent to support implementation during the first six months (demonstrate the behavior (Abraham, & Michie 2008)). To promote compliance with the goals, a phone discussion with each participant was performed after two and five months of the counselling. First general feelings were asked, after which the compliance to the goals was discussed. The participants were asked to self-evaluate the implementation of the goals with question "Did you do your best to achieve the goal?" which was rated on a scale of 1 to 5 (prompt review of behavioral goals (Abraham, & Michie 2008)). After this perceived barriers were asked and possible modifications to the goals were made (prompt barrier identification, provide instructions, prompt specific goal setting (Abraham, & Michie 2008)). Shortly after the six months measurements, feedback about the progress of a child's motor coordination in comparison to age-related peers was given to parents (Laukkanen et al. 2015) (provide feedback on performance (Abraham, & Michie 2008)).

The last six months of the study were identical for both groups containing nine and 12 month assessments without any counseling. At 12 months, after

the follow-up assessments were completed, the participants in the control group received a shortened version of the counseling.

4.4 Measurements and analyses

4.4.1 Anthropometrics

Participants' height, weight and waist circumference were measured to the nearest tenth unit. Waist circumference was measured while participants were standing with their feet shoulder width apart. The top of the crest of ilicum at the side of waist was located and the measurement tape was wrapped around the waist. The bottom of measurement tape was aligned with the top of the crest of ilicum and parallel to the floor along the entire length. Height and weight were measured twice and waist circumference three times and the means were used for further analysis.

Body composition including fat mass, lean mass and visceral fat, was measured with bioimbedance device (InBody 720, Biospace Ltd, Soul, Korea). In InPACT, body composition was measured additionally with dual-energy X-ray absorptiometry (DEXA, Lunar Prodigy, GE Healthcare).

4.4.2 Cardio-metabolic biomarkers

Blood pressure was measured twice on the left arm of the participants in supine position after five minutes resting period (Omron M6W, Omron Healthcare Co., Ltd. Kyoto, Japan). The means of repeated measurements were used. To represent a composite risk factor for hypertension (Franks et al. 2004), systolic and diastolic blood pressure levels were averaged [(systolic blood pressure + diastolic blood pressure) / 2 = blood pressure index] in Study II.

Blood samples were drawn from median cubital vein to 5/2ml EDTA vacuum tube (5/2 3.6 mg EDTA K2), 5/3 ml serum gel tube (SST II Advance 5/3 ml serum gel) and 5/2 ml Na-fluoride-K-oxalate tube (5/2 Na fluoride-K-oxalate 4 mg) (sample tubes by Becton Dickinson, Franklin Lakes, NJ USA). Complete blood count (white blood cell count, white blood cell differential, red blood cell count, hemoglobin, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width) was done from the EDTA vacuum tube by Sysmex KX 21N analyzer (Sysmex Co., Kobe, Japan). Serum gel tube was centrifuged with 3500rpm for 10 min and analyzed with Konelab 20 XTi analyzer (Thermo-Fisher, Espoo, Finland) for serum cholesterol, serum HDL cholesterol and serum triglycerides. Serum LDL cholesterol was calculated using Friedenwald's formula. Plasma glucose was analyzed from centrifuged Na-fluoride-oxalate tube with Konelab 20 XTi analyzer.

In InPACT, insulin was analyzed with chemilumometric enzyme immunoassay (Immulite 2000 XPi, Siemens Healthcare). Homeostasis model assess-

ment (HOMA) was used to calculate hepatic insulin resistance (HOMA-IR) and basal insulin secretion (HOMA-%B) from fasting glucose and insulin samples (Matthews et al. 1985). Additionally, all serum samples collected in InPACT were stored and sent to a different laboratory for further metabolomics analysis. A high-throughput serum NMR metabolomics platform (Soininen et al. 2009) was used to analyse apolipoproteins and mean diameter of lipoproteins. Professional laboratory personnel did all measurements and analyses.

4.4.3 EMG shorts

EMG was measured bilaterally from quadriceps and hamstring muscles with shorts made of knitted fabric similar to elastic clothes used for sport activities or functional underwear, with the exception of the capability to measure EMG from the skin surface (Myontec Ltd, Kuopio and Suunto Ltd, Vantaa, Finland, Figure 8). To measure average rectified EMG signal, bipolar electrode pairs are located on the distal part of the quadriceps and hamstrings, and the reference electrodes are located longitudinally along the left and right lateral sides (over tractus iliotibialis). The electrodes located on quadriceps muscles collect data from m. vastus lateralis, m. vastus medialis and m. rectus femoris. M. vastus intermedius is located deeply under the other muscles and thus will not be included. The electrodes located on hamstrings muscles gather signal from m. biceps femoris, m. semimembranos and m. semitendinosus. Detailed specifications of EMG shorts are provided in Table 1.

Figure 8 EMG was measured from quadriceps and hamstring muscles by textile electrodes sewed onto elastic shorts. The shorts could be worn as regular underwear with a small electronic module at the waist.

The EMG signal was stored to a 50 g electronic module attached to the waist. In this study, eight pairs of shorts (four different sizes) were used. In addition, using zippers located at the inner sides of short legs and adhesive elastic band in the hem ensured proper fit of shorts in every participant. Electrode paste (Redux Electrolyte Crème, Parker Inc., USA) was used on the electrode surfaces to improve and stabilize conductivity between the skin and electrodes. After every measurement day the shorts were washed after detaching the electronics module.

Table 1 EMG shorts specifications.

Electrodes	
Type	Bipolar
Material	Conductive textile
Width X height = area	XS Quadriceps: 11,0cm X 3,0cm = 33,0cm^2 XS Hamstring: 7,5cm X 3,0cm = 22,5cm^2 S Quadriceps: 11,5cm X 3,0cm = 34,5cm^2 S Hamstring: 8,0cm X 3,0cm = 24,0cm^2 M Quadriceps: 13,5cm X 3,5cm = 47,3cm^2 M Hamstring: 8,0cm X 3,5cm = 28,0cm^2 L Quadriceps: 14,5cm X 3,5cm = 50,8cm^2 L Hamstring: 8,0cm X 3,5cm = 28,0cm^2
Paste used	Yes
Shaving	No
Inter-electrode distance	XS 23mm, S 25, M 25mm, L 25mm
Measurement electrode location	Distal part of the left and right quadriceps and hamstring muscles
Reference electrode location	Longitudinally over the left and right tractus iliotibialis
Sampling	
Module weight	53,4g
Sample rate	Raw 1000 Hz
Frequency band	50 Hz - 200 Hz (-3dB)
Processed data output frequency	Averaged rectified value at 10 Hz (2005 model) or 25Hz (2010 model)
Battery life	~24 hours recording

4.4.4 EMG analysis

Data was downloaded to MegaWin-software (Megaelectronics Ltd, Kuopio, Finland) and was visually checked, cleaned and analyzed using MegaWin, Excel and Matlab (MathWorks Inc, version 7.11.0.587, Massachusetts) in different steps in an order presented below.

4.4.4.1 Data filtering and cleaning

Filtering for baseline-offset. The baseline was defined as zero EMG activity. However, sometimes the EMG baseline may offset in absence of physiological activity. To correct this fluctuation, the baseline of data was determined with a five-minute-long moving window with a custom made Matlab algorithm. The filter searched for the minimum value from this window and subtracted it from the data point preceding this window. This was repeated systematically throughout the recording period.

Qualitative selection of baseline-offset filter length. The length of the filter window was selected according to pilot analysis in laboratory conditions and

comparison of daily data analyzed by alternative filter lengths. The laboratory tests included a variety of controlled activities from low to high intensities that lasted up to one minute, and uncontrolled periods of standing and ambulatory activities that lasted several minutes. According to pilot analysis in five subjects, the longest continuous burst duration was on average 88 ± 16 s. For example, during a task which included a short period of sitting and three minutes of standing and ambulating while talking to phone, the longest continuous burst duration was 17 ± 9 seconds, although burst time was 71 ± 17% of the measurement time. This can be taken as illustration of the intermittent nature of EMG even while maintaining a posture. Although the five-minute window may affect real physiological data in long-term static muscle activations, these were assumed to be rare during normal daily life.

Quantitative influence of baseline-offset filter length. The five-minute baseline filter lowered average EMG amplitude on average by -4.6 ± 2.9 μV (-1.7 ± 1.2 %EMG_{MVC}), in laboratory test data files analyzed for Study I, which were lasting around 30 minutes. The effects of different filter lengths on EMG inactivity and activity variables measured during 42 normal days of 21 participants (average duration 12 hours) is presented in Table 2. The five-minute baseline filter lowered the average amplitude on average by 6.9 ± 5.3 μV. The most sensitive variable on minimum filter was the longest continuous burst duration, but other variables seemed not to be affected considerably when changing the baseline-offset filter window length (Table 2). However, without the baseline-offset filter EMG activity time was considerably higher as compared to objective measurements with accelerometer (Matthews et al. 2008), or as compared to results analyzed with any baseline filter. The five-minute window was considered not to shorten physiological muscle activity periods, but to effectively correct for possibly fluctuating baseline.

Data cleaning and channel removal. The EMG signal was visually evaluated for occasional artefacts (e.g. toilet visits when electrodes were displaced, short-term movement artefacts), which were manually removed in MegaWin software (Mega Electronics Ltd., Kuopio, Finland). These occasional artefacts usually appeared simultaneously in all channels, and the corresponding data period was deleted from every channel. Sometimes the amount of artefact increased during a long measurement because the electrode paste wears out during a long continuous wear time. If the artefact duration was >30 minutes, the whole channel including artefact was removed from the analysis. The artefacts were distinguished from the physiological signal based on comprehensive laboratory tests where artefacts were intentionally induced to the signal (Tikkanen et al. 2013). The effect of artifact on other channels was carefully evaluated, and only channels that contained physiological data were included in the analysis. In Study III, investigating efficacy of the intervention, only comparable channels from each day were included in the analysis to make the comparison between the two measurement days valid. For example, if artefact was evident in another hamstring muscle on day one, the respective channel was removed from day two even if it was artefact free.

Table 2 Results from a pilot analysis including 42 days of habitual EMG activity from 21 subjects analyzed by different baseline-offset filter window lengths.

Filter window	Muscle inactivity (%)	Average EMG (μV)	Bursts/min	Longest burst (min)	Longest inact. period (min)
No filter	15.1±23.4	12.1±6.7	14.9±26.0	404.3±330.8	2.3±3.7
60 min	63.0±10.7	7.5±5.9	22.8±17.1	7.7±10.1	9.9±5.6
30 min	64.0±10.6	7.3±5.8	22.8±16.5	6.3±8.2	10.2±5.7
10 min	65.1±10.3	7.1±5.5	22.8±15.5	4.0±3.1	10.4±5.8
5 min	65.7±10.1	6.9±5.3	22.9±15.2	3.1±1.9	10.4±5.8
3 min	66.1±10.0	6.8±5.2	23.0±15.0	2.3±1.1	10.5±5.8
1 min	67.3±9.6	6.5±4.8	23.9±15.1	1.2±0.4	10.5±5.8

Effects of channel removal. To take into account the possibility that results may vary depending on the number of muscle groups removed due to artefact, we investigated the differences between the averaged EMG containing all four channels, and the averaged EMG from which either one or more channels were removed (Table 3). This was done with daily EMG data measured from 13 subjects, who had artefact-free data on all four channels. The results were most sensitive on removal of hamstring muscles, but for example lack of both quadriceps muscle groups did not change inactivity time significantly if both hamstring muscle groups were included (Table 3). For all of the pilot tests of this chapter, inactivity threshold set at 90% of $EMG_{standing}$ was used (see paragraph Inactivity and activity thresholds).

Table 3 Percent difference in variables of averaged EMG from different constitution of channels compared to average EMG from all channels. N=13.

No of Q channels	No of H channels	Inactivity (%)	Average EMG (μV)	Aver of 5 longest inact. periods (min)	Number of bursts	Aver duration of bursts (s)
2	2	Ref.	Ref.	Ref.	Ref.	Ref.
1	2	-0.1±4.0	12.6±7.4***	-2.2±6.0	4.1±5.9	0.3±12.8***
2	1	5.6±4.8**	-6.4±7.6**	6.4±9.8*	13.0±10.2***	-16.8±9.0***
1	1	5.9±5.5**	5.2±6.7***	9.0±10.3**	22.8±16.1***	-24.6±11.3
0	2	0.8±8.9	33.6±12.8***	-4.8±11.0	9.5±10.6	-6.3±18.7***
2	0	20.3±13.1***	-23.9±13.1***	22.3±18.7***	10.0±24.2	-40.8±12.1***
1	0	24.5±14.6***	-20.1±13.3**	36.3±22.0***	18.8±34.0	-53.8±10.0***
0	1	8.0±10.3*	38.4±12.2***	9.1±19.8	28.7±25.1**	-32.6±16.7***

Q = quadriceps, H = hamstrings. Ref = reference data, * denotes to $P < 0.05$, ** to $P < 0.01$ and *** to $P < 0.001$.

Removal of inactivity periods during dynamic activities. Due to the intermittent nature of EMG signal some dynamic activities, e.g. walking, might include brief inactivity periods. Despite being briefly inactive, energy expenditure of muscles is considerably elevated during dynamic activities. For this reason inactivity periods occurring during dynamic activities were filtered by using a two-second moving average in Study II, which investigated cross-sectional associations between muscle inactivity and activity patterns and cardio-metabolic biomarkers. As compared to the results analyzed without the two-second moving average filter, the use of filter lengthened inactivity period durations and reduced number of bursts/min with only modest changes in other inactivity and activity variables (Table 4).

Table 4 EMG inactivity and activity results of Study II when analyzed with and without two-second moving average filter, which was used to remove muscle inactivity periods during dynamic physical activities. N = 150.

Muscle inactivity variables	No filter	2s filter	P value
Muscle inactivity (%)	68.9±12.2	65.2±12.9	**0.010**
Sum of 5 longest inact. periods (min)	45.1±21.9	67.7±30.5	**< 0.001**
Mean of inactivity period duration (s)	2.6±1.3	24.1±9.8	**< 0.001**
Muscle activity variables			
Light muscle activity (%)	18.6±9.8	21.3±10.6	**0.021**
Moderate muscle activity (%)	8.1±3.9	9.6±5.0	0.76
Vigorous muscle activity (%)	4.3±4.8	4.0±5.5	0.52
Mean amplitude (%EMG_{MVC})	2.9±2	2.9±2.0	1.00
Mean muscle activity amplitude (%EMG_{MVC})	8.2±4.2	7.3±3.6	**0.019**
No of bursts/min	20.8±13.5	1.8±0.5	**< 0.001**

Comparisons performed for log-transformed data with paired T-test.

4.4.4.2 Data chopping, normalization and averaging

Data chopping. Laboratory tasks and MVC periods were separated from laboratory data based on lab logs during the visual check in MegaWIN software. The data around the desired period were removed and the remaining data of the task was saved as a new file. A similar approach was used to separate the different domains (worktime, commuting and leisure time) from habitual physical activity based on diaries.

Data normalization. The most consistent one-second mean EMG from the MVC with highest force level was analyzed and used to normalize the data of each channel individually.

Channel averaging. In low intensity activities, like standing, some individual muscle groups might be active without activity in other channels because of different reciprocal and contra-lateral muscle coordination patterns. Thus the four normalized signals were further averaged to represent overall activity of

thigh muscles as $\%EMG_{MVC}$. The channel averaging enables the analysis of the global muscle activity in thigh muscle region, rather than focusing activity pattern of individual muscle group(s).

Data averaging. If EMG recordings were repeated on more than one days in a given time point from a participant, the results from different days were averaged to yield one result per participant. This was necessary only in the cross-sectional Study II.

4.4.4.3 Inactivity and activity thresholds

Inactivity thresholds. The aim of inactivity threshold selection was to classify participants active during standing and sedentary during sitting (Sedentary Behaviour Research Network 2012). Thus, four different MVC- or standing -based inactivity thresholds were considered for further use based on their ability to differentiate muscle inactivity time between sitting and standing in participants of Study I (Table 5). The standing-based inactivity thresholds were analyzed from the acute, static non-fatiguing 15-second period of standing, which is typical for static posturographic studies (Duarte, & Zatsiorsky 1999). Sitting was measured when the participants were sitting for 30 minutes at breakfast (see: Laboratory protocol).

Table 5 Comparison of Sitting (30 min), Standing (15 s) and Standing (15 s)-Sitting (30 min) –difference (pp, percentage points) in mean, min and max of muscle inactivity time analyzed with MVC-based and Standing-based inactivity thresholds. N = 84.

	MVC-based ($\%EMG_{MVC}$)		Standing-based ($\%EMG_{standing}$)	
	$1\%EMG_{MVC}$	$2\%EMG_{MVC}$	$60\%EMG_{standing}$	$90\%EMG_{standing}$
Sitting				
Mean	91.0±8.8	96.9±3.3	81.1±20.4	90.2±11.8
Min	55.9	82.6	10.6	35.3
Max	99.8	100.0	99.9	100.0
Standing				
Mean	45.0±39.7	76.2±35.3	9.6±11.5	40.5±12
Min	0.0	0.0	0.0	16.5
Max	100.0	100.0	65.0	75.3
Standing-Sitting				
Mean	46.0±38.0	20.7±34.8	71.5±23.6	49.7±17.9
Min	-21.3	-9.3	-23.6	-16.4
Max	99.8	99.2	99.2	77.4
*Inactive during Standing n (%)	15 (18)	36 (43)	1 (1)	2 (2)

*individuals having more inactivity during standing than sitting

All of the tested thresholds yielded high inactivity time during Sitting (Table 5). However, the Standing-based thresholds more effectively differentiated Sitting and Standing muscle inactivity time. Only less than 2% of participants had

higher inactivity time during Standing than Sitting when analyzed with Standing-based -thresholds, as compared to more than 18% with MVC-based thresholds. Thus, the thresholds of 60% and 90% of $EMG_{standing}$ were used for further testing.

A further analysis aimed at testing which physiological correlates have an influence on the measured muscle inactivity time during Sitting, since the aim of the threshold is to capture behavioral differences in habitual EMG inactivity and activity patterns, which are not influenced by the physiological correlates of the thresholds. This was done by examining partial correlations of Sitting muscle inactivity time (ln [100% − muscle inactivity time %]) with anthropometrics when adjusting for age, sex, knee extension strength and the individual inactivity threshold. Lower fat mass and higher lean mass were associated with higher muscle inactivity time during sitting when analyzed with 60% of $EMG_{standing}$ -threshold condition independent of sex, age, knee extension strength and the individual threshold, whereas Sitting inactivity analyzed with the threshold 90% of $EMG_{standing}$ showed no associations with anthropometric variables (Table 6). Thus, the threshold set at 90% of $EMG_{standing}$ was deemed suitable for further use, and has been used in previous studies measuring muscle inactivity time with EMG shorts (Tikkanen et al. 2013; Finni et al. 2014; Gao et al. 2016).

Table 6 Partial correlations between anthropometrics and the amount of time muscles were inactive during sitting as analyzed with different thresholds.

	ln (100% − muscle inactivity time %), 60%$EMG_{standing}$		ln (100% − muscle inactivity time %), 90%$EMG_{standing}$	
	Partial r	p value	Partial r	p value
Height (cm)	0.025	0.830[a]	0.015	0.896[a]
Weight (kg)	-0.181	0.118[a]	-0.168	0.146[a]
BMI (kg/m²)	-0.213	0.064[a]	-0.191	0.099[a]
Waist circumference (cm)	-0.215	0.062[a]	-0.194	0.093[a]
Fat mass (%)	-0.241	**0.036[a]**	-0.210	0.068[a]
Lean mass (%)	0.246	**0.033[a]**	0.214	0.064[a]
Fat mass (kg)	-0.222	0.054[a]	-0.196	0.090[a]
Lean mass (kg)	-0.015	0.898[a]	-0.030	0.798[a]

Adjusted for sex, age, knee extension strength and [a]additionally for the individual inactivity threshold. Because the muscle inactivity time was natural log transformed as follows: ln(100% − muscle inactivity time %), the directions of true associations are inverse to those reported at the table. Boldface font denotes to significance at $P < 0.05$.

Activity intensity thresholds. The threshold between light and moderate intensity muscle activity was defined individually as one-minute mean EMG value when walking at 5km/h. In Study III the threshold between moderate and vigorous muscle activity intensities was set at one minute mean EMG value when running at 10 km/h. In Study II the threshold between moderate and vigorous

muscle activity intensities was calculated as "light-to-moderate -threshold x 2", because the running protocol was different in EMG24 and InPACT (see: EMG test battery). These thresholds were selected since they correspond to the energy expenditure at 3 and 6 METs, respectively (Ainsworth et al. 2000; Tikkanen et al. 2014).

4.4.4.4 EMG outcomes

The final results were analyzed with a custom-made Matlab algorithm yielding the following results:

Muscle inactivity variables:
- Muscle inactivity time (% of measurement time)
- Sum of the duration of the longest to 5th longest muscle inactivity periods (min)
- Mean inactivity period duration (s)

Muscle activity variables:
- Light-intensity muscle activity time (% of measurement time)
- Moderate-to-vigorous -intensity muscle activity time (% of measurement time)
- Mean amplitude (over the whole measurement time, $\%EMG_{MVC}$)
- Mean muscle activity amplitude (average signal amplitude when the signal exceeded the inactivity threshold, $\%EMG_{MVC}$)
- Number of bursts per minute (number of occasions when the signal amplitude exceeded the inactivity threshold in a minute, i.e. Bursts/min)

4.4.4.5 Validity and repeatability

The EMG shorts have been tested for validity, repeatability and feasibility in our laboratory (Finni et al. 2007; Tikkanen et al. 2013) and can be used to accurately estimate energy expenditure (Tikkanen et al. 2014). In addition, paired t-tests were used to assess for systematic differences in laboratory tests between the days before and after intervention in participants of Study III. Day-to-day reliability in laboratory tests was evaluated with intra-class correlation coefficients (ICC), coefficient of variation (CoV) and limits of agreement (LoA) in Table 7.

Table 7 Repeatability of maximal voluntary contraction, EMG/force -relationship (MVC), walking at 5 km/h and running at 10 km/h between baseline and post-intervention (less than one month from baseline) measurements in InPACT. N=43.

	Pre	Post	%Diff	ICC (95% CI)	Mean CoV (%)	LoA
Maximal						
Knee extension strength (kg)	78.2±23.2	82.7±23.4	6.6±11.2***	0.95 (0.91-0.97)	10.6	2.08-6.51
Knee flexion strength (kg)	46.6±18.0	48.4±17.0	6.8±13.2***	0.96 (0.93-0.98)	12.1	0.81-3.70
EMG/force quadriceps (MVC)	3.9±1.5	3.8±1.5	-1.2±17.1	0.87 (0.76-0.93)	19.5	-0.37-0.18
EMG/force hamstrings (MVC)	6.9±3.1	7.1±3.5	4.5±28.2	0.85 (0.74-0.91)	30.3	-0.43-0.68
Submaximal						
90% of Standing (%EMG_{MVC})	1.6±0.9	1.5±0.9	-4.5±44.4	0.70 (0.51-0.82)	62.0	-0.38-0.05
Walking 5 km/h (%EMG_{MVC})	8.1±3.3	7.3±3.3	-7.5±23.1**	0.79 (0.64-0.88)	27.8	-1.45--0.08
Running 10 km/h (%EMG_{MVC})	24.1±9.5	22.4±9.3	-5.2±20.3*	0.81 (0.67-0.89)	24.4	-3.53-0.08

* denotes to p<.05, ** to p<.01 and *** to p<.001.

In the laboratory measurements, the maximal voluntary contraction increased significantly in post measurement ($P < 0.001$), but EMG/force -relationship assessed during maximal voluntary contraction remained the same. The intraclass correlation coefficient revealed moderate to high repeatability of the measured variables. The poorer repeatability of submaximal laboratory tests could be explained by the fact that some subjects reported being unfamiliar with testing conditions, e.g. walking and running on treadmill during the first measurements. Therefore, to assess the intervention efficacy of Study III, the results from the second laboratory tests were used to categorize the data of both measurement days.

4.4.5 Accelerometry

Device and data. Total sedentary time, light activity time and moderate-to-vigorous activity time were assessed with a waist-worn 2D accelerometer (dynamic range ±2.7 g, sample rate 75/s, resolution 8 bit, bandwidth 0-20 Hz, manufacturer Alive Technologies Ltd., Australia). This light-weight device was worn in a firm elastic band on the anterior right side of the waistline.

To calculate time spent at different activity intensities, the resultant of the raw accelerometer data was converted into general count value by summing the g-values (m/s^2) over 1-minute epochs. To enable use of validated count thresholds being exclusive for specific monitor models, calibration measurements were performed. A device-specific factor was derived from simultaneous re-

cordings with the Alive monitor and ActiGraph GT3X monitor (Actigraph LCC, Pensacola, FL, USA) in a calibration machine and in two adults at normal daily life conditions. The counts were categorized in sedentary (<100 counts/min), light (⩾100<2020 counts/min) (Matthews et al. 2008) and moderate-to-vigorous (⩾2020 counts/min) (Troiano et al. 2008) intensities and multiplied with calibrated factor to yield similar results for both devices. A break in sedentary time was defined as an interruption in sedentary time when the accelerometer counts rose up to or above 100 counts/min for a minimum of one-minute period. The number of breaks was then divided by sedentary time yielding breaks/sedentary hour (Breaks/Sedh) (Kozey-Keadle et al. 2011).

Wear time criteria. Targeted wear time was seven days. The device was removed during bathing, water-based activities, contact sports and for nights, which were logged in a diary. In addition, participants logged their working times and these logs were used to analyze total wear time, work time and weekday and weekend day leisure time. To improve comparability and minimize the variation in wear time, the outcomes were normalized to measurement duration at each domain (Healy et al. 2011a).

Requirements for a valid accelerometer data were decided based on a simulation analysis to ensure optimal reliability for the sedentary time (% of measurement time) in the full baseline dataset of Study IV (Table 8). Measurements including full seven days of data were included, and every possible combo of number of weekdays and weekend days was formulated by removing randomly the corresponding number of days was from every subject. The average weekday and weekend day sedentary time of each combo was weighted to number of days in a full week ((weekday sedentary time (%) x 5 + weekend day sedentary time (%) x 2) / 7). The correlation coefficient of % sedentary time between this modified day and the original day was calculated to yield the reliability of an incomplete measurement to a full seven days of measurement. This process was done for each combo and the process was repeated 1000 times. Thus for every combination 1000 correlation coefficients were calculated and the average of these is presented. To estimate reliability, the square of this averaged correlation coefficient was used. A moderate reliability of >0.6 was accepted for a combination to be included into the analysis meaning that a minimum of two days was accepted from which at least the other one needed to be a weekday (Table 8). In addition, a wear time criteria per day was set at 10 hours based on previous research (Matthews et al. 2008; Troiano et al. 2008). To be included for separate work time and weekday leisure time analysis, a minimum of one day with wear time of at least 5 hours were required. Respectively, a requirement for a valid weekend day was one day with 10-hour wear time.

Table 8 Reliability (r^2) of sedentary time (%) measured at the baseline of InPACT based on a simulation analysis, where the correlation between simulated incomplete days and complete seven days measurements were calculated. A minimum of two days measurement including at least one weekday, as emphasized with bold letters, ensured accepted reliability of > 0.6.

Weekend days (n)	Weekdays (n)					
	5	4	3	2	1	0
2	**0.98**	**0.95**	**0.91**	**0.85**	**0.74**	0.54
1	**0.95**	**0.93**	**0.88**	**0.82**	**0.68**	0.39
0	**0.89**	**0.86**	**0.80**	**0.72**	0.55	0.00

4.4.6 Questionnaires and diary

Activity diary included pre-set forms to report waking up time, start and end of working time, and time when the device was detached in evening. In addition, participants were instructed to fill in times when the device was removed during bathing, water-based activities, contact sports or any other occasions when the device was not worn. In addition, any abnormal behaviors, like staying at home because kids were sick, or having an exercise evening at work were to be reported.

Physical activity questionnaire asked number, duration and intensity of different physical activities (e.g. walking to work, jogging) in a month-by-month basis during the last year. Corresponding MET-values were coded for each activity (Ainsworth et al. 2000). The reported activity time at $\geqslant$ 3 METs intensity was multiplied with the MET-values yielding volume of moderate-to-vigorous physical activity as average METh / week. This is presented as self-reported physical activity.

Diet quality was assessed from food records of three weekdays and one weekend day at the baseline and end line of the study. Nutri Flow software (Nutri Flow Oy, Oulu, Finland) was used to analyze intakes of total energy and energy-yielding nutrients as percentage of total energy intake.

Health questionnaire included questions about detailed health and socioeconomic status, as well as working time and self-reported sitting time at work.

4.5 Statistical analyses

Descriptive statistics are presented as means ± standard deviations. Statistical significance of $P < 0.05$ was tested in PASW version 20.0 (IBM Inc.) or in statistical programming language R (R 3.0.1, NLME package, the R foundation for Statistical Computing).

Muscle inactivity and activity patterns during sitting, standing, walking and habitual life were compared separately between sexes adjusting for BMI, and be-

tween normal weight and overweight participants adjusting for sex with One-way ANOVA. The bursts/min, amplitude analysis variables and muscle inactivity time were transformed with natural logarithm for P-value testing but non-transformed results are presented. Covariates age and knee extension strength were used in all analyses because of their effects on energy cost (Byrne et al. 2005, Tompuri 2015) and EMG amplitude (Harwood et al. 2008) of a given physical activity. Further, the analyses were additionally adjusted for the inactivity threshold to yield comparisons independent of the individual threshold.

Cross-sectional associations between muscle inactivity and activity patterns and biomarkers. For non-normally distributed variables, logarithmic transformations (natural logarithm) were performed. After the transformation all variables met the criteria of normal distribution based on the Shaphiro-Wilk test or had skewness and kurtosis values between -1 and 1. The associations between muscle inactivity and activity subcomponents and individual phenotypes of cardiometabolic risk were modeled in separate forced-entry linear regression models. Potential confounders including sex, age, BMI, smoking (yes/no), education status (primary school/high school/vocational school/university degree), season (winter/summer), number of measured days, recording time and number of included channels were used as covariates in every model (excl. BMI when BMI was the dependent variable). Because the total muscle inactivity time is highly sensitive to the threshold used (Klein et al. 2010), the models were also adjusted for the thresholds used in respective comparisons (inactivity–to-light and/or light-to–moderate-to-vigorous). The independent effects of muscle inactivity time from physical activity level and vice versa were ensured by using muscle moderate-to-vigorous activity/muscle inactivity time as covariates. Data is presented as standardized beta coefficients to make the comparison of the different muscle inactivity and activity variables possible. For every model, residuals were normally distributed and homoscedastic, and VIF -value remained below three suggesting lack of multicollinearity. Finally, estimated marginal means between inactivity quartiles were analyzed to assess the effect sizes. Levene's test was used to ensure the equality of variances between the quartiles.

Short-term intervention efficacy on EMG outcomes. Differences between the groups at baseline were tested with independent samples T-test or the Mann-Whitney test. The effect of the intervention on EMG variables was assessed using repeated measures ANOVA with measurement time and baseline values of variables as covariates. Not normally distributed variables (total and leisure time average EMG and leisure time vigorous muscle activity time) were tested with the Mann-Whitney test by comparing within-group changes (post-values - pre-values) between the groups, after which within-group changes were tested with the Wilcoxon Signed-Rank test. The differences between % inactivity and activity time before and after the intervention were calculated as the arithmetic difference (% of measurement time post - % of measurement time pre) yielding a percentage point (pp).

Effectiveness of long-term intervention on accelerometer-derived outcomes and biomarkers. Differences between the groups at baseline were assessed by inde-

pendent samples t-test, Mann-Whitney test or Chi-square test. Intervention effectiveness was tested with linear mixed-effects model fit by REML in statistical programming language R (R 3.0.1, NLME package, the R foundation for Statistical Computing). The analysis was based on a three level hierarchy, where the random grouping variables participants (n = 133) where nested within families (n=89), and families were nested within the clusters (n = 7). Covariates age and self-reported work-time per week were selected based on being differently distributed background variables between the groups at baseline. Likelihood ratios were used to test the effectiveness separately for the intervention period (three and six months) and for the whole year for the primary outcomes, and for the whole year for secondary outcomes. Intention-to-treat principle was used meaning that all participants having acceptable baseline data, including those who dropped out later, were retained in the analysis. As a sensitivity analysis, intervention effectiveness on sedentary and physical activity outcomes was additionally tested with a more conservative wear time criteria. The models testing the effects on health outcomes were additionally adjusted for moderate-to-vigorous physical activity and energy intake and those testing effects on blood-drawn biomarkers were further adjusted for fat mass or lean mass (%). The changes in self-reported total physical activity, commuting activity and sitting time at work and leisure time between baseline and end-line were tested with Kruskal-Wallis and Wilcoxon tests in PASW version 20.0 (IBM Inc.). As a secondary analysis, the background characteristics of responders (reducing objectively measure sedentary time >2% between baseline and endline) were compared to those of non-responders (reducing objectively measured sedentary time ≤2% between baseline and endline) with Mann-Whitney test. Furthermore, their changed objectively measured sedentary time throughout the year was compared with repeated measures ANOVA in PASW version 20.0.

5 RESULTS

5.1 Participants

Background characteristics of eligible participants at each sub-study are presented in Table 9. On average, the participants were normal weight, young and physically active adults. The age ranged from 20 to 76 years and BMI ranged from 17.0 to 34.9 kg/m^2. Self-reportedly the participants were active more than 26 METh/week and over 75% met the current recommendation of 7.5 METh/week of aerobic physical activity at >3 METs intensity. However, since sedentary occupation was one of the inclusion criteria, participants were self-reportedly sitting the majority of their working hours. As compared to female, a higher proportion of male in Studies I and II were overweight, accompanied with their other anthropometric differences. However, their physical activity levels were the same (Table 9).

Table 9 Background characteristics of study participants.

	Study I		Study II		Study III		Study IV	
	Female	Male	Female	Male	Intervention	Control	Intervention	Control
N	44	40	85	65	24	24	71	62
Age (years)	36.7±4.8	38.9±5.1*	38.5±11.4	39.3±9.6	37.0±5.5	39.0±5.4	36.6±5	39.6±5.3**
Height (cm)	164.5±6.2	179.2±6.8***	165.9±6	178.8±6.4***	171.1±10.3	171.1±9.0	170.7±9.7	171.3±8.2
Weight (kg)	62.9±8.9	82.5±12.9***	63±8.7	80.5±12.2***	73.2±17.6	71.9±13.8	72±15.4	71.8±14
Waist circumference (cm)	85.6±8.6	93.5±10.1***	81.6±9.6	90.5±9.6***	90.9±10.8	89.6±10.4	89.9±10.1	89.5±10.7
BMI (kg/m²)	23.2±2.9	25.7±3.6**	22.9±2.8	25.1±2.9***	24.7±3.7	24.5±3.9	24.5±3.5	24.4±4.1
Overweight N (%)	8 (18)	19 (48)**	14 (16)	32 (49)**	9 (38)	9 (38)	26 (37)	19 (31)
Knee extension strength (kg)	65.8±15.1	98.1±20.1***			78.0±25.5	77.9±22.6		
Self-reported sitting at work (% work time)					80.8±14.4	84.5±12.4	85.7±12.0	83.5±13.8
Self-reported total physical activity (METh/week)	31.4±21.2	30.8±19.4	34.5±29.1	35±26.2	26.5±18.3	32.2±15.7	29.9±22.6	35.1±19.8
Recommended activity level of 7.5 METh/week at >3 METs (%)	38 (86)	33 (83)	74 (93)	58 (97)	20 (83)	24 (100)	53 (75)	53 (85)

Data are means ± standard deviations or N.

5.2 Muscle inactivity and activity patterns during sitting, standing, walking and habitual life (Studies I and II)

5.2.1 Sitting, standing and walking in laboratory (Study I)

On average, EMG amplitude was 0.5 ± 0.3% of EMG_{MVC} during sitting (range 0.1 to 1.3%EMG_{MVC}), 1.6 ± 1.4%EMG_{MVC} during standing (range 0.1 to 8.2% of EMG_{MVC}) and 9.6 ± 5.7% of EMG_{MVC} during walking at 5 km/h (range 3.0 to 30.7% of EMG_{MVC}). During 30 min sitting, the amount of time muscles were inactive was on average 90.2 ± 11.8% (range 35.3 to 100.0%), which was accumulated in an average of 9.0 ± 18.2 s periods (range 0.2 to 144.9 s) separated by an average of 12.9 ± 13.4 bursts/min (range 0.1 to 62.6).

Comparisons between groups revealed that male had longer mean inactivity period duration during sitting as compared to female ($P < 0.05$) without other differences between sexes (Table 10). When compared by overweight status, the overweight had higher EMG amplitude during standing ($P < 0.05$) and more muscle inactivity time during sitting ($P < 0.01$) than the normal weight independent of their age, sex, knee extension strength, and the individual threshold (Table 11). Figure 8 shows that there was considerable heterogeneity in muscle inactivity variables during sitting when examined across all participants.

Table 10 Differences in Sitting, Standing and Walking EMG amplitudes, and in muscle inactivity and no. of breaks per minute during Sitting between female and male.

	Female N = 44	Male N = 40	P-value
Sitting (30 min) mean amplitude (%EMG_{MVC})	0.5±0.3	0.5±0.2	0.17
Standing (15 s) mean amplitude (%EMG_{MVC})	1.6±1.7	1.6±1.1	0.41
Walking (1 min) mean amplitude (%EMG_{MVC})	11.0±6.4	8.0±4.4	0.18
Sitting (30 min) muscle inactivity (%)	89.1±12.4	91.4±11.1	0.45[a]
Sitting (30 min) mean inactivity period duration (s)	6.1±10.6	12.2±23.7	**0.016[a]**
Sitting (30 min) no. of bursts per minute	14.5±13.0	11.2±13.8	0.14[a]

Comparisons adjusted for age, BMI, knee extension strength and [a]additionally for the individual inactivity threshold at 90% of $EMG_{standing}$ where appropriate.

Table 11 Differences in Sitting, Standing and Walking EMG amplitudes, and in muscle inactivity and no. of breaks per minute during Sitting between overweight and normal weight participants.

	Normal weight N = 57	Over-weight N = 27	P-value
Sitting (30 min) mean amplitude (%EMG_{MVC})	0.5±0.2	0.5±0.3	0.57
Standing (15 s) mean amplitude (%EMG_{MVC})	1.4±1.4	1.9±1.5	**0.042**
Walking (1 min) mean amplitude (%EMG_{MVC})	9.6±5.6	9.5±6.1	0.31
Sitting (30 min) muscle inactivity (%)	88.7±12.1	93.3±10.8	**0.003**[a]
Sitting (30 min) mean inactivity period duration (s)	5.4±7.4	16.5±29.2	0.67[a]
Sitting (30 min) no. of bursts per minute	13.4±13.5	11.9±13.5	0.10[a]

Comparisons adjusted for age, sex, knee extension strength and [a]additionally for the individual inactivity threshold at 90% $EMG_{standing}$ where appropriate.

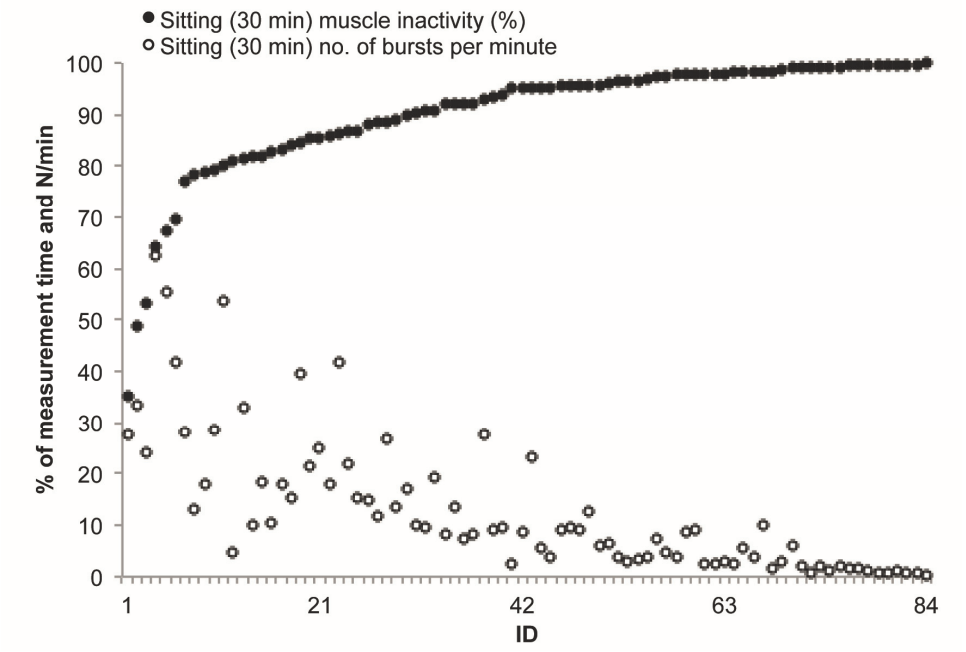

Figure 9 Heterogeneity of muscle inactivity and no. of bursts per minute during 30 minutes of sitting across participants ranked by muscle inactivity time.

5.2.2 Habitual life (Study II)

On average, thigh muscles were inactive for 68.9 ± 12.2% of the measurement time (range 37.8 to 93.5%) and on average 2.9 ± 2.0% of EMG_{MVC} of the muscle's voluntary contractile capacity was used (range 0.7 to 16.0% of EMG_{MVC}). The sum of five longest inactivity periods was on average 45.1 ± 21.9 minutes (range 1.6 to 138.1 min). Despite the high inactivity time, the mean inactivity period duration was only 2.6 ± 1.3 seconds (range 0.2 to 7.0 s), which was because subjects had on average 20.8 ± 13.5 bursts per minute (range 7.5 to 105.8). Thigh muscles were active at light intensity for 18.6 ± 9.8% (range 3.0 to 50.4%) and at moderate-to-vigorous intensity for 12.4 ± 7.4% of time (range 1.8 to 45.2%). When active, average amplitude was 8.2 ± 4.2 $\%EMG_{MVC}$ (range 2.4 to 25.7% of EMG_{MVC}) corresponding closely to that of walking at 5km/h (7.0 ± 3.5% of EMG_{MVC}, range 1.8 to 20.6% of EMG_{MVC}).

Table 12 Sex-specific differences in muscle inactivity and activity variables measured during daily life.

	Female N=85	Male N=65	P-value
Muscle inactivity variables			
Muscle inactivity (%)	66.7±11.9	71.9±11.9	**0.013**[a]
Sum of the five longest inactivity periods (min)	44.1±20.9	46.4±23.2	0.38[a]
Mean of the inactivity period duration (s)	2.4±1.1	2.9±1.4	**< 0.001**[a]
Muscle activity variables			
Light intensity muscle activity (%)	20.4±10.2	16.3±8.8	0.11[a,b]
Mod-to-vig intensity muscle activity (%)	12.9±7.4	11.9±7.4	0.06[b]
Mean amplitude ($\%EMG_{MVC}$)	3.3±2.2	2.5±1.7	**< 0.001**
Mean muscle activity amplitude ($\%EMG_{MVC}$)	8.7±4.5	7.6±3.6	**0.038**
No. of bursts per minute	22.2±15.8	18.9±9.3	**0.007**[a]
Recording covariates			
No of days	1.6±0.7	1.6±0.7	0.78
Recording time/day	11.7±1.3	12.1±1.7	0.29
No of channels	2.8±1.1	2.9±1.1	0.70
Inactivity threshold 90% $EMG_{standing}$ ($\%EMG_{MVC}$)	2.0±1.6	2.0±1.2	0.47
Mod-to-vig threshold $EMG_{walking}$ ($\%EMG_{MVC}$)	7.6±3.7	6.2±3.0	**0.001**

Comparisons adjusted for age, BMI and the recording covariates as appropriate. Adjustment for thresholds indicated as follows: [a]adjusted for inactivity threshold, [b]adjusted for moderate-to-vigorous muscle activity -threshold.

Comparisons between sexes revealed that female had less muscle inactivity ($P < 0.05$) and a shorter mean inactivity period duration ($P < 0.001$) as compared to male (Table 12). In addition, female had more bursts per minute ($P < 0.01$) and higher EMG amplitude on average ($P < 0.001$) and when active ($P < 0.05$). Despite their higher moderate-to-vigorous muscle activity threshold ($P = 0.001$), female had similar moderate-to-vigorous muscle activity time as compared to male. Comparisons between normal weight and overweight showed that the overweight had higher inactivity threshold as compared to the normal weight ($P < 0.01$). However, the overweight had shorter mean inactivity period duration ($P < 0.05$) and more bursts per minute ($P < 0.05$) independent of the inactivity threshold and the other covariates, as compared to the normal weight (Table 13). Heterogeneity in daily muscle inactivity and activity patterns across subjects is illustrated in Figure 10.

Table 13 Differences in muscle inactivity and activity variables between normal weight and overweight subjects measured during daily life.

	Normal weight N=102	Over-weight N=48	P-value
Muscle inactivity variables			
Muscle inactivity (%)	68.7±12.2	69.4±12.3	0.24[a]
Sum of the 5 longest inactivity periods (min)	46.0±22.3	43.2±21.1	0.21[a]
Mean of the inactivity period duration (s)	2.7±1.3	2.4±1.3	**0.038**[a]
Muscle activity variables			
Light muscle activity (%)	19.1±9.9	17.5±9.7	0.35[a,b]
Mod-to-vig activity (%)	12.1±7.3	13.1±7.8	0.37
Mean amplitude (%EMG_{MVC})	3.3±2.2	2.5±1.7	0.12
Mean muscle activity amplitude (%EMG_{MVC})	2.7±1.6	3.3±2.7	0.13
No. of bursts per minute	19.9±13.5	22.7±13.3	**0.026**[a]
Recording covariates			
No of days	1.6±0.7	1.5±0.7	0.22
Recording time/day	11.8±1.6	11.9±1.2	0.48
No of channels	2.8±1.1	2.7±1.1	0.42
Inactivity threshold 90% $EMG_{standing}$ (%EMG_{MVC})	1.8±1.3	2.4±1.6	**0.009**
Mod-to-vig threshold $EMG_{walking}$ (%EMG_{MVC})	6.9±3.5	7.1±3.5	0.96

Comparisons adjusted for age, sex and the recording covariates as appropriate. Adjustment for thresholds indicated as follows: [a]adjusted for inactivity threshold, [b]adjusted for moderate-to-vigorous muscle activity -threshold.

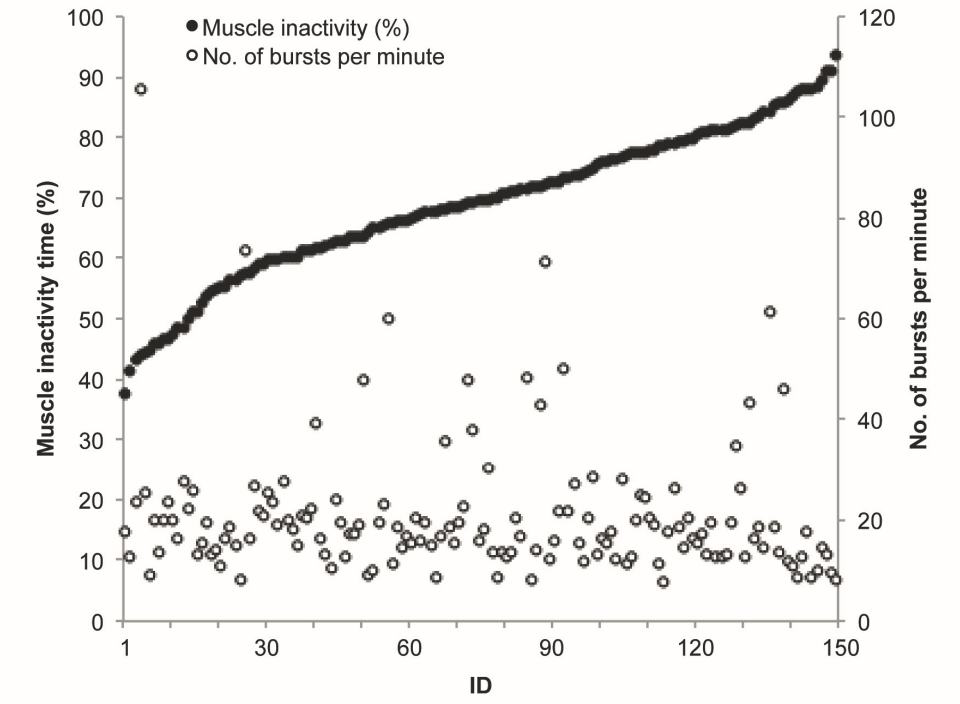

Figure 10 Muscle inactivity time and no. of bursts per minute across participant's normal daily life ranked by their muscle inactivity time.

5.2.3 Associations between sitting, standing, walking and daily EMG patterns (Studies I and II)

Correlations between muscle inactivity and activity patterns measured in the laboratory were studied with those measured during daily life by combining the 66 subjects having data in both Studies I and II. As presented in Table 14, muscle inactivity time and mean inactivity period duration during sitting were positively associated with mean muscle activity amplitude ($P < 0.01$) and sitting mean inactivity period duration was negatively associated with light muscle activity time during habitual life ($P < 0.01$). Conversely, sitting no. of bursts per minute was negatively associated with mean inactivity period duration ($P < 0.05$) and positively with light muscle activity time measured during habitual life ($P < 0.01$). Sitting mean amplitude was negatively associated with mean inactivity period duration ($P < 0.05$), and positively with mean amplitude ($P < 0.01$), mean muscle activity amplitude ($P < 0.01$) and no of bursts per minute of habitual living ($P < 0.01$). Standing mean amplitude was negatively associated with light muscle activity time ($P < 0.05$) and positively with mean amplitude ($P < 0.05$) as well as mean muscle activity amplitude ($P < 0.001$) of habitual life. Finally, walking mean amplitude was positively associated with habitual EMG mean amplitude ($P < 0.05$) (Table 14).

Table 14 Partial correlations between muscle inactivity and activity measured at laboratory and during normal daily life

		Partial Correlation Coefficient (P -value)					
		EMG during laboratory measurements					
		Sitting (30 min) muscle inactivity	Sitting (30 min) mean inactivity period duration	Sitting (30 min) no. of bursts per minute	Sitting (30 min) mean amplitude	Standing (15 s) mean amplitude	Walking (1 min) mean amplitude
EMG during habitual life	Muscle inactivity	0.10 (0.430)	0.21 (0.119)	-0.25 (0.060)	0.03 (0.833)	0.14 (0.295)	-0.20 (0.127)
	Sum of the 5 longest inactivity periods	-0.01 (0.914)	0.11 (0.412)	-0.11 (0.410)	-0.11 (0.410)	-0.09 (0.485)	-0.19 (0.158)
	Mean of the inactivity period duration	0.13 (0.320)	0.16 (0.219)	**-0.27 (0.036)**	**-0.30 (0.022)**	-0.07 (0.583)	-0.20 (0.126)
	Light muscle activity	-0.23 (0.077)	**-0.36 (0.005)**	**0.36 (0.005)**	0.03 (0.816)	**-0.27 (0.041)**	0.19 (0.141)
	Moderate-to-vigorous muscle activity	0.06 (0.629)	0.17 (0.207)	-0.01 (0.954)	-0.16 (0.220)	-0.02 (0.875)	-0.19 (0.144)
	Mean amplitude	0.20 (0.127)	0.09 (0.498)	0.05 (0.730)	**0.35 (0.006)**	**0.28 (0.029)**	**0.31 (0.017)**
	Mean muscle activity amplitude	**0.39 (0.002)**	**0.33 (0.012)**	-0.23 (0.081)	**0.39 (0.002)**	**0.49 (< 0.001)**	0.25 (0.055)
	No. of bursts per minute	-0.11 (0.415)	-0.09 (0.484)	0.20 (0.122)	**0.37 (0.004)**	0.16 (0.219)	0.15 (0.247)

Comparisons adjusted for age, sex, BMI, number of days, number of channels, recording time and knee extension strength.

5.3 Cross-sectional associations between habitual muscle inactivity and activity patterns and cardio-metabolic biomarkers (Study II)

Table 15 summarizes the standardized regression coefficients of muscle inactivity and activity variables with cardio-metabolic outcomes. The total muscle inactivity time was positively associated with triglycerides ($P = 0.001$) and negatively with HDL ($P < 0.05$) and light intensity muscle activity time was negatively associated with fasting plasma glucose ($P < 0.05$) independent of BMI and moderate-to-vigorous muscle activity time. Sum of the five longest inactivity

periods ($P < 0.05$) and mean inactivity period duration ($P < 0.001$) were negatively associated with BMI, and no. of bursts per minute was positively associated with BMI ($P < 0.001$) independent of moderate-to-vigorous activity time. After further adjustment for self-reported physical activity at >3 METs intensity during the previous year, the association between light intensity muscle activity and glucose turned insignificant ($P = 0.10$) without effect on other associations. Mean amplitude was positively associated with BMI independent of muscle inactivity time ($P < 0.05$), and with fasting plasma glucose independent of muscle inactivity time and BMI ($P < 0.01$).

Figure 11 presents the means for BMI, triglycerides, fasting plasma glucose, HDL cholesterol and systolic and diastolic blood pressure between the quartiles of total muscle inactivity time. As compared to those in the lowest total muscle inactivity quartile, those in the third quartile had 0.44 mM higher triglycerides ($P < 0.01$), and those in the fourth quartile had 0.38 mM lower HDL cholesterol ($P < 0.01$) (Figure 11).

Table 15 Standardized regression coefficients of muscle inactivity and activity variables with continuous cardiometabolic risk markers in 150 individuals.

	BMI		Blood pressure index		Fasting serum triglycerides		Fasting plasma glucose		HDL cholesterol	
	Standardized ß (95% CI)	P	Standardized ß (95% CI)	P	Standardized ß (95% CI)	P	Standardized ß (95% CI)	P	Standardized ß (95% CI)	P
Muscle inactivity time[a]	-0.13 (-0.34 to 0.08)	0.211	-0.13 (-0.34 to 0.08)	0.228	0.36 (0.15 to 0.57)	**0.001**	0.16 (-0.05 to 0.37)	0.145	-0.25 (-0.46 to -0.05)	**0.015**
Sum of the five longest inactivity periods[a]	-0.20 (-0.37 to -0.04)	**0.017**	-0.04 (-0.21 to 0.13)	0.632	0.11 (-0.07 to 0.28)	0.232	-0.02 (-0.19 to 0.15)	0.825	-0.08 (-0.25 to 0.08)	0.320
Mean inactivity period duration[a]	-0.31 (-0.46 to -0.15)	**<0.001**	-0.10 (-0.27 to 0.07)	0.234	0.16 (-0.02 to 0.34)	0.083	-0.02 (-0.20 to 0.15)	0.798	-0.01 (-0.19 to 0.16)	0.867
Light muscle activity[c]	0.10 (-0.14 to 0.34)	0.407	0.19 (-0.05 to 0.43)	0.114	-0.18 (-0.42 to 0.07)	0.154	-0.27 (-0.50 to -0.03)	**0.029**	0.16 (-0.07 to 0.39)	0.182
Mod-to-vig muscle activity[b]	-0.01 (-0.23 to 0.20)	0.914	0.08 (-0.14 to 0.29)	0.478	0.09 (-0.13 to 0.31)	0.409	0.12 (-0.09 to 0.34)	0.254	0.08 (-0.13 to 0.29)	0.445
Bursts/min[a]	0.31 (0.16 to 0.45)	**<0.001**	0.05 (-0.11 to 0.21)	0.526	-0.08 (-0.25 to 0.09)	0.345	0.00 (-0.17 to 0.16)	0.982	0.01 (-0.15 to 0.17)	0.878
Mean amplitude[b]	0.21 (0.01 to 0.41)	**0.043**	0.16 (-0.05 to 0.37)	0.124	0.09 (-0.13 to 0.31)	0.432	0.28 (0.08 to 0.48)	**0.008**	0.03 (-0.18 to 0.24)	0.794

All models adjusted for sex, age, smoking (yes/no), education status (primary school/high school/vocational school/university degree), season (winter/summer), number of measured days, recording time and number of included channels. All models except BMI were adjusted for BMI.
a = additionally adjusted for inactivity threshold and moderate-to-vigorous muscle activity time
b = additionally adjusted for moderate-to-vigorous activity threshold and muscle inactivity time
c = additionally adjusted for inactivity threshold, moderate-to-vigorous activity threshold and moderate-to-vigorous muscle activity time

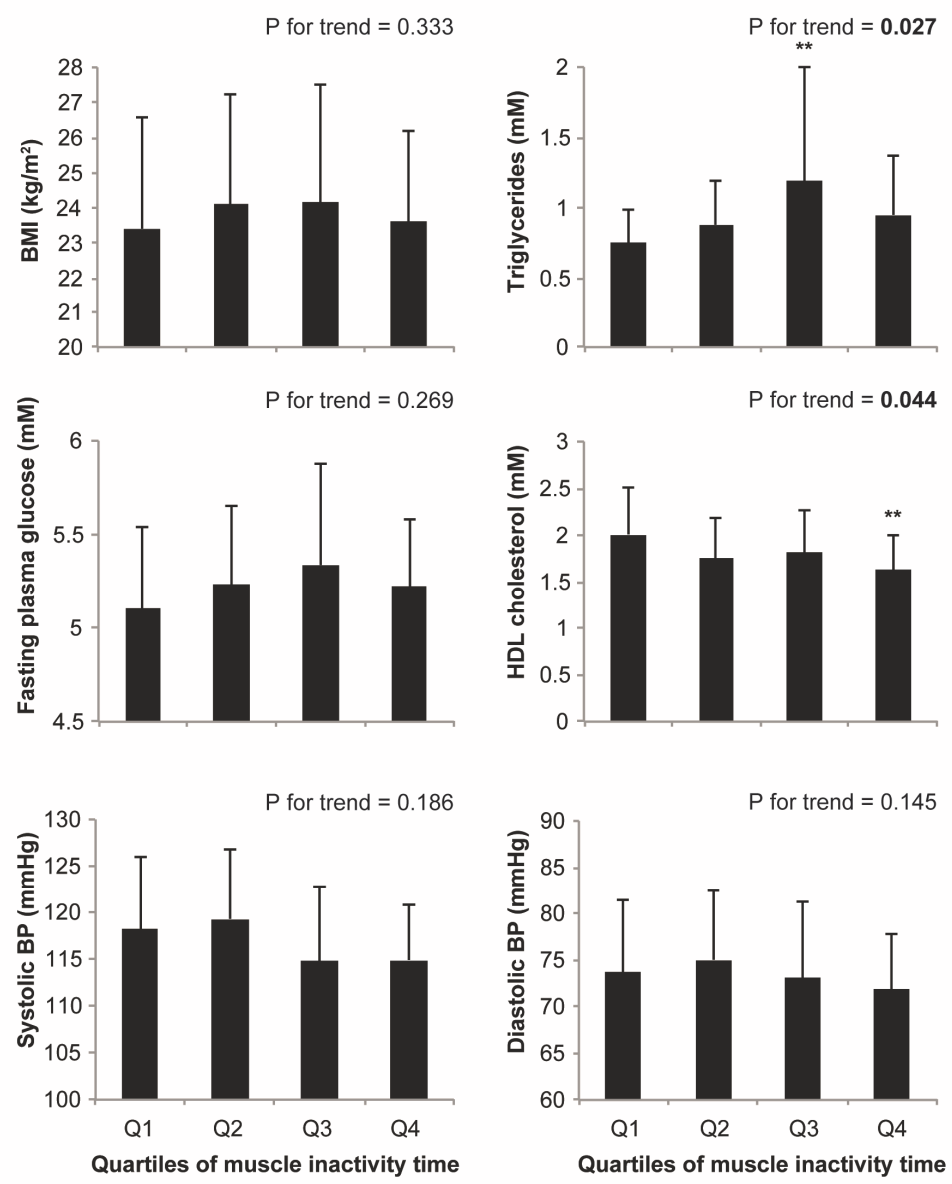

Figure 11 Means of BMI, triglycerides, fasting plasma glucose, HDL cholesterol, as well as systolic and diastolic blood pressure between quartiles of muscle inactivity time. Cut points for quartiles were 61.1%, 69.5% and 78.4% of muscle inactivity time as percent of measurement time. Models were adjusted for sex, age, BMI (except the model with BMI as dependent variable), education, smoking, winter/summer, number of days, recording time, number of channels, inactivity threshold and moderate-to-vigorous-intensity muscle activity time. For triglycerides and systolic blood pressure the statistical tests were performed for log-transformed data but the non-transformed data is presented here.

5.4 Acute changes in muscle inactivity and activity patterns following intervention (Study III)

Total muscle inactivity and activity variables in intervention and control group before and after intervention are presented in Table 16. Muscle inactivity time ($P < 0.05$) and the sum of the 5 longest inactivity periods ($P< 0.05$) decreased with concomitant increases in light muscle activity ($P < 0.05$) and the number of bursts per minute ($P < 0.05$) in the intervention group compared to the controls. Despite the significant group*time –interaction, the number of bursts per min did not change significantly within the intervention group (Table 16). **Virhe. Viitteen lähdettä ei löytynyt.** illustrates that the intervention targeting decreased sedentary time and increased breaks was successful in decreasing muscle inactivity and inactivity period durations without affecting higher intensity muscle activities.

Table 16 Total muscle inactivity and activity patterns in intervention and control group before and after intervention.

		Pre	Post	Difference	Group* time P
Total					
Muscle inactivity time (%)	Int.	69.1±8.5	64.6±10.9	**-4.5±9.7***	**0.042**
	Cont.	69.2±13.4	69.9±12.8	0.7±8.0	
Sum of the five longest muscle inactivity periods (min)	Int.	35.6±14.8	29.7±10.1	**-5.8±9.9***	**0.027**
	Cont.	37.9±17.4	42.6±22.7	4.7±17.7	
Mean of the inactivity period duration (s)	Int.	2.4±1.1	2.0±0.9	**-0.4±0.8***	**0.009**
	Cont.	2.2±1.2	2.6±1.7	0.4±1.0	
Light muscle activity time (%)	Int.	22.2±7.9	25.0±9.7	**2.8±7.2***	**0.023**
	Cont.	21.7±11.9	20.3±10.7	-1.3±5.1	
Mod-to-vig muscle activity time (%)	Int.	8.7±4.3	10.4±4	**1.7±4.6***	0.43
	Cont.	9.1±5.0	9.8±3.8	0.6±4.3	
Mean amplitude (%EMG_{MVC})	Int.	2.5±1.3	3.1±2.2	0.6±2.2	0.19
	Cont.	2.2±1.8	2.1±0.9	-0.1±1.6	
No of bursts per minute	Int.	22.5±14.9	24.8±15.3	2.3±7.5	**0.027**
	Cont.	24.3±15.2	22.6±15.3	-1.7±7.6	

* denotes to significance at $P < 0.05$, ** to $P < 0.01$ and *** to $P < 0.001$ * for within group changes.

Domain-specific muscle inactivity and activity variables in intervention and control group before and after intervention are presented in Table 17. The only difference between the groups at baseline was the greater amount of moderate-to-vigorous muscle activity during commuting time among the controls compared with that in the participants in the intervention group. During work time, a decrease in muscle inactivity time ($P < 0.05$) was accompanied by an increase in light muscle activity ($P < 0.01$) and average EMG amplitude ($P < 0.05$) in the intervention group compared to the controls. Compared to the control group,

muscle inactivity time (P < 0.05) and the sum of the 5 longest inactivity periods (P < 0.01) decreased and light muscle activity time (P < 0.05) increased in the intervention group during leisure time. Compared to the intervention group the sum of the 5 longest inactivity periods decreased during commuting in the control group (P < 0.01) (Table 17).

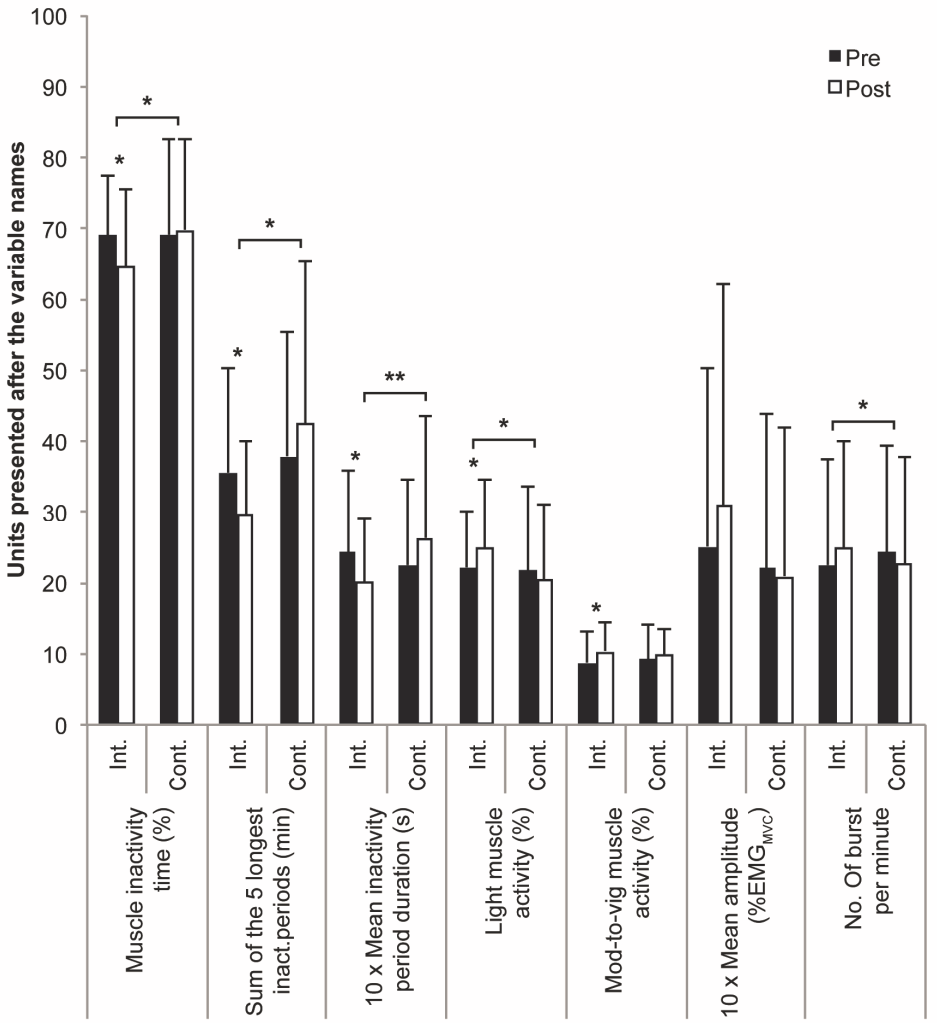

Figure 12 Muscle inactivity and activity outcomes in intervention and control groups during pre and post measurements. Units for each variable are presented after the variable names. Please note10 x scaling in mean inactivity period duration and mean amplitude to clarify the changes in these outcomes. Int. = intervention group, cont. = control group. *Denotes significance at P < 0.05.

Table 17 Domain-specific muscle inactivity and activity patterns in intervention and control group before and after intervention.

		Pre	Post	Difference	Group* time P
Work					
Muscle inactivity time (%)	Int.	79.7±8.9	75±11.2	**-4.6±6.9****	**0.023**
	Cont.	77.6±12.6	77.9±12.6	0.3±7.1	
Sum of the 5 longest muscle inactivity periods (min)	Int.	29.7±13.1	26±8.9	-3.7±9.1	0.12
	Cont.	33.6±18.5	34.2±17.1	0.5±11.7	
Mean of the inactivity period duration (s)	Int.	4.1±3.2	2.9±1.7	**-1.2±1.9****	**0.049**
	Cont.	3.5±2.2	3.8±2.7	0.3±1.5	
Light muscle activity time (%)	Int.	15.6±7.7	19.3±10	**3.7±6.0*****	**0.008**
	Cont.	16.8±10.7	16.4±10.9	-0.4±5.4	
Mod-to-vig muscle activity time (%)	Int.	4.8±2.7	5.7±2.8	0.9±2.5	0.51
	Cont.	5.6±3.7	5.7±3.3	0.1±3.2	
EMG amplitude (%EMG_{MVC})	Int.	1.6±0.9	1.8±1	**0.3±0.6***	**0.045**
	Cont.	1.4±0.6	1.3±0.7	0.0±0.5	
No of bursts per minute	Int.	19±14.5	20.9±12.2	**1.8±7.6***	0.09
	Cont.	19.8±14.5	20.9±16.4	1.1±6.9	
Commuting					
Muscle inactivity time (%)	Int.	48±21.1	49.5±19.1	1.5±17.4	0.08
	Cont.	40.5±22	36±22.6	-4.6±16.4	
Sum of the 5 longest muscle inactivity periods (min)	Int.	8.5±6.5	7.8±5.7	-0.7±5.1	**0.005**
	Cont.	7.2±7.1	4.1±4.7	**-3.1±5.6*****	
Mean of the inactivity period duration (s)	Int.	1.4±1	1.3±0.7	-0.1±0.8	0.14
	Cont.	1.1±1	0.8±0.6	-0.2±0.9	
Light muscle activity time (%)	Int.	35.5±15.8	32.5±12.6	-3.0±11.8	0.12
	Cont.	30±11.6	32.9±13	2.9±7.3	
Mod-to-vig muscle activity time (%)	Int.	16.4±9.2	17.9±10.6	1.5±7.7	0.18
	Cont.	29.5±17.7	31.1±15.4	1.6±13.2	
EMG amplitude (%EMG_{MVC})	Int.	4.2±2.6	4.7±3.2	0.5±2.3	0.60
	Cont.	5.2±3.2	5.3±2.7	0.1±2.3	
No of bursts per minute	Int.	26.2±16.9	30.1±21.7	3.9±16.6	0.36
	Cont.	36.3±30.9	33.1±28.4	-3.2±19.6	
Leisure					
Muscle inactivity time (%)	Int.	59.1±11.9	49.8±17.3	**-9.3±18.5****	**0.015**
	Cont.	64.1±18.6	63.4±16.6	-0.7±17.6	
Sum of the 5 longest muscle inactivity periods (min)	Int.	26.4±13.9	18.2±10.2	**-8.2±11.8*****	**0.005**
	Cont.	24.7±9.9	30.6±19.7	5.9±15.3	
Mean of the inactivity period duration (s)	Int.	1.8±1.1	1.3±0.8	**-0.5±1.2***	**0.008**
	Cont.	1.9±1.2	2.4±2.3	0.5±1.9	
Light muscle activity time (%)	Int.	29.1±11.6	33.3±12.8	**4.2±11.5***	**0.022**
	Cont.	25.8±15.4	24.5±13.1	-1.4±12.7	
Mod-to-vig muscle activity time (%)	Int.	11.8±6.7	16.9±9.4	**5.1±10.4****	0.08
	Cont.	10.1±9	12.1±6.6	2.0±9.2	
EMG amplitude (%EMG_{MVC})	Int.	3.3±1.9	5±5.3	**1.6±5.5***	0.07
	Cont.	2.7±4	2.7±1.7	0.0±3.9	
No of bursts per minute	Int.	27±18.9	30.6±23.6	3.6±13.8	0.10
	Cont.	27.5±16.8	23.9±15.2	-3.6±12.1	

* denotes to significance at $P < 0.05$, ** to $P < 0.01$ and *** to $P < 0.001$ * for within group changes.

5.5 Effectiveness of intervention during one year (Study IV)

5.5.1 Participants

A total of 300 individuals (150 from intervention and 150 from control clusters) expressed their interest on the study by returning the recruitment form and were assessed for eligibility (Figure 5). From these, 71 participants from intervention (47%) and 62 from the control (41%) clusters met the inclusion criteria and were measured at baseline.

The age range of the participants was 28-53 years and 71% of them had university education as compared to 35% mean at the recruitment regions. On average, the participants were normal weight and rather active with self-reported activity of around 30 MET hours per week. On the other hand, self-reportedly, they were sitting almost 85% of the work time (Table 9).

The primary and secondary outcomes at baseline are presented in Table 18 and Table 19, respectively. The participants were normolipidemic, normoglycemic and spent about 55% of the whole day in sedentary behaviors while the moderate-to-vigorous activity time was less than four percent based on objective measurement.

Table 18 Primary outcomes of intervention and control group participants at baseline.

	Intervention (N=71)	Control (N=62)
Total		
Sedentary time (%)	56.7±8.6	54.9±8.4
Breaks per sedentary hour	10.3±2.6	10.5±2.8
Light activity time (%)	39.9±8.1	41.5±8.5
Moderate-to-vigorous activity time (%)	3.6±2.1	3.7±1.9
Work time		
Sedentary time (%)	70.7±11.9	70.1±13.6
Breaks per sedentary hour	8.4±4.3	8.2±4.6
Light activity time (%)	26.9±11.6	27.4±13.2
Moderate-to-vigorous activity time (%)	3.5±2.1	3.6±1.6
Leisure time		
Sedentary time (%)	50.7±8.1	47.1±9.1
Breaks per sedentary hour	11.7±2.6	12.7±3.3
Light activity time (%)	45.2±8.0	48.2±8.4
Moderate-to-vigorous activity time (%)	5.3±3.0	5.9±3.6
Weekends		
Sedentary time (%)	49.9±12.3	48.3±12.6
Breaks per sedentary hour	11.8±3.8	11.9±3.7
Light activity time (%)	46.7±12.1	48.6±13.0
Moderate-to-vigorous activity time (%)	4.4±3.3	4.2±2.9

Table 19 Secondary outcomes of intervention and control group participants at baseline.

	Intervention (N=71)	Control (N=62)
Anthropometrics		
Weight (kg)	72.0±15.4	71.8±14.0
BMI (kg/m^2)	24.5±3.5	24.4±4.1
Total fat mass (%)	28.6±7.5	26.9±8.7
Arms fat mass (%)	2.6±0.8	2.5±1.0
Legs fat mass (%)	10.1±3.4	9.4±4.0
Trunk fat mass (%)	15.0±4.6	14.1±4.6
Total lean mass (%)	67.1±7.6	69.1±8.9
Arms lean mass (%)	7.6±1.6	8.0±1.7
Legs lean mass (%)	22.3±2.8	23.0±3.3
Trunk lean mass (%)	32.1±3.5	32.9±4.0
Biomarkers		
SBP (mmHg)	116.6±10.7	117.3±10.4
DBP (mmHg)	73.8±8	74.6±8.4
Total cholesterol (mM)	4.8±0.8	4.8±0.9
HDL cholesterol (mM)	1.8±0.5	1.7±0.4
LDL cholesterol (mM)	2.6±0.9	2.7±0.8
Triglycerides (mM)	1.0±0.6	1.0±1.1
FPG (mM)	5.3±0.5	5.2±0.5
Fasting serum insulin (pM)	40.7±29.1	34.9±19.7
HOMA-IR	1.6±1.3	1.4±0.8
HOMA-%B	75.6±44.9	71.8±41.6
Mean diameter of VLDL (nm)	35.6±1.5	35.4±0.8
Mean diameter of LDL (nm)	23.7±0.1	23.7±0.1
Mean diameter of HDL (nm)	10.0±0.3	10.0±0.2
apoA-1 (g/l)	1.5±0.2	1.6±0.2
apoB (g/l)	0.8±0.2	0.8±0.2
Ratio of apoB to apoA-1	0.5±0.1	0.5±0.1
Diet		
Energy (kcal/d)	2057±494	2146±520
Protein (E%)	18.2±3.4	18.1±3.2
Carbohydrate (E%)	43.3±5.6	44.2±6.6
Fat (E%)	34.1±5.6	33.6±6.4
Saturated fat (E%)	11.9±3.1	11.4±2.8
Monounsaturated fat (E%)	10.8±2.3	10.4±2.9
Polyunsaturated fat (E%)	4.9±1.2	4.9±1.4
Alcohol (g/d)	5.6±9.6	4.7±9.2

Between the baseline and 12 month follow-up, seven participants dropped out from the control group (11%) and nine from the intervention group (13%), from which two (3%) withdrew before the allocated intervention (Figure 5). Between those who adhered to the study and those who dropped out, there were no differences in gender (women 56% / 62%), age (37.9 ± 5.4 years / 38.3 ± 5.9 years), worktime/week (37.2 ± 5.6 h / 36.2 ± 6.0 h), professional status distribution (data not shown), BMI (24.4 ± 3.6 kg/m^2 / 25.4 ± 5.1 kg/m^2) or objectively meas-

ured sedentary (55.3 ± 9.3% / 55.1 ± 9.2%) and moderate-to-vigorous activity time (3.6 ± 2.0% / 3.7 ± 2.0 %).

5.5.2 Data quality

At baseline 7 participants had improper data (corrupted data or too short measurement) and 4 participants did not complete the baseline measurements leaving 89% and 95% of the intervention and control participants, respectively, for the intention-to-treat analysis (Figure 5). A total of 547 measurement points (90% of those included) were analyzed, while 42 (7%) were missing due to drop-outs and 16 (3%) due to improper measurement. On average, a measurement point included 5.5 ± 1.6 valid days. Of the analyzed measurement points 19 (3%) included only two days of data while 423 (77%) included five or more days. The average recording time at baseline was 14.5 ± 1.1 hours/day in both groups without changes over time.

A total of 53 (85% of those analyzed for total accelerometer time) intervention and 58 (98%) control participants were included for worktime, 59 (95%) and 58 (98%) for weekday leisure time and 56 (90%) and 49 (83%) for weekend day analysis, respectively. Valid data was obtained from 85%, 90% and 69% of the worktime, weekday leisure time and weekend day measurements, respectively. The reasons for data exclusion were improper reporting of work time and leisure time making separation impossible, problems with measurement resulting in improper data, or drop-out. The recording times per day were 7.5 ± 1.3 hours at work time (3.7 ± 1.2 days), 8.8 ± 1.8 hours during leisure time (3.8 ± 1.2 days), and 13.4 ± 1.6 hours during weekends (1.6 ± 0.6 days) without significant group x time -interaction.

5.5.3 Behavioral effectiveness

Effectiveness of the intervention on the primary outcomes is presented in Table 20 and Figure 13. The group x time -interactions during leisure time were significant for sedentary time at three ($P < 0.001$), six and 12 months ($P < 0.05$), for breaks/sedentary hour and light activity time at three months ($P < 0.001$) and for MVPA at three and six months ($P < 0.05$). During the first three months sedentary leisure time decreased within the intervention group ($P < 0.05$) and leisure breaks/sedentary hour ($P = 0.05$) and light activity time ($P = 0.06$) tended to increase. In contrast, control group's sedentary leisure time tended to increase at three and 12 months ($P = 0.07$) and leisure MVPA decreased at three months ($P < 0.05$). Adjusting for baseline group-difference in sedentary leisure time had no effect on the results. The sedentary leisure time at three months decreased 5.2% ($P < 0.01$) more in intervention group as compared to control group, which was reallocated to 3.8% of light and 1.5% of MVPA ($P < 0.05$ for both), respectively, in favor of the intervention group. At 12 months, the sedentary leisure time decreased 2.4% ($P = 0.20$) more in intervention group as compared to control group and was reallocated to 2.1% ($P = 0.24$) of light activity time and 0.4% ($P = 0.79$) of MVPA in favor of the intervention group. The

change in leisure breaks/sedentary hour was 1.0 (P = 0.07) and 0.7 (P = 0.27) greater in intervention group as compared to control group at three and 12 months, respectively. Intervention had no effect on sedentary and physical activity time on total measurement time, work time or weekends (Table 20).

After applying a more conservative wear-time criteria accepting only days with ⩾ 720 min wear time and measurements with at least three days (one day with ⩾ 360 min wear time for work and leisure time, one day with ⩾ 720 min wear time for weekends), the main effects for primary outcomes remained unchanged. However, the group x time –effect for total moderate-to-vigorous physical activity at six months turned significant (P = 0.018).

Responders vs. non-responders. Twenty of the intervention group participants (36% of those completing the 12 months measurements) were able to reduce the total sedentary time more than 2% of the measurement time between baseline and end line and were analyzed as responders. The distribution of gender, age, BMI, work time, professional status and number of children were the same between responders and non-responders (data not shown), but the responders were self-reportedly sitting 79.3 ± 14.2% of their work time as compared to non-responders' 87.0 ± 11.2% self-report (P < 0.05). However, there were no differences in objectively measured sedentary and activity variables at any domain at baseline. On average, the responders reduced their sedentary time by 8.5 ± 5.7%. The proportion of responders who reduced work sedentary time was 79% resulting in a group mean decrease of 7.0 ± 8.8% (P < 0.01, P < 0.01 as compared to non-responders), and 90% of responders reduced leisure sedentary time resulting in a group mean decrease of 6.5 ± 7.5% (P < 0.001, P < 0.001 as compared to non-responders). All of the responders reduced weekend sedentary time with a mean of 14.3 ± 8.8% (P < 0.001, P < 0.001 as compared to non-responders). However, when all measurement points were included the responders reduced significantly only total, leisure and weekend sedentary time as compared to non-responders (P < 0.001) without effect at work time.

Table 20 Changes (95% CI) in accelerometer-derived outcomes within and between groups between baseline and three, six and 12 months of the study.

	Out-come	Time (mo)	Mean change (95% CI)		Mean group diff. (95% CI)	P-values	
			Intervention	Control	Intervention-Control	Time	Group x Time
Total (n=122)	Seden-tary (%)	0-3	−1.1 (−3.1, 0.8)	0.2 (−1.9, 2.2)	−1.3 (−4.1, 1.6)	0.92	0.13
		0-6	1.0 (−1.0, 3.1)	−0.5 (−2.6, 1.5)	1.5 (−1.4, 4.4)	0.29	0.79
		0-12	−0.1 (−2.1, 1.9)	0.5 (−1.5, 2.6)	−0.6 (−3.5, 2.2)	0.76	0.39
	Breaks/ Sedh	0-3	0.6 (−0.1, 1.2)	0.6 (−0.1, 1.3)	0.0 (−0.9, 0.9)	0.22	0.41
		0-6	−0.1 (−0.7, 0.6)	0.6 (−0.0, 1.3)	−0.7 (−1.6, 0.2)	0.76	0.71
		0-12	0.2 (−0.5, 0.8)	0.5 (−0.1, 1.2)	−0.4 (−1.3, 0.6)	0.17	0.40
	Light (%)	0-3	1.1 (−0.8, 3.1)	0.5 (−1.4, 2.5)	0.6 (−2.1, 3.4)	0.69	0.16
		0-6	−0.7 (−2.6, 1.3)	0.7 (−1.2, 2.7)	−1.4 (−4.2, 1.4)	0.50	0.78
		0-12	0.2 (−1.8, 2.2)	−0.3 (−2.3, 1.7)	0.5 (−2.2, 3.3)	0.58	0.61
	MVPA (%)	0-3	0.0 (−0.5, 0.5)	**−0.7 (−1.2, −0.2)***	**0.7 (0.0, 1.4)***	0.71	0.45
		0-6	−0.3 (−0.8, 0.2)	−0.2 (−0.8, 0.3)	−0.1 (−0.8, 0.6)	0.32	0.07
		0-12	−0.1 (−0.6, 0.4)	−0.2 (−0.7, 0.3)	0.1 (−0.6, 0.9)	0.77	0.18
Work (n=111)	Seden-tary (%)	0-3	−2.6 (−5.7, 0.6)	−2.1 (−5.2, 1.0)	−0.5 (−4.9, 3.9)	**0.022**	0.67
		0-6	−1.4 (−4.6, 1.8)	−2.0 (−5.1, 1.1)	0.6 (−3.9, 5.0)	0.12	0.74
		0-12	−1.9 (−5.0, 1.3)	−1.6 (−4.7, 1.6)	−0.3 (−4.7, 4.1)	0.30	0.95
	Breaks/ Sedh	0-3	0.8 (−0.2, 1. 8)	**1.1 (0.2, 2.1)***	−0.4 (−1.8, 1.0)	**0.031**	0.73
		0-6	0.2 (−0.8, 1.2)	**1.0 (0.0, 2.0)***	−0.8 (−2.2, 0.6)	0.17	0.97
		0-12	0.5 (−0.6, 1.4)	**1.0 (0.0, 2.0)***	−0.6 (−1.9, 0.8)	0.08	0.86
	Light (%)	0-3	2.6 (−0.4, 5.6)	2.6 (−0.3, 5.6)	0.0 (−4.3, 4.2)	**0.013**	0.75
		0-6	1.6 (−1.5, 4.6)	2.3 (−0.7, 5.2)	−0.7 (−4.9, 3.6)	0.06	0.77
		0-12	2.0 (−1, 5.1)	1.6 (−1.4, 4.6)	0.5 (−3.7, 4.7)	0.16	0.96
	MVPA (%)	0-3	0.0 (−0.5, 0.5)	**−0.6 (−1.1, 0)****	**0.6 (−0.2, 1.3)***	0.96	0.22
		0-6	−0.2 (−0.7, 0.4)	−0.3 (−0.8, 0.3)	0.1 (−0.6, 0.8)	0.12	0.17
		0-12	−0.1 (−0.7, 0.4)	0.0 (−0.5, 0.5)	−0.2 (−0.9, 0.6)	0.23	0.08
Leisure (n=117)	Seden-tary (%)	0-3	**−2.8 (−5.4, −0.3)***	2.4 (−0.2, 5.0)[a]	**−5.2 (−8.9, −1.6)****	0.29	**<0.001**
		0-6	0.4 (−2.1, 3)	0.2 (−2.4, 2.9)	0.2 (−3.4, 3.8)	0.97	**0.022**
		0-12	0.0 (−2.6, 2.5)	2.4 (−0.3, 5.0)[a]	−2.4 (−6.1, 1.2)	0.60	**0.020**
	Breaks/ Sedh	0-3	0.8 (−0.1, 1.7)[a]	−0.1 (−1.0, 0.8)	1.0 (−0.3, 2.2)	0.78	**0.015**
		0-6	0.3 (−0.6, 1.2)	0.5 (−0.4, 1.4)	−0.2 (−1.4, 1.1)	0.48	0.42
		0-12	0.5 (−0.4, 1.4)	−0.2 (−1.1, 0.7)	0.7 (−0.5, 2.0)	0.57	0.26
	Light (%)	0-3	2.4 (0.0, 4.9)[a]	−1.3 (−3.8, 1.1)	**3.8 (0.3, 7.2)***	0.43	**0.001**
		0-6	0.0 (−2.4, 2.5)	0.4 (−2.1, 2.9)	−0.3 (−3.8, 3.2)	0.91	0.10
		0-12	0.4 (−2, 2.9)	−1.7 (−4.2, 0.9)	2.1 (−1.4, 5.6)	0.80	0.10
	MVPA (%)	0-3	0.4 (−0.4, 1.3)	**−1.1 (−1.9, −0.3)***	**1.5 (0.3, 2.7)***	0.42	**0.017**
		0-6	−0.5 (−1.3, 0.3)	−0.6 (−1.5, 0.2)	0.2 (−1, 1.3)	0.94	**0.034**
		0-12	−0.3 (−1.2, 0.5)	−0.7 (−1.6, 0.1)	0.4 (−0.8, 1.6)	0.21	0.16
Weekends (n=105)	Seden-tary (%)	0-3	−1.0 (−4.6, 2.7)	2.5 (−1.4, 6.5)	−3.5 (−8.9, 1.8)	0.81	0.14
		0-6	0.2 (−3.7, 4.1)	−1.0 (−4.9, 2.9)	1.3 (−4.3, 6.8)	0.52	0.38
		0-12	−2.3 (−6.0, 1.4)	0.0 (−4.1, 4.0)	−2.3 (−7.8, 3.3)	0.56	0.41
	Breaks/ Sedh	0-3	0.7 (−0.6, 1.9)	0.04 (−1.3, 1.4)	0.6 (−1.2, 2.4)	0.57	0.11
		0-6	−0.2 (−1.5, 1.1)	0.7 (−0.6, 2.1)	−0.9 (−2.8, 0.9)	0.35	0.42
		0-12	0.3 (−0.9, 1.6)	0.6 (−0.8, 2.0)	−0.3 (−2.1, 1.6)	0.84	0.35
	Light (%)	0-3	−1.0 (−4.6, 2.7)	−2.4 (−6.2, 1.4)	2.9 (−2.3, 8)	0.82	0.09
		0-6	0.2 (−3.7, 4.1)	0.8 (−3, 4.6)	−0.6 (−6.0, 4.7)	0.35	0.52
		0-12	−2.3 (−6, 1.4)	−0.4 (−4.3, 3.6)	2 (−3.3, 7.4)	0.51	0.66
	MVPA (%)	0-3	0.5 (−0.7, 1.8)	−0.2 (−1.5, 1.1)	0.7 (−1.0, 2.5)	0.75	0.41
		0-6	−0.3 (−1.6, 1.0)	0.2 (−1.1, 1.5)	−0.6 (−2.4, 1.3)	0.99	0.88
		0-12	0.7 (−0.5, 2.0)	0.4 (−0.9, 1.8)	0.3 (−1.5, 2.1)	0.89	0.20

P-values indicated as follows: a < 0.10, * < 0.05 and ** < 0.01. Group x Time = Group x time – interaction based on likelihood ratios adjusted for age and self-reported work-time per week at baseline.

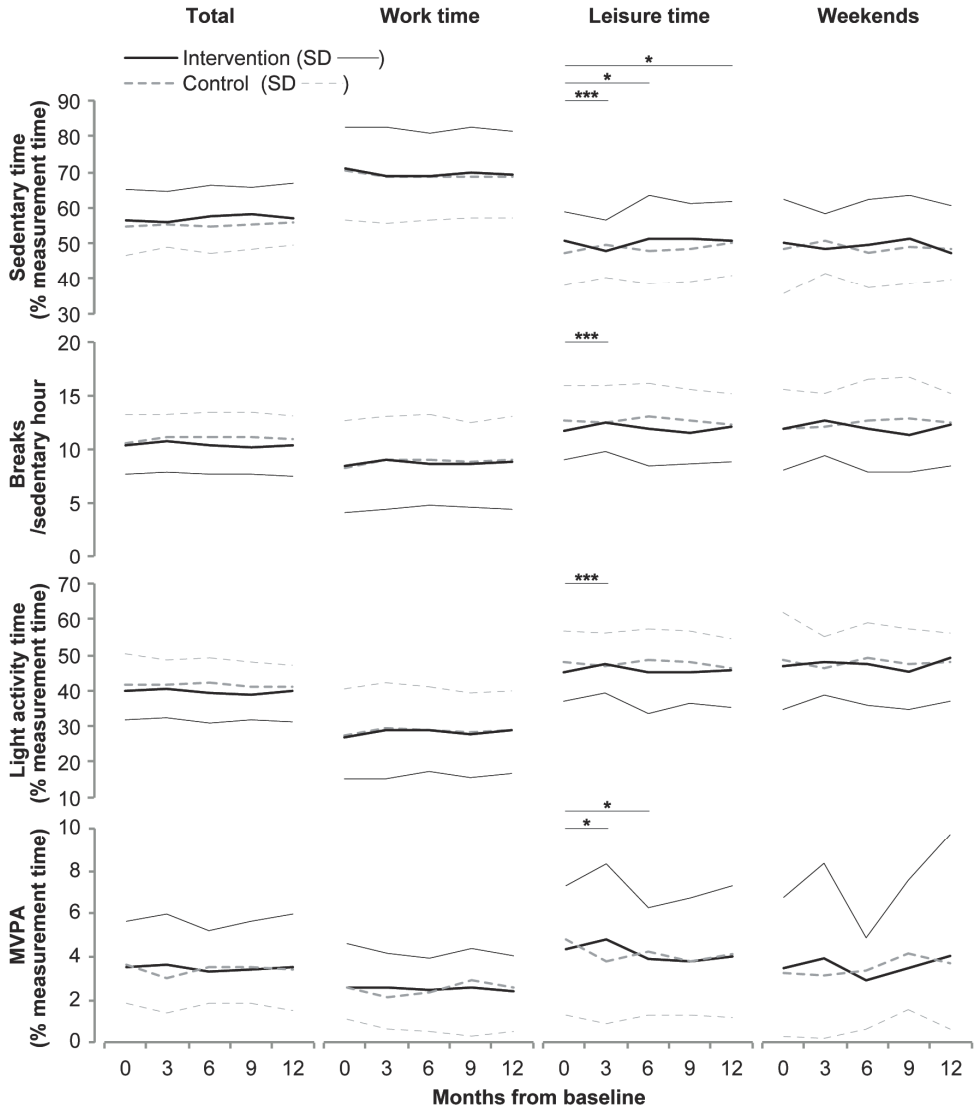

Figure 13 Effectiveness of intervention on primary outcomes in different domains throughout the study. Means and standard deviations (SD) are presented. Significances for likelihood ratios between models with and without group x time -interaction at different time periods are illustrated as follows: * = P < 0.05, *** = P < 0.001. MVPA = moderate-to-vigorous physical activity.

5.5.4 Cardio-metabolic effectiveness

The effectiveness of the intervention on secondary outcomes during the whole year is presented in Table 21 and Table 22. From anthropometrics, the group x time -effect was significant for legs lean mass independent of MVPA and energy intake ($P < 0.05$), and those for arms and legs fat mass and total lean mass were close to significant ($P < 0.068$) (Table 21). Between baseline and 12 months, total fat mass decreased within intervention group as compared to control group ($P < 0.05$). At the same time weight, BMI and arms fat mass increased ($P < 0.01$) and total and legs lean mass decreased ($P < 0.001$) within control group as compared to intervention group ($P < 0.05$, for BMI $P = 0.051$). Between groups, the mean differences in changes of weight (−0.9 kg), total fat mass (−0.7%), arms fat mass (−0.1%), total lean mass (1.0%) and legs lean mass (0.5%) favored intervention group (P for all < 0.05). All other effects, except those on total fat mass ($P = 0.09$) and arms fat mass ($P = 0.13$), remained independent of MVPA and energy intake (Table 21).

From biomarkers, the group x time -effect was significant for the ratio of apoB to apoA-1 (Table 22). Between baseline and 12 months, the apoB to apoA-1 -ratio decreased and apoA-1 increased ($P < 0.05$) within intervention group as compared to control group ($P < 0.05$). Between groups, the mean differences in changes of apoA-1 (0.05 g/l) and ratio of apoB to apoA-1 (−0.04) favored intervention group (P for both < 0.05) and remained independent of MVPA and energy intake. Adjustment for fat mass or lean mass removed the group difference in ApoA-1 ($P < 0.08$), but the apoB/apoA-1 -ratio -results remained unchanged (Table 22). There were no other significant between group differences for the other biomarkers.

Table 21 Changes (95% CI) in anthropometrics and diet within and between groups from baseline to 12-month follow-up.

Outcome	Mean change (95% CI)		Mean difference between groups (95% CI)	P-values			
	Intervention	Control	Intervention-Control	Time	Group x Time	Group x Time + MVPA	Group x Time + MVPA + EI
Anthropometrics							
Weight (kg)	0.27 (−0.37, 0.92)	**1.18 (0.54, 1.83)*** **	**−0.91 (−1.71, −0.11)***	**0.038**	0.14	0.45	0.24
BMI (kg/m²)	0.06 (−0.14, 0.26)	**0.32 (0.11, 0.52)*** **	−0.26 (−0.52, 0.00)[a]	0.14	0.24	0.60	0.31
Total fat mass (%)	**−0.50 (−0.99, 0.00)***	0.17 (−0.34, 0.68)	**−0.65 (−1.30, 0.00)***	0.43	0.28	0.50	0.32
Arms fat mass (%)	−0.01 (−0.07, 0.05)	**0.09 (0.03, 0.15)*** **	**−0.10 (−0.17, −0.02)***	**0.037**	0.06	0.23	0.22
Legs fat mass (%)	**−0.22 (−0.39, −0.06)*** **	−0.04 (−0.21, 0.13)	−0.18 (−0.39, 0.03)	0.17	0.05	0.10	0.16
Trunk fat mass (%)	−0.25 (−0.57, 0.07)	0.11 (−0.23, 0.44)	−0.36 (−0.79, 0.07)	0.63	0.58	0.68	0.48
Total lean mass (%)	0.01 (−0.56, 0.59)	**−1.05 (−1.64, −0.45)*** **	**1.06 (0.30, 1.82)*** **	0.21	0.07	0.12	0.07
Arms lean mass (%)	0.03 (−0.05, 0.12)	−0.01 (−0.10, 0.08)	0.04 (−0.07, 0.15)	0.90	0.91	0.96	0.89
Legs lean mass (%)	−0.07 (−0.29, 0.15)	**−0.54 (−0.77, −0.32)*** **	**0.47 (0.18, 0.77)*** **	**0.005**	**0.021**	**0.043**	**0.021**
Trunk lean mass (%)	0.12 (−0.21, 0.44)	−0.32 (−0.66, 0.02)	0.43 (−0.01, 0.88)	0.96	0.20	0.24	0.17
Diet							
Energy (kcal/d)	−121.2 (−257.7, 15.3)	−171.5 (−315.0, −27.9)	50.2 (−146.6, 247.0)	**0.002**	0.18	0.22	NA
Protein (E%)	0.44 (−0.87, 1.75)	0.49 (−0.89, 1.87)	−0.05 (−1.94, 1.84)	0.15	0.44	0.82	0.68
Carbohydrate (E%)	−1.09 (−3.56, 1.37)	−2.31 (−4.89, 0.28)	1.21 (−2.35, 4.77)	0.24	0.84	0.82	0.84
Fat (E%)	1.71 (−0.7, 4.11)	2.16 (−0.36, 4.67)	−0.45 (−3.92, 3.02)	0.31	0.94	0.94	0.94
Saturated fat (E%)	0.07 (−1.18, 1.32)	1.2 (−0.12, 2.51)	−1.13 (−2.93, 0.68)	0.75	0.28	0.55	0.56
Monounsaturated fat (E%)	0.91 (0.28, 1.55)	0.99 (0.32, 1.65)	−0.08 (−0.99, 0.84)	**<0.001**	0.27	0.31	0.31
Polyunsaturated fat (E%)	1.60 (0.46, 2.75)	1.99 (0.8, 3.19)	−0.39 (−2.05, 1.26)	**<0.001**	0.55	0.48	0.52
Alcohol (g/d)	−2.05 (−4.22, 0.12)	−0.32 (−2.6, 1.95)	−1.73 (−4.86, 1.41)	**<0.001**	0.76	0.63	0.57

P-values indicated as follows: a < 0.10, * < 0.05 and ** < 0.01. Group x Time = Group x time –interaction based on likelihood ratios adjusted for age and self-reported work-time per week at baseline. Group x Time + MVPA = Group x time –interaction based on likelihood ratios after additional adjustment for moderate-to-vigorous physical activity. Group x Time + MVPA + EI = Group x time –interaction based on likelihood ratios after additional adjustment for moderate-to-vigorous physical activity and energy intake.

Table 22 Changes (95% CI) in biomarkers within and between groups from baseline to 12-month follow-up.

Outcome	Mean change (95% CI)		Mean difference between groups (95% CI)	P-values			
	Intervention	Control	Intervention-Control	Time	Group x Time	Group x Time + MVPA	Group x Time + MVPA + EI
Biomarkers							
SBP (mmHg)	1.61 (−0.13, 3.34)	**2.34 (0.54, 4.14)***	−0.73 (−3.18, 1.71)	**0.020**	0.87	0.89	0.97
DBP (mmHg)	**−2.18 (−3.4, −0.96)***	**−1.62 (−2.88, −0.36)*****	−0.56 (−2.26, 1.15)	**<0.001**	0.96	0.84	0.72
Total cholesterol (mM)	−0.11 (−0.25, 0.02)	0.02 (−0.12, 0.16)	−0.13 (−0.32, 0.06)	0.36	0.63	0.15	0.18
HDL cholesterol (mM)	**−0.12 (−0.19, −0.05)*****	**−0.12 (−0.19, −0.05)****	0.00 (−0.1, 0.09)	**<0.001**	0.44	0.37	0.15
LDL cholesterol (mM)	0.00 (−0.13, 0.14)	0.14 (0.00, 0.28)	−0.14 (−0.33, 0.06)	0.26	0.66	0.21	0.18
Triglycerides (mM)	0.07 (−0.02, 0.16)	0.03 (−0.07, 0.12)	0.05 (−0.09, 0.18)	0.59	0.97	0.96	0.75
FPG (mM)	0.03 (−0.09, 0.16)	**0.17 (0.04, 0.3)****	−0.14 (−0.32, 0.04)	**<0.001**	0.10	0.15	0.26
Fasting serum insulin (pM)	0.95 (−4.94, 6.83)	2.31 (−4.5, 9.13)	−1.37 (−10.28, 7.54)	**0.038**	0.46	0.61	0.52
HOMA−IR	0.03 (−0.22, 0.29)	0.15 (−0.15, 0.45)	−0.12 (−0.5, 0.27)	**0.047**	0.25	0.38	0.37
HOMA−%B	3.70 (−9.5, 17)	−4.3 (−19.5, 10.9)	8.10 (−12, 28.1)	**0.008**	0.23	0.27	0.42
Mean diameter of VLDL (nm)	−0.11 (−0.41, 0.19)	0.03 (−0.29, 0.34)	−0.14 (−0.57, 0.29)	0.76	0.85	0.94	0.82
Mean diameter of LDL (nm)	**−0.05 (−0.09, −0.02)****	−0.03 (−0.07, 0.01)	−0.03 (−0.08, 0.03)	**0.008**	0.38	0.65	0.56
Mean diameter of HDL (nm)	0.00 (−0.04, 0.03)	−0.02 (−0.06, 0.02)	0.02 (−0.04, 0.07)	0.73	0.67	0.91	0.98
apoA−1 (g/l)	**0.05 (0.01, 0.09)****	0.00 (−0.04, 0.03)	**0.05 (0.00, 0.11)***	0.24	0.25	0.44	0.33
apoB (g/l)	−0.01 (−0.04, 0.02)	0.02 (−0.01, 0.05)	−0.03 (−0.07, 0.01)	0.79	0.58	0.35	0.41
Ratio of apoB to apoA−1	**−0.02 (−0.04, −0.01)***	0.02 (0, 0.04)	**−0.04 (−0.07, −0.01)****	0.87	**0.038**	**0.025**	**0.024**

P-values indicated as follows: a < 0.10, * < 0.05 and ** < 0.01. Group x Time = Group x time –interaction based on likelihood ratios adjusted for age and self-reported work-time per week at baseline. Group x Time + MVPA = Group x time –interaction based on likelihood ratios after additional adjustment for moderate-to-vigorous physical activity. Group x Time + MVPA + EI = Group x time –interaction based on likelihood ratios after additional adjustment for moderate-to-vigorous physical activity and energy intake.

6 DISCUSSION

One of the driving hypotheses of the sedentary behavior field is that frequent light intensity engagement of anti-gravity muscles, particularly large muscle groups of the lower limbs, helps in short-circuiting the adverse physiological effects of prolonged muscle inactivity thus contributing to healthy metabolism. However, so far the field has lacked information about the muscle inactivity patterns at the low end of physical activity spectrum. Most of the evidence proposing adverse health effects of sedentary time are from observational studies measuring sedentary exposure with questionnaires or accelerometers without knowledge if muscles are inactive or active.

This study aimed to give insights on the field by directly quantifying detailed muscle inactivity and activity patterns during sedentary behavior, normal daily life, as well as following an intervention aimed to decrease sedentary time. The long-term effectiveness of intervention was studied by measuring accelerometer-derived sedentary time, anthropometrics and blood-drawn cardiometabolic biomarkers to report if short-term changes in muscle inactivity patterns translate to long-term behavioral and physiological benefits.

The main findings of this study were that the muscle inactivity patterns of sitting are highly heterogeneous. However, a simple tailored counseling was able to decrease muscle inactivity time across participants without affecting high intensity activities. This suggests that targeting reduced sedentary time changes muscle activity patterns as hypothesized and significant reductions in muscle inactivity can be achieved at very low levels of muscular effort. A lecture, tailored counseling and two follow-up calls during the first six months of the intervention were successful in changing weekday sedentary leisure time beneficially in the intervention group as compared to control group throughout the whole year. This resulted also in some modest positive changes in biomarkers at the end of the year independent of changes in moderate-to-vigorous physical activity and energy intake supporting the causal role of reduced muscle inactivity time on improved metabolic profile. This study is one of the first ones targeting sedentary behaviors in ecologically valid settings with a long follow-up and having strategies applicable to practice.

6.1 Muscle inactivity and activity patterns

Sedentary behavior can be defined as a seated/reclining posture accompanied by low energy expenditure (Sedentary Behaviour Research Network 2012). As physical activity is defined as any bodily movement produced by skeletal muscles that requires energy expenditure (Caspersen et al. 1985), specifically muscle activity is needed to change the sedentary time to physical activity. Because a given posture or level of energy expenditure may coexist with an unknown mixture of volume, intensity and frequency of muscle activity, it is important to measure the exposure of a treatment in this outcome. To illustrate the differences in sitting and standing muscle inactivity and activity patterns across individuals, the EMG of these activities was measured in laboratory conditions. Although the average muscle activity amplitude was threefold higher in standing than sitting, the participants had almost 13 bursts per minute also when seated and some participants had higher mean amplitude during sitting than standing. These results suggest that considerable amount of muscle activity can accumulate also during sedentary behavior. Even though it is common to measure sedentary exposure only by quantifying posture or lack of impacts near the center of body mass (Gibbs et al. 2015) with an assumption that muscles are inactive during sitting and active during upright activities, the direct thigh muscle EMG recordings of this study showed that EMG activity during sitting and standing can be tenfold different between individuals.

During habitual life the muscles were inactive (on average at < 2.0% of EMG_{MVC}) for almost 70% of time despite nearly all of the participants met the current physical activity recommendations. The high muscle inactivity time as measured in this study is in agreement, or even slightly higher as compared to the accelerometer-derived population level sedentary time of 55% to 70% per day (Matthews et al. 2008; Hagströmer et al. 2010; Colley et al. 2011). However, the accelerometer-derived sedentary time accumulates in six minute periods on average (Healy et al. 2008b), but muscle inactivity time is broken up every third second on average. In essence, the definition of "break" may be very different depending on the measure used. The short mean duration of inactivity periods implies that many of the muscle inactivity breaks occurred while participants were sitting. Measuring muscle activity might thus give new insights for sedentary behavior research beyond the impact- or posture-derived sedentary behavior. One could consider if the muscle activity as measured during sedentary behavior should actually be accepted as physical activity (Mansoubi et al. 2015).

The comparisons between sexes revealed that female had shorter mean inactivity period duration during sitting than male. This difference was evident also during their habitual life, where female had shorter mean inactivity period duration, more bursts and lower total muscle inactivity time than the male. This suggests that muscle inactivity patterns measured during sitting may have an influence for the habitual muscle inactivity. The correlations showed that the mean inactivity period duration during sitting was negatively associated with

light muscle activity during habitual life. In contrast, number of bursts during sitting showed a negative association with mean inactivity period duration, and a positive association with light muscle activity time measured during habitual life. Thus, even though sitting is considered very passive as compared to the abundant light activities of daily living, the fidgeting-like activities during sitting contribute significantly to accumulated light muscle activity time during the whole day. In contrast, although the overweight had more muscle inactivity during sitting, during habitual life their muscle inactivity time was similar and they had more bursts and shorter mean inactivity periods than the normal weight. This suggests that the overweight accumulate their higher number of breaks when upright, not during the sitting time, which is a dissimilar pattern to female whose muscle inactivity periods were shorter both during sitting and habitual life.

Other differences between groups were that the overweight had higher mean amplitude during standing than the normal weight, but their mean amplitude during habitual life was similar. In contrast, female had similar mean amplitudes during sitting, standing and walking, but higher mean and mean muscle activity amplitude during habitual life as compared to male. These differences might have interesting implications on how total daily activity volume is accumulated between these groups. Because the overweight had more muscle inactivity during sitting, they might need to accumulate more activity time outside sitting to achieve the similar total muscle inactivity time than the normal weight. In contrast, because their standing mean amplitude was higher, the overweight can accumulate a higher total muscle activity volume during similar light intensity activities as compared to the normal weight. Similarly, the overweight may achieve a similar total activity volume from activities which are of lower absolute intensity, than is required from the normal weight. Thus, part of the similarities between the overweight and normal weight subjects' cumulative habitual muscle inactivity and activity patterns might be explained by their different sitting and standing muscle activity patterns. This is supported by the fact that sitting, standing and walking mean amplitudes were positively associated with the mean amplitude measured during habitual life. In contrast, because female had similar mean amplitudes during sitting, standing and walking, but higher mean and mean muscle activity amplitude during habitual life than male, female simply were more active to achieve their higher habitual mean amplitude as compared to male.

The heterogeneity in muscle inactivity and activity of sitting and standing could be explained by methodological, biological or behavioral factors. It is well acknowledged that higher body mass poses additional load for postural support against gravity during standing (Hue et al. 2007), which in this study was verified by the higher standing mean amplitude in overweight as compared to normal weight. However, the lack of difference between females and males suggests that females might increase their mean amplitude from sitting to standing similarly to males despite their lower body weight. One explanation might be that female have generally higher fat percentage as compared to male,

suggesting that females need to support their higher proportional fat mass with similar EMG activity as compared to males, despite their lower total body weight. Other factors might include differences in activation patterns, coordination and technique, issues related to EMG as a method to measure muscle activity, or behavioral differences like fidgeting, among other factors (Levine et al. 2000; Farina et al. 2004; Enoka, & Duchateau 2015). Previous studies have shown that overweight people are habitually standing less than normal weight people, which could be determined by their different biological propensity towards sedentariness as compared to normal weight people (Levine et al. 2005). This might include the fact that the overweight need to use a higher proportion of their physiological capacity during standing than the normal weight, making their standing more demanding and fatiguing. This study provided evidence that the overweight are also more inactive during sitting as compared to the normal weight. However, provided the high heterogeneity between individuals, the different patterns between sexes and BMI groups in laboratory than daily life could be solely explained by the fact that these studies included partly different subjects. In addition, it is important to note that the present study focused on short-term static standing, which ignores shifting and fidgeting like activities (Duarte, & Zatsiorsky 1999). A longer measurement time might emphasize the differences between the groups in their cumulated muscle activity during standing. It would be beneficial to measure habitual muscle activity concomitantly with inclinometer to report if the muscle activity differences during sitting and standing as measured in laboratory are evident during normal daily life. The practical significance of high inactivity time during sitting and high mean amplitude during standing might have implications on the feasibility of behavior-targeted or the efficacy of biomarker–targeted anti-sedentary interventions.

6.2 Muscle inactivity and cardio-metabolic biomarkers

There were clinically significant differences in HDL cholesterol and triglycerides independent of BMI and muscle's moderate-to-vigorous activity time between participants having high vs. low total muscle inactivity time. Further, muscle light activity time was negatively associated with fasting plasma glucose with same adjustments. These findings are consistent with previous research showing independent associations of total sedentary time and breaks in sedentary time with cardio-metabolic outcomes independent of moderate-to-vigorous physical activity in non-clinical population in cross-sectional (Healy et al. 2007; Healy et al. 2008a; Healy et al. 2008b; Healy et al. 2011b) and longitudinal designs (Helmerhorst et al. 2009; Koster et al. 2012; Wijndaele et al. 2014b; Fishman et al. 2016; Schmid et al. 2016). However, the participants in these previous studies have been on average older and had bigger waist circumference as compared to the present sample suggesting that these associations can be seen already in young, healthy and physically active participants.

Another main difference is the measurement of muscle activity, which reflects the activity periods also during sedentary behavior and quantifies the exposure in relation to individual's physiological capacity, as discussed previously. Even though previous studies have emphasized the importance of breaking up sedentary time, the results of this study revealed no associations between neither long nor mean muscle inactivity periods and biomarkers independent of BMI. Instead, the longest and the mean inactivity period duration were negatively associated with BMI, which is in disagreement to a reported positive association between accelerometer-derived breaks and waist circumference (Healy et al. 2008a). This discrepancy could be explained by the fact that the majority of EMG-derived breaks might actually occur during dynamic physical activities when energy expenditure is considerable elevated also during the brief muscle inactivity periods. To give insights to this issue, in the results of the original publication II filtering the signal with a two-second moving average removed the inactivity periods during physical activity (Pesola et al. 2015). By doing this, the association between mean inactivity periods and BMI disappeared. Instead, the mean inactivity period duration was positively associated with triglycerides, but also negatively with blood pressure index. This might suggest that sustained activity benefits lipid metabolism, but poses a hemodynamic challenge. Longitudinal research is needed to confirm whether the minimal reduction in total or uninterrupted muscle inactivity time yields clinically significant outcomes in long term.

6.3 Effectiveness of the intervention

Because reducing muscle inactivity time has been proposed to be the key physiological stimulus for improved metabolic profile following anti-sedentary interventions, the acute efficacy of the present intervention was quantified by measuring directly muscle EMG activity. To fully elucidate the independent role of reduced muscle inactivity from increased high-intensity muscle activity, it is important to measure the efficacy on the total physical activity spectrum. A onetime lecture and face-to-face tailored counseling aimed at reducing and breaking up sedentary time and increasing non-exercise physical activity time reduced muscle inactivity time 37 minutes, resulted in 4 more bursts per minute, shortened mean muscle inactivity periods 0.8 seconds and long inactivity periods 10.5 minutes. There were no significant intervention effects in other activity variables suggesting that the counseling was able to change the muscle inactivity patterns acutely as hypothesized. The main intervention message of reducing prolonged sedentary time and increasing non-exercise physical activity was thus well transferred to the muscle level and to the variables which were shown to be associated with cardio-metabolic biomarkers in Study II. The intervention was thus well set to study longitudinal independent associations of reduced muscle inactivity time.

The present results emphasize the incidental and highly domain-specific nature of habitual sedentary time. In contrast to the hypothesis, the initial decrease in total sedentary time was not maintained throughout the year. Rather than a sustained decrease, the intervention participants were able to reduce sedentary leisure time in the first months of the study, followed by a modest increase towards midline, and then maintain the level until end of the study. The trend in the control group participants was almost the opposite in the beginning, accompanied with a modest increasing trend in the sedentary leisure time towards the end of the study resulting in a beneficial intervention effect. The intervention effectiveness observed in the first months of this study is in line with previous literature showing that prolonged sitting time can be reduced acutely (Otten et al. 2009; Gardiner et al. 2011; Kozey-Keadle et al. 2012; Raynor et al. 2013; Aadahl et al. 2014). Generally, the short-term reduction has ranged from approximately 30 minutes to 2 hours, which is slightly more than the 15 minutes reduction (adjusted for wear time) in accelerometer-derived sedentary leisure time observed in the first 3 months of this study. The mean difference in change between groups at 3 months was −27 minutes in favor of the intervention group, as compared to −48 minutes reported by a recent meta-analysis (Martin et al. 2015). However, the authors of this meta-analysis reported that the only evidence available from interventions targeting sedentary time as a primary outcome is from studies lasting less than three months (Martin et al. 2015) with few exceptions (Aadahl et al. 2014; Healy et al. 2016), which is a major gap in the literature. The results of this 12 months study suggest that despite the positive acute effect, the intervention methods being successful at short term may not induce long-term positive effects, though they might prevent unfavorable behavior change over time.

Sitting is a predominant activity in multiple domains of daily life. Because the potential to decrease sedentariness through behavioral intervention might be very different between these domains, interventions targeting reduced sedentary time in these domains should be highly context-specific. Although the present intervention was targeted to change both work time and leisure time behaviors, the only changes were seen at sedentary leisure time, for many possible reasons. Generally, the effect size of a given intervention is bigger when multiple domains and contexts are intervened and when social, cultural and environmental aspects are considered (Owen et al. 2011). A typical example is a workplace intervention, in which changes in physical environment and targeting the whole workplace community (Healy et al. 2013), instead of intervening merely an individual (Evans et al. 2012), have been found to be beneficial. The focus on families rather than workplace community clearly favored leisure time changes, emphasizing the context-specificity of this approach. A recent review identified restructuring of the social or physical environment among the most promising behavior change techniques to reduce sedentary time (Gardner et al. 2016). In addition, their analysis distinguished different functions across worksite and non-worksite settings with a notion that worksite sedentary behavior may appear as more routinized as compared to non-worksite sedentary

time, which may explain the lack of effectiveness at work time in the present study. Positive long-term changes at worksite might benefit from environmental restructuring, which would maximize opportunities for light intensity physical activity alongside of the worksite routines and make the active choice more socially acceptable and even appealing. However, although changing the physical environment would increase the efficacy of a given intervention, it is important to develop scalable and low cost interventions for wide-scale, equal and achievable public health impact. For example, targeting increased motivation through information provision have been suggested for pursuit of significant population-level decreases in sedentary behavior (Gardner et al. 2016). It is important to note that information provision, along the other intervention techniques used in this study, affected only leisure time behaviors. Although high sitting time at work exposes people to high total sedentary time (Jans et al. 2007), this study provided hope that beneficial changes in leisure sedentary time can be achieved even without changes in total or worktime sedentary time.

Across the intervened contexts, the degree of volition on sedentary behavior vs. physical activity may vary. For example, an individual may choose to walk for lunch to a distant restaurant, but she needs to sit at the desk while working because of no option for standing workstations. However, at home she may choose jogging over the television. The "responders" at the present study were sitting less at work at baseline, which might illustrate their routines as less sedentary-time dependent enabling a positive behavior change without changes in social or physical environment. However, even the responders were unsuccessful in reducing worksite sedentary time throughout the year. Workplace has been suggested to be the key place to target interventions at, because the majority of total sitting hours are accumulated at work (Parry, & Straker 2013). However, the results of our intervention showed that changes were more effective during leisure time, where the sitting time was readily low. Future interventions should consider the behavioral potential for change beyond merely the measured amount of sedentary time.

Interestingly, although the intervention was unsuccessful at decreasing total sedentary time, some positive changes in cardio-metabolic health markers were seen at the end of the study. Based on epidemiological evidence, the sedentary periods undertaken at different domains are unequally associated with health effects suggesting that the constituents, rather than the simple total sitting time, mediate these associations. Several studies have associated negative health outcomes to leisure sedentary time (Grøntved et al. 2011), but to a lesser extent to worksite sedentary time (Chau et al. 2012). Indeed, the participants were able to decrease leisure sedentary time at the beginning of the study with a sufficient magnitude to produce some cardio-metabolic benefits (Dunstan et al. 2012; Alkhajah et al. 2012; Duvivier et al. 2013; Thorp et al. 2014; Blankenship et al. 2014). One reason for the inequality between domains is that leisure sedentary time might coexist with some confounding unhealthy behaviors, like TV viewing and snacking, which add to the risk (Heinonen et al. 2013). However, there were no differences in change of diet between groups, and most changes

in biomarkers remained independent of energy intake. Another reason might be the different trade-off between activities at work and leisure time. During leisure time, unlike during worktime routines, the sitting might replace more active leisure time behaviors like moderate-to-vigorous exercise for fitness. Recent studies using isotemporal substitution modeling have shown that replacing sitting with moderate-to-vigorous activity than light intensity activity is more beneficial for cardio-metabolic outcomes (Stamatakis et al. 2015). In the present study, some of the decreased sedentary time was reallocated to moderate-to-vigorous activity, which could be seen as a beneficial outcome. However, the initial efficacy study (Study III) showed that the intervention decreased muscle inactivity time in the absence of changes in muscle moderate-to-vigorous activity both during work and leisure time. Additionally, the beneficial changes in body weight, BMI, leg's lean mass and apoB/apoA-1 -ratio remained significant after adjustment for moderate-to-vigorous physical activity suggesting that the beneficial cardio-metabolic changes were not solely due to increased leisure time exercise, for example.

6.4 Physiological mechanisms

In short-term experimental laboratory studies reallocation of sitting to light intensity activity in bouts of different length has been beneficial for glucose metabolism (Dunstan et al. 2012; Peddie et al. 2013) and to some extent on lipid metabolism (Duvivier et al. 2013). The proposed acute mechanisms include improved muscle-activity -mediated glucose transport, decreased post-prandial glycemic load and improved plasma triglyceride catabolism among others (Bey, & Hamilton 2003; Bienso et al. 2012; Dunstan et al. 2012). Importantly, some of these key mechanisms related to substrate utilization and insulin resistance, like GLUT4 transporter expression and translocation (Gibala et al. 2012; Richter, & Hargreaves 2013), lipoprotein lipase activity (Bey, & Hamilton 2003) and post-prandial lipidemia (Peddie et al. 2012), are sensitive to muscle activity volume, intensity and frequency partly independent of cellular energy status. These mechanisms were reflected in cross-sectional associations between muscle inactivity patterns and biomarkers of this study. It may be that lack of muscular contractions throughout the day suppresses systemic lipid transport, lipid transport to muscle, and lipid oxidation in the muscle, resulting in high circulating triglycerides and low HDL cholesterol concentrations. The concentration of fasting triglycerides is affected by the total volume of physical activity energy expenditure of the days preceding blood sampling (Peddie et al. 2012). Low HDL cholesterol concentrations are often observed in combination with hypertriglyceridemia, because triglyceride enriched HDL is rapidly catabolized in a high triglyceride environment (Lamarche et al. 1999). This mechanism is further promoted during muscle inactivity, when the decreased muscle lipoprotein lipase activity suppresses the transport of circulating triglycerides into the muscle cell thus contributing to increased blood triglyceride concentration and low

HDL cholesterol concentration (Bey, & Hamilton 2003). In addition, physical activity is able to independently increase circulating HDL concentrations especially in those having low baseline HDL concentrations (< 0.9 mM), albeit this effect is rather modest (mean response +0.05 mM) (Leon et al. 2000; Bouchard, & Rankinen 2001). Because the subjects of Study II were healthy and physically active and their HDL cholesterol concentrations were on average high (1.8 ± 0.5 mM), muscle inactivity may have an influence on HDL cholesterol concentrations even at high concentrations and at high physical activity levels. However, it is more likely that the HDL -association is driven by triglycerides, because they are more responsive to light intensity activities in short-term interventions (Duvivier et al. 2013).

Low amount of light intensity muscular contractions were associated with high fasting plasma glucose concentrations in Study II. Systemic and local lipid abundance (as evidenced by the association between muscle inactivity and triglycerides), as well as lack of muscular contractions, induce muscle insulin resistance and high circulating concentrations of glucose which might be one possible physiological explanation for the observed association (Bergouignan et al. 2011; Bergouignan et al. 2013). Acute experimental studies have been effective in improving postprandial glucose tolerance by replacing sitting with light-intensity activity suggesting that the observed association might be causal and at hypothesized direction (Dunstan et al. 2012; van Dijk et al. 2013; Peddie et al. 2013; Buckley et al. 2014; Thorp et al. 2014; Bailey, & Locke 2014; Henson et al. 2015). Even though only fasting glucose was measured in Study II, both fasting and postprandial glucose are interconnected and show similar associations with cardiovascular disease risk in non-diabetic glucose range, albeit the postprandial glucose appears to be a stronger surrogate marker of cardiovascular disease risk (Blaak et al. 2012). Even though the subjects of Study II had on average normal fasting glucose concentrations (on average 5.2 mM, whereas the threshold for raised fasting plasma glucose is > 5.6 mM (Alberti et al. 2006)), the relationship between plasma glucose and cardiovascular risk extends below the diabetic threshold (DCCT Research Group 1996). Thus, the association between muscle inactivity and fasting plasma glucose might be clinically relevant even in the healthy subjects of Study II. However, postprandial measures of glucose tolerance might be more closely related to peripheral insulin sensitivity and thus physical inactivity, whereas peripheral insulin sensitivity does not markedly influence glucose concentrations in fasted state (Færch et al. 2009). Unfortunately we did not have insulin samples available from all subjects, so the association of muscle inactivity to insulin sensitivity remains unknown. In some studies light intensity physical activity has affected only insulin sensitivity without effects on glucose tolerance (Duvivier et al. 2013; Aadahl et al. 2014) suggesting that the associations of muscle inactivity with glucose tolerance or insulin sensitivity may not be straightforward. Circulating triglycerides and cathecholamines inhibit insulin signaling, whereas insulin inhibits fatty acid oxidation and uptake by muscle. Circulating triglycerides and catecholamines, adipose tissue fatty acid mobilization, hepatic insulin resistance, hepatic glucose

production, muscle insulin resistance, muscle glycogen and lipid stores, the prevailing energy balance among other factors, have independent, but interconnected and partly opposing effects on whole body insulin sensitivity. For example, even if an increased muscle activity would improve muscle insulin sensitivity, a simultaneous availability of lipids in bloodstream might increase the use of fats in energy production, which inhibits insulin signaling and may thus appear as decreased whole body insulin sensitivity. Whether the metabolic state resulting from a given intervention relies more on carbohydrate or lipid oxidation, the amount of cardio-metabolic risk markers as measured from bloodstream might differ. This reliance does not depend only from the intensity of a given activity, but also from the availability of carbohydrates or lipids, which can be either endogenous or exogenous depending on the prevailing metabolic state. All of these factors remain unknown in our cross-sectional observational study measuring only fasting samples, lacking insulin, and unadjusting for energy intake.

The aforementioned acute mechanisms were not reflected in longitudinal outcomes of the intervention. Instead, at 12 months the intervention was successful in slightly decreasing fat mass as hypothesized, preventing an increase in weight and a decline in lean mass, and improving apoA-1 concentration and apoB/apoA-1 ratio. The changes in weight, lean mass and apoA-1 and apoB/apoA-1 -ratio were independent of MVPA and energy intake. ApoA-1 accounts for the majority of protein in HDL particles and is responsible for the gathering of excess cholesterol into HDL particle from peripheral cells and induces anti-inflammatory and antioxidant effects, with apoB inducing atherogenic mechanisms in LDL subclasses. As such, apoB/apoA-1 -ratio appears to be a better marker for cardiovascular diseases than traditional lipids or lipid ratios (Walldius, & Jungner 2006). In a study by Duvivier et al. (2013), light intensity physical activity that reduced total sitting time reduced triglycerides and insulin response, but also apoB concentrations (Duvivier et al. 2013). Even though the apoB/apoA-1 -ratio was not reported, the result supports our notion that also long-term changes in sedentary time may influence apolipoproteins. In a six-month intervention of Aadahl et al. (2014), increased standing time resulted in decreased fasting insulin and also decreased waist circumference (Aadahl et al. 2014). Thus, also in their study spanning over several months, the several biomarkers being changed at short-term studies, like triglycerides and glucose, remained unchanged even though a positive change in adiposity was evident. It is possible that the slightly increased energy expenditure over several months resulted in the anthropometric change, but the minor increase in physical activity (0.21 hours/day) was not enough to affect other fasting cardio-metabolic markers than insulin – the same may apply for apolipoproteins in the present study.

In the present intervention both groups self-reportedly decreased their energy intake between baseline and 12 months, but total weight increased significantly within the control group without change in intervention group. Total fat mass and legs fat mass decreased only in intervention group, whereas total lean

mass and leg's lean mass decreased and arms fat mass increased within control group. This is obviously possible through their differently changed activity profiles. Intervention group decreased sedentary time and increased light activity time in the first months of the study, but the control group became more sedentary throughout the year. Because of their supposedly decreased total energy expenditure and the increased weight, the control group participants were probably in positive energy balance throughout the year despite reporting decreased energy intake between baseline and end line. In contrast, the preferably changed activity profile in the intervention group resulted in lost fat mass and maintained lean mass. It is possible that the adverse trend in control group's sedentary time affected the flow of dietary nutrients to adipose stores instead of being oxidized in muscles, and a decrease in contractile activity resulted in lost lean mass of muscles. Instead, in intervention group the flow of nutrients was more directed to muscles, where they were oxidized and used for muscle mass maintenance. Although the changes were small, this finding is novel and important provided that decreased physical activity level across the adulthood has been associated with sarcopenic ageing (Evans 2010). This study showed that already small changes in daily physical activities at low intensities, or even a maintained sedentary time, can prevent an increase in weight and a decrease in lean mass during one year.

The findings of this study are supported by experimental evidence. Reduced daily stepping results in a quick decline of lean mass due to decreased muscle protein synthesis (Breen et al. 2013). The clearest changes were seen in leg's lean mass supporting the possibility that the increase in control group's sedentary leisure time is accompanied with reduced leg's muscle loading resulting in loss of lean mass in these unloaded muscles. In an experimental setting, only two weeks reduction of daily ambulatory activities results in a decline of legs' lean mass without changes in upper body lean mass (Krogh-Madsen et al. 2010) providing some support for the observation of this study. It is important to note that usually weight gain causes an increase in lean mass, but a decrease in weight is more strongly associated with lost lean mass at least at old age (Newman et al. 2005). Because the changes in lean mass remained independent of energy intake, the supposed positive energy balance of control group was not enough to counteract the decline in their lean mass, but the lack of muscular contraction lead to the adverse changes. Instead, the intervention group participants were able to maintain their lean mass despite losing some fat mass while maintaining their weight.

An unexpected finding was that the cardio-metabolic benefits were evident during the one year follow-up even though the intervention group's sedentary time returned to the baseline level at the end of the study. Instead, that of the controls tended to increase towards the end of the study. Thus, it appears that even a slight initial decrease of sedentary time accompanied with a long-term maintained level, as witnessed in the intervention group, provides cardio-metabolic benefits as compared to a slight increase in sedentary time, as seen in the controls. This finding is supported by the intervention by Krogh-Madsen et

al. (2010) where a two-week increase in sedentary time had rapid and significant effects on insulin sensitivity, maximal oxygen consumption and legs' lean mass (Krogh-Madsen et al. 2010). In addition, compositional data-analyses have shown that an increase in sedentary time is more deleteriously associated with cardio-metabolic health markers than a decrease in sedentary time of similar magnitude brings benefits (Chastin et al. 2015b). One reason for this asymmetric effect might be that increasing sedentary time may replace a proportionally sizeable amount of other activities even though the proportional increase in sedentary time was small. Instead, an absolutely similar decrease in sedentary time is proportionally of small magnitude as compared to the high daily total sedentary time. Therefore, it seems that impairing health with sedentary lifestyle is easier than gaining benefits from increasing activity. Bed-rest studies have shown that reversing the deleterious effects of complete muscle inactivity is hard or even impossible with subsequently increased physical activity (Alibegovic et al. 2010), but preventing these adverse changes is very easy (Sun et al. 2004; Krogh-Madsen et al. 2010). Thus, part of the effectiveness of the present intervention might be attributable to the deleterious effects seen in control group, which resulted from their increased leisure sedentary time. Future interventions should consider whether actually only maintenance of the current level of sedentary behavior by preventing an unfavorable change could be an achievable, feasible and ultimately effective goal. Taken together, beneficial changes in sedentary time patterns might induce some cardio and muscle mass -protective long-term outcomes even in the absence of, and through different mechanisms than acute changes, independent of changes in MVPA and energy intake and even without actually decreasing sedentary time if that of controls increases.

6.5 Methodological considerations and limitations

Unlike accelerometers, inclinometers, pedometers or heart rate monitors, EMG measures directly the activity of muscle by placement of surface electrodes. Although the use of EMG is the explicit strength of this study, several factors need to be considered when measuring EMG activity. To minimize the effects of inter-individual differences in subcutaneous tissue and muscle properties on the signal quality, the results are typically presented as a fraction of the maximal EMG measured during isometric maximal voluntary contractions (Burden 2010). Thus, EMG normalized to MVC represents an effort relative to a muscle group's force production capacity. In this study, the group comparisons were adjusted to maximal knee extension strength to yield results which are independent of differences in muscle strength, which is relevant for sit-stand interventions. EMG shorts measure only thigh muscle region, although many other muscle groups are activated during standing, including important antigravity muscles soleus and erector spinae (Panzer et al. 1995). However, their activity is of similar magnitude during standing than that of vasti muscles (Panzer et al.

1995), suggesting that the results of this study are representative of different activity patterns between individuals. In addition, EMG shorts were found to reflect total body energy expenditure throughout different intensities of a versatile treadmill activity protocol (Tikkanen et al. 2014). The estimate was superior to accelerometer-derived energy expenditure estimate at low loads, if individual calibrations were performed as in the present study (Tikkanen et al. 2014). This suggests that measurement of thigh region muscle activity gives a valid gross estimate of total body physical activity. Future studies should perform repeated measurements for sitting and standing and include other relevant muscle groups to ensure the consistency of behavioral differences in EMG activity patterns, and combine them to cardio-metabolic and behavioral measures to study their clinical significance. Measuring standing for a longer period of time could reveal individual fidgeting activities and incorporate the effects of fatigue, which could reveal more inter-individual differences in standing EMG activity.

In addition to different electrodes, muscles and activities studied, the muscle inactivity and burst results are highly sensitive to the inactivity threshold chosen (Klein et al. 2010). Some of the previous studies measuring habitual EMG activity have used an inactivity threshold fixed into 2% of EMG_{MVC} (Harwood et al. 2008; Harwood et al. 2011). However, the present study showed that the mean amplitude during standing is only 1.6% of EMG_{MVC}. In practice, an inactivity threshold of 2% of EMG_{MVC} would classify 43% of participants inactive during standing (Table 5). Using a functional (%$EMG_{standing}$) instead of fixed (%EMG_{MVC}) inactivity thresholds is beneficial based on the fact that it most effectively classifies participants active during standing and is justified because standing is defined as physical activity (Sedentary Behaviour Research Network 2012). However, the different inactivity threshold between overweight and normal weight could have an influence on the comparisons. To provide a robust comparison, different inactivity thresholds were tested in the original publication of Study I (Pesola et al. 2016). Three out of four threshold conditions tested showed consisted results with the chosen threshold at 90% of $EMG_{standing}$. Further, the individual inactivity threshold was adjusted for in every comparison suggesting its limited influence on the observed differences.

The use of the present EMG method enabled measurement of only one day in a row because of memory limitations and a relatively challenging setup. This resulted in an average of 1.6 valid days (range = 1–3 valid days) per participant in Study II and two days per participants in Study III (one day before and one after intervention), whereas 3–5 days have been suggested to give a reliable estimate of habitual physical activity measured by accelerometers (Trost et al. 2005). To counteract this limitation, the participants were asked to select a typical workday to be measured. Self-reported abnormal days were not included. It is suggested that the activity of people having sedentary occupations (82% of the sample) is highly consistent across days (Baranowski et al. 2008). In addition, because in the Study II participants having more than one valid day the total muscle inactivity time did not differ significantly between the days ($P = 0.27$) and was highly correlated (Pearson $r = 0.78$, $P < 0.001$, reported in (Pesola et al.

2015)), we believe that the present data represent a typical sedentary time and habitual physical activity behavior measured directly from the main locomotor muscles. In Study III, having a control group and selecting only self-reportedly typical workdays in the analysis, the effect of between-day variability on the results was minimized. In addition, the moderate to high between-day reliability of standing EMG activity and EMG/force -ratio as presented in Table 7 ensured that EMG shorts can be used to estimate intervention efficacy. On the other hand, many participants were excluded on the basis of the criterion "self-reportedly typical workdays". These ''atypical'' days included, for example, organized exercise evenings at workplace, giving visitors a grand tour of the workplace, or staying at home because kids were sick. Because of device availability and study schedule, we were not able to replicate these measurements, resulting in reduced sample and limited power in some variables. Previous long-term EMG recordings of habitual physical activity have used measurements of similar or even shorter length compared to those used in the present study (Nordander et al. 2000; Kern et al. 2001; Klein et al. 2010). Even though this approach would be suitable to measure muscle activity over some days, we were unable to follow the change in muscle inactivity patterns during the one year follow up and consequently could not estimate their effect on biomarker changes. Longitudinal EMG recordings coupled with cardio-metabolic outcomes provide an interesting research setup for future studies aiming to elucidate causal associations between muscle inactivity and activity patterns and health.

In Study III, the control group showed a decrease in the longest inactivity periods during commuting compared with that in the intervention group. This may be explained by their more active commuting habits at baseline in combination with participation in a study entitled ''Daily Activity'' that included an informed consent, which potentially provided a cognitive intervention to the participants. On the other hand, there were no differences in the change in total muscle inactivity or activity parameters during commuting between the groups.

Although widely utilized, a clear drawback of the long-term intervention (Study IV) was the use of waist-worn accelerometer to assess the primary outcomes. The primary results thus illustrate changes between non-movement and movement, but bear no information about postures like standing, although standing increases muscle activity and is beneficial for health (Ploeg et al. 2014). Another limitation was that the a-priori planned sample size was not reached (Finni et al. 2011). The reason to stop recruitment before reaching the planned sample size related to the fact that we increased the number of measurement time points following suggestion from the funding agency. This caused logistic and feasibility problems in the execution of the measurements with the given framework of time and funding. However, the significant findings in some of the primary outcomes suggest that the sample was big enough to test the primary hypothesis, and the frequent measurement interval improved the assessment of changes throughout the year. The strengths of the long-term intervention were objective assessment of primary outcomes in several time points, sep-

aration of different domains in intervention message and analysis, assessment of dietary outcomes, long follow-up, robust statistical methods and no-treatment control group. Thus, this intervention was well set up to study the independent long-term effects of reducing sedentary time and the results can be compared for studies using the similar measure of exposure.

Although the observed changes may have a minor clinical significance, it is important to note that the participants in this study were healthy, normal weight, relatively young and physically active, whereas previous sedentary-time -targeted studies have focused on at-risk participant groups (Aadahl et al. 2014). Taken together, the present results provide a conservative estimate of the long-term effectiveness of sedentary-time targeted intervention, which could be improved by assessing posture and including high risk participant groups.

6.6 Practical implications and future directions

The field of sedentary behavior research has lacked the fundamental information on the muscle activity levels of a given individual at the low end of physical activity spectrum which may have prevented us from understanding the mechanisms of how the different aspects of physical activity might mitigate the health hazards of sedentary time. Thus, the direct muscle activity recordings of this study provide a promising launchpad for future studies and implications aiming to elucidate how to prevent the health hazards of prolonged muscle inactivity. Muscle activity recordings showed that some individuals might be more active during sitting than others are during their habitual life. Therefore, beyond measuring only postures or impacts near the center of body mass, future studies should assess how the observed heterogeneity in muscle activity might mediate the associations of time spent at different postures and health outcomes. Moreover, interventions should incorporate the measures of muscle activity to elaborate their quantification of exposure beyond only the time spent sitting or standing, for example. It would be interesting to test if experimentally changing specific muscle activity patterns, like increasing number of muscle activity bursts, would induce metabolic benefits even if it happened while sitting. This might be relevant for occupations were prolonged sitting is unavoidable, like in transportation. In addition, not all people can activate their lower body muscle groups. It would be of high importance to study if activating upper body muscles might improve the metabolic profile of paralyzed people. Even though the standing posture itself does not increase energy expenditure without muscle activity (Chang et al. 2005), the standing posture might activate sympathetic nervous system activity to adapt the vascular system for upright posture (Supiano et al. 1990). Sympathetic activity increases catabolism e.g. through epinephrine secretion, which increases lipolysis, availability of blood lipids and thus fat use as a substrate (Coppack et al. 1994; Snitker et al. 1998), which can be also achieved by increasing muscle activity (Bergouignan et al. 2011). Thus, the effects of changed posture from changed muscle activity should

be separated experimentally. This would also help in defining which aspects of physical activity are effective replacements for sitting. A hypothesis might be put forward that either a low muscle activity during sitting, or a high muscle activity during standing, increase the potential to gain cardio-metabolic benefits from replacing sitting with standing. Interventions should also test if increasing muscle activity during sitting is possible, feasible and beneficial. Several studies have tested efficacy of novel office equipment in increasing energy expenditure while seated (McAlpine et al. 2007; Beers et al. 2008; Ellegast et al. 2012; Grooten et al. 2013), but their practical long-term usability and clinical significant remain unclear.

Another important extension of the future sedentary-time targeted studies should be the incorporation of activities performed throughout the 24 hour time, including sleep. This is important because wear time and sleep contribute to the total sedentary time measured, and sleep is an important component of healthy lifestyle (Tremblay et al. 2007). Preferably, the studies should measure different postures, intensities of physical activity, as well as their muscle activity, to elucidate which constituents of the total physical activity and sedentary patterns are of importance. The EMG measurements should be combined with other sensors, like inclinometers, to measure muscle activity during habitual postures, like sitting and standing. It might be that the muscle activity of sitting might vary in different occasions, such that watching television is more passive than sitting at the office, which might explain their different associations with health outcomes. Determining the mechanisms through which sedentary behavior influences health will help in determining which features of this total palette should be prioritized in measurement. It would be helpful to develop a taxonomy which would include all different aspects of sedentary and physical activity behavior and thus streamline the reporting and synthetize the evidence (Garber et al. 2011; Thompson, & Batterham 2013). It is also important to consider not only these classes themselves, but also the exchange between these classes following a given intervention. Perhaps strikingly, it has been shown that replacing sedentary time with sleep might provide cardio-metabolic benefits even though sleep is the most passive behavior of the day (Buman et al. 2014). In addition to the total time, the association of breaks in sedentary time with cardio-metabolic outcomes has gained supporting evidence from observational, but also from experimental studies. However, the fundamental definition of a "break" which brings the greatest cardio-metabolic benefits is still unknown (Kim et al. 2015). The muscle activity results of this study do not make defining a "break" any easier. Moreover, because breaking up sedentary time naturally decreases total sedentary time, it still remains unclear how breaks contribute to metabolic health independent of changes in total sedentary time. Some preliminary studies have elucidated into this issue by testing the effects of different duration/intensity of breaks. For example, total activity volume was shown to be important for post-prandial effects, but long-term glycemic variability was decreased more effectively with frequent long breaks as compared to single bout of exercise with similar energy expenditure (Blankenship et al. 2014).

Instead, other studies have shown that breaks themselves, not their energy expenditure, are important also for postprandial effects (Dunstan et al. 2012; Duvivier et al. 2013). In this respect it should be also remembered that prolonged standing may induce also adverse effects (Gregory, & Callaghan 2008; Nelson-Wong et al. 2008; Nelson-Wong et al. 2010; Garcia et al. 2015). More research is needed to suggest an implementable activity pattern with the greatest benefits but minimal harm.

Even though the inter-individual variability of muscle activity was high, the acute intervention of Study III showed that significant reductions in muscle activity can be achieved at very low levels of muscular effort. The average muscle loading remained below 3% of muscle maximal voluntary contractile capacity suggesting that the execution of this change is safe and feasible across numerous settings. Moreover, because the long-term intervention showed that already maintaining the sedentary time may be enough for cardio-metabolic benefits, the beneficial change required might be very small and can be executed with minimal effort. Because the effects remained independent of moderate-to-vigorous physical activity, these minimal changes can provide additional benefits for the recommended amounts of physical exercise. These results give important support for the sedentary behavior recommendations, which have been lately published in several countries (Tremblay et al. 2011; Department of Health 2014; Ministry of Social Affairs and Health Finland 2015; Buckley et al. 2015). The proposed message "sit less, stand up, move more" is thus preliminary well grounded. The next step would be to evaluate how long sitting is "too much" and what combinations of sedentary time and physical activity would bring comparable benefits. It is possible that several different patterns of daily physical activity behavior could provide comparable benefits and thus could be achieved by people having different preferences and possibilities for physical activity during their everyday lives. As with moderate-to-vigorous physical activity, the proposed physiological mechanisms only take effect if people are able to reduce, break up or maintain their daily sedentary time. The science of sedentary behavior provides an appealing alternative to physical exercise at high intensities, which has remained out of reach for the most of population. Playing with kids, walking in a park, gardening, or any everyday light intensity activity which activates muscles, could provide a first step to physically active lifestyle which preferably opens the path towards more intense activities with additional benefits. It should be always kept in mind that other reasons than the acquired health benefits may be the actual reason why people will start physically active lifestyle. It would be inspiring to advice people that standing up from the chair is the first step towards physically active lifestyle, and already these small activities can bring health benefits, but also joy, well-being and inspiration for every day.

Provided that the definition of physical activity includes muscle activity as its central component, it is logical to propose that also lack of physical activity, i.e. sedentary behavior, should include muscle inactivity as its defining component. I hope that the next update of the sedentary behavior definition would

include a notion of muscle inactivity for example as follows: “sedentary time is defined as any waking behavior accompanied with a seated/reclined posture when the major locomotor muscles are inactive resulting in low energy expenditure”.

7 MAIN FINDINGS AND CONCLUSIONS

Replacing sitting by standing has been hypothesized to reduce the health risks of sitting with an assumption that muscles are passive during sitting and active during standing. However, the main findings of this study were that the muscle inactivity patterns of sitting and standing are highly heterogeneous and the daily muscle inactivity is adversely associated with cardio-metabolic outcomes. Despite this heterogeneity, a simple tailored counseling was able to decrease muscle inactivity time across participants without affecting high intensity activities. This suggests that targeting reduced sedentary time changes muscle activity patterns as hypothesized and significant reductions in muscle inactivity can be achieved at very low levels of muscular effort. A family-based tailored counseling was successful in changing weekday sedentary leisure time beneficially in the intervention group as compared to control group throughout the whole year. This resulted also in some modest positive changes in biomarkers at the end of the year independent of changes in moderate-to-vigorous physical activity and energy intake supporting the causal role of reduced muscle inactivity time on improved cardio-metabolic and anthropometric profile. This study is one of the first ones targeting sedentary behaviors in ecologically valid settings with a long follow-up and having strategies applicable to practice. The main findings and conclusions of this thesis are summarized as follows:

1) EMG activities of sitting, standing and habitual life are highly heterogeneous. While the average muscle activity amplitude was threefold higher in standing (1.6% of EMG_{MVC}) than sitting (0.5% of EMG_{MVC}), the inter-individual differences were tenfold. Overweight were more inactive during sitting, but had higher EMG amplitude when standing than normal weight. Despite nearly all of participants self-reportedly met the current aerobic physical activity recommendations, their muscles were inactive for almost 70% of the recording time during their habitual life.

2) To the best of our knowledge, this was the first study examining the association between cardio-metabolic biomarkers with directly measured muscle inactivity. Between participants having high vs. low total muscle inactivity time, there were clinically significant differences in HDL cholesterol and triglycerides independent of BMI and muscle's moderate-to-vigorous activity time. Further, muscle light activity time was negatively associated with fasting plasma glucose with same adjustments. Even physically active individuals may benefit from light intensity activities which reduce the ubiquitous muscle inactivity time.

3) A onetime lecture and face-to-face tailored counseling aimed at reducing and breaking up sedentary time and increasing non-exercise physical activity time reduced muscle inactivity time 37 minutes and increased 4 bursts per minute without changes in high intensity muscle activities suggesting that the main intervention message was well transferred to the muscle level. Provided that the average EMG amplitude remained at 3.1% of $EMG_{MVC,}$ significant reductions in muscle inactivity can be achieved at very low levels of muscular effort.

4) This family-based cluster randomized controlled trial changed weekday leisure sedentary time beneficially in intervention group as compared to controls without affecting total, weekend or work time sedentary time. The behavioral methods induced a desired decrease in weekday leisure sedentary time at short term (-27 minutes in favor of intervention group at three months) and prevented unfavorable behavior change trend during one year (+13 minutes within control group, no change within intervention group), which resulted in improved apoA-1, apoB/apoA-1 balance, preserved total and legs muscle mass and prevented an increase in weight independent of changes in moderate-to-vigorous physical activity and energy intake. At long term, a change in only sedentary leisure time might induce positive health outcomes independent of change in total sedentary time, moderate-to-vigorous physical activity or energy intake, and can be achieved by simple behavioral counseling targeting families. Reducing sedentary time produces causal health benefits and may bear public health potential.

YHTEENVETO (FINNISH SUMMARY)

Lihasten inaktiivisuuden ja sedentaariajan vähentäminen tuo kardiometabolisia hyötyjä: perhelähtöisen klusterisatunnaistetun intervention vaikuttavuus vuoden aikana.

Viime vuosina lisääntynyt epidemiologinen näyttö on osoittanut liiallisen istumisen olevan terveysriski, vaikka henkilö täyttäisi nykyiset liikuntasuositukset. Keskeinen istumisen haitallisuutta selittävä konsepti liittyy lihasten passiivisuuteen. Pienenkin istumisen passiivisuutta katkovan lihasaktiivisuuden on ehdotettu ennaltaehkäisevän terveyshaittoja, jota pitkäaikainen passiivisuus aiheuttaa. Tähän asti istumisen haitallisuutta on kuitenkin tutkittu epäsuorilla menetelmillä, kuten mittaamalla liikkeen vähäisyyttä kiihtyvyysantureilla tai kysymällä television katsomiseen käytettyä aikaa. Lisäksi suurin osa istumisen terveyshaittoja tukevasta tiedosta on peräisin seurantatutkimuksista tai lyhytaikaisista laboratoriointerventioista, jotka eivät kerro istumisen vähentämisen kausaalisista terveyshyödyistä pitkällä aikavälillä. Vaikka sedentaaritutkimuksen keskeinen hypoteesi liittyy lihasten passiivisuuteen, tätä yhteyttä ei ole osoitettu mittaamalla suoraan lihasten passiivisuutta. Tämän tutkimuksen tarkoituksena oli tarjota uutta tietoa istumisen, seisomisen sekä normaalin elämän aikaisesta lihasten passiivisuudesta käyttämällä uutta puettavaa EMG-teknologiaa. EMG-aktiivisuuden muutoksia mitattiin istumisen vähentämiseen tähtäävän perhelähtöisen neuvonnan seurauksena selvittääksemme miten istumisen vähentäminen vaikuttaa lihasten passiivisuuteen. Neuvonnan pitkäaikaisvaikuttavuutta tutkittiin kiihtyvyysanturimenetelmällä sekä mittaamalla veriarvojen sekä kehon koostumuksen muutosta vuoden aikana. Tavoitteenamme oli selvittää onko sedentaariajan vähentäminen mahdollista vuoden aikana ja onko mahdollisilla muutoksilla vaikutusta terveyteen pitkällä aikavälillä.

Tämä tutkimus koostui neljästä osajulkaisusta, joihin data kerättiin pääasiassa kaksi vuotta kestäneestä perheiden sedentaariajan vähentämiseen tähtäävästä InPACT -projektista (ISRCTN28668090) (Finni et al. 2011). Tähän kaksihaaraiseen klusteri-satunnaistettuun kontrolloituun interventiotutkimukseen valittiin Jyväskylästä seitsemän kaupunginosaparia, jotka vastasivat toisiaan sosioekonomisen taustan ja ympäristön liikuntamahdollisuuksien perusteella, ja arvottiin koe- ja kontrolliryhmiin. Rekrytointi tehtiin näiden alueiden päiväkotien ja koulujen kautta. Yhteensä 133 aikuista istumatyöntekijää (BMI < 30 kg/m^2), joilla oli 3-9 vuotiaita lapsia, osallistui alkumittauksiin. Alkumittausten jälkeinen interventio koostui puolen tunnin asiantuntijaluennosta, jossa kerrottiin istumisen haitoista ja arkiliikunnan hyödyistä. Luentoa seurasi kasvokkain suoritettu perhelähtöinen neuvonta, jossa osallistujat asettivat itselleen ja perheelleen tavoitteita istumisen vähentämiseksi ja tauottamiseksi työ- ja vapaa-aikana. Suosituimmat tavoitteet sisälsivät yksinkertaisia, pieniä päivittäisiä valintoja. Työaikana haluttiin juoda kahvit seisten, kävellä portaita enemmän ja käydä työkaverin luona setvimässä työasiat kasvotusten chatin käyttämisen

sijasta. Vapaa-ajan tavoitteisiin sisältyi perheen yhteisiä juttuja. Kauppamatkoja alettiin taittaa pyörällä, lapset haettiin päiväkodista kävellen ja järjestivätpä jotkut perheet jopa yhteisiä tanssi-iltoja television katsomisen sijasta. Tärkeintä neuvonnassa oli, että koehenkilöt asettivat tavoitteiksi asioita, jotka he kokivat itselleen sopiviksi ja motivoiviksi. Lisäksi interventioon kuului kaksi puhelua ensimmäisen puolen vuoden aikana, jolloin keskusteltiin tavoitteiden saavuttamisesta ja mahdollisista muutoksista.

Tutkimuksen alussa koehenkilöiden lihasten passiivisuutta ja aktiivisuutta mitattiin istuessa, seistessä, sekä yhden normaalin päivän aikana ennen ja jälkeen neuvonnan. Sedentaariaikaa (<100 counts/min) sekä keski-kovatehoista aktiivisuutta mitattiin kiihtyvyysanturiteknologialla seitsemän päivän ajan viisi kertaa vuoden aikana kolmen kuukauden välein. Kardio-metaboliset muuttujat (perinteiset biomarkkerit sekä metabolomiikka) mitattiin vastaavissa aikapisteissä ja antropometriset muuttujat (paino, rasvaprosentti, lihasmassaprosentti) mitattiin kolme kertaa vuoden aikana kuuden kuukauden välein. Kolmen arkipäivän ja yhden viikonloppupäivän ruokavaliokysely toteutettiin alussa ja lopussa, ja yhden arkipäivän ruokavaliokysely tehtiin kolme kertaa vuoden aikana. Intervention vaikuttavuus analysoitiin lineaarisella yhteisvaikutusmallilla REML –sovituksella intention-to-treat –periaatteella. Lisäksi 64 henkilön 1–3 päivän lihasaktiivisuus sekä kardio-metaboliset muuttujat mitattiin osana EMG24 -projektia, ja tuo data yhdistettiin intervention lähtötason datan kanssa poikittaisanalyysejä varten (n = 150). Päivittäistä lihasaktiivisuutta verrattiin miesten ja naisten sekä normaalipainoisten ja ylipainoisten kesken, ja yhteyksiä kardio-metabolisiin muuttujiin tutkittiin regressioanalyysin avulla.

Laboratoriomittausten perusteella istumisen ja seisomisen lihasaktiivisuus vaihteli huomattavasti yksilöiden kesken. Vaikka seisominen oli kolme kertaa kuormittavampaa kuin istuminen, olivat yksilöiden väliset erot kymmenkertaisia. Ylipainoiset olivat passiivisempia istuessa, mutta heidän lihasaktiivisuutensa oli suurempaa seistessä verrattuna normaalipainoisiin. Vaikka lähes kaikki koehenkilöistä täyttivät itse raportoidusti nykyiset liikuntasuositukset aerobisen liikunnan osalta, heidän lihaksensa olivat passiivisena lähes 70 % päivittäisen elämän aikana. HDL kolesterolin määrä oli korkeampi, ja triglyseridien määrä matalampi henkilöillä, joilla oli vähän lihasten passiivisuutta (<62 % mittausajasta) verrattuna niihin, joilla oli paljon lihasten passiivisuutta (>78 % mittausajasta). Yhteys säilyi merkitsevänä keski-kovatehoiseen lihasten aktiivisuuteen sekä painoindeksiin vakioinnin jälkeen.

Ennen neuvontaa koehenkilöiden lihakset olivat passiivisena työajalla lähes 80 % ja vapaa-ajalla hieman yli 60 % mittausajasta. Kahdeksan tunnin työaikana passiivisuutta pääsee kertymään siis jo lähes kuusi ja puoli tuntia. Neuvonnan tuloksena passiivisuuden määrä laski 37 minuuttia päivässä ja samalla pitkien passiivisuusjaksojen kesto lyheni interventioryhmällä verrattuna kontrolliryhmään. Suurin osa muutoksesta saavutettiin vapaa-ajalla, jossa istumista oli jo valmiiksi vähemmän. Tämä saattaa kertoa työajan vaatimuksista, jolloin istumista on vaikea vähentää töiden kärsimättä. Toisaalta vapaa-aika voi

tarjota perheen kanssa mielekästä aktiivista tekemistä, sekä ympäristön jossa istumisen vähentäminen on mahdollista.

Intervention seurauksena vapaa-ajan sedentaariaika muuttui edullisesti interventioryhmällä kontrolliryhmään verrattuna vuoden aikana. Interventio vähensi vapaa-ajan sedentaariaikaa kolmen kuukauden kohdalla (-27 minuuttia interventioryhmän eduksi) ja esti epäedullisen muutoksen vuoden aikana (ei muutoksia interventioryhmällä, +13 minuuttia kontrolliryhmällä,). Kokonais-sedentaariajassa tai työn ja viikonlopun sedentaariajassa ei havaittu muutoksia. Vuoden aikana apoB/apoA-1 -suhde parani ja paino sekä koko kehon ja jalkojen lihasmassa pysyivät muuttumattomana interventioryhmällä, kun kontrolli-ryhmän paino nousi sekä koko kehon ja jalkojen lihasmassa laskivat itsenäisesti keski-kovatehoisen aktiivisuuden tai energiansaannin muutoksista. Pitkällä aikavälillä jo vapaa-ajan sedentaariajan muutos voi tuoda itsenäisiä positiivisia terveyshyötyjä, ja ne voidaan saavuttaa perheille suunnatulla istumisen vähentämiseen tähtäävällä neuvonnalla.

Tämän väitöskirjan tulokset osoittavat, että vaikka päivittäinen lihasten aktiivisuus voi koostua hyvin erilaisista aktiivisuusmalleista jopa istuessa, voi istumista vähentämällä vähentää lihasten passiivisuutta. Vuoden aikana seden-taariajan pienikin vähentäminen auttaa ylläpitämään painoa ja lihasmassaa, sekä parantamaan kardio-metabolisia muuttujia jopa fyysisesti aktiivisilla ihmi-sillä itsenäisesti liikunnan harrastamisesta tai energiansaannista. Tämä väitös-kirja antaa tukea useille vasta julkaistuille kansainvälisille suosituksille istumi-sen vähentämiseksi, jotka tarjoavat perinteisten liikuntasuositusten rinnalle ta-van edistää terveyttä helposti arjen lomassa. Istumisen vähentäminen tuo ter-veyshyötyjä sekä ihmisille jotka täyttävät nykyiset liikuntasuositukset, mutta varsinkin niille jotka syystä tai toisesta eivät harrasta kuntoliikuntaa suositusten mukaisesti.

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ORIGINAL PAPERS

I

HETEROGENEITY OF MUSCLE ACTIVITY DURING SEDENTARY BEHAVIOR

by

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ARTICLE

Heterogeneity of muscle activity during sedentary behavior

Arto J. Pesola, Arto Laukkanen, Olli Tikkanen, and Taija Finni

Abstract: Replacing sitting by standing has been hypothesized to reduce the health risks of sitting, based on the assumption that muscles are passive during sitting and active during standing. Interventions have been more effective in overweight (OW) than in normal weight (NW) individuals, but subjects' muscle activities have not been quantified. This study compared quadriceps and hamstring muscle electromyographic (EMG) activity between 57 NW (body mass index (BMI) 22.5 ± 1.5 kg/m^2, female n = 36) and 27 OW (BMI 28.4 ± 2.9 kg/m^2, female n = 8) subjects during non-fatiguing standing (15 s, $EMG_{standing}$) and sitting (30 min). EMG amplitude was normalized to EMG measured during maximal isometric knee extension and flexion (% EMG_{MVC}), and sitting muscle inactivity and bursts were determined using 4 thresholds (60% or 90% $EMG_{standing}$ and 1% or 2% EMG_{MVC}). Comparisons were adjusted for sex, age, knee extension strength, and the individual threshold. Standing EMG amplitude was 36% higher in OW (1.9% ± 1.5% EMG_{MVC}) than in NW (1.4% ± 1.4% EMG_{MVC}, $P < 0.05$) subjects. During sitting, muscles were inactive 89.8% ± 12.7% of the measurement time with 12.7 ± 14.2 bursts/min across all thresholds. On average, 6% more activity was recorded in NW than in OW individuals for 3 of the 4 thresholds ($P < 0.05$ for 60% or 90% $EMG_{standing}$ and 1% EMG_{MVC}). In conclusion, the OW group had higher muscle activity amplitude during standing but more muscle inactivity during sitting for 3/4 of the thresholds tested. Interventions should test whether the observed heterogeneity in muscle activity affects the potential to gain cardiometabolic benefits from replacing sitting with standing.

Key words: sedentary time, sitting, standing, muscle inactivity, electromyography, textile electrodes, body composition.

Résumé : Le remplacement de la position assise par la position debout devrait hypothétiquement diminuer le risque pour la santé, car, selon le postulat, les muscles sont passifs en position assise et actifs en position debout. Les interventions documentées sont plus efficaces chez les personnes en surpoids (« OW ») que chez celles de poids normal (« NW »), mais on n'a pas quantifié l'activité musculaire. Cette étude compare l'activité myoélectrique du quadriceps et des ischio-jambiers chez 57 NW (indice de masse corporelle (« IMC ») 22,5 ± 1,5 kg/m^2, n = 36 femmes) et 27 OW (IMC 28,4 ± 2,9 kg/m^2, n = 8 femmes) en position debout sans fatigue (15 s, EMG_{debout}) et assise (30 min). On normalise l'amplitude EMG par rapport à EMG_{MVC} mesurée au cours d'une contraction isométrique maximale du genou en extension et en flexion (% EMG_{MVC}) et on évalue l'inactivité musculaire et les salves en position assise en fonction de quatre seuils : 60 ou 90 % EMG_{debout} et 1 ou 2 % EMG_{MVC}. On ajuste les comparaisons en fonction du sexe, de l'âge, de la force du genou en extension et des seuils individuels. L'amplitude EMG en position debout est de 36 % plus forte chez OW (1,9 ± 1,5 % EMG_{MVC}) comparativement à NW (1,4 ± 1,4 % EMG_{MVC}, $P < 0.05$). En position assise, les muscles sont inactifs 89,8 ± 12,7 % du temps mesuré et présentent 12,7 ± 14,2 salves/min pour tous les seuils. On enregistre en moyenne 6 % plus d'activité chez NW par rapport à OW dans 3 des 4 seuils ($P < 0,05$ pour 60 ou 90 % EMG_{debout} et 1 % EMG_{MVC}). En conclusion, les sujets OW présentent une plus grande amplitude d'activité musculaire en position debout, mais plus d'inactivité musculaire en position assise dans 3/4 seuils testés. Les interventions ultérieures devraient vérifier si l'hétérogénéité de l'activité musculaire observée a un impact sur le potentiel d'obtenir des bienfaits cardiométaboliques en substituant la position debout à la position assise. [Traduit par la Rédaction]

Mots-clés : temps sédentaire, assis, debout, inactivité musculaire, électromyographie, électrodes textiles, composition corporelle.

Introduction

Sedentary behavior encompasses a range of daily activities performed in a seated or reclined posture and requiring little energy expenditure (Sedentary Behaviour Research Network 2012). The high prevalence (Matthews et al. 2008) and the health risks (Tremblay et al. 2010; Matthews et al. 2012; Pesola et al. 2015) of excessive sedentary time justify the need for interventions aiming to reallocate sedentary time to light-intensity physical activities over the course of the day. By definition, either upright posture or energy expenditure reaching a level of >1.5 metabolic equivalents (METs) (Sedentary Behaviour Research Network 2012) is hypothesized to result in beneficial changes across individuals. Nonetheless, tested interventions have been more effective in overweight (OW, Thorp et al. 2014) than in normal weight (NW, Miyashita et al. 2013; Bailey and Locke 2015) subjects, suggesting that the exposure of changing sitting to standing might vary between individuals.

Body weight represents a load that needs to be supported in upright posture. Consequently, the absolute energy cost of

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weight-bearing activities is generally higher in OW than in NW individuals because of their higher body weight (Howell et al. 1999). Energy expenditure of a task is commonly presented as METs to standardize the amount and intensity of physical activities (Tompuri 2015). As per the definition of sedentary time, energy expenditure exceeding 1.5 times the resting metabolic rate (i.e., 1.5 METs) in an upright posture ends a sedentary bout (Sedentary Behaviour Research Network 2012). However, a recent study exploring the adequacy of this threshold found that obese people did not exceed the standardized MET threshold when standing still and were thus defined as sedentary (Mansoubi et al. 2015). In contrast, their lean counterparts were defined as active based on their standing MET values (Mansoubi et al. 2015). Well acknowledged explanations for this discrepancy include the use of total weight of a subject and an assumed resting constant of energy expenditure in scaling, which lead to underestimated energy expenditure of a given task in overweight people and make estimating the true exposure of a given treatment difficult (Byrne et al. 2005; Tompuri 2015). Furthermore, it is often overlooked that sitting can also be active, even more so as compared with standing still, and the resulting energy gap between sitting and standing is small (Mansoubi et al. 2015). These conflicting results suggest that the higher efficacy of standing to decrease the health risks of sitting in OW than in NW individuals is not explained by their energy expenditure (Miyashita et al. 2013; Thorp et al. 2014; Bailey and Locke 2015).

Distinct from the contemporary definition of sedentary behavior (Sedentary Behaviour Research Network 2012), the driving hypothesis of the sedentary behavior field is that frequent activity in antigravity muscles short-circuits the detrimental physiological processes associated with sedentary time, resulting in a better cardiometabolic risk profile (Hamilton et al. 1998, 2007). For example, recent trials replacing sitting time with different activities in both NW and OW individuals produced different responses in glucoregulation even though energy balance was retained, suggesting that muscle contraction-mediated mechanisms may be involved (Stephens et al. 2011; Duvivier et al. 2013; Blankenship et al. 2014). Because a given posture or level of energy expenditure may coexist with an unknown mixture of volume, intensity, and frequency of muscle activity, which these mechanisms are sensitive to (Peddie et al. 2012), it is important to measure these aspects of a treatment. A hypothesis might be put forward that either low muscle activity during standing or high muscle activity during sitting reduces the potential to gain cardiometabolic benefits when reducing sitting time by standing.

Currently the field of sedentary behavior research lacks fundamental information on the muscle activity levels of a given individual at the low end of the physical activity spectrum, which may prevent us from understanding how the different aspects of physical activity might mitigate the health hazards of sedentary time. Thus, the first aim of this study was to quantify differences in thigh muscle activity between NW and OW individuals during non-fatiguing standing using shorts with built-in electrodes. The second aim of the study was to determine differences in sitting muscle activity, inactivity, and bursts between NW and OW individuals. Because several thresholds have been used to assess muscle inactivity time (Harwood et al. 2008, 2011; Tikkanen et al. 2013; Finni et al. 2014; Pesola et al. 2014, 2015; Gao et al. 2016) and the measured electromyographic (EMG) activity is highly sensitive to the chosen inactivity threshold (Klein et al. 2010), EMG activity during sitting was analyzed at several thresholds to provide a comprehensive comparison between the groups. We hypothesized that compared with sitting, standing increases muscle activity amplitude more in OW than in NW individuals because of the required support for higher body weight, but sitting is very passive in both groups regardless of the chosen threshold for determining muscle inactivity. Finally, EMG variables during sitting and standing were regressed against subject characteristics to gain insights into possible determinants of the observed differences.

Materials and methods

The data for this study were collected at baseline of a randomized controlled trial targeting sedentary time in 2011–2013 (Finni et al. 2011). EMG was measured from the quadriceps and hamstring muscles with EMG shorts (shorts with built-in EMG electrodes), because these large muscles are involved in postural support and their activation contributes to the healthy metabolism of non-sedentary activity. At baseline, EMG was measured from 121 individuals in the laboratory, of which 34 were removed because of artifacts at any of the channels and 3 because of missing data. The final sample consisted of 84 individuals having artifact-free EMG signals during laboratory measurements on all 4 channels. The project was approved by the ethics committee of the Central Hospital District of Central Finland and participants signed an informed consent form prior to the measurements.

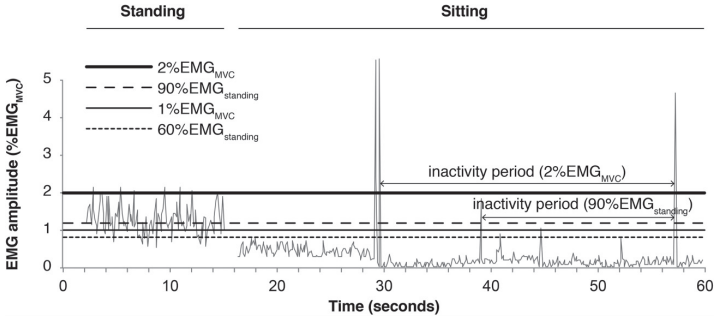
The participants were asked to wear comfortable clothes and shoes that enabled light activities such as walking and jogging for a short period (Pesola et al. 2014). In the morning, in a fasted state, participants' height, weight, waist circumference, and lean and fat body mass were measured by dual-energy X-ray absorptiometry (LUNAR Prodigy, GE Healthcare, Chicago, Ill., USA). Subjects changed into appropriately sized EMG shorts (Finni et al. 2007; Tikkanen et al. 2013) with recording electrodes positioned bilaterally on the distal part of the quadriceps and hamstring muscles. Subjects then sat at a table for breakfast, where general instructions regarding the study were provided and questionnaires were administered for approximately 30 min while participants remained seated (sitting). After participants were asked to sit down at a table, no instructions for how to sit were given. All participants sat on the same lobby chairs with light cushioning (model: Jokke, Asko, Lahti, Finland). Next, the participants were asked to stand still casually for 15 s, with weight on both legs (standing).

After sitting and standing, EMG signals were normalized to those measured during maximal voluntary isometric knee flexion and extension (David 220 dynamometer, David Health Solutions Ltd., Helsinki, Finland) with a knee angle of 140° (Pesola et al. 2014). After a warm-up, 3 maximal efforts of 3–5 s with strong verbal support were performed with 1 min of rest between trials. If the torque improved more than 5%, additional trials were performed.

EMG was measured bilaterally from quadriceps and hamstring muscles with EMG shorts (Myontec Ltd., Kuopio and Suunto Ltd., Vantaa, Finland; supplementary file S1[1]), providing valid and repeatable data (Finni et al. 2007; Tikkanen et al. 2014; Pesola et al. 2014). The analysis workflow was as follows and is presented in supplementary file S1 accordingly: (1) baseline correction, (2) data chopping, (3) data normalization, (4) data averaging, (5) threshold determination, and (6) Matlab analysis. Briefly, the baseline of the whole data file was corrected for possible non-physiological baseline fluctuations (Pesola et al. 2014). Next, data were separated into sitting, standing, and maximum voluntary contraction (MVC) periods based on lab logs. The most consistent 1-s mean EMG from the MVC repetition with the highest force level was analyzed, and sitting and standing signals from the 4 muscle groups were normalized individually to the respective maximal 1-s mean EMG amplitude. The 4 signals were then averaged to represent overall inactivity or activity of thigh muscles. The final results were

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/apnm-2016-0170

Fig. 1. Schematic illustration of the 4 inactivity thresholds and 2 examples of the respective inactivity periods. The bursts in electromyographic (EMG) amplitude during sitting break up the inactivity periods, depending on the burst amplitude and the threshold used. MVC, maximum voluntary contraction.



analyzed with a custom-made Matlab (MathWorks Inc., version 7.11.0.587) algorithm as follows.

Standing (15 s) amplitude analysis

A 15-s period for standing was used because the aim of this study was to investigate EMG activity in acute, non-fatigued conditions, which is typical for static posturographic studies (Duarte and Zatsiorsky 1999) and for previous studies using standing as the inactivity threshold (Tikkanen et al. 2013; Finni et al. 2014; Pesola et al. 2014, 2015; Gao et al. 2016). The analysis yielded average EMG amplitude during standing (% EMG$_{MVC}$).

Sitting amplitude, inactivity, and burst analysis (30 min)

Similar to that during standing, the average EMG amplitude during sitting is presented as % EMG$_{MVC}$. Because inactivity time is highly sensitive to the inactivity threshold (Klein et al. 2010), 4 different inactivity thresholds were used, based on previous research, to improve the sensitivity and comparability of the sitting inactivity and burst analysis (Fig. 1). The thresholds included those obtained from the standing (15 s) amplitude analysis. The 4 thresholds were as follows:

(1) 60% EMG$_{standing}$: 60% of EMG amplitude measured during standing. This particular threshold was used because it yielded the biggest difference in muscle inactivity time between sitting and standing (supplementary file S1).
(2) 90% EMG$_{standing}$: 90% of EMG amplitude measured during standing (Tikkanen et al. 2013; Finni et al. 2014; Pesola et al. 2014, 2015; Gao et al. 2016).
(3) 1% EMG$_{MVC}$. This threshold was included to enable group comparisons at a fixed threshold (1% EMG$_{MVC}$) versus an individual threshold (90% EMG$_{standing}$), which both yielded similar group averages of muscle inactivity time during sitting (supplementary file S1).
(4) 2% EMG$_{MVC}$ (Harwood et al. 2008, 2011).

Subsequently, the following variables were analyzed for sitting (30 min, Fig. 1):

(1) muscle inactivity time: the amount of time EMG remained under the inactivity threshold, presented as percentage of measurement time;
(2) number of bursts per minute: the number of occasions in a minute when the EMG amplitude exceeded the inactivity threshold.

Statistical analysis

Subject characteristics were compared between NW and OW participants with independent-samples *t* tests for continuous variables and χ^2 tests for categorical variables. The bursts per minute and amplitude analysis variables were natural log transformed. Because the muscle inactivity time approached 100%, it was transformed as follows: ln(100% – muscle inactivity time (%)). The covariates sex, age, and knee extension strength were used in all analyses because of their effects on energy cost (Byrne et al. 2005; Tompuri 2015) and EMG amplitude (Harwood et al. 2008) of activity. When the 60% EMG$_{standing}$ or 90% EMG$_{standing}$ inactivity threshold was used, the analysis was additionally adjusted for the threshold (Table 2) to yield comparisons independent of the individual threshold. A one-way ANOVA was conducted that examined the effect of overweight status on the standing amplitude as well as the sitting amplitude, inactivity, and bursts. Partial correlations were performed to examine the associations of EMG-derived variables with anthropometrics when adjusting for age, sex, knee extension strength, and the individual inactivity threshold (where appropriate). Those EMG variables showing significant associations with any of the anthropometric variables were used as dependent variables in hierarchical multiple linear regression. The covariates sex, age, knee extension strength, and individual inactivity threshold (where appropriate) were entered into every model, and inclusion of those anthropometric variables having a significant partial correlation with EMG variables was tested in a stepwise manner. The effects of each independent variable adjusted for the effects of all other independent variables were analyzed by forward stepwise multiple linear regression against the same dependent variables, and the anthropometric variables showing independent significant effects were included in the final models. Residual normality and homoscedasticity as well as lack of multicollinearity were ensured. Significance was set at a level of $P < 0.05$. Analyses were performed with PASW version 20.0.

Results

Analyzed data were from 57 NW (36 female) and 27 OW (8 female) participants with sedentary work (Finni et al. 2011, Pesola et al. 2014). Age, weight, and body mass index (BMI) ranged from 29 to 50 years, from 49.5 to 120.8 kg, and from 18.3 to 34.9 kg/m^2, respectively. Table 1 shows the characteristics of NW and OW subjects without adjustment for sex. The proportion of females was higher in the NW group than in the OW group. The OW participants were taller, heavier, and had higher BMI, waist circumference, fat mass (% and kg), lean mass (% and kg), and knee extension strength than the NW participants ($P < 0.05$, Table 1). After adjustment for sex, the differences in height ($P = 0.91$) and knee extension strength ($P = 0.12$) became insignificant.

Standing (15 s) amplitude analysis

On average, EMG amplitude was 1.6% ± 1.4% of EMG$_{MVC}$ during standing (Table 1, range 0.1%–8.2% EMG$_{MVC}$). The OW group had

Table 1. Characteristics of normal weight and overweight participants.

	Normal weight, $n = 57$	Overweight, $n = 27$	Total, $n = 84$	P
Female *n* (%)	36 (63)	8 (30)	44 (52)	**0.004**
Age, y	38.0±5.0	37.4±5.1	37.8±5.0	0.616
Height, cm	169.9±9.8	175.0±9.1	171.5±9.8	**0.025**
Weight, kg	65.2±9.3	87.1±12.8	72.2±14.7	**<0.001**
BMI, kg/m^2	22.5±1.5	28.4±2.9	24.4±3.4	**<0.001**
Waist circumference, cm	84.5±6.3	99.5±9.1	89.2±10.1	**<0.001**
Fat mass, %	25.6±8.0	30.4±6.2	27.1±7.8	**0.007**
Lean mass, %	70.1±8.3	65.8±6.4	68.7±7.9	**0.020**
Fat mass, kg	16.4±5.0	26.5±7.0	19.7±7.4	**<0.001**
Lean mass, kg	46.0±9.9	57.3±9.7	49.6±11.2	**<0.001**
Knee extension strength, kg	75.4±21.5	92.1±25.0	80.5±23.8	**0.003**

Note: Values are means ± SD unless otherwise stated. Boldface indicates statistical significance at $P < 0.05$. BMI, body mass index.

36% higher standing EMG amplitude as compared with the NW group, independent of sex, age, and knee extension strength.

Sitting (30 min) amplitude, inactivity, and burst analysis

During 30 min of sitting, the average EMG amplitude was 0.5% ± 0.3% of EMG_{MVC} (range 0.1%–1.3% EMG_{MVC}) and did not differ between the groups. The average amount of time the muscles were inactive varied from 81.1% ± 20.4% (inactivity threshold 60% $EMG_{standing}$; range 10.6%–99.9%) to 96.9% ± 3.3% (inactivity threshold 2% EMG_{MVC}, range 82.6%–100.0%), depending on the threshold used (Table 2, Fig. 2). Similarly, the average number of bursts per minute varied from 20.9 ± 19.9 (60% $EMG_{standing}$; range 0.4–90.9) to 5.3 ± 5.3 (2% EMG_{MVC}; range 0.0–24.0). The OW group had, on average, 9.4% (60% $EMG_{standing}$, $P < 0.01$), 5.2% (90% $EMG_{standing}$, $P < 0.01$), and 2.5% (1% EMG_{MVC}, $P < 0.05$) more muscle inactivity time during sitting for 3 of the 4 thresholds, independent of sex, age, knee extension strength, and the individual threshold (Table 2).

Partial correlations

Higher weight (partial $r = 0.307$, $P < 0.01$), BMI (partial $r = 0.248$, $P < 0.05$), fat mass (kg, partial $r = 0.243$, $P < 0.05$), and lean mass (kg, partial $r = 0.248$, $P < 0.05$) were associated with higher EMG amplitude during standing, independent of sex, age, and knee extension strength. Higher fat mass (Partial *r* between fat mass (%) and ln(100% – muscle inactivity time %) = –0.241, $P < 0.05$) and lower lean mass (Partial *r* between lean mass (%) and ln(100% – muscle inactivity time %) = 0.246, $P < 0.05$) were associated with higher muscle inactivity time during sitting when analyzed at the 60% $EMG_{standing}$ threshold, independent of sex, age, knee extension strength, and the individual threshold. Sitting EMG amplitude, bursts, and inactivity analyzed at the other thresholds showed no associations with anthropometric variables. All partial correlations are provided in supplementary file S2.

Multivariate models

In multiple stepwise regression analyses in which sex, age, and knee extension strength were entered in the model, only weight remained a significant predictor of standing EMG amplitude in addition to knee extension strength, whereas BMI, fat mass, and lean mass dropped out. Table 3 shows that the final multivariate model explained 14% of the variance in standing EMG amplitude ($P < 0.05$). The model explaining variance in sitting muscle inactivity time analyzed at the 60% $EMG_{standing}$ threshold was initially adjusted for sex, age, knee extension strength, and the individual threshold. In the stepwise analysis, lean mass (%) remained a significant predictor in the model, while fat mass (%) was left out. The final multivariate model explained 51% of the variance in sitting muscle inactivity time, and both the individual inactivity threshold and lean mass (%) were significant independent predictors in this model (Table 3).

Discussion

Sitting and standing are conventionally regarded as dichotomous replacement activities for each other. In practice, the time spent standing has been reported as the exposure variable without knowing the heterogeneity in muscle activity of sitting and standing between individuals. The direct thigh muscle EMG recordings in this study showed that the average muscle activity amplitude was threefold higher during standing than sitting; however, there were up to tenfold differences between individuals. It is well acknowledged that higher body mass poses an additional load for postural support against gravity during standing (Hue et al. 2007), which in this study was verified by the positive correlation between weight and the standing EMG as well as by the higher standing EMG in the OW as compared with the NW group. In contrast to the hypothesis that muscle inactivity time would be high during sitting in both groups, the NW group had, on average, 5% less muscle inactivity during sitting as compared with the OW group at 3 of the 4 thresholds used. This difference is larger than that achieved by an effective intervention (Pesola et al. 2014). Although NW and OW individuals can execute sedentary and light-activity tasks with similar energy expenditure (Mansoubi et al. 2015), these results imply that their muscle activity can be significantly different during both sitting and standing. Investigators using reallocation of sitting to standing as their intervention should be aware of the effects of body weight on differences in EMG activity, which are not detected by accelerometers or inclinometers but which may influence the desired dose of activity and subsequently the efficacy of the intervention. These differences should be confirmed by measuring other relevant muscles with a larger sample size. In addition, sitting and standing should be measured for longer periods of time and in more normal living environments to test whether these results apply to habitual sitting and standing.

Standing upright is a recommended means to exceed the energy expenditure threshold of 1.5 METs, which is hypothesized to reduce the health hazards of sitting across individuals. However, the standing posture itself does not increase energy expenditure without muscle activity (Chang et al. 2005), and the actual protective mechanisms are hypothesized to be muscle-contraction mediated (Hamilton et al. 2008). Importantly, some key mechanisms related to substrate utilization and insulin resistance, such as GLUT4 transporter expression and translocation (Gibala et al. 2012; Richter and Hargreaves 2013), lipoprotein lipase activity (Bey and Hamilton 2003), and postprandial lipidemia (Peddie et al. 2012), are sensitive to muscle activity volume, intensity, and frequency partly independently of cellular energy status. Intervention studies targeting sedentary time and relying on a count-based proxy for metabolic cost or a posture-related classification may not reveal the true exposure of their treatment in relation to the hypothesized mechanisms at the muscle level. During discrete tasks and normal daily life, individuals may be activating these mechanisms through different pathways depending on their individual muscle activity patterns. Future studies should assess whether the reductions in muscle inactivity time per se, or the heterogeneous EMG amplitudes during sitting versus standing across individuals, yield further insights into the mechanistic associations between sedentary time and health (Tremblay et al. 2010). It is still unclear whether the interindividual differences at this low level of muscle activity are clinically relevant. For example, significant reductions in muscle inactivity can be achieved at very low levels of muscular effort (Pesola et al. 2014), and lower muscle inactivity time is associated with clinically relevant cardiometabolic benefits in physically active adults regardless of moderate-to-vigorous muscle activity (Pesola et al. 2015). Hence, interventions targeting reduced muscle inactivity time bear health-enhancing potential, which might be mediated through an individual's reallocation of time between muscle inactivity and light activity.

Table 2. Differences in standing and sitting EMG amplitudes and in muscle inactivity and breaks during sitting between normal weight and overweight subjects.

	Normal weight, $n = 57$	Overweight, $n = 27$	Total, $n = 84$	P
Standing EMG amplitude, % EMG_{MVC}	1.4±1.4	1.9±1.5	1.6±1.4	**0.042**
Sitting EMG amplitude, % EMG_{MVC}	0.5±0.2	0.5±0.3	0.5±0.3	0.565
Muscle inactivity during sitting, %				
60% $EMG_{standing}$	78.7±20.5	86.1±19.6	81.1±20.4	**0.007**[a]
90% $EMG_{standing}$	88.7±12.1	93.3±10.8	90.2±11.8	**0.003**[a]
1% EMG_{MVC}	90.3±8.2	92.6±9.9	91.0±8.8	**0.049**
2% EMG_{MVC}	96.3±3.6	97.9±2.3	96.9±3.3	0.097
Bursts/min during sitting				
60% $EMG_{standing}$	21.5±17.7	19.6±24.2	20.9±19.9	0.197[a]
90% $EMG_{standing}$	13.4±13.5	11.9±13.5	12.9±13.4	0.100[a]
1% EMG_{MVC}	11.2±9.9	16.0±20.0	12.8±14	0.838
2% EMG_{MVC}	5.5±4.7	5.1±6.4	5.3±5.3	0.466

Note: Values are means ± SD. Boldface indicates statistical significance ($P < 0.05$). Adjusted for sex, age, and MVC extension. EMG, electromyographic; MVC, maximum voluntary contraction.
[a]Adjusted additionally for the individual inactivity threshold.

Fig. 2. Individual sitting inactivity time (% of measurement time, threshold 1% EMG_{MVC}) and bursts per minute ranked by inactivity time. Raw data samples from the sitting period are shown from cut points of every quartile, from the most active to the most passive. EMG, electromyographic.

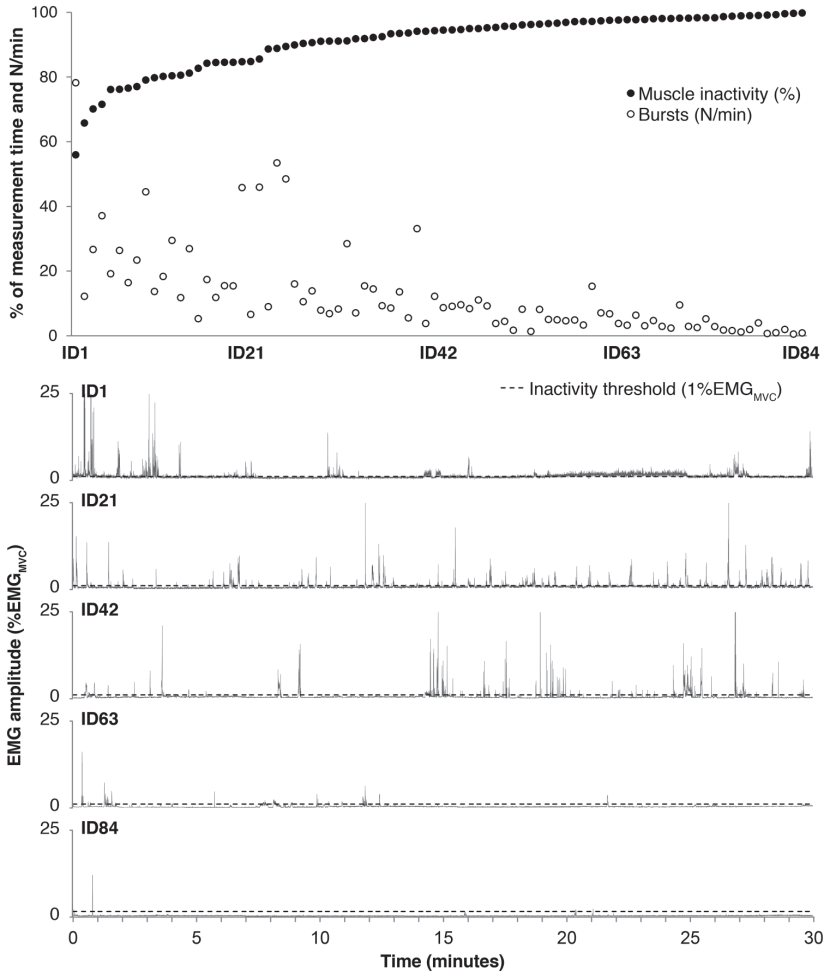


Table 3. Adjusted stepwise multiple linear regression models having standing EMG amplitude (ln) and sitting muscle inactivity time (ln(100% – muscle inactivity time %), threshold 60% $EMG_{standing}$) as dependent variables as informed by partial correlations.

	R^2	Adjusted R^2	Standardized β coefficients (95% CI)	*P*
ln standing EMG amplitude, % EMG_{MVC}	0.135	0.090		**0.024**
Sex, F = 0, M = 1			–0.002 (–0.323, 0.320)	0.991
Age, y			0.097 (–0.123, 0.318)	0.385
Knee extension strength, kg			–0.356 (–0.666, –0.045)	**0.027**
Weight, kg			0.430 (0.126, 0.733)	**0.006**
ln(100% – muscle inactivity time %), threshold 60% $EMG_{standing}$	0.506	0.473		**<0.001**
Sex, F = 0, M = 1			–0.244 (–0.488, 0.000)	0.051
Age, y			–0.094 (–0.264, 0.075)	0.275
Knee extension strength, kg			–0.072 (–0.295, 0.151)	0.526
ln inactivity threshold, 60% $EMG_{standing}$			–0.610 (–0.776, –0.445)	**<0.001**
Lean mass, %			0.207 (0.019, 0.394)	**0.032**

Note: Boldface denotes significance at $P < 0.05$. The covariates sex, age, knee extension strength, and the individual inactivity threshold were entered into the models and the anthropometric variable was selected in a stepwise analysis. Because the muscle inactivity time was natural log transformed as follows: ln(100% – muscle inactivity time %), the directions of true associations are inverse to those reported at the table. EMG, electromyographic; F, female; ln, natural logarithm; m, male.

It is interesting to speculate whether differences in muscle activity between individuals in intervention studies that reallocate sitting to standing could mediate the efficacy of the intervention on cardiometabolic outcomes. For example, not all individuals benefit from reallocating sitting to standing. In a study by Miyashita et al. (2013), regular standing breaks (6 × 45 min for 6 h) were ineffective at improving postprandial glucose, insulin, or triglyceride levels over those measured during prolonged sitting in young, healthy males. In contrast, Thorp et al. (2014) observed improved postprandial glucose levels with alternating 30-min bouts of sitting and standing for 8 h versus prolonged sitting in overweight or obese adults. In the present study, the standing EMG amplitude of NW males, which represent a similar group of participants as in the study of Miyashita et al. (2013), was 1.4% EMG_{MVC}. However, when the results for OW females and males in the present study were averaged, yielding similar participants as in the study of Thorp et al. (2014), the standing EMG amplitude was 1.9% EMG_{MVC}. Assuming that the cumulative integrated muscle activity could be calculated by simply multiplying intensity difference by time, the difference in increased muscle activity volume would be 36% between these groups over a similar time period. Although the intervention of Thorp et al. (2014) included 15 min less standing per hour, the exposure in terms of cumulative integrated muscle activity was more than 20% higher in their overweight or obese participants than in the normal weight males studied by Miyashita et al. (2013), which could partly explain the difference in the efficacy of the interventions. Yet, it should be noted that several other mechanisms that regulate glucose tolerance and are mediated by the degree of overweight, such as the baseline level of glucose intolerance (Kelley and Goodpaster 1999), could contribute to the different efficacies of these interventions.

A novel finding of this study was that the measured muscle activity during sitting varied greatly between individuals. On average, participants had almost 13 bursts/min during sitting, and NW individuals were more active than OW individuals. Furthermore, some participants had higher EMG amplitude during sitting than standing. The lack of correlations between the sitting inactivity and burst analysis parameters and the anthropometric measures suggests that factors other than body weight or composition explain the high interindividual differences. These factors could include differences in activation patterns, coordination, and technique; issues related to EMG as a method to measure muscle activity; or behavioral differences such as fidgeting, among other factors (Levine et al. 2000; Farina et al. 2004; Enoka and Duchateau 2015). Previous studies have shown that overweight people habitually stand less than normal weight people, which could be determined by their different biological propensity towards sedentariness as compared with normal weight people (Levine et al. 2005). This study provides evidence that OW individuals are also more inactive when seated as compared with NW individuals, which could imply that the same mechanisms also induce less activity during sitting. However, the standing EMG amplitude in the OW group was higher than that in the NW group, suggesting that the higher added activity during standing might compensate for the higher inactivity time during sitting and shorter upright time per day (Levine et al. 2005) in terms of cumulative muscle activity. It is important to note that the present study focused on short-term static standing, which ignores activities such as shifting and fidgeting (Duarte and Zatsiorsky 1999). Thus, a longer measurement time might emphasize the difference in cumulative muscle activity during standing between NW and OW individuals. The high inactivity time during sitting and high EMG amplitude during standing in OW people might have implications for the feasibility of behavior-targeted interventions or the efficacy of biomarker-targeted interventions to reduce sedentary time.

Methodological and protocol differences make direct comparisons with previous EMG studies somewhat challenging. In addition to the different electrodes, muscles, and activities studied, the muscle inactivity and burst results are highly sensitive to the inactivity threshold chosen (supplementary file S1 and Klein et al. (2010)). Some of the previous studies measuring habitual EMG activity have used a fixed inactivity threshold of 2% of EMG_{MVC} (Harwood et al. 2008, 2011). However, the present laboratory study showed that the average EMG amplitude during standing is only 1.6% of EMG_{MVC}. In practice, an inactivity threshold of 2% of EMG_{MVC} would classify 43% of participants as inactive during standing (supplementary file S1). Using a functional (% $EMG_{standing}$) instead of a fixed (% EMG_{MVC}) inactivity threshold is beneficial because it most effectively classifies participants as active during standing and is justified because standing is defined as physical activity (Sedentary Behaviour Research Network 2012). Furthermore, the standing EMG amplitude, and thus the individual inactivity threshold, was adjusted for, suggesting that it has limited influence on the observed differences. However, the threshold 60% $EMG_{standing}$ was a strong significant predictor of sitting muscle inactivity time in the multiple regression analysis, suggesting that caution should be used in future studies if this particular threshold is selected.

Unlike accelerometers, inclinometers, pedometers, or heart rate monitors, EMG directly measures muscle activity by placement of surface electrodes. Although the use of EMG is the explicit strength of this study, several factors need to be considered when comparing EMG activity between individuals. The effects of interindividual differences in subcutaneous tissue and muscle properties on signal quality are typically minimized by presenting results as a fraction of the maximal EMG measured during isometric maximal voluntary contractions (Burden 2010). Thus, EMG normalized to MVC represents an effort relative to a muscle's force production capacity. In this study, the group comparisons were adjusted to maximal knee extension strength. Thus, the results yield insights into the added exposure during standing, independent of differences in muscle strength, which is relevant for sit–stand interventions. EMG shorts measure only the thigh muscle region, although many other muscle groups are activated during standing, including the important antigravity muscles soleus and erector spinae (Panzer et al. 1995). However, the activity of these muscles during standing is of similar magnitude to that of vasti muscles (Panzer et al. 1995), suggesting that the results of this study are representative of different activity patterns between individuals. A repeated measurement in a subsample of this study revealed strong between-day reliability for standing and EMG/force ratio (Pesola et al. 2014), suggesting that the measured activity likely represents true behavioral differences between individuals rather than methodological variance. However, future studies should collect repeated measurements for sitting and standing and include other relevant muscle groups to ensure the consistency of behavioral differences in EMG activity patterns, and they should combine these measures with cardiometabolic and behavioral measures to study their clinical significance. Measuring standing for a longer period of time could reveal individual fidgeting activities and incorporate the effects of fatigue, which could reveal more interindividual differences in standing EMG activity.

Although the act of standing up is considered a simple means to reduce the health hazards of prolonged sitting, this cross-sectional laboratory study showed that interindividual differences in muscle activity during sitting and standing are significant. The OW group had higher muscle activity amplitude during standing but more muscle inactivity during sitting at 3 of the 4 thresholds tested. Interindividual variability in standing EMG amplitude was partly explained by differences in body weight. Future studies should determine whether low muscle activity during sitting or high muscle activity during standing increases the potential to gain cardiometabolic benefits from replacing sitting with standing beyond the differences in energy expenditure.

Conflict of interest statement

The authors have no financial conflict of interest to declare.

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MUSCLE INACTIVITY IS ADVERSELY ASSOCIATED WITH BIOMARKERS IN PHYSICALLY ACTIVE ADULTS

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EPIDEMIOLOGY

Muscle Inactivity Is Adversely Associated with Biomarkers in Physically Active Adults

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[1]*Department of Biology of Physical Activity, Neuromuscular Research Center, University of Jyväskylä, FINLAND;* [2]*Department of Sport Sciences, University of Jyväskylä, FINLAND; and* [3]*Department of Health Sciences, Gerontology Research Center, University of Jyväskylä, FINLAND*

ABSTRACT

PESOLA, A. J., A. LAUKKANEN, O. TIKKANEN, S. SIPILÄ, H. KAINULAINEN, and T. FINNI. Muscle Inactivity Is Adversely Associated with Biomarkers in Physically Active Adults. *Med. Sci. Sports Exerc.*, Vol. 47, No. 6, pp. 1188–1196, 2015. **Purpose**: While the lack of muscular activity is a proposed trigger for metabolic alterations, this association has not been directly measured. We examined the associations between EMG-derived muscle inactivity and activity patterns and cardiometabolic biomarkers in healthy, physically active adults. **Methods**: Data for this cross-sectional study were pooled from two studies (EMG24 and InPact), resulting in a sample of 150 individuals without known chronic diseases and with high-quality EMG data (female $n = 85$, male $n = 65$, age = 38.8 ± 10.6 yr, body mass index = 23.8 ± 3.1 $kg \cdot m^{-2}$). EMG was measured during one to three typical weekdays using EMG shorts, measuring quadriceps and hamstring muscle EMG. Muscle inactivity time and moderate- to vigorous-intensity muscle activity were defined as EMG amplitude below that of standing still and above that of walking 5 $km \cdot h^{-1}$, respectively. Blood pressure index, waist circumference, fasting plasma glucose, HDL cholesterol, and triglycerides were measured, and long-term exercise behaviors were assessed by questionnaire. **Results**: In a group of physically active participants, muscles were inactive for 65.2% ± 12.9% of the measurement time in an average of 24.1 ± 9.8-s periods. Compared to those in the lowest muscle inactivity quartile (<55.5% of measurement time), those in the highest quartile (≥74.8% of measurement time) had 0.32 $mmol \cdot L^{-1}$ lower HDL cholesterol ($P < 0.05$) and 0.30 mM higher triglycerides ($P < 0.05$) independent of muscle's moderate- to vigorous-intensity activity. **Conclusions**: Clinically significant differences in HDL cholesterol and triglycerides were found, favoring participants having low muscle inactivity time, independent of moderate- to vigorous-intensity muscle activity. Even physically active individuals may benefit from light-intensity activities that reduce ubiquitous muscle inactivity time. **Key Words:** SEDENTARY TIME, MUSCLE INACTIVITY PERIODS, NONEXERCISE PHYSICAL ACTIVITY, EMG, TEXTILE ELECTRODES, METABOLIC SYNDROME

An essential concept in sedentary behavior research is the balance between sedentary time and light-intensity physical activity, which collectively cover most of the waking hours (13,26,34). Accelerometers have been widely used to assess this balance, generally measuring sedentary time as a lack of impacts near the center of body mass. Long periods of minimal impacts are associated with several detrimental health outcomes independent of moderate- to vigorous-intensity activity (14,15,19). When sedentary time is measured subjectively, this association exists even among people participating in recommended levels of exercise (16,27), warranting further study with objective measures.

One of the actual proposed mechanisms for the association between sedentary time and health outcomes is the lack of activity on the muscle level (13). Prolonged muscle inactivity results in a series of alterations in metabolic flexibility, including reduced insulin action, glucose intolerance, and impaired lipid metabolism (4). In experimental settings, these detrimental effects have been reversed by frequently activating antigravity muscles (6,8,29), supporting the biological plausibility of sedentary behavior as a distinct health

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risk to lack of exercise. Specifically, the lack of change in hepatic insulin action followed by an inactivity-induced decrease in peripheral and whole body insulin sensitivity support the role of muscular inactivity as at least one of the potential causes for these detrimental changes (32,33).

Sedentary behavior can be defined as a seated/reclining posture accompanied by low energy expenditure (31). Because physical activity is defined as any bodily movement produced by skeletal muscles that requires energy expenditure (5), specifically muscle activity is needed to change the sedentary time to physical activity. Enzymatic processes leading to substrate utilization and improved insulin sensitivity are initiated by muscle activity, not by physical impacts as generally measured by accelerometers. Despite the proposed causal role of muscular inactivity on metabolic changes (13), it is unknown whether and to what extent the association between health outcomes and habitual sedentary time exists when measured directly from the muscles. To the best of our knowledge, this is the first study to investigate associations between cardiometabolic biomarkers and directly measured muscular inactivity time from quadriceps and hamstring muscles, which are key muscles for moving and upright position. We hypothesized that total muscle inactivity time and long periods of muscle inactivity are associated with cardiometabolic biomarkers independent of the muscle's moderate- to vigorous-intensity activity.

RESEARCH DESIGN AND METHODS

Data for this cross-sectional study were pooled from two projects measuring muscle activity during normal daily life and cardiometabolic biomarkers. The EMG24 project conducted in 2007–2012 (9) was a cross-sectional study to quantify muscle loading during normal daily life. The InPact project (11) was a sedentary time–targeted randomized controlled trial including families with sedentary behaviors. Data were collected between 2011 and 2013. The EMG activity was measured from a subsample before the intervention, and only these baseline data were used for the present study. The EMG24 project was approved by the ethics committee of the University of Jyväskylä, and the InPact project was approved by the ethics committee of the Central Hospital District of Central Finland. The participants signed an informed consent before the measurements.

Methods for these studies have been previously published (30,34). Initially, a total of 545 participants were assessed for eligibility based on respective study criteria (EMG24, n = 245; InPact, n = 300). An eligible sample of 241 participants (EMG24 109/InPact 132) consisted of those who had no reported chronic (diabetes, cardiovascular diseases, cancer, hypertension, rheumatism, or osteoporosis), those who had no related medications affecting daily ambulatory activity or cardiometabolic markers, and women who were not pregnant. From this eligible sample, EMG was measured in 226 participants (EMG24, n = 109; InPact, n = 117) for a minimum of a 1-d period. The participants having prolonged (>30 min) artifact (nonphysiological signal caused by, e.g., movement of electrodes in relation to skin or close proximity to electronic devices, masking the physiological signal) on all four channels (40/13) or <8 h of EMG data from self-reported typical weekdays (3/17) were excluded from the analysis. The excluded untypical days were, for example, as follows: *having day off*, *having organized exercise evening at work*, or *staying at home because kids were sick*. Of the remaining 153 individuals, one participant's fasting plasma glucose (FPG) values were not available, one participant had exceptionally high blood pressure (176/93), and one had exceptionally high serum triglyceride levels (8.4 mM). These participants were excluded from the analysis, giving a final sample of 150.

The study protocol consisted of laboratory measurements including biochemical, anthropometric, and behavioral assessments, as well as structured laboratory test patterns for EMG normalization (11,30). After the laboratory measurements, participants were asked to continue normal daily living and to wear the EMG shorts during waking hours. When applicable in terms of the study protocol and device availability, the EMG measurements were repeated on a second day.

Before arriving to the laboratory in the morning, participants were asked to fast for a minimum of 10 h and refrain themselves from vigorous-intensity exercise the day before. Subject's height, weight, and triplicate waist circumference were measured using standard procedures. Blood pressure was measured twice on the left arm of the participants in supine position after 5 min of resting period (Omron M6W; Omron Healthcare, Co., Ltd., Kyoto, Japan), and the means of the repeated measurements were used. Systolic and diastolic blood pressure levels were averaged [(systolic blood pressure + diastolic blood pressure) / 2 = blood pressure index], representing a composite risk factor for hypertension (12). Professional laboratory personnel measured and analyzed FPG, HDL cholesterol, and triglycerides using standardized procedures (Konelab 20 XTi analyzer; ThermoFisher, Espoo, Finland).

After the fasting assessments, the EMG shorts were put on and set to record. First, participants were offered breakfast while sitting at a table and filling in the physical activity questionnaire where the number, duration, and intensity of different physical activities (e.g., walking to work, jogging) were assessed month by month during the last year. From this questionnaire, the average MET-hours per week at ≥3-METs intensity was calculated for each participant (self-reported physical activity). Subsequently, the study protocol was discussed briefly with each participant, and instructions for filling in the other questionnaires and diary were given. Questions about education and smoking were included, which were used as covariates in the analysis. The participants were asked to fill in a physical activity diary during the measurement day as well as questionnaires concerning their detailed health status and socioeconomic status on the Internet or on paper afterward.

After breakfast, laboratory measurements continued with the measurement of EMG during standing (15 s on both legs

and 15 s on each leg individually), walking at 5, 6, and 7 $km \cdot h^{-1}$ (1-min loads), and stair ascending and descending. Finally, maximal voluntary EMG activity was measured in a knee flexion/extension machine (David 220; David Health Solutions, Ltd., Helsinki, Finland) with a knee angle of 140° in both flexion and extension. After a warm-up, three 3- to 5-s maximal efforts with strong verbal support were performed with 1 min of rest between trials. If the torque improved by >5%, additional trials were performed.

After the laboratory measurements, participants were expected to continue normal living while wearing the EMG shorts and to report any abnormal tasks or behaviors (e.g., abnormal working tasks) to include only typical days for analysis.

EMG data collection and analysis. EMG was measured from quadriceps and hamstring muscles using shorts, similar to elastic sport clothes, with embedded textile electrodes sewed into its inner surface (Myontec, Ltd., Kuopio, Finland, and Suunto, Ltd., Vantaa, Finland). Bipolar electrodes were located on the distal part of the quadriceps and hamstrings, and reference electrodes were lying longitudinally over the tractus iliotibialis on both sides. Four different sizes of shorts (XS, S, M, and L) and electrode paste (Redux Electrolyte Crème; Parker, Inc., Fairfield, NJ) were used to optimize the skin–electrode contact. The EMG shorts have been tested for validity, repeatability, and feasibility (10) and can be used to accurately estimate energy expenditure (35). Detailed descriptions of this method have been reported previously (10,34).

After the measurements, EMG data were checked visually for occasional artifacts (e.g., toilet visits, short-term movement artifacts), and the corresponding data periods were manually removed from every channel. On the occasions of measurement device malfunction or impedance problems between skin and electrodes, the artifact was prolonged and the channels having artifact periods longer than 30 min were removed from the analysis. The artifacts were distinguished from the physiological signal based on comprehensive laboratory tests where we had intentionally induced artifacts to the signal (34). EMG was measured for a total of 241 d, including 964 channels of daily data (left and right quadriceps muscles and left and right hamstring muscles). If multiple days were measured from a given participant, data from these days were averaged to represent one result per individual.

The individual channels from quadriceps and hamstring muscles were normalized to maximal EMG amplitude measured during bilateral MVC contractions averaged for a 1-s window. To reflect the overall inactivity and activity periods, the normalized data from the four muscles were averaged to a mean thigh muscle EMG. To exclude muscle inactivity periods occurring during dynamic activities such as walking, the EMG data were averaged with a 2-s moving filter (see Document, Supplemental Digital Content 1, additional details of the use of a 2-s moving filter, http://links.lww.com/MSS/A445). EMG baseline was defined as zero activity. The EMG during sitting was quantified while participants were sitting for 30 min during breakfast. The inactivity threshold was set individually at 90% of the mean EMG amplitude measured during standing still for 15 s at the laboratory. The threshold between light- and moderate-intensity activities was defined individually as a 1-min mean EMG value when walking at 5 $km \cdot h^{-1}$. To further separate moderate- and vigorous-intensity activities, the moderate- to vigorous-intensity activity threshold was calculated as "light- to moderate-intensity threshold × 2." These thresholds were selected because they correspond to the energy expenditure at 3 and 6 METs, respectively (1,35). EMG data were analyzed with a custom-made MATLAB (The MathWorks, Inc., version 7.11.0.587) algorithm (for details, see Pesola et al. [30]).

Statistical analysis. Descriptive statistics are presented as means ± SD, and differences between sexes were calculated using Mann–Whitney *U* test. For non–normally distributed variables, logarithmic transformations (natural logarithm) were performed. After transformation, all variables met the criteria for normal distribution based on the Shapiro–Wilk test or had skewness and kurtosis values between −1 and 1. Correlations between muscle inactivity and activity patterns, self-reported physical activity level, and age were studied with Pearson correlation coefficients. The associations between muscle inactivity and activity subcomponents and individual phenotypes of cardiometabolic risk were modeled in separate forced-entry linear regression models. Potential confounders including sex, age, smoking (yes/no), education status (primary school/high school/vocational school/university degree), season (winter/summer), number of measured days, recording time, and number of included channels were used as covariates in model 1. Because the total muscle inactivity time is highly sensitive to the inactivity threshold (24), the individual threshold (inactivity to light and/or light to moderate to vigorous) was also used as a covariate in respective models. To assess the mediating effect of body composition on the results (excluding waist circumference as dependent variable), waist circumference was added as a covariate into model 2. The independent effects of muscle inactivity time from physical activity level were tested by adding moderate- to vigorous-intensity muscle activity as a covariate in addition to model 1 covariates (model 3). In addition, the effect of self-reported physical activity level was tested in model 3. Data are presented as standardized β coefficients to make the comparison of the different muscle inactivity and activity variables possible. For every model, residuals were normally distributed and homoscedastic. Finally, estimated marginal means between inactivity quartiles were analyzed to assess the effect sizes. Levene test was used to ensure the equality of variances between the quartiles. Statistical significance was set at $P < 0.05$. Statistical analyses were conducted with PASW version 20.0.

RESULTS

Table 1 reports the sex-specific sociodemographic, metabolic, and muscle-derived inactivity and activity characteristics.

TABLE 1. Sex-specific characteristics of study participants.

Characteristics	Women	Men	Total	*P* for Sex Difference
n	85	65	150	
Age (yr)	38.5 ± 11.4	39.3 ± 9.6	38.8 ± 10.6	0.304
Weight (kg)	63.0 ± 8.7	80.5 ± 12.2	70.6 ± 13.5	**<0.001**
Height (cm)	165.9 ± 6.0	178.8 ± 6.4	171.5 ± 8.9	**<0.001**
BMI ($kg \cdot m^{-2}$)	22.9 ± 2.8	25.1 ± 2.9	23.8 ± 3.1	**<0.001**
Self-reported physical activity ($MET \cdot h \cdot wk^{-1}$)	34.5 ± 29.1	35.0 ± 26.2	34.7 ± 27.8	0.429
Reaching recommended activity level (%)	74 (93)	58 (97)	132 (94)	0.293
University/further education (%)	59 (69)	38 (58)	97 (65)	0.164
Sedentary work (%)	71 (84)	52 (80)	123 (82)	
Physically active work (%)	10 (12)	9 (14)	19 (13)	0.849
Not working or unknown (%)	4 (5)	4 (6)	8 (5)	
Recording covariates				
No. days	1.6 ± 0.7	1.6 ± 0.7	1.6 ± 0.7	0.686
Recording time per day (h)	11.7 ± 1.3	12.1 ± 1.7	11.9 ± 1.5	0.294
No. channels	2.8 ± 1.1	2.9 ± 1.1	2.8 ± 1.1	0.697
Measured during winter	35 (41)	22 (34)	57 (38)	0.359
Sitting ($\%EMG_{MVC}$)	1.0 ± 1.0	0.9 ± 0.6	1.0 ± 0.9	0.428
Standing ($\%EMG_{MVC}$)	2.3 ± 1.8	2.2 ± 1.3	2.2 ± 1.6	0.610
Walking 5 $km \cdot h^{-1}$ ($\%EMG_{MVC}$)	7.6 ± 3.7	6.2 ± 3.0	7.0 ± 3.5	**0.011**
Muscle inactivity variables				
Muscle inactivity (% recording time)	62.9 ± 12.4	68.2 ± 13.0	65.2 ± 12.9	**0.014**
Sum of the five longest inactivity periods (min)	66.6 ± 27.6	69.1 ± 34.2	67.7 ± 30.5	0.995
Mean of inactivity period (s)	22.9 ± 8.8	25.7 ± 10.9	24.1 ± 9.8	0.181
Muscle activity variables				
Light-intensity muscle activity (%)	23 ± 10.9	19.1 ± 9.8	21.3 ± 10.6	**0.022**
Moderate-intensity muscle activity (%)	9.9 ± 5.0	9.2 ± 5.1	9.6 ± 5.0	0.368
Vigorous-intensity muscle activity (%)	4.2 ± 5.8	3.6 ± 5.1	4.0 ± 5.5	0.899
Mean amplitude ($\%EMG_{MVC}$)	3.3 ± 2.2	2.5 ± 1.7	2.9 ± 2.0	**0.001**
Mean muscle activity amplitude ($\%EMG_{MVC}$)	7.7 ± 3.9	6.6 ± 3.2	7.3 ± 3.6	**0.049**
No. bursts per minute	1.8 ± 0.5	1.8 ± 0.6	1.8 ± 0.5	0.723
Cardiometabolic biomarkers				
Waist (cm)	81.6 ± 9.6	90.5 ± 9.6	85.5 ± 10.5	**<0.001**
Systolic BP (mm Hg)	114.6 ±10.8	119.7 ± 9.9	116.8 ± 10.7	**0.001**
Diastolic BP (mm Hg)	72.4 ± 7.6	74.8 ± 6.9	73.4 ± 7.4	**0.027**
Blood pressure index[a]	93.5 ± 8.9	97.2 ± 8.0	95.1 ± 8.7	**0.002**
FPG (mM)	5.1 ± 0.4	5.4 ± 0.4	5.2 ± 0.4	**<0.001**
HDL (mM)	2.0 ± 0.5	1.6 ± 0.4	1.8 ± 0.5	**<0.001**
Triglycerides (mM)	0.9 ± 0.5	1.1 ± 0.5	0.9 ± 0.5	**0.001**

Data are means ± SD or *n* (%). Muscle inactivity time (percentage of measurement time at $<0.9\ EMG_{standing}$), light-intensity muscle activity time (percentage at $0.9\ EMG_{standing}$ − $<EMG_{5\ km \cdot h^{-1}}$ walking), moderate-intensity muscle activity time (percentage at $EMG_{5\ km \cdot h^{-1}}$ walking − $<2\ EMG_{5\ km \cdot h^{-1}}$ walking), and vigorous-intensity muscle activity time (percentage at $\geq 2\ EMG_{5\ km \cdot h^{-1}}$ walking). Boldface font denotes to significance at $P < 0.05$.

[a]Blood pressure index was calculated as follows: (systolic BP + diastolic BP) / 2.

BMI = body mass index; BP = blood pressure; FPG = fasting plasma glucose, HDL = high-density lipoprotein cholesterol.

Participants' age ranged from 20 to 76 yr and body mass index (BMI) ranged from 17 to 33 $kg \cdot m^{-2}$. On the basis of metabolic syndrome criteria (2), 37.3% of the participants had one elevated risk marker and 22.7% of the participants had two or more elevated risk markers, with men having a worse metabolic risk profile compared to women (Table 1). Only one of the participants was smoking. Compared to the included participants, the eligible participants having improper EMG did not differ in terms of sex (women; 57%/49%), age (38.9 ± 10.7 yr/37.1 ± 9.7 yr), BMI (23.8 ± 3.1 $kg \cdot m^{-2}$/24.3 ± 4.0 $kg \cdot m^{-2}$), waist circumference (85.5 ± 10.8 cm/88.1 ± 12.4 cm), or self-reported physical activity level (37.1 ± 36.0 $MET \cdot h \cdot wk^{-1}$/36.3 ± 27.4 $MET \cdot h \cdot wk^{-1}$), respectively.

Average EMG amplitudes during sitting, standing, and walking at 5 $km \cdot h^{-1}$ are presented in Table 1. EMG activity during standing still was 193.1% ± 284.6% higher ($P < 0.001$) than sitting at the breakfast table. Compared to sitting and standing, EMG activity during walking at 5 $km \cdot h^{-1}$ was 918% ± 844% ($P < 0.001$) and 348% ± 447% ($P < 0.001$) higher, respectively. Muscle inactivity and activity patterns were generally correlated with each other (Table 2), including high correlations between total muscle inactivity time and mean muscle inactivity period duration and muscle light-intensity activity time. Moderate- to vigorous-intensity muscle activity time was moderately correlated with total muscle inactivity time, weakly correlated with self-reported physical activity time, but not correlated with light-intensity muscle activity time (Table 2). In participants having more than one valid day, the total muscle inactivity time did not differ significantly between the days ($P = 0.27$) and was highly correlated (Pearson $r = 0.78$, $P < 0.001$).

Nearly all (94%) of the participants met the criteria of $\geq 7.5\ MET \cdot h \cdot wk^{-1}$ of physical activity at ≥3 METs intensity (self-reported physical activity). However, muscle inactivity time was 65% of the measurement time, and on average, less than 3% of the muscle's voluntary contractile capacity was used during the day, with men being more inactive and having lower mean amplitude (Table 1). The duration of the five longest inactivity periods was almost 70 min, and on average, the inactivity periods lasted 24 s in both sexes. When active, the mean muscle activity intensity was similar

TABLE 2. Pearson correlation coefficients for muscle inactivity and activity patterns, self-reported physical activity level, and age.

	Pearson Correlation Coefficient (*P* Value)							
	Muscle Inactivity Time	Mean Inactivity Period Duration	Sum of the Five Longest Inactivity Periods	Light-Intensity Muscle Activity Time	Moderate- to Vigorous-Intensity Muscle Activity Time	No. of Bursts per Minute	Average EMG	Self-reported Physical Activity
Mean inactivity period duration	**0.68 (<0.001)**							
Sum of the five longest inactivity periods	**0.57 (<0.001)**	**0.61 (<0.001)**						
Light-intensity muscle activity time	**−0.68 (<0.001)**	**−0.52 (<0.001)**	**−0.49 (<0.001)**					
Moderate- to vigorous-intensity muscle activity time	**−0.53 (<0.001)**	**−0.38 (<0.001)**	**−0.30 (<0.001)**	−0.06 (0.447)				
No. bursts per minute	**−0.17 (0.035)**	**−0.84 (<0.001)**	**−0.40 (<0.001)**	**0.19 (0.024)**	0.13 (0.116)			
Average EMG	**−0.60 (<0.001)**	**−0.46 (<0.001)**	**−0.30 (<0.001)**	**0.23 (0.004)**	**0.56 (<0.001)**	**0.18 (0.030)**		
Self-reported physical activity ($MET \cdot h \cdot wk^{-1}$)	0.01 (0.931)	0.01 (0.926)	0.10 (0.242)	−0.15 (0.084)	**0.22 (0.010)**	0.01 (0.918)	−0.04 (0.665)	
Age	−0.09 (0.289)	0.02 (0.788)	**0.16 (0.048)**	−0.01 (0.889)	0.11 (0.202)	−0.09 (0.277)	**0.30 (<0.001)**	0.07 (0.410)

Boldface font denotes to significance at $P < .05$.

to that measured during walking at 5 $km \cdot h^{-1}$. When active, women had greater EMG amplitude ($\%EMG_{MVC}$, $P < 0.05$) and had more light-intensity muscle activity ($P < 0.05$) than men did. Moderate- and vigorous-intensity muscle activity covered, on average, 10% and 4% of the days, respectively, in both sexes (Table 1 and Fig. 1).

In the adjusted linear regression model 1, the total muscle inactivity time was negatively associated with HDL (standardized $\beta = -0.16$ [−0.33 to 0.00], $P = 0.047$, adjusted $R^2 = 0.15$) and positively associated with triglycerides (standardized $\beta = 0.24$ [0.07 to 0.41], $P = 0.005$, adjusted $R^2 = 0.09$). These associations persisted after adjusting for waist circumference, and the association with triglycerides persisted after adjusting for moderate- to vigorous-intensity muscle activity time (Table 3). The mean muscle inactivity period duration was positively associated with triglycerides (standardized $\beta = 0.20$ [0.03 to 0.37], $P = 0.021$, adjusted $R^2 = 0.08$) and negatively associated with the blood pressure index (standardized $\beta = -0.18$ [−0.34 to −0.01], $P = 0.034$, adjusted $R^2 = 0.18$). The light-intensity muscle activity time was negatively associated with the FPG (standardized $\beta = -0.25$ [−0.45 to −0.05], $P = 0.016$, adjusted $R^2 = 0.19$) and positively with the blood pressure index (standardized $\beta = 0.28$ [0.08 to 0.48], $P = 0.006$, adjusted $R^2 = 0.19$). These associations remained significant after adjusting for waist circumference or moderate- to vigorous-intensity muscle activity time (Table 3). Further adjustment for self-reported physical activity removed the significance between mean muscle inactivity period and both blood pressure index and triglycerides without effect on other associations.

The average EMG amplitude was positively associated with the blood pressure index in model 1 (standardized $\beta = 0.18$ [0.02 to 0.34], $P = 0.032$, adjusted $R^2 = 0.18$), but adjusting for waist circumference or total muscle inactivity time (Table 3) attenuated the association. The average EMG amplitude was negatively associated with triglycerides (standardized $\beta = -0.17$ [−0.34 to 0.00], $P = 0.047$, adjusted $R^2 = 0.15$) and positively associated with HDL (standardized $\beta = 0.16$ [0.00 to 0.33], $P = 0.046$, adjusted $R^2 = 0.21$) independent of waist circumference. However, the average EMG amplitude was positively associated with FPG when adjusted for total muscle inactivity time (Table 3). While none of the muscle inactivity and activity variables were associated with waist circumference, the association between waist circumference and self-reported physical activity was negative with model 1 covariates (standardized $\beta = -0.26$ [−0.41 to −0.10], $P < 0.001$, adjusted $R^2 = 0.30$).

Figure 2 presents the means for waist, FPG, HDL, and triglycerides between the quartiles of total muscle inactivity time. Compared to those in the lowest total muscle inactivity quartile, those in the highest quartile had 0.32 mM lower HDL and 0.30 mM higher triglycerides (estimated marginal means analyzed for log-transformed triglycerides to meet equality of variances). In waist circumference (Fig. 2) and

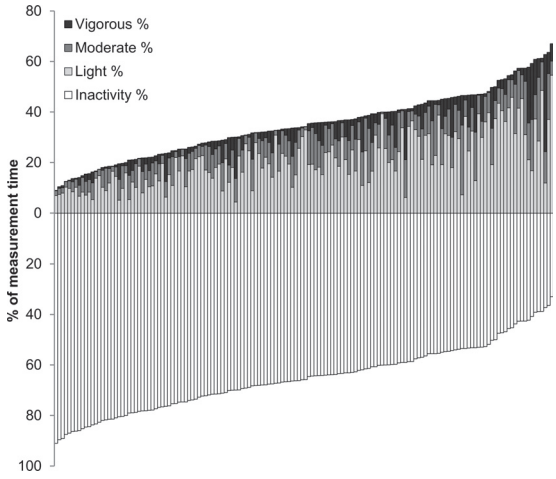

FIGURE 1—Individual distribution of muscle inactivity and light-, moderate-, and vigorous-intensity activities across the study population. Each *bar* sums up to 100% of measurement time.

EPIDEMIOLOGY

TABLE 3. Standardized regression coefficients of muscle inactivity and activity variables with continuous cardiometabolic risk markers in 150 individuals adjusted for baseline covariates and moderate- to vigorous-intensity [a]muscle activity time or [b]muscle inactivity time.

	Blood Pressure Index		Waist Circumference		FPG		HDL		Triglycerides	
	Standardized β (95% CI)	P	Standardized β (95% CI)	P	Standardized β (95% CI)	P	Standardized β (95% CI)	P	Standardized β (95% CI)	P
Muscle inactivity time[a]	−0.15 (−0.35 to 0.04)	0.122	−0.11 (−0.29 to 0.07)	0.236	0.14 (−0.05 to 0.34)	0.144	−0.16 (−0.35 to 0.04)	0.112	0.28 (0.08 to 0.48)	**0.006**
Mean inactivity period duration[a]	−0.18 (−0.35 to 0.00)	**0.049**	−0.13 (−0.30 to 0.03)	0.116	0.01 (−0.17 to 0.19)	0.946	0.00 (−0.18 to 0.18)	0.983	0.20 (0.02 to 0.39)	**0.033**
Sum of the five longest inactivity periods[a]	−0.08 (−0.26 to 0.11)	0.405	−0.16 (−0.34 to 0.01)	0.059	0.05 (−0.13 to 0.24)	0.562	0.03 (−0.15 to 0.22)	0.734	0.12 (−0.07 to 0.31)	0.226
Light-intensity muscle activity time[a]	0.27 (0.07 to 0.48)	**0.010**	0.15 (−0.05 to 0.34)	0.142	−0.25 (−0.46 to −0.05)	**0.017**	0.11 (−0.10 to 0.32)	0.307	−0.11 (−0.33 to 0.10)	0.302
Moderate- to vigorous-intensity muscle activity time[b]	0.00 (−0.21 to 0.21)	0.993	−0.14 (−0.34 to 0.07)	0.184	0.10 (−0.11 to 0.31)	0.366	0.06 (−0.15 to 0.28)	0.575	0.01 (−0.21 to 0.23)	0.938
No. bursts per minute[b]	0.10 (−0.06 to 0.27)	0.214	0.08 (−0.08 to 0.24)	0.330	0.09 (−0.08 to 0.25)	0.310	−0.11 (−0.28 to 0.06)	0.195	−0.06 (−0.24 to 0.11)	0.475
Average EMG[b]	0.15 (−0.06 to 0.35)	0.153	0.12 (−0.08 to 0.31)	0.246	0.27 (0.07 to 0.47)	**0.008**	0.06 (−0.15 to 0.27)	0.576	−0.01 (−0.22 to 0.21)	0.952

Boldface font denotes to significance at $P < .05$.
[a]Adjusted for baseline covariates and moderate- to vigorous-intensity muscle activity time.
[b]Adjusted for baseline covariates and muscle inactivity time.
FPG = fasting plasma glucose, HDL = high-density lipoprotein cholesterol.

blood pressure index, there were no differences between any of the total muscle inactivity quartiles.

DISCUSSION

One of the driving hypotheses of the sedentary behavior research is that the lack of the frequent engagement of the antigravity muscles, particularly the large muscles of the lower limbs, results in detrimental physiological processes and adverse cardiometabolic profile (13). However, the association between muscle inactivity time and cardiometabolic outcomes has not been shown with direct measures. To the best of our knowledge, this is the first study to examine this association by using EMG to measure muscular activity. We found that, in physically active people, the thigh muscles were inactive (on average at <2.0% of EMG_{MVC}) for 65% of the day. Between participants having high versus low total muscle inactivity time, there were clinically significant differences in HDL cholesterol and triglycerides independent of muscle's moderate- to vigorous-intensity activity time. Whereas the act of standing up increases muscle activity compared to the muscle activity during sitting (from 1.0% to 2.2% of EMG_{MVC}), these results give further support for the message "stand up, sit less, move more" (7) to promote metabolic health as a complement to current exercise guidelines.

These findings are consistent with previous research showing independent associations of total sedentary time and breaks in sedentary time with cardiometabolic outcomes independent of moderate- to vigorous-intensity physical activity in a nonclinical population in cross-sectional (14,15,17,19) and longitudinal designs (20,37). However, participants in these previous studies have been, on average, older and had bigger waist circumference compared to the present sample, suggesting that these associations can be seen already in young, healthy and physically active participants. Another main difference is the method used to assess sedentary behavior. Conventionally, sedentary time is measured as a lack of impacts (accelerometer) or as a systemic response to movement (heart rate) or as a postural difference over a certain period. In the present study, however, sedentary time was defined as a lack of any muscular activity in major locomotor muscles, providing a measure that is the primary source for the outcomes measured with the conventional methods. Whereas the contemporary definition of sedentary time is based on the measures of posture and energy expenditure (31), the actual proposed mechanism for adverse metabolic changes is the lack of muscle activity (13). Although the energy expenditure of standing still is only ~10% higher as compared to sitting (25), the thigh muscle activity is increased by ~190% to 2.2% ± 1.6% of EMG_{MVC} by this simple postural change as demonstrated by the present study. Yet, it should be kept in mind that muscles can be activated occasionally also during sitting and lying down. Activities performed at very low energy expenditure may thus contribute substantially to the actual underlying

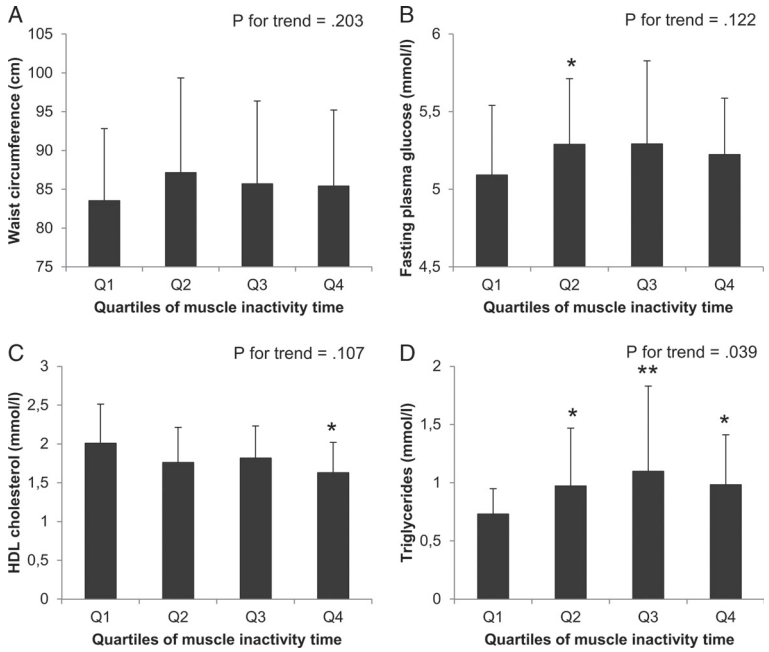

FIGURE 2—Means of waist circumference (A), FPG (B), HDL (C), and triglycerides (D) between the quartiles of muscle inactivity time. Cut points for quartiles were 55.5%, 65.8%, and 74.8%. Models were adjusted for age, sex, education, smoking, winter/summer, number of days, recording time, number of channels, inactivity threshold, and moderate- to vigorous-intensity muscle activity. For triglycerides, the statistical tests were performed for log-transformed data to avoid heterogeneity of variances, but the nontransformed data are presented here.

mechanisms of substrate utilization and insulin resistance and are even linked to reduced mortality (22). The measurement of muscle activity provides a method to directly assess this primary contributor.

While breaking up accelerometer-derived prolonged inactivity periods are proposed to be of particular importance (14), it is interesting that there were no significant associations between the duration of the five longest EMG-derived inactivity periods and any of the biomarkers. However, the present data show that it is the mean, rather than the longest, muscle inactivity period and the total muscle inactivity time that were adversely associated with some biomarkers. While the biological plausibility of reduced sedentariness on metabolic health has gained further support from experimental studies, showing improved insulin sensitivity, glycemic control, and lipid metabolism with frequent ambulatory activities that reduced total sedentary time (6,8,29), the effect of different durations and intensities of breaks independent of the total duration of activity warrants further study. In essence, the definition of "break" might be very different depending on the measure used. In the present study, the inactivity periods measured directly from the muscles were lasting, on average, only less than 30 s, being markedly shorter than the accelerometer-derived sedentary periods (~5.7 min) (14). Although we used 90% of standing EMG activity as the inactivity threshold, the short mean duration of inactivity periods implies that many of the muscle inactivity breaks occurred while participants were sitting. Measuring muscle activity might thus give new insights for sedentary behavior research beyond the effects of impact- or posture-derived breaks. However, longitudinal research is needed to confirm whether the minimal reduction in total or uninterrupted muscle inactivity time yields clinically significant outcomes in the long term.

From a behavioral point of view, very simple strategies have the potential to affect the balance between muscle inactivity and activity. In the present study, the differences in triglycerides and HDL between the lowest and highest quartiles of muscle inactivity time were clinically significant, with approximately 2-h difference in total muscle inactivity time. A workplace randomized controlled trial was able to induce an effect of this magnitude to accelerometer-derived sedentary time through a combination of strategies (18). In addition, a recent randomized controlled trial using behavioral strategies was able to reduce total muscle inactivity time by ~30 $\text{min}\cdot\text{d}^{-1}$ while the average muscle loading remained <3% of muscle maximal voluntary contractile capacity (30). While a reduction in muscle inactivity of this magnitude may already promote metabolic health (6,29), the extremely low muscular effort required makes the execution of this change safe and feasible across numerous settings.

While it is well known that moderate physical activity reduces blood pressure (21), the present data showed that light-intensity muscle activity time was positively associated

with the blood pressure index, independent of moderate- to vigorous-intensity physical activity. However, the negligible differences between the inactivity quartiles at the blood pressure index were neither statistically nor clinically significant (data not shown).

The main limitation of the study is the short measurement period. The use of the present EMG method enabled measurement of only 1 d in a row because of memory limitations and a relatively challenging setup. This resulted in an average of 1.6 valid days (range = 1–3 valid days) per participant, whereas 3–5 d have been suggested to give a reliable estimate of habitual physical activity measured by accelerometers (36). To counteract this limitation, the participants were asked to select a typical workday to be measured. Self-reported abnormal days were not included. Because it is suggested that the activity of people having sedentary occupations (82% of the sample) is highly consistent across days (3) and the repeated measurements were highly correlated, we believe that the present data represent a typical sedentary time and habitual physical activity behavior measured directly from the main locomotor muscles. Previous long-term EMG recordings of habitual physical activity have used measurements of similar or even shorter length compared to those used in the present study (23,24,28).

However, given the varied and periodical nature of exercise behavior, the short measurement time might be a reason for the nonsignificant associations between moderate- to vigorous-intensity muscle activity time and cardiometabolic biomarkers. To fully explore the independence of muscle inactivity time from exercise, the measurement period should be long enough to capture the whole range of these behaviors. This limitation was counteracted by using self-reported physical activity levels in addition to objectively measured data, which was the only independent variable associated with waist circumference. The use of long-term exercise as a covariate did not affect the associations between muscle inactivity time and triglycerides and between light-intensity muscle activity time and FPG, supporting the independence of these associations.

Another important limitation is the lack of dietary data. Although some experimental evidence has pointed out energy balance–independent mechanisms of sedentary time on cardiometabolic outcomes (32), the quality of diet needs to be assessed in future studies. In addition, a larger sample size is required to enable stratification by sex, age, etc., to find out the differences between these categories. Although sedentary time may increase at a later age (26), total muscle inactivity time was not correlated with age at the present sample, suggesting its limited influence on the observed findings.

An essential concept in sedentary behavior research is the balance between muscle inactivity and activity. By directly measuring muscle EMG, we examined the associations between the proposed trigger for metabolic changes, muscle inactivity time, and cardiometabolic outcomes. Even in healthy, physically active people, the thigh muscles were inactive (on average at <2.0% of EMG_{MVC}) for 65% of the day. Total muscle inactivity time was adversely associated with some cardiometabolic biomarkers, with clinically significant differences in HDL cholesterol and triglycerides, independent of the muscle's moderate- to vigorous-intensity activity time. Future interventions aiming at the reallocation of sedentary time to activities of different muscular exposure may benefit from these results, specifically among physically active people.

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The authors have no financial conflict of interest to declare.

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III

MUSCLE INACTIVITY AND ACTIVITY PATTERNS AFTER SEDENTARY-TIME TARGETED RANDOMIZED CONTROLLED TRIAL

by

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EPIDEMIOLOGY

Muscle Inactivity and Activity Patterns after Sedentary Time-Targeted Randomized Controlled Trial

ARTO J. PESOLA[1], ARTO LAUKKANEN[1,2], PIIA HAAKANA[1], MARKO HAVU[1], ARJA SÄÄKSLAHTI[2], SARIANNA SIPILÄ[3], and TAIJA FINNI[1]

[1]*Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, FINLAND;* [2]*Department of Sport Sciences, University of Jyväskylä, Jyväskylä, FINLAND; and* [3]*Gerontology Research Center, Department of Health Sciences, University of Jyväskylä, Jyväskylä, FINLAND*

ABSTRACT

PESOLA, A. J., A. LAUKKANEN, P. HAAKANA, M. HAVU, A. SÄÄKSLAHTI, S. SIPILÄ, and T. FINNI. Muscle Inactivity and Activity Patterns after Sedentary Time-Targeted Randomized Controlled Trial. *Med. Sci. Sports Exerc.*, Vol. 46, No. 11, pp. 2122–2131, 2014. **Purpose**: Interventions targeting sedentary time are needed. We used detailed EMG recordings to study the short-term effectiveness of simple sedentary time-targeted tailored counseling on the total physical activity spectrum. **Methods**: This cluster randomized controlled trial was conducted between 2011 and 2013 (InPact, ISRCTN28668090), and short-term effectiveness of counseling is reported in the present study. A total of 133 office workers volunteered to participate, from which muscle activity data were analyzed from 48 (intervention, $n = 24$; control, $n = 24$). After a lecture, face-to-face tailored counseling was used to set contractually binding goals regarding breaking up sitting periods and increasing family based physical activity. Primary outcome measures were assessed 11.8 ± 1.1 h before and a maximum of 2 wk after counseling including quadriceps and hamstring muscle inactivity time, sum of the five longest muscle inactivity periods, and light muscle activity time during work, commute, and leisure time. **Results**: Compared with those in the controls, counseling decreased the intervention group's muscle inactivity time by 32.6 ± 71.8 min from 69.1% ± 8.5% to 64.6% ± 10.9% (whole day, $P < 0.05$; work, $P < 0.05$; leisure, $P < 0.05$) and the sum of the five longest inactivity periods from 35.6 ± 14.8 to 29.7 ± 10.1 min (whole day, $P < 0.05$; leisure, $P < 0.01$). Concomitantly, light muscle activity time increased by 20.6 ± 52.6 min, from 22.2% ± 7.9% to 25.0% ± 9.7% (whole day, $P < 0.05$; work, $P < 0.01$; leisure, $P < 0.05$), and during work time, average EMG amplitude (percentage of EMG during maximal voluntary isometric contraction (MVC) ($\%EMG_{MVC}$)) increased from 1.6% ± 0.9% to 1.8% ± 1.0% ($P < 0.05$) in the intervention group compared with that in the controls. **Conclusions**: A simple tailored counseling was able to reduce muscle inactivity time by 33 min, which was reallocated to 21 min of light muscle activity. During work time, average EMG amplitude increased by 13%, reaching an average of 1.8% of EMG_{MVC}. If maintained, this observed short-term effect may have health-benefiting consequences. **Key Words:** SEDENTARY TIME, NONEXERCISE PHYSICAL ACTIVITY, ELECTROMYOGRAPHY, TEXTILE ELECTRODES, INTERVENTION

Physical activity patterns illustrating typical daily life of modern people include a large proportion of sitting with relatively idle muscles, whereas the other dominant part consists of nonexercise physical activity, and only a fraction of the day can be categorized as more intense exercise (18). The sedentary part of this pattern has been recognized as an independent predictor of adverse health outcomes (11) even in people doing regular moderate-to-vigorous physical activity (25). The underlying cause of the independence of exercise and inactivity may be the different metabolic pathways that have been identified to convey the effects of inactivity compared with those of exercise (5). Similarly, muscle activities required for standing and walking slowly have been found to improve postprandial glucose and insulin responses (12) and to prevent the effects of complete inactivity (5,35,40), which emphasizes the importance of nonexercise activities. The driving concept behind these findings is the so-called inactivity physiology paradigm, which states that "the brief, yet frequent, muscular contraction throughout the day may be necessary to short-circuit unhealthy molecular signals causing metabolic diseases"

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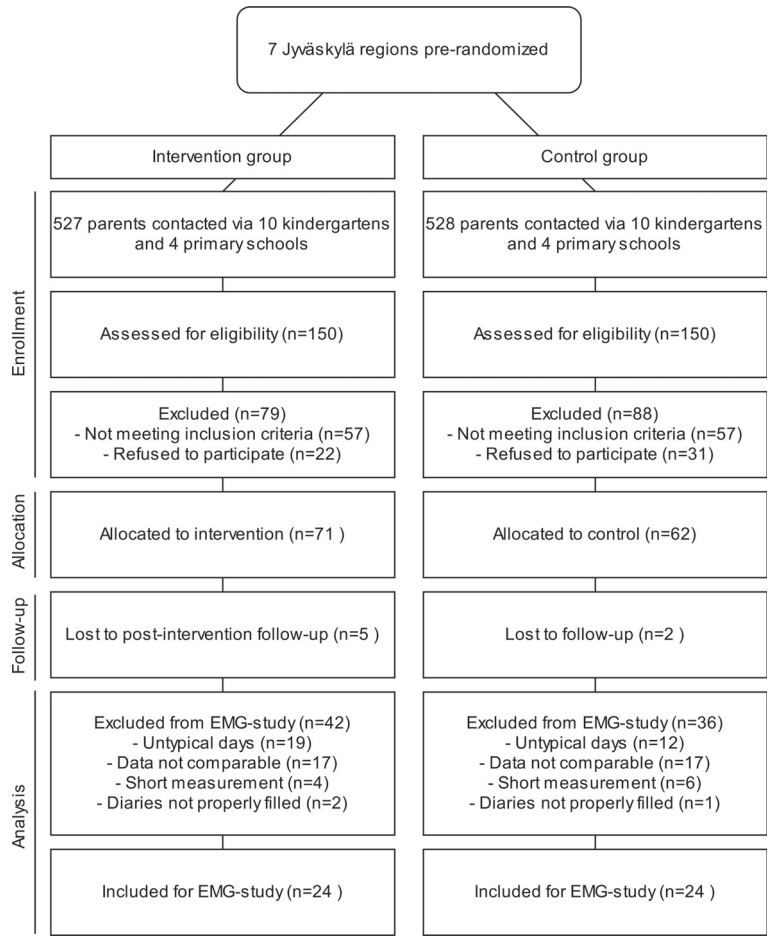

FIGURE 1—Randomization and recruitment procedure for EMG analysis in the present InPact project.

(18). Therefore, understanding and improving this typical physical activity pattern of modern people requires quantification of the whole physical activity continuum with careful evaluation of the balance between the two most dominant components: nonexercise physical activity and sedentary time, which in this article is defined as a lack of any muscular activity of the locomotor muscles.

In addition to different metabolic pathways, exercise and inactivity have been shown to be independent factors of behavior (6,14,27), which highlights the need for promotion of reduced sitting in addition to traditional exercise guidelines. Despite a promising hypothesis, there is paucity of randomized controlled trials (RCT) that have assessed sedentary time instead of physical activity as a primary outcome. In addition, workplace interventions promoting increased physical activity through mixed behavioral approaches have been shown to be ineffective at decreasing self-reported sitting time (7). Because of the potential health benefits of replacing sitting with light-intensity activities, promotion of this small change as a primary intervention goal could be an accessible, viable, and effective method for busy and sedentary target groups including people in sedentary occupations (38) and parents of young children (31).

To gain further insight into interventions targeting sedentary time, the effect of behavioral change needs to be studied across the whole physical activity spectrum with objective measures. Given that the key mechanism proposed for the associations of sedentary time with health is lack of muscular activity, it is important to measure the changes in this outcome. By using novel wearable textile electrodes, it is possible to measure muscle activity from the main locomotor muscles with similar or even better repeatability compared with that in traditional bipolar electrodes (15) across the whole physical activity spectrum (36).

The purpose of this study was to examine whether tailored counseling designed to reduce and break up sedentary time decreases muscle inactivity and increases muscle activity as measured by EMG from the quadriceps and hamstring

muscles, which are some of the main locomotor muscles. We hypothesized that this specific counseling would decrease muscle inactivity time without increasing moderate-to-vigorous muscle activity.

METHODS

As a part of a year-long RCT "A Family Based Tailored Counseling to Increase Nonexercise Physical Activity in Adults with a Sedentary Job and Physical Activity in Their Young Children" (InPact project, (16)), this study investigated the short-term (within 2 wk of the counseling) main outcomes of the RCT. The study was approved by the ethics committee of the Central Hospital, District of Central Finland, on March 25, 2011 (Dnro 6U/2011), and the participants signed an informed consent before measurements.

Recruitment and study sample. Sampling was performed in the Jyväskylä region located in central Finland, with a population of 133,000. Jyväskylä has a surface area of 1171 km^2 with a relatively small city center, is near lakes and forests, and has numerous opportunities for active commuting with an extensive network of bike paths and sidewalks in the city region. Homogeneous regions around the city were identified in terms of socioeconomic status and environmental possibilities for outdoor physical activities, and cluster randomization was done within these regions. These seven regions included eight schools and 20 kindergartens (2–5 schools or kindergartens per region). The recruitment was done in three phases, where recruitment forms asking profession, percentage of sitting time at work, health status, and contact information were delivered to parents via kindergartens and primary schools in spring 2011, autumn 2011, and spring 2012. In total, 1055 recruitment forms were delivered including information about the study, inclusion and exclusion criteria, and an incentive to get diverse information about personal health, diet and physical activity, and motor skills of their children. Inclusion criteria were as follows: healthy men and women with children 3–8 yr old, parental occupation where they self-reportedly sat more than 50% of their work time, and children in all-day day care in kindergarten or in the first grade of primary school. Exclusion criteria were as follows: body mass index >35 $kg \cdot m^{-2}$, self-reported chronic diseases, families with a pregnant mother at baseline, children with disorders that delay motor development, and concurrent participation in another study. No monetary incentive was offered to the participants.

We received 300 responses. People fulfilling the criteria were contacted by phone and invited to an information lecture, where the procedures were explained in detail. If people were unable to attend the lecture, details of the project were explained on the phone. Finally, 133 participants were measured at baseline. Figure 1 summarizes the recruitment and randomization process.

Of the 133 participants, 48 were selected for the EMG analysis on the basis of the following criteria: 1) measured days were self-reportedly typical and identical in terms of occupational tasks, workday duration, and leisure time activities (31 excluded), 2) both days included artifact-free EMG signal from the same muscles recorded with the same electrodes (34 excluded), 3) length of measurement >9 h (10 excluded), and 4) diaries were returned properly filled (three excluded). In addition, seven participants dropped out before the second measurement day. The final study sample included 24 participants in the intervention group and 24 in the control group.

Study protocol. Muscle activity from the quadriceps and hamstring muscles was recorded during a structured laboratory test protocol and during daily measurements from two workdays before and after the counseling intervention. The participants were asked to select two measurement days that were as similar and typical as possible in terms of working schedule and duties. On the first day, the participants' height and weight were measured after arriving at the laboratory in the morning. Subsequently, EMG shorts (Myontec Ltd., Kuopio, Finland) were put on. To measure the minimum level of EMG, the laboratory test protocol began by asking the participants to sit in front of a table while informing them about the diaries and questionnaires to be filled in. After the sitting period, a treadmill (OJK-1; Telineyhtymä, Kotka, Finland) protocol including walking at 5, 6, and 7 $km \cdot h^{-1}$ and running at 10 $km \cdot h^{-1}$ with 1-min steps was performed. Next, muscle activity was measured while standing still, while standing on each leg individually, and while walking up and down the stairs twice. Standing in different positions was measured for 15 s per task, and participants were asked to stand still as they usually do, except that the weight was first supported by both legs and then by one leg at a time. For the stair walking, the participants were instructed to step on every step and to use their normal pace. EMG amplitudes (percentage of EMG during maximal voluntary isometric contractions (MVC) ($\%EMG_{MVC}$)) from these tests are presented in Figure 2. Participants then performed bilateral MVC in a knee extension/flexion machine (David 220; David Health Solutions, Helsinki, Finland) with a 140° knee angle in both flexion and extension. After thorough familiarization and warm-up, at least three 3- to 5-s maximal efforts with strong verbal encouragement were performed with 1-min rest periods between trials. If torque improved by more than 5% in the last trial, more trials were performed.

After the laboratory experiments, the participants left for work and were expected to continue normal living while wearing the shorts and keeping a diary of commuting, working, and leisure time. Any abnormal tasks and behaviors (e.g., abnormal working tasks, working time, or leisure activities) were to be reported to include only structurally similar days for analysis. After baseline measurements, the intervention group received tailored counseling. The postintervention measurements were performed within 2 wk of the counseling session.

Description of intervention. The intervention was designed on the basis of previous knowledge of effective

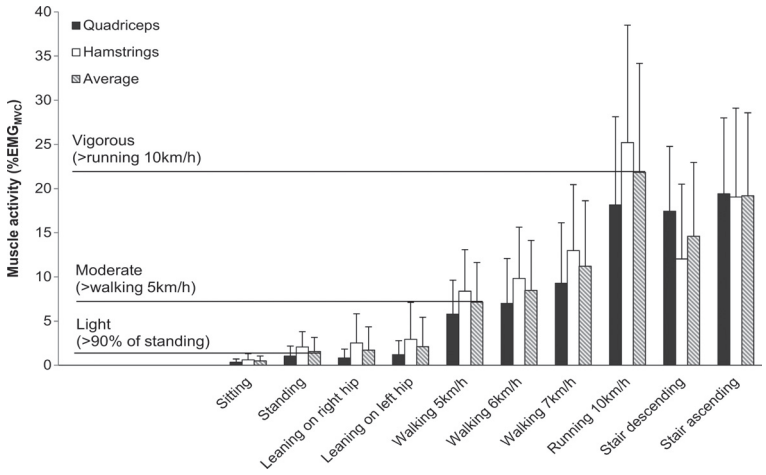

FIGURE 2—Mean and SD of %EMG$_{MVC}$ measured during various laboratory tests. The *horizontal lines* show thresholds for intensity classification, which was done individually for each participant.

interventions (9) and theory of planned behavior (1). Briefly, the intervention consisted of a common 30-min lecture for a maximum of six participants at a time followed by face-to-face discussions with the researchers (16). The lecture included research-based information about health hazards of prolonged sitting and encouragement to incorporate even the smallest physical activities into everyday routines to overcome these health problems. In the face-to-face discussions, the participants were first asked to describe their normal daily routines during commuting, working hours, and leisure time. Regarding leisure time, routines of the entire family were discussed because they are relevant to the individual's behavior. During the discussion, participants were encouraged to think of feasible ways to reduce long sitting periods, to increase nonexercise physical activity, and to increase family-based activities from their personal premises, accompanied by ideas from the researcher. The participants set small-step goals, which were written into a contract signed by the researcher and the participant. An example from the contract of one participant is as follows:

My goals to decrease sitting time and to increase nonexercise physical activity during working time are:

- *I stand up from my chair every half an hour;*
- *When answering the phone, I stand up from the chair;*
- *Instead of calling, I walk to my colleague's room;*
- *I take the stairs instead of the elevator; and*
- *I walk for lunch and once a week choose a restaurant that is farther away.*

Mine and my family's goals to decrease sitting time and to increase physical activity during leisure time are:

- *At least once a day, we go out as a family in order to replace family sitting activity;*
- *We cycle to work whenever the weather permits us to do so;*
- *Instead of taking the car, we walk or bicycle to the grocery shop more often as a family;*
- *We organize family dancing sessions; and*
- *We will work hard with snow removal, using child labour together with us ☺.*

The goals related to occupational tasks and leisure time activities were printed for participants to place them in a visible location at home and at work. In addition, participants were given a material about simple break exercises, local outdoor activities, and simple games suitable for the whole family. The materials were gathered to a project web page (perheliikunta.nettisivu.org), from which the participants were encouraged to find the relevant information.

Assessment of outcomes. The primary study outcomes were EMG-derived muscle inactivity time, duration of the five longest inactivity periods, and light muscle activity time assessed during working time, commute, and leisure time. The domains were separated on the basis of diaries. Because differences in EMG wear time may affect the time spent at different physical activity intensities (20), an equal recording time was analyzed for both measurements on the basis of the shorter measurement.

EMG recordings. EMG was measured with shorts made of knitted fabric similar to elastic clothes used for sport activities or functional underwear, with the exception of the capability to measure EMG from the skin surface of the quadriceps and hamstring muscles (Myontec Ltd., Kuopio and Suunto Ltd., Vantaa, Finland). Bipolar electrode pairs are located on the distal part of the quadriceps and hamstrings, and the reference electrodes are located longitudinally along the left and right lateral sides (over the tractus iliotibialis). The EMG signal was stored in a 50-g electronic module attached to the waist. In this study, eight pairs of shorts (four different sizes) were used. Electrode

paste (Redux Electrolyte Crème; Parker, Inc.) was used on the electrode surfaces to improve and stabilize conductivity between the skin and electrodes. After every measurement day, the shorts were washed after detaching the electronics module. The EMG shorts have been tested for validity, repeatability, and feasibility in our laboratory, and detailed descriptions of the recording devices and analysis software have been reported previously (15,36).

Signal processing and categorizing. The individual channels from the right and left quadriceps and hamstring muscles were normalized to EMG amplitude measured during bilateral MVC. The repetition with the highest force level was chosen, from which the most consistent 1-s mean EMG amplitude was used for each channel. To reflect the overall inactivity or activity of thigh muscles, the normalized channels from the quadriceps and hamstring muscles were averaged. The threshold between inactivity and light activity was set individually at 90% of the mean $\%EMG_{MVC}$ measured while standing still for 15 s in the laboratory (Fig. 2). This approach enabled determination of inactivity periods in the main locomotor muscles. The thresholds between light and moderate and moderate and vigorous muscle activity intensities were defined individually as a 1-min mean EMG value when walking at 5 $km \cdot h^{-1}$ and running at 10 $km \cdot h^{-1}$, respectively. Because some of the participants reported being unfamiliar with walking and running on a treadmill and MVC increased on the second laboratory test ($P < 0.001$), the values from the second measurement were used for both days to minimize the effect of learning on the thresholds. Adequate repeatability of the EMG–force relation ($0.74 \leq ICC \leq 0.93$) (see table, Supplemental Digital Content 1, http://links.lww.com/MSS/A387: supplemental Table 4—Repeatability of MVC) ensured the consistency of EMG signals between days. Detailed descriptions of signal processing, artifact removal, and MATLAB analysis procedures are presented in Supplemental Digital Contents 1, http://links.lww.com/MSS/A387, and 2, http://links.lww.com/MSS/A388 (additional details on EMG analysis procedures and EMG channel averaging and baseline correction, respectively).

Statistical analyses. The initial sample size calculations for the entire intervention have been reported previously (16), and for this particular sample of EMG study, the calculated *post hoc* statistical powers and effect sizes (eta squared, η^2) for the outcomes are reported. Effect sizes can be interpreted as follows: small, >0.01; medium, >0.06; and large, >0.14. Statistical analyses were performed with PASW Statistics version 18.0 (SPSS Inc., Chicago, Ill). Data are presented as mean ± SD. The Shapiro–Wilk test was used to evaluate whether the data were normally distributed. Where data were not normally distributed, log transformation was used, and normality was retested. Differences between the groups at baseline were tested with independent samples *t*-test, the Mann–Whitney test, or chi-square test. The effect of the intervention on EMG variables was assessed using repeated-measures ANOVA, with measurement time and baseline values of variables as covariates. Not normally distributed variables (total and leisure time average EMG and leisure time vigorous muscle activity time) were tested with the Mann–Whitney test by comparing within-group changes (postvalues − prevalues) between the groups; after which, within-group changes were tested with the Wilcoxon test. The differences between percentages of inactivity and activity time before and after the intervention were calculated as the arithmetic difference (percentage of measurement time after − percentage of measurement time before), yielding a percentage point (pp). Significance level was set at $P < 0.05$.

RESULTS

Participants. The study groups were comparable in terms of anthropometry, profession, weekly work time, and self-reported sitting at work (Table 1). There were no significant differences between the participants in the EMG study as compared with the remaining InPact study sample

TABLE 1. Basic characteristics of the subjects.

	Intervention			Control			All
	All (n = 24)	Female (n = 15)	Male (n = 9)	All (n = 24)	Female (n = 13)	Male (n = 11)	n = 48
Anthropometrics							
Age (yr)	37.0 ± 5.5	34.8 ± 4.0	40.7 ± 6.2	39.0 ± 5.4	38.2 ± 5.9	40.1 ± 4.8	38.0 ± 5.5
Height (cm)	171.1 ± 10.3	165.3 ± 7.0	180.8 ± 7.1	171.1 ± 9.0	165.2 ± 6.1	178.2 ± 6.3	171.1 ± 9.5
Weight (kg)	73.2 ± 17.6	63.2 ± 8.3	90.0 ± 16.4	71.9 ± 13.8	64.6 ± 9.3	80.4 ± 13.5	72.6 ± 15.7
BMI ($kg \cdot m^{-2}$)	24.7 ± 3.7	23.1 ± 3.0	27.4 ± 3.4	24.5 ± 3.9	23.6 ± 2.6	25.4 ± 4.9	24.6 ± 3.7
MVC extension (kg)	78.0 ± 25.5	63.9 ± 14.7	101.6 ± 22.4	77.9 ± 22.6	65.4 ± 14.9	92.6 ± 21.5	77.9 ± 23.8
Professional group, n (%)							
Employee	6 (25)	6 (40)	0 (0)	5 (21)	3 (23)	2 (18)	11 (23)
Official	1 (4)	0 (0)	1 (11)	4 (17)	3 (23)	1 (9)	5 (10)
Managerial employee	13 (54)	7 (47)	6 (67)	13 (54)	5 (38)	8 (73)	26 (54)
Entrepreneur	2 (8)	1 (7)	1 (11)	0 (0)	0 (0)	0 (0)	2 (4)
Undefined	2 (8)	1 (7)	1 (11)	2 (8)	2 (15)	0 (0)	4 (8)
Work time per week, n (%)							
<34 h	4 (17)	4 (27)	0 (0)	1 (4)	1 (8)	0 (0)	5 (10)
35–39 h	12 (50)	8 (53)	4 (44)	11 (46)	5 (38)	6 (55)	23 (48)
≥40 h	8 (33)	3 (20)	5 (56)	12 (50)	7 (54)	5 (45)	20 (42)
Self-reported sitting at work (%)	80.8 ± 14.4	80.2 ± 15.4	82.1 ± 12.9	84.5 ± 12.4	82.4 ± 10.6	86.7 ± 14.3	82.7 ± 13.4

There were no significant differences within the genders between the groups and between the group means.
BMI, body mass index.

(females EMG study: 58%, age = 38.0 ± 5.5 yr, BMI = 24.6 ± 3.7 $kg \cdot m^{-2}$, managerial employees = 54%, work time per week = 37.6 ± 5.6 h, self-reported sitting at work = 82.7% ± 13.4%; females InPact study: 54%, age = 37.9 ± 5.3 yr, BMI = 24.4 ± 3.8 $kg \cdot m^{-2}$, managerial employees = 41%, work time per week = 38.0 ± 14.7 h, self-reported sitting at work = 85.8% ± 12.5%). As compared with the recruitment region's mean, a higher proportion of InPact study participants had university education (35% vs 71%, respectively).

Recording time. The total recording time was 11.8 ± 1.1 h on both days. The duration of work time increased from 5.9 ± 1.2 to 6.7 ± 1.0 h ($P < 0.001$), whereas the duration of leisure time decreased from 5.0 ± 1.3 to 4.2 ± 1.2 h ($P < 0.001$), with no differences between the groups. The commuting time was 0.9 ± 0.4 h on both days.

Baseline observations. At baseline, there were no differences between the groups in any of the muscle inactivity variables. Detailed group, gender, and domain-specific baseline values are presented at Supplemental Digital Content 1, http://links.lww.com/MSS/A387, supplemental Table 1. Both groups were inactive for an average of 69.1% ± 11.1% of the whole day, and the sum of the five longest inactivity periods was, on average, 36.7 ± 16.0 min. During working hours, an average 78.6% ± 10.8% of signals fell below the inactivity threshold and the duration of the five longest inactivity periods averaged 31.7 ± 16.0 min. During commuting and leisure time, muscle inactivity times were, on average, 44.3% ± 21.6% and 61.6% ± 15.6% of measurement time, and the durations of the five longest inactivity periods were, on average, 7.8 ± 6.8 and 25.6 ± 12.0 min, respectively.

At baseline, light muscle activity covered 21.9% ± 10.0% of the whole day, with values of 16.2% ± 9.3% during work, 32.8% ± 14.0% during commuting, and 27.5% ± 13.6% during leisure time. Less than 8% (7.4% ± 3.2%) of the whole day involved moderate muscle activity, consisting of 4.5% ± 2.7%, 19.1% ± 12.8%, and 8.7% ± 4.5% during work, commuting, and leisure time, respectively. On average, only 1.5% ± 2.6% of the whole day included vigorous muscle activity. The lowest value, 0.7% ± 1.0%, was measured during work time, whereas during commuting and leisure time, the vigorous muscle activity times were 3.9% ± 5.4% and 2.2% ± 5.1%, respectively. The only difference between the groups at baseline was the greater amount of moderate muscle activity during commuting time among the controls (24.1% ± 14.5%) compared with that in the participants in the intervention group (14.1% ± 8.4%, $P < 0.05$).

At baseline, $\%EMG_{MVC}$ was, on average, 2.4% ± 1.6% of EMG_{MVC} during the whole day, with a value of only 1.5% ± 0.8% of EMG_{MVC} measured during working hours. During commuting and leisure time, the $\%EMG_{MVC}$ were 4.7% ± 2.9% and 3.0% ± 3.1% of EMG_{MVC}, respectively. Both groups had, on average, 23.4 ± 14.9 muscle activity bursts per minute during the whole day. During work time, commuting, and leisure time, the number of bursts per minute was 19.4 ± 14.3, 31.3 ± 25.1, and 27.2 ± 17.7, respectively. At baseline, there were no differences between the groups in either $\%EMG_{MVC}$ or the number of bursts per minute.

Intervention effects. Table 2 and Figures 3 and 4 summarize the effects of intervention on EMG inactivity and EMG activity variables in the intervention group compared with those in the control group. During the whole day, muscle inactivity time ($P < 0.05$, power = 0.54, $\eta^2 = 0.09$) and the sum of the five longest muscle inactivity periods ($P < 0.05$, power = 0.61, $\eta^2 = 0.11$) decreased with concomitant increases in light muscle activity time ($P < 0.05$, power = 0.63, $\eta^2 = 0.11$) and the number of bursts per minute ($P < 0.05$, power = 0.61, $\eta^2 = 0.11$) in the intervention group compared with those in the controls. Despite the significant group–time interaction, the number of bursts per minute did not change significantly

TABLE 2. Changes of EMG inactivity and activity variables in intervention and control groups in different domains.

		Total		Work		Commute		Leisure	
		Difference	Group–Time *P*	Difference	Group–Time *P*	Difference	Group–Time *P*	Difference	Group–Time *P*
EMG inactivity									
Muscle inactivity time (pp)	Int	**−4.5 ± 9.7***	**0.042**	**−4.6 ± 6.9****	**0.023**	1.5 ± 17.4	0.08	**−9.3 ± 18.5****	**0.015**
	Cont	0.7 ± 8.0		0.3 ± 7.1		−4.6 ± 16.4		−0.7 ± 17.6	
Sum of the five longest muscle inactivity periods (min)	Int	**−5.8 ± 9.9***	**0.027**	−3.7 ± 9.1	0.12	−0.7 ± 5.1	**0.005**	**−8.2 ± 11.8*****	**0.005**
	Cont	4.7 ± 17.7		0.5 ± 11.7		**−3.1 ± 5.6*****		5.9 ± 15.3	
EMG activity									
Light muscle activity time (pp)	Int	**2.8 ± 7.2***	**0.023**	**3.7 ± 6.0*****	**0.008**	−3.0 ± 11.8	0.12	**4.2 ± 11.5***	**0.022**
	Cont	−1.3 ± 5.1		−0.4 ± 5.4		2.9 ± 7.3		−1.4 ± 12.7	
Moderate muscle activity time (pp)	Int	**1.4 ± 3***	0.57	0.9 ± 2.0	0.42	0.8 ± 5.5	0.08	**4.1 ± 6.5*****	0.08
	Cont	0.7 ± 3.3		0.1 ± 2.7		**1.8 ± 10.8***		2.1 ± 6.1	
Vigorous muscle activity time (pp)	Int	0.2 ± 2.2	0.84	−0.1 ± 0.9	0.38	0.7 ± 3.8	0.29	1.0 ± 5.0	0.74
	Cont	−0.1 ± 2.3		0.0 ± 0.6		**−0.1 ± 4.4***		0.0 ± 5.5	
$\%EMG_{MVC}$	Int	0.6 ± 2.2	0.19	**0.3 ± 0.6***	**0.045**	0.5 ± 2.3	0.60	1.6 ± 5.5*	0.07
	Cont	−0.1 ± 1.6		0.0 ± 0.5		0.1 ± 2.3		0.0 ± 3.9	
No. of bursts per minute	Int	2.3 ± 7.5	**0.027**	**1.8 ± 7.6***	0.09	3.9 ± 16.6	0.36	3.6 ± 13.8	0.10
	Cont	−1.7 ± 7.6		1.1 ± 6.9		−3.2 ± 19.6		−3.6 ± 12.1	

Data marked with asterisks (*) represent within-group changes. For group–time interactions, *P* values are presented. For clarity, all significant changes are emphasized with bold letters.
*Denotes significance at $P < 0.05$.
**Denotes significance at $P < 0.01$.
***Denotes significance at $P < 0.001$.
Cont, control group; int, intervention group.

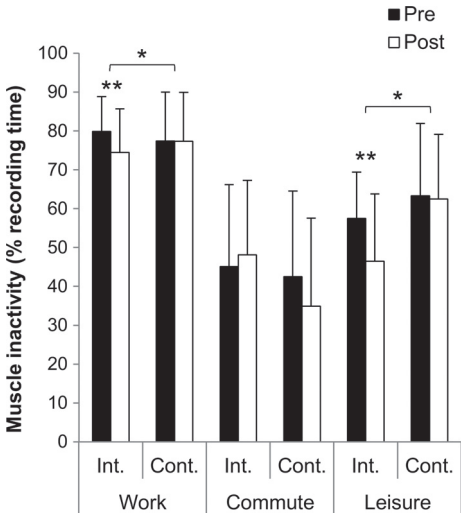

FIGURE 3—EMG-derived muscle inactivity time in intervention and control groups across different domains in pre- and postmeasurements. Cont, control group; int, intervention group. *Denotes significance at $P < 0.05$ and **$P < 0.01$.

within the intervention group (Table 2). During work time, a decrease in muscle inactivity time ($P < 0.05$, power = 0.63, $\eta^2 = 0.11$) was accompanied by an increase in light muscle activity time ($P < 0.01$, power = 0.77, $\eta^2 = 0.15$) and average $\%EMG_{MVC}$ ($P < 0.05$, power = 0.52, $\eta^2 = 0.09$) in the intervention group compared with those in the controls. Compared with those in the control group, muscle inactivity time ($P < 0.05$, power = 0.70, $\eta^2 = 0.13$) and the sum of the five longest muscle inactivity periods ($P < 0.01$, power = 0.83, $\eta^2 = 0.17$) decreased and light muscle activity time ($P < 0.05$, power = 0.64, $\eta^2 = 0.11$) increased in the intervention group during leisure time. Compared with that in the intervention group, the sum of the five longest muscle inactivity periods decreased during commuting in the control group ($P < 0.01$, power = 0.83, $\eta^2 = 0.17$).

DISCUSSION

In this intervention, a one-time lecture and face-to-face tailored counseling aimed at reducing and breaking up sitting time and increasing nonexercise physical activity time led to decreases in muscle inactivity time and long inactivity periods with concomitant increases in light muscle activity time. The effects were achieved partly during work time and more profoundly during leisure time. However, given the minimal use of muscle MVC capacity (1.5% of EMG_{MVC} during working hours), these changes resulted in a significant increase in average $\%EMG_{MVC}$ during working hours of office workers. In other activity variables, there were no significant group–time changes during the whole day, suggesting that this specific counseling changed muscle inactivity and activity patterns, as hypothesized. Reallocation of muscle inactivity to ambulatory activity of the observed magnitude (approximately 30 min) have been shown to decrease metabolic risk factors in short-term interventions (12,30).

According to a previous review (7), strategies aimed at promoting physical activity are often not able to reduce self-reported sitting time despite increasing physical activity in various workplace interventions. However, behavioral interventions targeted specifically at reducing sedentary behavior in overweight office workers (23) and in the elderly (17) showed similar results to those of the present study, i.e., reductions of sedentary time with simultaneous increases in light-intensity physical activity time. In addition, a lecture and a specific prompt program for office workers of normal

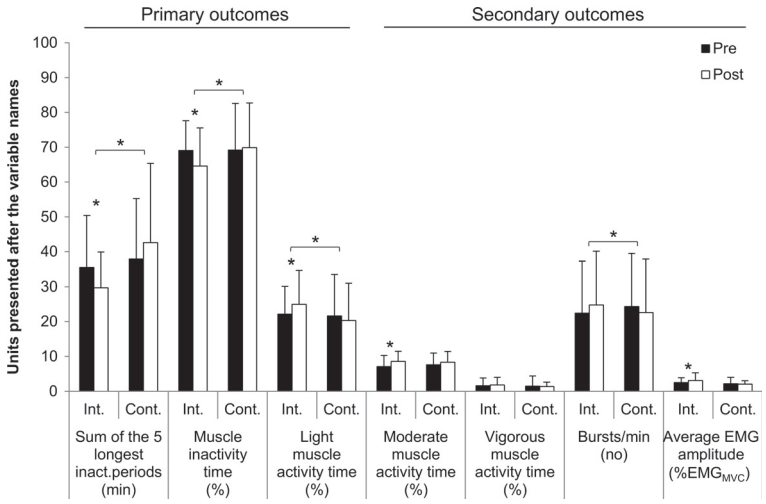

FIGURE 4—EMG-derived muscle inactivity and activity variables in the intervention and control groups during the whole day in pre- and postmeasurements. Units for each variable are presented after the variable names. *Denotes significance at $P < 0.05$.

weight (13), a television lockout system for overweight and obese individuals (26), and the implementation of sit–stand workstations (2,19) were able to reduce sedentary behavior by changing the physical environment, resulting mostly in more substantial changes as compared with those in behavioral intervention alone. These results illustrate that to reduce sedentary time, the specific physical and social contexts that modify participation in sedentary activities must be modified, and these factors are likely different from factors related to physical activity (28).

In the present study, both the intervention instructions and data analysis were classified into commute, work time, and leisure time to emphasize the effect of the intervention within these domains. The changes during leisure time were approximately twofold bigger as compared with those in the work time, whereas no intervention effects were observed during commuting. The specific contexts affecting sedentary behavior are likely various and present throughout the day. Specifically, a potential to decrease sedentariness through behavioral intervention is different between these domains. Even though workplace settings include challenges for behavioral interventions in terms of structured time use, social norms, and environment, among others, the present behavioral intervention was effective in participants from various professional backgrounds. We also tested the potential confounding effect of occupational status by using it as a covariate in the statistical tests, but the results remained largely unchanged, suggesting that the intervention was independent of professional background within our study population. On average, the magnitude of the change induced by this simple intervention is rather modest and may benefit from environmental support and a multilevel approach at the workplace. In addition, given the high education level of the study participants, these results may not be fully generalizable. However, because sedentary work seems to be most prevalent in highly educated people (8), there might be a need for sedentary time-targeted intervention within this particular group.

Leisure time, on the other hand, offers a more flexible environment for behavioral changes, as evidenced by a twofold bigger decrease in sedentary time as compared with that in the work time. In particular, the family based approach, which incorporates educational and parental aspects in addition to individual priorities, may have exposed the motivation toward nonexercise activity through the desire for activities that are important for children. About 40% of Finnish families have children, 50% of whom have children under the age of 6 (34). The findings of this study show the potential of family based intervention in a population representing busy stage of life and low daily physical activity level (31). To increase the effectiveness of future interventions targeting sedentary time, workplace settings might benefit from environmental support and commuting time may require a more powerful and wide-ranging intervention (28).

In addition to different domains, it is also important to consider changes in behavior across the entire physical activity spectrum. For example, an increase in high-intensity physical activity may occur independently of inactivity (10,14) or may even be paralleled by a decrease in light-intensity physical activity (32), changing the interpretation of findings. From the perspective of sedentary time, laboratory studies have revealed different metabolic pathways that are activated by physical inactivity and by reallocation of inactivity to light or to more intense activities (4,18,24). Because of these differences, it is important to consider not only the change but also the reallocation of sedentary time. In the present study, the intervention achieved the stated goals because the only significant group–time interactions during the whole day were seen in muscle inactivity time, sum of the five longest muscle inactivity periods, and light muscle activity time, which were the primary outcome variables. The main intervention message of reducing prolonged sedentary time and increasing nonexercise physical activity was thus well transferred to the muscle level.

The beneficial effects of reduced sedentary time have been suggested by cross-sectional and prospective studies, but evidence from long-term interventions is lacking. However, short-term experimental studies have induced a positive change on postprandial glucose and insulin responses with regular activity breaks of 1 min 40 s to 2 min, totaling approximately 30-min reallocation of sitting to ambulatory activity a day (12,30), a change of similar magnitude as that seen in this study. In the long term, a 2-h reduction in objectively measured sedentary time was associated with a favorable change in cardiometabolic biomarkers, reflecting 7% lower risk of cardiovascular events (21,39). When adjusted to similar wear time, 21% of the participants in the present study achieved a change of this magnitude. Although the results of this study show potential in terms of clinical significance, more research is needed to confirm the required minimum reduction in sedentary time yielding clinically significant end point in the long term.

The limitations of the present study include 1-d measurement periods and a systematic increase in the working time between the measurement days. This is likely due to longer duration of laboratory measurements on the first day, whereas on the second day, the participants had fewer questionnaires to fill in and instruction time was shorter. By having a control group and selecting only self-reportedly typical workdays in the analysis, the effect of between-day variability on the results was minimized. On the other hand, many participants were excluded on the basis of this criterion. These "atypical" days included, for example, organized exercise evenings at workplace, giving visitors a grand tour of the workplace, or staying at home because kids were sick. Because of device availability and study schedule, we were not able to replicate these measurements, resulting in reduced sample and limited power in some variables. During commuting, the control group showed a decrease in the longest inactivity periods compared with that in the intervention group. This may be explained by their more active commuting habits at baseline in combination with participation in a study entitled "Daily

Activity" that included an informed consent, which potentially provided a cognitive intervention to the participants. On the other hand, there were no differences in the change in total muscle inactivity or activity parameters during commuting between the groups.

The main strength of this study was the use of EMG, which shows both the duration and intensity of muscle activity with high precision (36,37). Classifying the EMG signal, and accelerometer counts (22), merely by threshold values makes it impossible to determine whether the participants were actually sitting, standing, or moving. However, because the inactivity threshold was set individually to be between the values of sitting and standing, it is likely that the inactivity time presented in this study reflects the actual sitting time accompanied with complete inactivity periods from the quadriceps and hamstring muscles. Concerning associations between physical activity and health, the underlying enzymatic processes related to insulin resistance and substrate use are initiated by muscle activity, not physical impact, measured by accelerometer counts or the posture itself. The definition of sedentary behavior has gained wide attention, but consensus is yet to be reached (3,27,29,33). With these considerations in mind, the present study focused on complete inactivity and activity periods measured directly from the locomotor muscles, which we believe is the most insightful method for the measurement of physical inactivity and activity.

Only a small fraction (2.4%) of the muscle's maximal voluntary strength capacity is used in normal daily life, and the main locomotor muscles are inactive almost 70% of the day. Tailored counseling was effective in decreasing muscle inactivity time by 33 min (4.5 pp), with concomitant increases in light muscle activity by 21 min (2.8 pp). This resulted in 13% increase in work time average $\%EMG_{MVC}$ without increases in high-intensity EMG. These results reveal the potential of behavioral interventions targeting decreased sedentary time, rather than merely increased physical activity time, to decrease muscle inactivity time.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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IV

OBJECTIVELY MEASURED SEDENTARY WORK, LEISURE TIME AND CARDIO-METABOLIC BIOMARKERS: EFFECTIVENESS OF A ONE-YEAR FAMILY-BASED CLUSTER RANDOMIZED CONTROLLED TRIAL

by

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