The metabolic cost of overground and treadmill walking in healthy young and older adults

Sauvik Das Gupta

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Contact: sauvik@okstate.edu

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Sauvik Das Gupta

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promotoren:	prof.dr. J.H. van Dieen prof.dr. I. Jonkers
copromotoren:	dr. M.F. Bobbert dr. D.A. Kistemaker
promotiecommissie:	prof.dr. G.A. Gaesser dr. V. Cornelissen prof.dr. M. Hopman prof.dr. H. Houdijk prof.dr. H.A.M. Daanen

KU Leuven
Biomedical Sciences Group
Faculty of Movement and Rehabilitation Sciences
Department of Movement Sciences



DOCTORAL SCHOOL
BIOMEDICAL SCIENCES



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Sauvik DAS GUPTA

Jury: dr. V. Cornelissen

Supervisor KU Leuven: prof.dr. I. Jonkers
Supervisor Vrije Universiteit Amsterdam: prof.dr. J.H. van Dieen
Co-supervisor(s): dr. M.F. Bobbert, dr. D.A. Kistemaker Vrije Universiteit Amsterdam
Chair examining committee: prof.dr. H.A.M. Daanen
Chair public defense: prof.dr. J.J.G. Geurts
Jury members: prof.dr. G.A. Gaesser
dr. V. Cornelissen
prof.dr. M. Hopman
prof.dr. H. Houdijk
prof.dr. H.A.M. Daanen

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18.04.2023

"Be the change that you wish to see in the world"

Arguably attributed to Mohandas Karamchand Gandhi

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Foreword

1. Introduction

If we look at the data from the United Nations we see that the world population is generally aging, especially in the global West of Europe and North America. Globally, the number of people over 65 years of age (generally considered as elderly) is actually growing faster than those below them. Worldwide in 2018, for the first time, elderly who are at least 65 years old or above outnumbered children under 5 years of age and in 2019, one in eleven persons in the world was 65 years old or above. In contrast, by 2050 one in four persons living in Europe and North America, and globally one in six persons could be 65 years or older. If we look at the absolute numbers, octogenarians or older are projected to triple from 143 million in 2019 to about 426 million in 2050. All in all, these figures show the rapid growth of an elderly population in the human society. It is generally known from the literature that mobility and gait become challenging with aging and mobility loss with aging is a vast and rapidly growing global socioeconomic problem. For example, we see that mobility loss is accompanied by general degradation of sensory, motor and central nervous system functions with aging leading to numerous gait problems. In elderly we observe markedly poor balance control, increased chance of falling (van Dieën JH et al., 2017, Pijnappels, Bobbert, & Van Dieën, 2005; Pijnappels, van der Burg, Reeves, & van Dieën, 2008; Andersen, 2012) (https://ec.europa.eu/eurostat/cache/digpub/ageing/) and an increased Metabolic Cost of Walking (*MCoW*) (Gaesser, Tucker, Sawyer, Bhammar, & Angadi, 2018; Horiuchi, Endo, Horiuchi, & Abe, 2015; Hortobágyi, Finch, Solnik, Rider, & DeVita, 2011; Jones, Waters, & Legge, 2009; Mian, Thom, Ardigo, Narici, & Minetti, 2006; Ortega, Fehlman, & Farley, 2008; Peterson & Martin, 2010; Waters, Hislop, Perry, Thomas, &

Campbell, 1983; Waters, Lunsford, Perry, & Byrd, 1988). This thesis, which is part of the 'MOVE-AGE' program of the Erasmus Mundus Joint Doctoral scheme of the European Commission, is an attempt to quantify the elevation in *MCoW* with a systematic review and meta-analysis and then see if there is an increased *MCoW* overground for elderly compared to young, since overground walking is ecologically relevant.

Routine physical activity like walking accounts for up to 30% of an adult's total daily energy expenditure (McArdle, Katch, & Katch, 1986). From the common physical activities performed, walking is the most prevalent physical activity for most of the people and it is also a sub-maximal task, meaning it does not require extreme physical effort. To reach point B from point A by walking, humans need to move using their feet and lower limbs. *MCoW*, defined as the metabolic energy expended per meter travelled, is an important variable in daily human life. If *MCoW* becomes higher when we grow old, we will expend more energy to traverse the same distance compared to when we were young. As a result, we will become fatigued for a given distance walked, which will then limit our overall functional possibilities when we grow old. Increased fatigue and limiting of functional possibilities can have many bad repercussions for us at an older age such as less social contacts, reduced loading of musculoskeletal and cardiorespiratory systems and increased sedentarism, among others. *MCoW* is typically expressed either in terms of Gross Cost of Walking (*GCoW*) which is the total amount of metabolic energy consumed per kilogram of body mass per meter travelled on a whole-body level, or in terms of Net Cost of Walking (*NCoW*). *NCoW* is calculated by subtracting the Resting Metabolic Rate (*RMR*) from *GCoW*, where *RMR* is the amount of energy expended when the human body is at complete rest. *NCoW* is typically interpreted as the amount of metabolic energy consumed per kilogram of body mass per

meter travelled, solely due to walking. It has long been hypothesized that human walking minimizes a cost function, under a given set of constraints. From an evolutionary perspective, *MCoW* is believed to be minimized by humans, by optimizing our gait parameters.

If we look at the body of literature involving *MCoW*, there are numerous studies (Hortobágyi, Finch, Solnik, Rider, & De Vita, 2011; Malatesta et al., 2003; Mian, Thom, Ardigò, Narici, & Minetti, 2006; Pincheira, Stenroth, Avela, & Cronin, 2017; Waters, Hislop, Perry, Thomas, & Campbell, 1983; Waters, Lunsford, Perry, & Byrd, 1988) reporting *MCoW* to be 20-30% higher in Older Adults (*OA*) than in Young Adults (*YA*). In this thesis, we consider persons at least 65 years old or above to be *OA*, and persons between 18-40 years old to be *YA*. In the literature it has been shown that an elevated *MCoW* is accompanied by other gait differences between *OA* and *YA*. For example, *OA* have different kinematics and kinetics, and more antagonistic muscle co-activation (Aboutorabi, Arazpour, Bahramizadeh, Hutchins, & Fadayevatan, 2016; Donelan, Kram, & Kuo, 2001; Hortobágyi et al., 2011; Mian, Thom, Ardigò, et al., 2006; Ortega & Farley, 2015; Peterson & Martin, 2010; Piche et al., 2021; Tudor-Locke et al., 2021), especially around the ankle joint (Benjuya, Melzer, & Kaplanski, 2004; Gebel, Lüder, & Granacher, 2019; Iwamoto, Takahashi, & Shinkoda, 2017). It is currently unclear why *MCoW* is higher in *OA* compared to *YA*. It has been hypothesized that *OA* might have a higher need for stabilization, leading to higher muscle activity, resulting in more metabolic energy consumption, or/and expend more metabolic energy to generate a given amount of positive muscle work due to lower muscle efficiency (Dean, Alexander, & Kuo, 2007; Ortega & Farley, 2007, 2015).

2. Schematic outline of the main chapters

For this thesis, first we wanted to see whether we can observe this commonly reported elevation in *MCoW* for *OA* over *YA*, and if so, quantify the elevation. Taking a look at the literature, we could not find a summarized quantitative analysis on the differences in *MCoW* between *YA* and *OA* and so we decided to make our own. In order to have the summarized quantification of the elevation in *MCoW* for *OA* over *YA*, we started with a thorough systematic review and meta-analysis on the published literature in Chapter 1 and concluded that there is an elevation in *MCoW* for *OA* over *YA* at similar speeds. However, the studies we reviewed in this chapter were a mixture of overground and treadmill studies, with the majority of them being on a treadmill. Furthermore, there were a few studies in which there were differences in anthropometrics between their *YA* and *OA* or/and which had not explicitly mentioned the physical activity and fitness status of their participating subjects. So we were not sure as to whether the elevation in *MCoW* for *OA* over *YA* was caused due to age *per* se or due to other differences in anthropometrics, physical activity and fitness status or/and due to the fact that there was an interaction between age and treadmill walking. Hence, we wanted to find out whether there would still be an elevation in *MCoW* for *OA* over *YA* if they were similar in anthropometrics, physical fitness and activity status, and while walking overground and on a treadmill with sufficient familiarization. Based on the findings of the systematic review and meta-analysis and in order to answer our research questions, for this thesis, next we designed subsequent experimental studies involving healthy *YA* and *OA* on both overground and treadmill walking, which were carried out in a controlled laboratory setting.

In our first experimental study we studied *MCoW* of *OA* and *YA*, both overground and on a treadmill. In this study we wanted to see whether there are

differences between overground and treadmill *MCoW* for both *OA* and *YA* and whether there was an elevation in *MCoW* for *OA* over *YA.* We reported on this study in Chapter 2. In this study, we found no statistically significant differences in *PWS* and *MCoW* (either overground or on a treadmill) between our *YA* and *OA.* Furthermore, we found that *NCoW* was elevated on the treadmill compared to overground for *OA* in the morning session, but not for *YA*. The elevation was not statistically significant in the afternoon session for *OA*. So from this study it seems that *YA* and *OA* reacted differently to the treadmill, especially so in the morning session, which was the first time they encountered walking on a treadmill during our experiments. It could be that *OA* were more anxious while walking on a treadmill for the first time and that led them to react differently to the treadmill and having a higher *MCoW*.

From the study described in Chapter 2, we were unable to understand the reasons why there was an elevation in treadmill *NCoW* compared to overground for *OA*, especially in the morning session. We hypothesized that it could be due to anxiety (since they were encountering the treadmill for the first time in the morning and not all of them had prior treadmill walking experience), which could lead to gait differences in *OA*, resulting in an elevated *MCoW* compared to overground. In the literature, it has been reported that walking heart rate (relative to rest) is higher on treadmills than overground (Greig, Butler, Skelton, & Mahmud, 1993; Parvataneni, Ploeg, Olney, & Brouwer, 2009) and this led to suggestions that heart rates could be a biomarker for anxiety and thus anxiety could be increased on treadmills compared to overground. Additionally, the treadmill that we had used in the study in Chapter 2 was at a height of 60cm from the ground and fear of height solely could induce anxiety in humans. We wanted to first find out whether self-reported anxiety caused an elevation in treadmill *MCoW* compared to

overground for our *OA.* Secondly, we wanted to find out whether this self-reported anxiety was due to the increased height of our treadmill in the study in Chapter 2.

In order to answer these two questions and also to try and replicate the results that we saw for our *OA* in the study in Chapter 2, we conducted another experimental study and reported on it in Chapter 3. In this chapter, we only had *OA* (who had previous experience of walking on treadmills) as our subjects during overground walking and on two different treadmills. To test whether there is anxiety at all and if it is there, whether fear of height induced the anxiety, we used a second treadmill at the floor level. In line with the results described in Chapter 2, we saw statistically significant elevation of *MCoW* (both *GCoW* and *NCoW*) and heart rates on both the treadmills compared to overground, thereby also replicating the result of elevated *MCoW* for *OA* on the treadmill compared to overground in the study described in Chapter 2. However, self-reported anxiety was statistically significantly elevated only on the high treadmill compared to overground and on the high treadmill compared to the floor level treadmill. There was no statistically significantly elevated self-reported anxiety on the floor level treadmill compared to overground. Hence from this chapter we could show that anxiety (as interpreted from the self-reported anxiety measures) is not the cause for the elevated *MCoW* on treadmills compared to overground that we saw for our *OA* in this study and in the previous study. This is because self-reported anxiety was not coupled to *MCoW* and *MCoW* was elevated on both the treadmills compared to overground, regardless of anxiety.

3. Layout of the thesis

The purpose of this foreword is to give a brief description of the field of research in *MCoW* for *OA* and *YA* both overground and on treadmills and to provide a brief snapshot of the main research work undertaken in this thesis. The foreword is followed

by the three main chapters – namely Chapters 1, 2 and 3. Each chapter explains in detail how each of the studies was conceptualized, what methods were followed, what the key results were and which conclusions we have drawn from the results. These three main chapters are followed by a chapter titled Summary and General Discussion (Chapter 4) where we have given a summarized overview of all the main findings from the studies with their general implications and then highlighted some of the limitations of this thesis with some future directions in which this research can be continued. This chapter is followed by the Acknowledgment section, where apart from the general acknowledgments I have also briefly reflected upon my valorization activities that I have performed independently from my research team. Finally, I have concluded the thesis with a list of my journal publications and conference presentations, a list of grants awarded to me during my valorization activities, a list of courses and activities that I undertook as part of my doctoral training, and my brief bio sketch.

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Chapter 1

The metabolic cost of walking in healthy young and older adults – a systematic review and meta analysis

(Adapted from) Sauvik Das Gupta, Maarten F. Bobbert and Dinant A. Kistemaker. The Metabolic Cost of Walking in healthy young and older adults – A Systematic Review and Meta Analysis. *Scientific Reports*, 9, 1-10. [9956] (2019). https://doi.org/10.1038/s41598-019-45602-4

The Metabolic Cost of Walking (*MCoW*) is an important variable of daily life that has been studied extensively. Several studies suggest that *MCoW* is higher in Older Adults (*OA*) than in Young Adults (*YA*). However, it is difficult to compare values across studies due to differences in the way *MCoW* was expressed, the units in which it was reported and the walking speed at which it was measured. To provide an overview of *MCoW* in *OA* and *YA* and to investigate the quantitative effect of age on *MCoW*, we have conducted a literature review and performed two meta-analyses. We extracted data on *MCoW* in healthy *YA* (18-41 years old) and healthy *OA* (≥59 years old) and calculated, if not already reported, the Gross (*GCoW*) and Net *MCoW* (*NCoW*) in J/kg/m. If studies reported *MCoW* measured at multiple speeds, we selected those values for *YA* and *OA* at which *MCoW* was minimal. All studies directly comparing *YA* and *OA* were selected for meta-analyses. From all studies reviewed, the average *GCoW* in *YA* was 3.4 ± 0.4 J/kg/m and 3.8± 0.4 J/kg/m in *OA* (~12% more in *OA*), and the average *NCoW* in *YA* was 2.4 ± 0.4 J/kg/m and 2.8 ± 0.5 J/kg/m in *OA* (~17% more in *OA*). Our meta-analyses indicated a statistically significant elevation of both *GCoW* and *NCoW* ($p<0.001$) for *OA*. In terms of *GCoW*, *OA* expended about 0.3 J/kg/m more metabolic energy than *YA* and about 0.4 J/kg/m more metabolic energy than *YA* in terms of *NCoW*. Our study showed a statistically significant elevation in *MCoW* of *OA* over *YA*. However, from the literature it is unclear if this elevation is directly caused by age or due to an interaction between age and methodology. We recommend further research comparing *MCoW* in healthy *OA* and *YA* during "natural" over-ground walking and treadmill walking, after sufficient familiarization time.

Glossary

a. *WS* in [m/s]: the measured walking speed either over-ground or on a treadmill.

b. $\dot{V}O_2$ in [ml/kg/min]: the rate of oxygen consumption.

c. *EEq* of $\dot{V}O_2$ in [J/kg/min]: Energetic Equivalent of the oxygen consumed during walking.

d. *RMR* in [J/kg/min]: the rate of metabolic energy consumption during quiet standing or sitting (Resting Metabolic Rate).

e. *MCoW* in [J/kg/m]: metabolic energy consumed, per kilogram of body mass per meter travelled, typically expressed as gross or net values (Metabolic Cost of Walking).

f. *GCoW* in [J/kg/m]: *EEq* / (*WS* ×60) (Gross Cost of Walking).

g. *NCoW* in [J/kg/m]: (*EEq* – *RMR*) / (*WS* ×60) (Net Cost of Walking).

h. *RER* [dimensionless]: the amount of carbon dioxide produced divided by the amount of oxygen consumed (Respiratory Exchange Ratio).

i. *OA*, *YA*: (Old Adults, mean age ≥ 59 years) and (Young Adults, mean age 18-41 years).

1.1 Introduction

Walking is the most prevalent physical activity for humans and may account for up to thirty percent (30%) of an adult's daily energy expenditure (McArdle, Katch, & Katch, 1986). Hence, the Metabolic Cost of Walking (*MCoW*), defined as metabolic energy expended per meter travelled, is an important variable in daily life and has been studied extensively. It is generally presumed that humans, under normal circumstances, select their gait parameters (like step frequency and walking velocity) to minimize *MCoW* (see: (Donelan, Kram, & Kuo, 2001); (Bertram & Ruina, 2001); (Zarrugh, Todd, & Ralston, 1974)). *MCoW* has been studied in healthy children, teenagers, young and elderly adults, at preferred or fixed walking speeds and during both over-ground and treadmill walking. One major finding that has been reported by several studies is that *MCoW* is higher in healthy Older Adults (see: (Hortobagyi, Finch, Solnik, Rider, & DeVita, 2011); (Malatesta et al., 2003); (Mian, Thom, Ardigo, Narici, & Minetti, 2006)) (*OA*; to be consistent with the analysed literature, defined here as mean age $\geq$ 59 years) than in Young Adults (*YA*; here defined as mean age from 18-41 years), although a recent extensive study has found contradictory results (Gaesser, Tucker, Sawyer, Bhammar, & Angadi, 2018). An elevated *MCoW* may cause *OA* to become fatigued more quickly and walk less, which in turn may lead to functional limitations and reduced societal participations. Currently, no quantitative analyses are present in the literature that directly compare the results across studies on *MCoW* in *YA* and *OA*, or on the differences between these two groups. Such analyses may be important, for example to decide whether the elevation in *MCoW* of *OA* over *YA* is clinically relevant.

Directly comparing the results in the literature across different studies on *MCoW* is not without problems. A first difficulty comparing quantitative measures across studies arises from the ways in which *MCoW* was expressed. Several studies expressed

MCoW only in terms of Gross Cost of Walking (*GCoW*) (see: (Horiuchi, Endo, Horiuchi, & Abe, 2015); (Shimada et al., 2011)) which is the total amount of metabolic energy consumed per kilogram of body mass per meter travelled. Other studies expressed *MCoW* only in terms of Net Cost of Walking (*NCoW*) (see: (Peterson & Martin, 2010); (Hortobagyi et al., 2011); (Mian et al., 2006)) which is assumed to be the total amount of metabolic energy expended due to walking *per se* (e.g., due to cross-bridge cycling and the Ca^{2+} pump in the muscle) calculated by subtracting first the Resting Metabolic Rate (*RMR)* from the metabolic rate during walking, and then divided by the Walking Speed (*WS)*. *GCoW* and *NCoW* are different in nature and quantity, making it difficult to interpret results across studies that only reported either one of these. For example, an increase in *NCoW* does not necessarily mean an increase in *GCoW* (and vice-versa) as there is evidence in literature that *RMR* may be different between *YA* and *OA* (Peterson & Martin, 2010); (Ortega & Farley, 2015). In addition, some of these studies do not report *RMR* values making it impossible to convert *GCoW* to *NCoW* and vice versa. A second difficulty is that *MCoW* and (rate of) energy expenditure have been reported in various units such as ml of O_2/kg/m (Horiuchi et al., 2015), J/kg/m (Peterson & Martin, 2010) or W/kg (Ortega & Farley, 2007) and it is not trivial to convert one to another. For example, to convert from ml of O_2/kg/m to J/kg/m, Respiratory Exchange Ratio (*RER*) is required, which typically differs among subjects and is often not reported. A third problem arises from differences in walking speed at which *MCoW* was measured. For example, Stoquart et al. (Stoquart, Detrembleur, & Lejeune, 2008) reported *MCoW* in *YA* at a walking speed of 1.1m/s, whereas Davies and Dalsky (Davies & Dalsky, 1997) reported *MCoW* in *OA* at a speed of 1.5m/s. This is a problem because it is known that *MCoW* depends on walking speed (see: (Ralston, 1960); (Zarrugh et al., 1974); (Waters & Mulroy, 1999)). Some studies used an identical

fixed speed for *YA* and *OA*, but the values at which speed was fixed varied across studies, (e.g., 1.0 m/s in (Hortobagyi et al., 2011) and 1.3 m/s in (Ortega, Fehlman, & Farley, 2008)). All of these difficulties mentioned above hamper the direct comparison of the results across different studies and therewith the observation of the actual values of *MCoW* in *YA* and *OA*.

Recently, Aboutorabi et al. (Aboutorabi, Arazpour, Bahramizadeh, Hutchins, & Fadayevatan, 2016) performed a literature review on effects of aging on the temporo-spatial (gait) parameters, kinematics, kinetics and energetics of gait. They concluded that aging led to an increase in *MCoW*. However, their list of included studies was not exhaustive, they did not address the difficulties described above and, more importantly, they did not quantify the elevation identified.

The primary aims of the present study were to provide an overview of the literature on *MCoW* in *YA* and *OA* and to investigate quantitatively the elevation of *MCoW* related to aging on the basis of the published literature. To do so, we first carefully searched the literature on *MCoW* in both *YA* and *OA* and selected all relevant scientific articles using the Preferred Reporting Items for Systematic Review and Meta Analyses *(PRISMA)* method. We then statistically analysed group differences, and then selected studies in which both *YA* and *OA* were measured in the same experimental setup. To estimate the effect size of *MCoW* between *YA* and *OA* we subsequently performed two meta-analyses, one on *GCoW* and the other on *NCoW* on these selected studies, that directly compared *YA* and *OA*.

1.2 Methods

Inclusion and Exclusion Criteria

We considered only studies published in peer reviewed journals that reported *MCoW* in healthy humans. In this study, we focused on healthy young and elderly adults

and excluded all studies involving subjects with disabilities, comorbidities and a history of falling. We noted that the study of Malatesta et al., (2003) did not mention whether the value for *MCoW* was gross or net. Based on the reported values, we are fairly confident that they were *GCoW* values and have included the study in our analyses; the values for the two *OA* groups (G80 and G65) were averaged and used for our meta-analyses. We furthermore specifically comment on the exclusion of two studies from our analyses. First, Thomas et al. (Thomas, De Vito, & Macaluso, 2007) decided to pool the data of *MCoW* in their analysis as they did not find any significant difference in the Preferred Walking Speed (*PWS)* of healthy young and elderly women. Second, Pearce et al. (Pearce et al., 1983) also pooled *MCoW* for both *YA* and *OA* as they were only interested in the effect of walking surface on *MCoW*. The results of these studies are obviously unsuitable for our purpose of investigating an age-related elevation in *MCoW*.

Search Strategy

A review protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement (see: www.prisma-statement.org;(Liberati et al., 2009)). A comprehensive search was performed in the bibliographic databases PubMed, Embase.com, Ebsco/SPORTDiscus and Wiley/Cochrane Library in collaboration with a medical librarian. Databases were searched from inception up to 14 Aug 2018. The following terms were used (including synonyms and closely related words) as index terms or free-text words: "Aged", "Elderly", "Gait", "Walking", "Energy metabolism". The search was performed without date, language or publication status restriction. The full search strategies for all databases can be found in the Supplementary Information. The initial list of the papers was selected through evaluation of the Title, Keywords and Abstract. Then, a further selection based on screening of the Methodology, Protocol and the Aims of the studies

was conducted by all authors independently. A flow-diagram for *PRISMA* method is shown in Figure 1. Screening was done simultaneously using a manual search process undertaken in EndNote (Clarivate Analytics) and online by the open-source web application Rayyan QCRI (Qatar Foundation & Qatar Computing Research Institute).

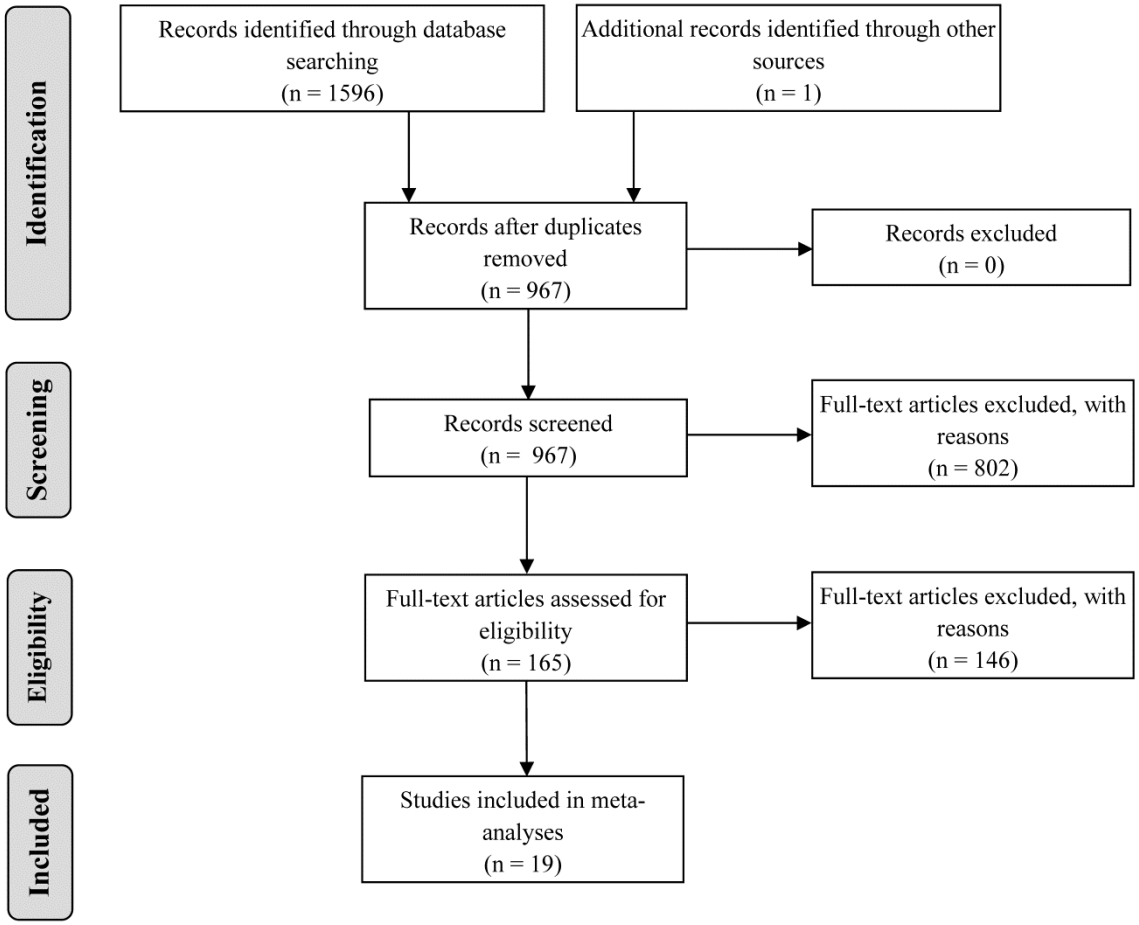

Figure 1. Flow-diagram of the search and selection strategy followed using PRISMA method.

Definition of age group

In our analyses we followed the definition of age group (*OA* vs *YA*) used by the authors of the individual studies. This resulted in a somewhat arbitrary overall definition of *OA* group of 59 years and older. To test for robustness against definition of the age group *OA*, we also performed statistical tests following the definition by the United

Nations (UN) of elderly, being of the age of 65 and older, which showed only a marginal influence of the difference in definition of *OA*. The only study in the meta-analyses that had *OA* below 65 was that of Jones et al. (Jones, Waters, & Legge, 2009).

Overview of analyses

From the selected studies, we extracted all data on *MCoW* and calculated, if not already reported, *GCoW* and *NCoW*. If the unit in which *MCoW* was reported was not in the *SI* unit of J/kg/m, we converted the reported values accordingly. Studies typically either report *MCoW* for self-selected or preferred walking speed. If studies reported *MCoW* measured at multiple speeds, we selected *MCoW* values for both *YA* and *OA* at the speeds at which *MCoW* values were minimal as it is known that under normal circumstances humans tend to self-select walking speeds in close proximity of the minimal *MCoW* speed. Using the selected data, we first reported *MCoW* in both *YA* and *OA* and statistically analysed group differences in walking speed (since the selected studies included both the experimentally fixed and preferred walking speeds) and *MCoW* between *YA* and *OA*. We then performed two meta-analyses, one on *GCoW* and one on *NCoW*, on the selected studies that directly compared *YA* and *OA*. We did this in order to account for the heterogeneity across the studies and differences in the experimental sample size and to calculate the effect size of *MCoW* between *YA* and *OA*.

Calculations of MCoW

Not all of the included studies reported all the variables of interest (being *GCoW, NCoW, RMR*, and *RER*). If any of these variables was not reported, we attempted to calculate its mean and standard deviation from the other variables reported. In general, calculating means is straightforward, but calculating standard deviations is not possible unless the individual sample data are known, which was generally not the case. Whenever standard deviations were not given, we estimated them (see Appendix A for

detailed overview of the calculated variables of interest and Discussion). In general, we used the following two equations to estimate standard deviations. For addition and subtraction of two quantities (say z = a + b or z = a - b), the estimated standard deviation of z ($\tilde{\sigma}_z$) was calculated as:

$$\tilde{\sigma}_z = \sqrt{(\sigma_a)^2 + (\sigma_b)^2} \qquad (1)$$

with σ_a and σ_b the known standard deviations of a and b. For multiplication and division of two quantities (say z = ab or z = a/b), the estimated standard deviation was calculated as:

$$\tilde{\sigma}_z = z\sqrt{\left(\frac{\sigma_a}{a}\right)^2 + \left(\frac{\sigma_b}{b}\right)^2} \qquad (2)$$

Finally, we note here that in some studies *RMR* was measured while sitting, whereas in other studies it was measured while standing. *RMR* is close to 14% lower during sitting than during standing (Weyand, Smith, & Sandell, 2009) and this may influence the values obtained for either *GCoW* or *NCoW* when only one of these was reported.

Statistical analysis

We checked for normality of the data using a Shapiro-Wilk hypothesis test along with violinplots and QQ plots. The result of the test and the visual inspection of the plots showed that the data was non-normally distributed. Additionally, Levene's test was used to check for the equality of variances and no violation of the equality was observed in the data. Based on these outcomes we chose to use a non-parametric Wilcoxon rank sum test.

We analysed whether walking speeds at which *MCoW* (for both *GCoW* and *NCoW)* was measured differed between *YA* and *OA* using an independent two-sided non-parametric Wilcoxon rank sum test. After that, we used an independent one-sided non-parametric Wilcoxon rank sum test to test for an elevation of *GCoW* and *NCoW* of *OA*

over *YA*. The open-source software JASP (version 0.9.0.1) was used for these statistical tests. However, from these analyses it is not possible to draw a definitive conclusion on the effect size of *GCoW* and *NCoW* between *YA* and *OA*. This is because not all selected studies directly compared *YA* and *OA*, and across the selected studies there were considerable differences in sample size, experimental methodology and various other factors like equipment, ethnicity and gender of subjects, etc. To be able to calculate the (pooled) effect size of *MCoW* between *YA* and *OA*, we selected only those studies that directly compared *YA* and *OA*, 19 in total (see Figure 1) and performed meta-analyses on them. We ended up with 13 studies for *GCoW* and 18 studies for *NCoW* (both being a large-scale meta-analysis (Mathes & Kuss, 2018)), because in 5 studies *RMR* was not mentioned and only *GCoW* or *NCoW* was reported. If some studies did not report *RMR* it became impossible to calculate *GCoW*/*NCoW* when only one was reported. Some of these studies measured *MCoW* at the same fixed walking speed in *YA* and *OA*, while others measured *MCoW* at individually preferred walking speeds, that differed between *YA* and *OA*.

To account for the possible heterogeneity across studies, we used a continuous random-effects model. The studies were weighed according to their respective sample sizes and standard deviations of their group mean values. The meta-analyses were performed using the open-source software packages Open Meta[Analyst] (Brown University of Public Health, Providence, USA) and JAMOVI (version 0.9.1.12). The two-arm metric of mean differences of individual studies was used to calculate the pooled effect size, the 95% confidence intervals and the 95% prediction intervals. Pooled mean differences were calculated as *MCoW* for *OA* minus *MCoW* for *YA* for both *GCoW* and *NCoW*. In addition, we calculated for both *GCoW* and *NCoW* the pooled

standardized mean difference, which is the (pooled) mean difference normalized relative to pooled standard deviation computed using Hedge's G.

Validation and sensitivity tests

To assess the validity of the a-priori assumption of heterogeneity in the meta-analyses, we used the three standard tests of heterogeneity (Cochran's Q, tau^2 and I^2). A sensitivity analysis was performed on each of the studies to check for the robustness of the meta-analysis using the Leave-One-Out method.

Risk of Bias Assessment

The appraisal tool for cross-sectional studies (AXIS (Downes, Brennan, Williams, & Dean, 2016)) was used as a tool to conduct the risk of bias assessment for the individual studies included in our meta-analyses. In short, all the studies were judged systematically on each of the specific sections of the articles starting from Introduction, Methods, Results to Discussion and other relevant parameters. Twenty specific questions were used to evaluate each of these sections of the articles with the option of answering them by a 'YES', 'NO' or 'DO NOT KNOW'. Additionally there is an option to leave specific comments explaining the answers provided for each of the questions.

1.3 Results

Figures 2A and 2B depict the mean *GCoW* and *NCoW* values as function of mean age of a group of studies reporting values for *GCoW* and/or *NCoW*. The data were then grouped into either *YA* (black dots) or *OA* (red dots) as was done in the selected studies. The mean walking speeds were found to be not statistically different between these groups (1.2 ± 0.2 m/s in *YA* vs 1.2 ± 0.2 m/s in *OA*, mean ± SD, p=0.58). It was found that the average *GCoW* was 12% higher in *OA* (3.8 ± 0.4 J/kg/m) than in *YA* (3.4 ± 0.4 J/kg/m) and that this elevation was statistically significant ($p<0.001$). The average *NCoW* was 17% higher in *OA* (2.8 ± 0.5 J/kg/m) than in *YA* (2.4 ± 0.4 J/kg/m); this

elevation was also statistically significant ($p<0.001$). These results indicate an elevation in *GCoW* and *NCoW* in *OA* compared to *YA*.

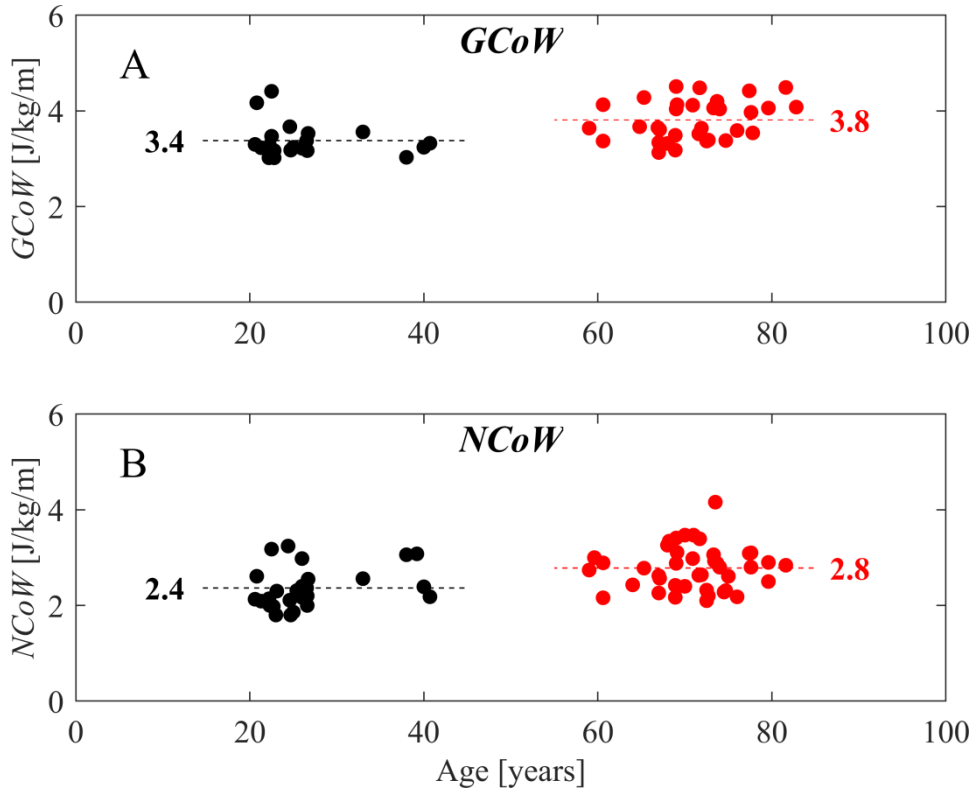

Figure 2. Metabolic Cost of Walking across the mean age in each study. A) Gross Cost of Walking across age. B) Net Cost of Walking across age. Black solid circles represent *YA* and the red solid circles *OA*. Each of the circles is a mean *GCoW* value or a mean *NCoW* value from a selected study. The dashed straight lines (black and red) show the mean metabolic cost of a particular age-group for both *GCoW* and *NCoW*.

After this, a selection of studies was made from the studies in Figure 2, which directly compared *YA* and *OA*. No statistically significant differences ($p=0.59$) were found in the overall walking speeds between *YA* (1.2 ± 0.2 m/s) and *OA* (1.2 ± 0.1 m/s) of these studies. We then used these studies to perform two meta-analyses to calculate mean difference in *MCoW* between *OA* and *YA*. Figures 3 and 4 depict forest plots for *GCoW* and *NCoW*, respectively, showing for each of the individual studies the mean difference (red squares) and 95% Confidence Interval (95% *CI*, black lines), as well as the estimated pooled mean difference (center of blue diamond and dashed black summary line), 95% *CI* (width of blue diamond) and 95% Prediction Interval (*PI*, blue

arrow). The size of the relative weight (*w*) of a particular study (indicated by the size of the squares in Figures 3 and 4) was dependent on the study sample size and standard deviation of the study sample mean (see (Cumming, 2012)). Both for *GCoW* and *NCoW* we found a pooled mean difference that was statistically significant (0.31J/kg/m and 0.38J/kg/m, respectively, both p<0.001). Note that the pooled mean difference of *NCoW* was 23% higher than that of *GCoW*. The 95% *CI* estimates the uncertainty of capturing the mean difference. By taking into account the heterogeneity across studies, the 95% *PI* estimates the interval in which an effect size from a future study assessing the same outcome will lie (Borenstein et al., 2009).

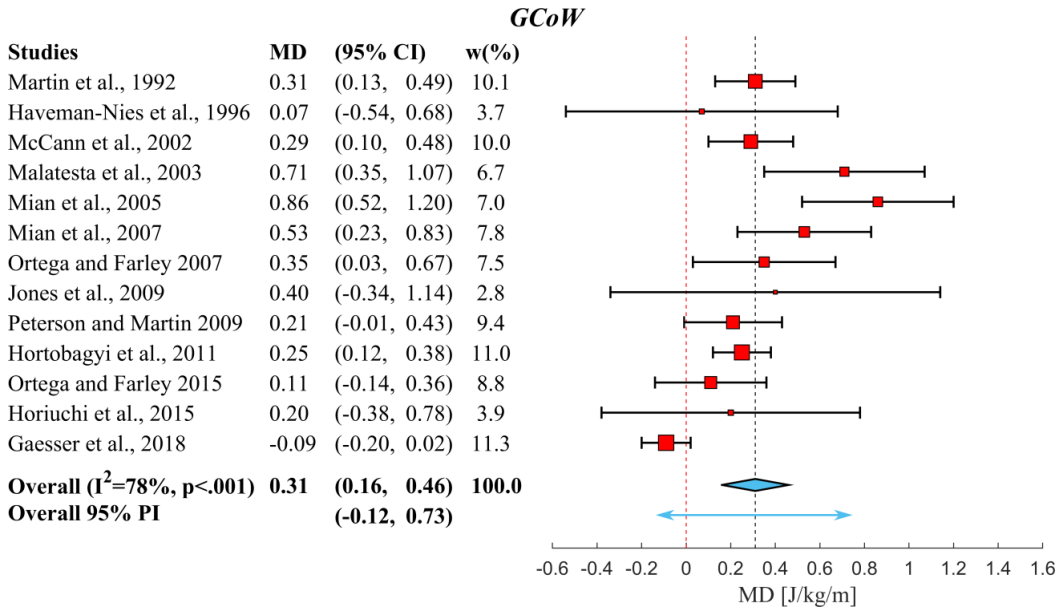

Figure 3. Overview of the results of the meta-analysis using a random effects model on the differences in Gross Metabolic Cost of Walking between *YA* and *OA*. A positive Mean Difference (*MD*) means that *OA* have higher *GCoW* than *YA*. The position of the red squares corresponds to the *MD* value per study and the horizontal black line to the 95% *CI*. The size of the square is proportional to the relative weight of that study w(%) to compute the overall *MD* (blue diamond and vertical black dashed line). The width of the diamond represents the 95% *CI* of the overall *MD* and the blue arrow represents the 95% *PI* of the overall *MD*. If a 95% *CI* spans zero (indicated by the red vertical dashed line), that study has found no significant elevation in *GCoW*. This meta-analysis shows a significant overall increase in *GCoW* of *OA* over *YA* (p<0.001). Note that the p-value mentioned in the forest plot is the p-value for the Cochran's Q heterogeneity test.

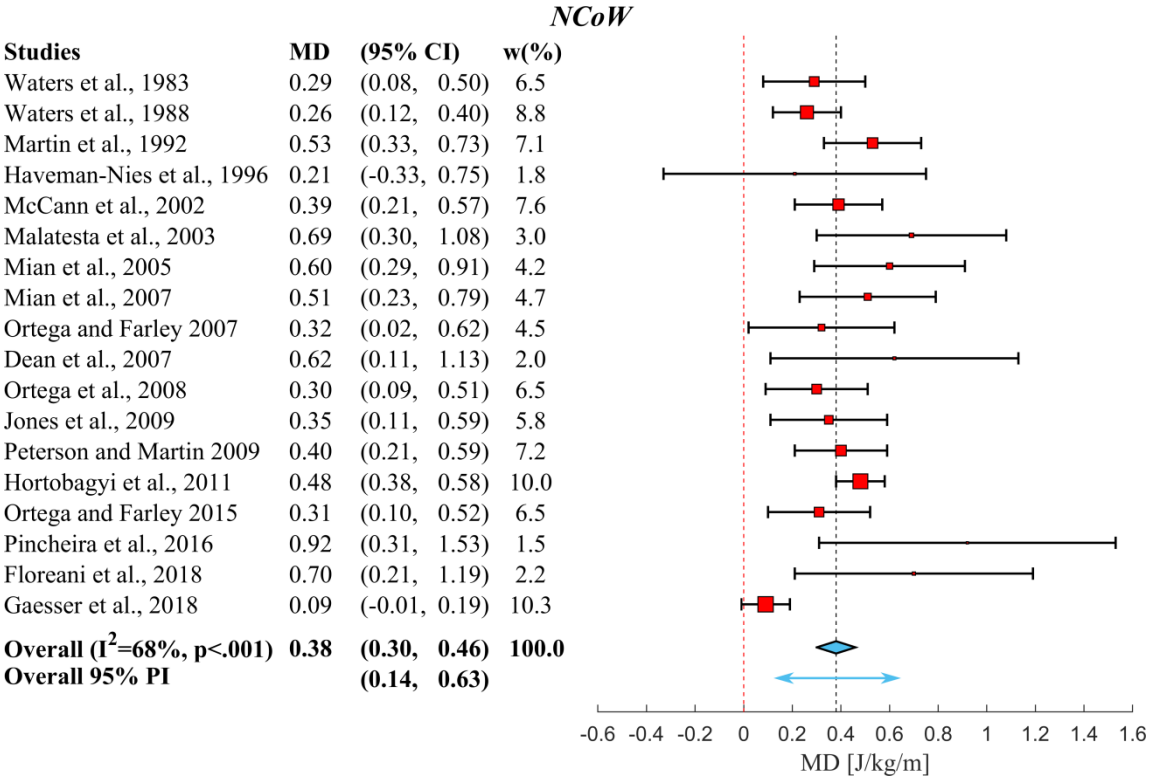

Figure 4. Overview of the results of the meta-analysis using a random effects model on the differences in Net Metabolic Cost of Walking between *YA* and *OA*. Symbols and conventions are the same as in Figure 3. This meta-analysis shows a significant overall increase in *NCoW* of *OA* over *YA* ($p<0.001$). Note again that the p-value mentioned in the forest plot is the p-value for Cochran's Q heterogeneity test.

In order to assess the magnitude of the (pooled) effect size, we calculated the pooled *standardized* mean difference (Hedge's G), which was 0.65 for *GCoW* and 1.00 for *NCoW*. Quantitatively, according to general guidelines (Cohen, 1969), the effect size was in the range of medium to large for *GCoW* and large for *NCoW*.

Validation and sensitivity tests

In order to assess the validity of our *a-priori* assumption of heterogeneity, we performed three standard statistical tests for heterogeneity. Firstly, we computed Cochran's Q, which is the weighted sum of squared differences between the individual study effects and the pooled effect across studies. Cochran's Q was 55.7 ($p<0.001$) and 53.2 ($p<0.001$) for *GCoW* and *NCoW*, respectively, indicating a statistically significant heterogeneity. Since Cochran's Q test has limited power to detect heterogeneity when

using a small number of studies (Higgins, Thompson, Deeks, & Altman, 2003), we secondly calculated I^2, which is a measure of variation in the pooled effect size proportional to the total amount of variance and is independent of the number of studies. The calculated values of I^2 were 78% and 68% for respectively *GCoW* and *NCoW* (0% meaning complete homogeneity). Even though there are no known concrete thresholds of I^2, these values indicate substantial heterogeneity (see (Higgins et al., 2003)). Thirdly, we calculated tau^2 values, which show the variance among the studies; these were 0.043 (with Standard error=0.028) for *GCoW* and 0.013 (with Standard error=0.01) for *NCoW* (a tau^2 value of greater than one indicates a meta-analysis should not be performed on the selected studies). The non-zero values of tau^2 again indicate that there was heterogeneity across studies. The overall results support our choice of using a random effects model for our meta-analyses.

Finally, we performed sensitivity analyses of the meta-analyses using the Leave-One-Out method, which involves repeating the meta-analyses leaving out one of the studies in turn (see Figures 5 and 6). Each red diamond in Figures 5 and 6 represents the estimated pooled mean difference (middle of diamond) and 95% *CI* (the width of the diamond) while leaving out the indicated study from the meta-analyses. The red arrow indicates the 95% *PI*. These figures show that leaving out any one of the studies would not have affected the statistical significance of the overall results of the meta-analyses ($p<0.001$ for both *GCoW* and *NCoW*). These figures furthermore show that the choice of definition of *OA* group did not influence our conclusions; by leaving out (Jones et al., 2009), the only study which defined *OA* to be below 65 years old (being >59), pooled *MD* only marginally changed. Note, however, that the 95% *PI* for leaving out (Gaesser et al., 2018) in the meta-analysis for *GCoW* does change; the interval does not span zero. This is because this study found a higher mean value for *YA* than *OA*.

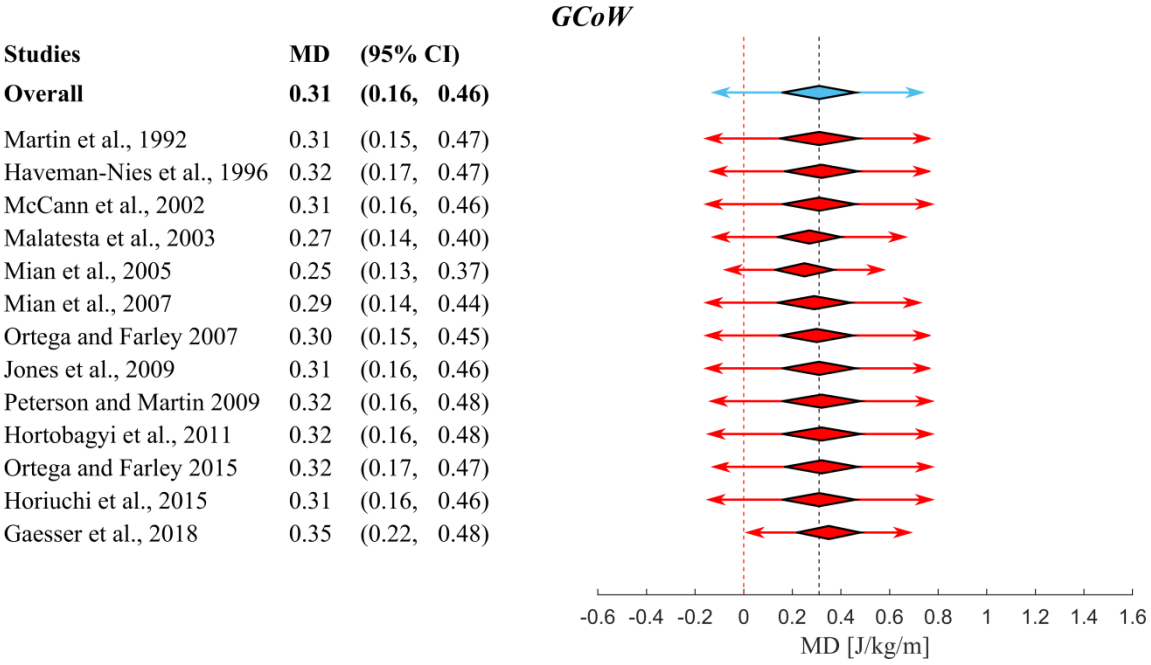

Figure 5. Leave-one-out plot of the meta-analysis on *GCoW*. The blue diamond now represents the pooled *MD* with its 95% *CI*, and the red diamonds represent the pooled *MD* along with its 95% *CI* when leaving out the study indicated. The arrows represent the 95% *PI* for the overall pooled *MD*. This figure shows that leaving out any of the studies does not cause the 95% *CI* to span zero for any of the red diamonds. Thus, the statistical significance of the elevation of GCoW in OA is not affected by leaving out any one of the studies.

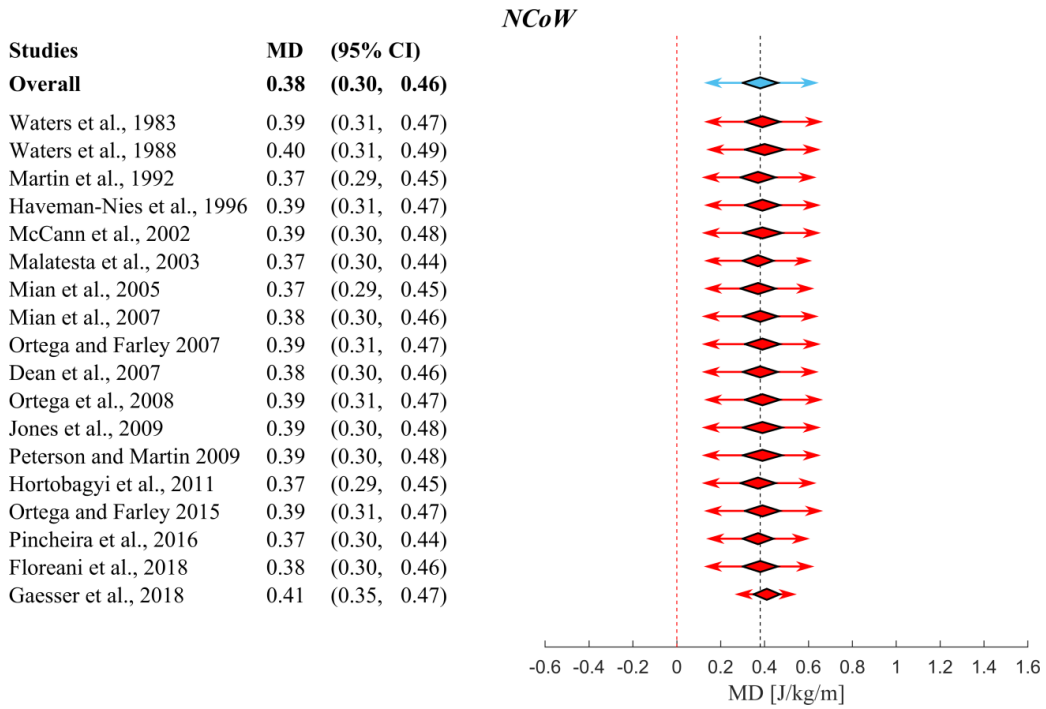

Figure 6. Leave-one-out plot of the meta-analysis on *NCoW*. Symbols and conventions are the same as in Figure 5. As with *GCoW*, the statistical significance of the elevation of *NCoW* in *OA* is not affected by leaving out any one of the studies.

Risk of Bias Assessment

The risk of bias assessment was done using the AXIS tool as mentioned in the Methods section. To summarize, none of the studies reported an a-priori statistical power calculation and few of the studies had a possible selection bias in recruiting human subjects for the experiments. Overall, the studies were found to be of good quality. For a detailed description of the assessment please see Appendix B.

1.4 Discussion

We set out to report and pictorially represent *MCoW* in healthy *YA* and *OA* and to investigate the quantitative effect of age on *MCoW* through a literature review and meta-analyses. We extracted all data on *MCoW* in healthy *YA* and healthy *OA* and calculated, if not already reported, *GCoW* and *NCoW* in units of J/kg/m. If studies reported *MCoW* measured at multiple speeds, we selected the speed and *MCoW* values for *YA* and *OA* at which *MCoW* was minimal. An analysis of the compilation of *GCoW* and *NCoW* values from the literature as a function of age (see Figure 2) showed a statistically significant elevation for *OA* over *YA*. To calculate the pooled effect size of *MCoW* between *YA* and *OA*, we performed two meta-analyses on the selected studies that directly compared *YA* and *OA*. In terms of *GCoW*, *OA* expended about 0.31 J/kg/m more than *YA* at similar walking speeds (walking speeds included in the meta-analyses were not significantly different between *OA* and *YA*). In terms of *NCoW*, *OA* expended about 0.38 J/kg/m more than *YA*. There was (~23%) more elevation for *NCoW* than *GCoW* for *OA* over *YA*. These results were found to be robust against choices made in statistical models and parameters. Figures 5 and 6 furthermore showed that leaving out any of the studies would not have affected the statistical significance of the overall results of the meta-analyses (in terms of pooled *MD* and 95% *CI*). These figures also show that the choice of definition of *OA* group did not influence our conclusions; even

leaving out (Jones et al., 2009), the only study in the meta-analyses that had *OA* below 65 years of age, only marginally changed the results. Although it is difficult to directly assess the clinical relevance from statistical results, the standardized effect sizes were medium to large, indicating that these differences are indeed clinically relevant (Page, 2014).

For our analyses, we have expressed *MCoW* in terms of both *GCoW* and *NCoW* and in units of J/kg/m. Since not all studies used in our analyses reported the variables of interest, or reported them in different units, we attempted to determine these from the other variables that were reported. In general, as stated in the *Methods,* calculating means is straightforward. However, calculating standard deviations is not possible unless the individual sample data are known, which were typically not reported. Since standard deviations are required to perform statistical tests we had to resort to estimating them. We estimated standard deviations conservatively (see: *Calculations of MCoW* in *Methods* and in Appendix), which decreased the likelihood of finding a statistically significant elevation. Nevertheless, our meta-analyses showed a significant overall elevation in both *GCoW* and *NCoW* of *OA* over *YA* and thus our results were not critically influenced by the estimation of the standard deviations.

In our analyses, we combined studies that measured *MCoW* at different walking speeds (e.g., at a range of fixed values versus self-selected/preferred walking speed). As stated before, when several fixed speeds were used in a particular study, we selected the speed accompanied by the lowest *MCoW*. Typically, *PWS* is measured in advance of the actual experiment and this speed is then also fixed during the experiment itself. Importantly, before conducting the analyses on *MCoW* we analysed group differences in walking speed and showed that the preferred walking speeds were not statistically

significantly different, and in fact were quite similar. All in all, we can say that it was safe to combine 'self-paced' and 'fixed-speed' conditions studies.

A general concern with meta-analyses is the risk of positive publication bias (Begg & Berlin, 2016), also known as the 'file-drawer' phenomenon. The general way of assessing a positive publication bias, is to make use of a Funnel plot and to test for asymmetry. In a Funnel plot, standard error is shown against mean difference (effect size). Based on funnel plots, there is no reason to assume the presence of positive publication bias for *GCoW* (see: Appendix C for details). For *NCoW* this is less clear as we found a statistically significant asymmetry in the Funnel plot, which can be caused by publication bias. However, the majority of the papers in the Funnel plot for *NCoW* are also in the Funnel plot for *GCoW*. It is therefore less likely, albeit not impossible, that a positive selection bias is present for *NCoW* only. In addition, several other factors also introduce asymmetry (e.g., heterogeneity within and among the selected studies and differences in methodologies of studies performed (Egger, Davey Smith, Schneider, & Minder, 1997)). Unfortunately, there is no decisive way to test for the presence of positive publication bias.

Having established that there is a difference in *MCoW* between *YA* and *OA*, a question arises whether the elevated *MCoW* in healthy *OA* is a direct effect of age, or is due to age-related confounders? There is indeed some evidence in the literature that points to a possible effect of age on *MCoW*. Malatesta et al., (2003) measured *MCoW* in ten *YA* and two groups of *OA* (ten subjects from 60-69 years old and ten subjects from 77-86 years old) and found that for all the five speeds measured, significant differences in *MCoW* were found between *YA* and the oldest *OA* group, and only for the two highest speeds between *YA* and youngest *OA* group. In addition, in a large study, Gaesser et al. (2018) measured *MCoW* in 96 healthy *YA* and in 94 healthy *OA* that were divided into

three age sub-groups: 31 subjects from 60-64 years old, 34 from 65-69 years old and 29 from >69 years old. As stated before, they found no statistically significant differences in *GCoW* between *YA* and any of *OA* groups (actually, the mean *GCoW* in *YA* was higher). However, for *NCoW* they reported a significantly elevated cost only for the oldest group (>69 years old, p=0.04). Be that as it may, such an age related influence does not explain the contradictory results of Gaesser et al. (2018) with our meta-analyses. Since we have grouped age ≥59 as *OA*, our mean difference should be, if anything, lower than the mean difference between *YA* and the oldest *OA* group from (Gaesser et al., 2018), which is clearly not the case. Thus, based on the literature, it cannot be determined whether the elevation found in our study is directly caused by age, or whether age-related confounders play a role.

A possible confounder is that *OA* need more familiarization time than *YA* in experimental settings. For example, it has been shown that *OA* need substantially more time than *YA* to find their natural gait on a treadmill (Schellenbach, Lövdén, Verrel, Krüger, & Lindenberger, 2010). Wass, Taylor, & Matsas, (2005) showed that *OA* require at least 15 minutes of familiarization time on a treadmill to approximate natural over-ground gait kinematics for a single walking speed at level grade of treadmill. Most of the studies in our meta-analyses were conducted on a treadmill and, unfortunately, often did not provide details about the familiarization time, rendering a correlational analysis between familiarization time and *MCoW* impossible. Based on reported total familiarization time across conditions, the average familiarization time *per walking condition* ranged from 1.2 minutes (Pincheira, Stenroth, Avela, & Cronin, 2017) to 10 minutes e.g., (Ortega & Farley, 2015) which may not have been enough to arrive at normal gait and *MCoW* (Schellenbach et al., 2010). Gaesser et al. (2018) performed their study on a treadmill at a fixed speed (1.34 m/s) and did not find an elevation of *MCoW*

in *OA* over *YA*. Interestingly, in that study though no details on the familiarization protocol were given in the article, but ample familiarization time was provided (personal communication). To conclude, at this point it is unclear if treadmill walking in combination with insufficient familiarization time is a confounding factor in the elevation in *MCoW* in *OA*.

If insufficient familiarization time in treadmill experiments is the true reason for elevation in *MCoW* of *OA* over *YA*, one would expect that the elevation in *MCoW* would not be present when measured during normal over-ground walking. In the two studies in our meta-analyses that measured *MCoW* during over-ground walking, (Waters, Hislop, Perry, Thomas, & Campbell, 1983);(Waters, Lunsford, Perry, & Byrd, 1988) *MCoW* was elevated in *OA* over *YA*, just as in the treadmill studies ; (first two studies listed in Figure 4). However, the preferred walking speed of *OA* group was significantly lower than that of *YA* group, which, as suggested by the authors, was the likely cause of the found elevation in *MCoW* of *OA* over *YA*.

Other possible confounders are differences in fitness level, activity level and anthropometrics. It is quite possible that the normal daily physical activity level differs between *YA* and *OA* and that this may influence the elevation in *MCoW* found in *OA*. Unfortunately, details about the daily physical activity level of participants are often not reported; typically the articles included in the meta-analyses report only that their subjects are all "fit and healthy" for both *YA* and *OA.* In addition, the body mass and body composition may also confound the results. Some of the studies in our meta-analyses statistically analysed the differences in body mass and Body Mass Index (*BMI*), and found statistical differences between *YA* and *OA*. In our analyses, we used reported mass-normalized values of *MCoW* for both *YA* and *OA*, assuming that all participants have the same proportion of muscle mass to body mass, which may thus well not be the

case and may influence the found results (see: (Packard & Boardman, 1999)) of our study. Since the studies analysed here did not report individual data, we were not able to control for the effect of body composition on *MCoW* using an Analysis of Covariance (*ANCOVA*) (Packard & Boardman, 1999).

To conclude, our analyses show a robust and statistically significant elevation in *MCoW* of *OA* over *YA* when walking at comparable speeds. However, it cannot be determined on the basis of the literature whether this elevation is caused directly by age or is caused by age-related confounders. This merits further research comparing *MCoW* in healthy, active and anthropometrically matched *OA* and *YA* during both "natural" over-ground walking and treadmill walking at fixed walking speeds with sufficient familiarization time.

List of studies used in Figure 2 that were not explicitly cited in the main text

(Martin, Rothstein, & Larish, 1992) (Weyand, Smith, Puyau, & Butte, 2010) (Silder, Besier, & Delp, 2012) (Ortega, 2005) (Valenti, Bonomi, & Westerterp, 2015) (Vannetti, Pasquini, Vitiello, & Molino-Lova, 2014) (Ortega, Beck, Roby, Turney, & Kram, 2014) (Schuch, Balbinot, Boos, Peyré-Tartaruga, & Susta, 2011) (Malatesta, Simar, Saad, Préfaut, & Caillaud, 2010) (Fiser et al., 2010) (Terracciano et al., 2013) (Thomas, Vito, & Macaluso, 2007) (Mian et al., 2007) (Dean, Alexander, & Kuo, 2007) (Haveman-Nies, Van Iperen, & Deurenberg, 1996) (McCann & Adams, 2002) (Floreani et al., 2018)

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Appendices

Appendix A - Calculations of *MCoW*

Calculation of MCoW from oxygen consumption

For those studies that did not report *MCoW*, but did report the oxygen consumption rate and *RER*, we used the Lusk Equation (Lusk, 1923) to calculate the mean of the Energy Equivalent (*EEq*) of the amount of oxygen consumed in units of J/kg/min:

$$EEq = (15962 + 5155 \cdot RER) \cdot (\dot{V}O_2 / 1000) \quad (1)$$

The estimated standard deviation of *EEq* was calculated by:

$$\tilde{\sigma}_{EEq} = EEq \sqrt{\left(\frac{\sigma_{\dot{V}O_2}}{\dot{V}O_2}\right)^2 + \left(\frac{\sigma_{RER}}{RER}\right)^2} \quad (2)$$

with $\dot{V}O_2$ and $\sigma_{\dot{V}O_2}$ the reported mean and standard deviation of oxygen consumption rate respectively, and *RER* and σ_{RER} the reported mean and standard deviation of *RER* respectively.

If *RMR* was not reported, but resting $\dot{V}O_2$ ($\dot{V}O_{2_rest}$) was reported, *RMR* was calculated using the Lusk Equation:

$$RMR = (15962 + 5155 \cdot RER_{rest}) \cdot (\dot{V}O_{2_rest} / 1000) \quad (3)$$

The standard deviation of *RMR* was calculated using

$$\tilde{\sigma}_{RMR} = RMR \sqrt{\left(\frac{\sigma_{RER_{rest}}}{RER_{rest}}\right)^2 + \left(\frac{\sigma_{\dot{V}O_{2_rest}}}{\dot{V}O_{2_rest}}\right)^2} \quad (4)$$

For the studies that reported the oxygen consumption rate but not *RER*, we estimated the mean *RER* to be equal to the average of the mean *RER*s from all the other studies that did report it. In that case we estimated the standard deviation of *RER* ($\tilde{\sigma}_{RER}$) using:

$$\tilde{\sigma}_{RER} = \sqrt{\sum_{i=1}^{n} {\sigma_{RER_i}}^2} \text{ and } \tilde{\sigma}_{RER_rest} = \sqrt{\sum_{i=1}^{n} {\sigma_{RER_{rest_i}}}^2} \quad (5)$$

for all *n* studies that reported *RER*.

Then, we calculated *GCoW* in units of J/kg/m using:

$$GCoW = \frac{EEq}{WS \times 60} \quad (6a)$$

and, similarly *NCoW* using :

$$NCoW = \frac{EEq - RMR}{WS \times 60} \quad (6b)$$

The estimated standard deviation of *GCoW* was calculated by:

$$\tilde{\sigma}_{GCoW} = GCoW \sqrt{\left(\frac{\tilde{\sigma}_{EEq}}{EEq}\right)^2 + \left(\frac{\sigma_{WS}}{WS}\right)^2} \quad (7a)$$

The estimated standard deviation of *NCoW* was then calculated by:

$$\tilde{\sigma}_{NCoW} = \sqrt{\tilde{\sigma}_{GCoW}{}^2 + \sigma_{RMC}{}^2} \quad (7b)$$

when $\dot{V}O_2$ was measured at a fixed speed, σ_{RMC} was simply:

$$\sigma_{RMC} = \frac{\sigma_{RMR}}{WS \times 60}$$

If speed differed among subjects, then the estimation of σ_{RMC} was more involved:

$$\sigma_{RMC} = \frac{RMR}{WS \times 60} \sqrt{\left(\frac{\sigma_{RMR}}{RMR}\right)^2 + \left(\frac{\sigma_{WS}}{WS}\right)^2}$$

Calculation of GCoW/NCoW

For those studies that did report *MCoW*, but only reported either *GCoW* or *NCoW* (and *RMR*), *NCoW* or *GCoW* was calculated. *GCoW* was calculated using:

$$GCoW = NCoW + \frac{RMR}{WS \times 60} \quad (8a)$$

NCoW was calculated using:

$$NCoW = GCoW - \frac{RMR}{WS \times 60} \quad (8b)$$

In case *RMR* was not reported, only the reported values of *GCoW* and *NCoW* were used, since *RMR* is needed to calculate one from the other.

The standard deviation of either *GCoW* or *NCoW* was estimated by:

$$\tilde{\sigma}_{GCoW} = \sqrt{\sigma_{NCoW}{}^2 + \sigma_{RMC}{}^2} \quad (9a)$$

$$\tilde{\sigma}_{NCoW} = \sqrt{\sigma_{GCoW}{}^2 + \sigma_{RMC}{}^2} \quad (9b)$$

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Appendix B - Risk of Bias Assessment

The risk of bias assessment (table B1) is shown for all the studies included in the two meta-analyses on *GCoW* and *NCoW*. Specific comments about all or particular studies are mentioned in the rightmost column of the table.

Table B1 Risk of Bias Assessment

Serial Number	Question	Yes	No	Don't know/Comment
Introduction				
1	Were the aims/objectives of the study clear ?	YES		
Methods				
2	Was the study design appropriate for the stated aim(s) ?	YES		
3	Was the sample size justified ?		NO	No details about *a-priori* power analysis or Bayesian statistical analysis were mentioned
4	Was the target/reference population clearly defined ? (Is it clear who the research was about ?)	YES		
5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation ?	YES		
6	Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation ?	Mostly YES		For some studies it is difficult to say as it seems convenience sampling might have been done to recruit participants
7	Were measures undertaken to address and categorise non-responders ?		NO	
8	Were the risk factors and outcome variables measured appropriate to the aims of the study ?	YES		
9	Were the risk factors and outcome variables measured correctly using instruments/measurements that had been trialled/piloted or published previously ?	YES		
10	Is it clear what was used to determine statistical significance and/or precision estimates ? (e.g., p-values, confidence intervals)		NO	Confidence intervals mostly not considered. Only Pincheira et al. (Pincheira et al., 2017) and Waters et al. (Waters et al., 1988) reports it. Mian et al. (Mian et al., 2006), Martin et al. (Martin et al., 1992) & Horiuchi et al. (Horiuchi et al., 2015) say p=0.05 to determine statistical significance, whereas it should be $p<0.05$ Waters et al. (Waters et al.,

				1983) does not mention the criteria for statistical significance in the Methodology section McCann & Adams (McCann & Adams, 2002) does not report the p or alpha value to determine statistical significance under statistical analyses under Methods section
11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated ?	YES		
Results				
12	Were the basic data adequately described ?	YES		
13	Does the response rate raise concerns about non-response bias ?		NO	Non-response bias not handled by the studies
14	If appropriate, was information about non-responders described ?		NO	
15	Were the results internally consistent ?		NO	(Waters et al., 1983) – Sample size in *OA* group not consistent in Table 1
16	Were the results presented for all the analyses described in the methods ?	YES		
Discussion				
17	Were the authors' discussions and conclusions justified by the results ?	YES		
18	Were the limitations of the study discussed ?	YES		
Other				
19	Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results ?		NO	
20	Was ethical approval or consent of participants attained ?		NO	No mention of ethical approval or informed consent in (Waters et al., 1983) and (Waters et al., 1988).

Appendix C - Funnel Plots

For potential publication bias assessment, funnel plots were plotted for the meta-analyses on *GCoW* and *NCoW* (Figure C1). These plots are simple scatterplots of the mean differences of the included studies in the horizontal axis against the standard errors of the same studies in the vertical axis. A regression test for funnel plot asymmetry was also carried out because the pooled effect sizes for our meta-analyses were computed as mean differences (see: https://handbook-5-1.cochrane.org/chapter_10/10_4_3_1_recommendations_on_testing_for_funnel_plot_asymmetry.htm).

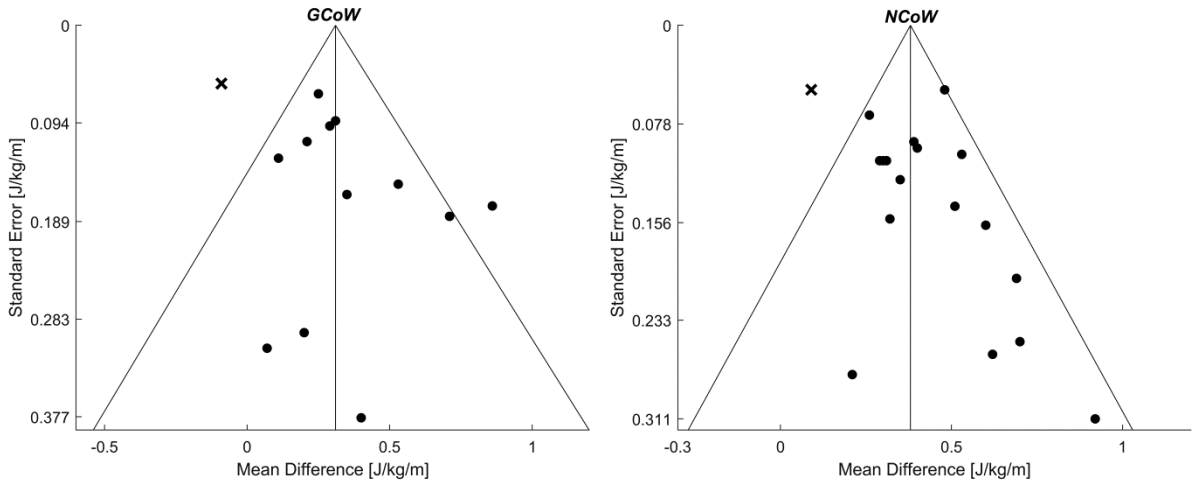

Figure C1. Funnel plot for all the studies included in the meta-analysis for *GCoW* (above left) and *NCoW* (above right). The standard errors of all the individual studies are plotted against their *MD*. The vertical black line in the center of the funnels represents the overall pooled *MD* and the two sides of the funnels represent the "pseudo" 95% *CI* of the overall pooled *MD*. The outliers are plotted automatically outside the funnels and in absence of any publication bias and/or heterogeneity, the number of studies should look symmetrical on both sides of the vertical black line. The black cross in the above figures denotes the study by Gaesser et al., 2018. That study has the lowest standard error and the highest relative weight among all the studies in our meta-analyses and is arguably the most precise on an individual level.

Appendix D – Search Strategy

The full search strategy with the query terms used for each of the four databases are detailed below.

PubMed Session Results (14 Aug 2018) 704 items

(("Energy Metabolism"[Mesh:noexp] OR "Oxidative Phosphorylation"[Mesh] OR energy metabolism[tiab] OR energy expenditure[tiab] OR metabolic cost*[tiab] OR energy cost*[tiab] OR energetic cost*[tiab] OR metabolic consum*[tiab] OR energy consum*[tiab]) AND ("Gait/physiology"[Mesh] OR "Walking/physiology"[Mesh] OR walk[tiab] OR walking[tiab] OR gait[tiab]) AND ("Aged"[Mesh] OR "Aged, 80 and over"[Mesh] OR "Geriatrics"[Mesh] OR "Geriatric Nursing"[Mesh] OR "Health Services for the Aged"[Mesh] OR elder*[tw] OR eldest[tw] OR geriatri*[tw] OR old age*[tw] OR oldest old*[tw] OR senior*[tw] OR senium[tw] OR very old*[tw] OR septuagenarian*[tw] OR octagenarian*[tw] OR octogenarian*[tw] OR nonagenarian*[tw] OR centarian*[tw] OR centenarian*[tw] OR supercentenarian*[tw] OR older people[tw] OR older subject*[tw] OR older patient*[tw] OR older age*[tw] OR older adult*[tw] OR older man[tw] OR older men[tw] OR older male[tw] OR older woman[tw] OR older women[tw] OR older female[tw] OR older population*[tw] OR older person*[tw])) NOT ("Animals"[Mesh] NOT "Humans"[Mesh])

Embase.com Session Results (14 Aug 2018) 722 items

('energy metabolism'/de OR 'oxidative phosphorylation'/exp OR 'energy metabolism':ab,ti,kw OR 'energy expenditure':ab,ti,kw OR 'metabolic cost*':ab,ti,kw OR 'energy cost*':ab,ti,kw OR 'energetic cost*':ab,ti,kw OR 'metabolic consum*':ab,ti,kw OR 'energy consum*':ab,ti,kw) AND ('walking'/exp OR walk:ab,ti,kw OR walking:ab,ti,kw OR gait:ab,ti,kw) AND ('aged'/exp OR 'geriatrics'/exp OR 'elderly care'/exp OR elder*:de,ab,ti,kw OR eldest:de,ab,ti,kw OR geriatri*:de,ab,ti,kw OR 'old

age*':de,ab,ti,kw OR 'oldest old*':de,ab,ti,kw OR senior*:de,ab,ti,kw OR senium:de,ab,ti,kw OR 'very old*':de,ab,ti,kw OR septuagenarian*:de,ab,ti,kw OR octagenarian*:de,ab,ti,kw OR octogenarian*:de,ab,ti,kw OR nonagenarian*:de,ab,ti,kw OR centarian*:de,ab,ti,kw OR centenarian*:de,ab,ti,kw OR supercentenarian*:de,ab,ti,kw OR 'older people':de,ab,ti,kw OR 'older subject*':de,ab,ti,kw OR 'older patient*':de,ab,ti,kw OR 'older age*':de,ab,ti,kw OR 'older adult*':de,ab,ti,kw OR 'older man':de,ab,ti,kw OR 'older men':de,ab,ti,kw OR 'older male':de,ab,ti,kw OR 'older woman':de,ab,ti,kw OR 'older women':de,ab,ti,kw OR 'older female':de,ab,ti,kw OR 'older population*':de,ab,ti,kw OR 'older person*':de,ab,ti,kw) NOT ([animals]/lim NOT [humans]/lim)

SPORTDiscus Session Results (14 Aug 2018) 125 items

(DE "ENERGY metabolism" OR DE "AEROBIC metabolism" OR DE "ANAEROBIC metabolism" OR DE "BASAL metabolism" OR DE "CALORIC expenditure" OR TI ("energy metabolism" OR "energy expenditure" OR "metabolic cost*" OR "energy cost*" OR "energetic cost*" OR "metabolic consum*" OR "energy consum*") OR AB ("energy metabolism" OR "energy expenditure" OR "metabolic cost*" OR "energy cost*" OR "energetic cost*" OR "metabolic consum*" OR "energy consum*")) AND (DE "WALKING" OR DE "GAIT in humans" OR TI (walk OR walking OR gait) OR AB (walk OR walking OR gait)) AND (DE "OLDER people" OR DE "GERIATRICS" OR TI (elder* OR eldest OR geriatri* OR "old age*" OR "oldest old*" OR senior* OR senium OR "very old*" OR septuagenarian* OR octagenarian* OR octogenarian* OR nonagenarian* OR centarian* OR centenarian* OR supercentenarian* OR "older people" OR "older subject*" OR "older patient*" OR "older age*" OR "older adult*" OR "older man" OR "older men" OR "older male" OR "older woman" OR "older women" OR "older female" OR "older population*" OR "older person*") OR AB

(elder* OR eldest OR geriatri* OR "old age*" OR "oldest old*" OR senior* OR senium OR "very old*" OR septuagenarian* OR octagenarian* OR octogenarian* OR nonagenarian* OR centarian* OR centenarian* OR supercentenarian* OR "older people" OR "older subject*" OR "older patient*" OR "older age*" OR "older adult*" OR "older man" OR "older men" OR "older male" OR "older woman" OR "older women" OR "older female" OR "older population*" OR "older person*"))

Cochrane Library Session Results (14 Aug 2018) 45 items

(("energy metabolism" or "energy expenditure" or "metabolic cost*" or "energy cost*" OR "energetic cost*" or "metabolic consum*" or "energy consum*") AND (walk or walking or gait) AND (elder* or eldest or geriatri* or "old age*" or "oldest old*" or senior* or senium or "very old*" or septuagenarian* or octagenarian* or octogenarian* or nonagenarian* or centarian* or centenarian* or supercentenarian* or "older people" or "older subject*" or "older patient*" or "older age*" or "older adult*" or "older man" or "older men" or "older male" or "older woman" or "older women" or "older female" or "older population*" or "older person*")):ti,ab,kw

Chapter 2

Metabolic cost in healthy fit older adults and young adults during overground and treadmill walking

(Adapted from) Sauvik Das Gupta, Maarten Bobbert, Herre Faber and Dinant Kistemaker. Metabolic cost in healthy fit older adults and young adults during overground and treadmill walking. *European Journal of Applied Physiology*, 121(10), 2787-2797 (2021). https://doi.org/10.1007/s00421-021-04740-2

The purpose of this study was to determine whether net metabolic cost of walking is affected by age *per se*. We selected 10 healthy, active older adults (mean age 75 yr) and 10 young adults (mean age 26 yr), and determined their preferred overground walking speed. On the same day, in a morning and afternoon session, we had them walk at that speed overground and on a treadmill while we measured oxygen consumption rate. From the latter we subtracted the rate in sitting and calculated net metabolic cost. We found that anthropometrics were not different between the groups, nor was preferred walking speed (1.27 $m \cdot s^{-1}$ both groups). There was no difference in net metabolic cost of overground walking between older and young adults (e.g., in the morning 2.64 and 2.56 $J\ kg^{-1}m^{-1}$, respectively, $p>0.05$). In the morning session, net metabolic cost of walking was higher on the treadmill than overground in our older adults by 0.6 $J\ kg^{-1}m^{-1}$ ($p<0.05$), but not in young adults. From this study we saw that there is no effect of age *per se* on metabolic cost of overground walking. Second, older adults tend to have higher metabolic cost of walking on a treadmill than walking overground at preferred speed, and adaptation may take a long time. The commonly reported age-related elevation of metabolic cost of walking may be due to confounding factors causing preferred walking speed to be lower in older adults, and/or due to older adults reacting differently to treadmill walking than young adults.

Abbreviations:

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BAI	Body Adiposity Index
BMI	Body Mass Index
D	Cohen's d
EMG	Electromyography
GCoW	Gross Cost of Walking
MCoW	Metabolic Cost of Walking
NCoW	Net Cost of Walking
OA	Older Adults
PWS	Preferred Walking Speed
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
SD	Standard Deviation
SEM	Standard Error of Mean
$\dot{V}O_2$	Oxygen consumption rate
W/H	Waist-to-Height ratio
YA	Young Adults

2.1 Introduction

The ability to walk is important for people to participate in society. Healthy young people walk seemingly without physical effort, but when people get older, walking can become a challenge. An important variable in studying walking is the Metabolic Cost of Walking (MCoW), defined as the metabolic energy expended per kilogram body mass per meter travelled. *MCoW* is typically calculated from measured oxygen consumption, carbon dioxide production and walking speed (Zarrugh & Radcliffe, 1978), and can be expressed in terms of Gross Cost of Walking (*GCoW*) and in terms of Net Cost of Walking (*NCoW*). *GCoW* relates to the total amount of metabolic energy consumed, whereas *NCoW,* calculated by subtracting from the metabolic rate during walking the resting metabolic rate, captures the amount of metabolic energy expended due to walking *per se* (i.e., due to walking-related cross-bridge cycling and Ca^{2+} pumping within muscles). *MCoW* has been studied across all age-groups in humans, from children to the elderly. Typically, healthy Young Adults (*YA*) walking at their Preferred Walking Speed (*PWS*) exhibit a *GCoW* of about 3.4 J $kg^{-1}m^{-1}$ and a *NCoW* of about 2.4 J $kg^{-1}m^{-1}$. When published results on differences between *YA* and Older Adults (*OA*) for *MCoW* are summarized, *OA* have a statistically significantly elevated *MCoW* compared to *YA;* the pooled mean *GCoW* was ~0.3 J $kg^{-1}m^{-1}$ (d=0.65) higher in *OA* than in *YA,* and the pooled mean *NCoW* was ~0.4 J $kg^{-1}m^{-1}$ (d=1.00) higher in *OA* than in *YA* (Das Gupta, Bobbert, & Kistemaker, 2019).

In the literature, the increase in *MCoW* in *OA* has been attributed to age-induced physiological changes in muscles and the cardiovascular system (Gaesser, Tucker, Sawyer, Bhammar, & Angadi, 2018), changes in gait coordination (Donelan, Kram, & Kuo, 2001), and/or higher coactivation reflected in Electromyography (*EMG*) activity (Peterson & Martin, 2010). However, in our meta-analysis (Das Gupta et al., 2019) we

indicated several potential confounding factors that made it difficult to judge whether the difference in *MCoW* found in the literature is a direct effect of age.

A first possible confounder is *PWS*. In two studies in which overground walking was studied, *OA* were reported to have a higher *MCoW* than *YA* (Waters, Hislop, Perry, Thomas, & Campbell, 1983; Waters, Lunsford, Perry, & Byrd, 1988), and also had a lower *PWS* than *YA.* It is generally known from the literature that *MCoW* depends on walking speed (Ralston, 1958; Zarrugh, Todd, & Ralston, 1974), and that *YA* prefer to walk at the speed at which *MCoW* is minimized (Ralston, 1958). Due to an age-related reduction of fitness, or disease, *OA* may prefer a walking speed which is lower than the speed at which *MCoW* is minimized (Sanseverino et al., 2018). It cannot be decided to what extent the difference in *MCoW* between *OA* and *YA* in the two studies on overground walking is due to age *per se* and to what extent it is due to a difference in walking speed; potential scenarios for a confounding effect of walking speed are presented in Fig. 1A-B.

A second possible confounder in studying age effects on *MCoW* is the use of a treadmill. Possibly to avoid a confounding effect of *PWS* in overground walking, all other studies that we found on the difference in *MCoW* between *OA* and *YA* have been on treadmill walking at fixed speeds (Dean, Alexander, & Kuo, 2007; Floreani et al., 2018; Jones, Waters, & Legge, 2009; Martin, Rothstein, & Larish, 1992). However, *OA* have a higher metabolic requirement at *PWS* on a treadmill than overground (Parvataneni, Ploeg, Olney, & Brouwer, 2009). It has been suggested that *OA* need more familiarization time than *YA* to restore ‘normal’ walking kinematics (Schellenbach, Lövdén, Verrel, Krüger, & Lindenberger, 2010; Wass, Taylor, & Matsas, 2005), and thus require more time to arrive at their steady state *MCoW*. It is possible that the investigators may not have provided *OA* with enough time to familiarize to treadmill

walking *per walking condition* (see: (Das Gupta et al., 2019)), which could have resulted in an elevated *MCoW* compared to *YA.* Hence, if one is interested in the ecologically valid difference in *MCoW* between *OA* and *YA* in overground walking, the use of a treadmill may itself be a confounder.

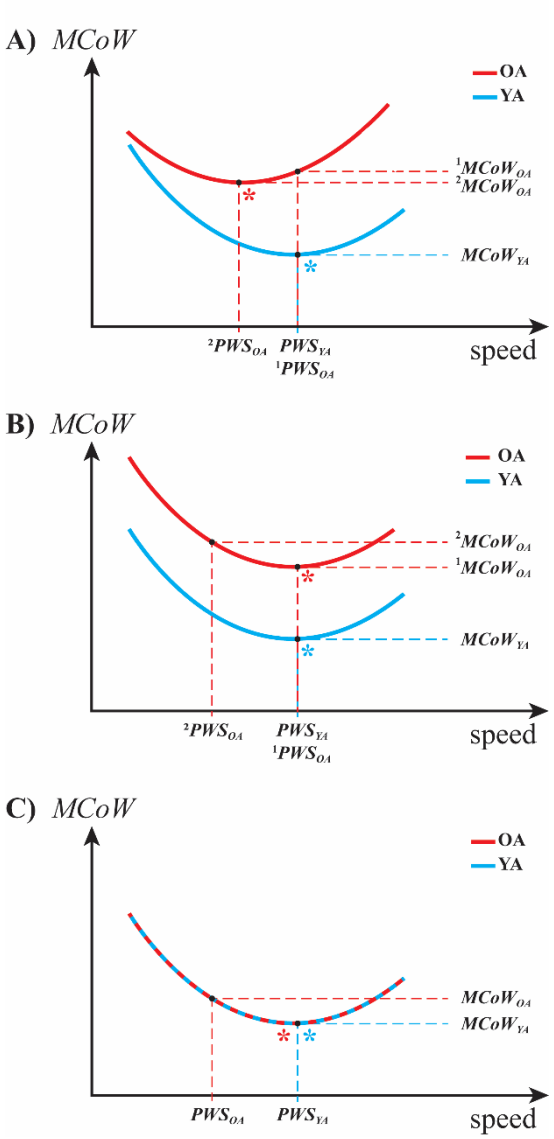

Figure 1. Possible scenarios underlying an elevated metabolic cost of walking (*MCoW*) in older adults (*OA*) compared to Young Adults (*YA*). At a given *MCoW* curve, the asterisk (*) indicates the optimal walking speed, i.e., the speed at which *MCoW* is minimal. In scenario A the *MCoW* curve is shifted leftward and upward in *OA* compared to *YA*, in scenario B it is only shifted upward, and in scenario C it is not shifted at all. In scenario A, two cases are shown leading to an elevated *MCoW* in *OA*: in the first case Preferred Walking Speed (*PWS*) of *OA* is sub optimal ($^1PWS_{OA}$) yet similar to that of *YA* resulting in $^1MCoW_{OA}$, and in the second case $^2PWS_{OA}$ is optimal and resulting in $^2MCoW_{OA}$. In scenario B, *MCoW* is elevated in *OA*, both when *OA* are selecting a *PWS* that is sub-optimal ($^2PWS_{OA}$) and when they select a *PWS* that is optimal/similar to that of *YA* ($^1PWS_{OA}$). In scenario C, there is no (statistically) significant difference in *MCoW* curves and only if *OA* select a lower (sub-optimal) *PWS* than *YA*, their *MCoW* would be higher. Selecting a (for *NCoW*) sub-optimal *PWS* in *OA* may, for example, be caused by increased stability demands or insufficient familiarization time.

The third possible confounder is physical fitness. It is known that due to aging there is a general decline of the physical activity and fitness, which in turn may affect *PWS* (Haveman-Nies, Van Iperen, & Deurenberg, 1996; Jones et al., 2009; Malatesta et al., 2003; Martin et al., 1992; Pincheira, Stenroth, Avela, & Cronin, 2017; Waters et al., 1983,1988). A reduction in *PWS* of *OA* due to a decline in fitness may lead to an elevated *MCoW* for *OA* compared to their fitter *YA* counterparts, even if the (U-shaped) relationship between *MCoW* and walking speed itself is the same for *YA* and *OA*; this possible scenario is shown schematically in Fig. 1C.

The fourth possible confounder is a difference in anthropometrics. *MCoW* is commonly reported in mass-normalized values, which implicitly assumes that all participants have the same proportion of muscle mass to body mass. However, particularly in sedentary elderly this proportion might be smaller than in active young participants. It has been suggested in the literature that normalizing physiological variables to body mass may substantially influence the (statistical) outcomes; it may actually introduce a methodological bias and lead to inflated type I errors (Packard & Boardman, 1999).

The purpose of the present study was to determine whether net metabolic cost of walking is affected by age *per se*. We set out to measure *MCoW* in *YA* and *OA* while attempting to control the possible confounders mentioned above. To end up with anthropometrically matched groups of physically active, fit and healthy *OA* and *YA*, we used strict inclusion criteria for potential participants. To investigate the potential influence of mass-normalization of physiological variables (see: (Packard & Boardman, 1999)), we performed an *ANCOVA*. We determined overground *PWS*, and we measured *GCoW* and *NCoW* at each individual's *PWS* both overground and on a treadmill, and both in a morning session and in an afternoon session on the same day. In the afternoon

session, we additionally measured kinematics, kinetics and *EMG*, but in this study we report only *GCoW* and *NCoW*. We focussed on the following questions: (1) Is *MCoW* in overground walking higher in *OA* than in *YA*? (2) Is *MCoW* in treadmill walking higher than in overground walking in *OA* and in *YA*? (3) Is *MCoW* in treadmill walking higher in *OA* than in *YA*?

2.2 Methods

Characteristics of participants and Ethics statement

For this study, we recruited 10 healthy *OA* (mean age 75.3 ± 6.3 years, 5 males and 5 females) and 10 healthy *YA* (mean age 25.5 ± 3.4 years, 4 males and 6 females). The sample size per group was estimated from published studies (as also done in (Horiuchi, Endo, Horiuchi, & Abe, 2015) and comparable to that in other studies (Dean et al., 2007; Horiuchi et al., 2015; Hortobágyi, Finch, Solnik, Rider, & De Vita, 2011; Malatesta et al., 2003, 2004; Ortega & Farley, 2007, 2015; Ortega, Fehlman, & Farley, 2008; Peterson & Martin, 2010; Pincheira et al., 2017). We excluded participants who had chronic heart disease, diabetes, past surgeries or prosthesis on the lower limbs, or neuromuscular disabilities, or had experienced a fall in the past six months. In soliciting potential participants, we stressed that they should be fit and physically active, carrying out their normal day-to-day activities without any assistance. We ended up accepting only those participants who met these criteria. None of the participants was actively involved in any kind of special strength or endurance training. The inclusion/exclusion criteria were exactly similar for both *YA* and *OA* and thus we believe that they have not introduced new confounding factors. We asked all our participants to specify their general day-to-day activity patterns and in hindsight feel safe to say that there was no selection bias between our *YA* and *OA.* All participants signed a written informed consent. We measured standard anthropometrics; body mass, height, waist and hip

circumferences and lower limb lengths up to malleolus and foot from the greater trochanter. Body Adiposity Index (*BAI*) was calculated according to the following equation (Bergman et al., 2011):

$$BAI(\%) = \left(100 \times c_{hip} / h^{3/2}\right) - 18 \quad (1)$$

where c_{hip} is the circumference measured at the height of the hip and h is the body height, both in *m*.

The ethical review committee of the Faculty of Behavioural and Movement Sciences of the Vrije Universiteit Amsterdam approved the experimental protocol.

Protocol

Pre-experiment factors

Detailed instructions regarding diet and exercise were given to all *YA* and *OA* participants. In short, participants were to have a light meal for both their breakfast and lunch (i.e., moderate intake of carbohydrates and fats, minimal intake of protein and fiber rich foods; food intake was listed). They were also instructed to refrain from consuming any caffeine or alcohol-containing products and from smoking tobacco until the experiments were finished. To negate the thermic effect of food, we ensured that there was an interval of at least two hours between their meals and measurement of any metabolic data. In total, there was at least a three-hour interval between the two experimental sessions. Within each session, there were at least 20 minutes between the overground trial and the treadmill trial.

Preparation

The participants were fitted with a portable Cosmed K4b2 breath-by-breath indirect calorimetry system (Cosmed, Rome, Italy) and a face-mask to measure rates of oxygen consumption and carbon dioxide production. The Cosmed K4b2 was calibrated before the start of each experimental session according to the manufacturer's guidelines.

Morning & Afternoon session

We had our participants walk in a morning session and in an afternoon session on the same day. We chose to have two sessions in order to allow our *OA* to familiarize themselves with walking in our laboratory, and in particular on our treadmill. This was based on studies showing that *OA* need more time (~15 minutes) to arrive at kinematics that are similar to those observed during overground walking (Schellenbach et al., 2010; Wass et al., 2005) than *YA*. The morning session consisted of two trials; one overground trial (9 min) and one treadmill trial (15 min). The afternoon overground trial was of similar duration as the morning overground trial. The afternoon treadmill trial consisted of 9 min walking and not 15 min like the morning session, because *OA* had indicated in pilot studies that a 15 min treadmill trial at the end of the day was too tiring.

Prior to every walking trial, Resting Metabolic Rate (*RMR*) was measured while the participants were seated in a chair for 5 minutes in a relaxed state (see: Fig. 2). In several other studies, *RMR* was measured during standing. The *RMR* measured during standing is about 1.16 times the *RMR* measured during resting; the additional metabolic cost is attributed to activation of leg and trunk muscles to maintain an upright posture, support the body weight and to balance (Weyand, Smith, & Sandell, 2009). The same cost is also involved in walking and in our opinion is part of the cost of walking. Hence, we feel that when using *NCoW* to estimate the metabolic cost attributed to walking itself, it is more meaningful to subtract *RMR* in a non-standing posture to estimate *NCoW*.

All participants then walked continuously (without any pause) at their preferred speeds inside a large indoor laboratory along an oval track with 32 m straights interconnected by two half-circles of 4 m radius (Fig. 2). They did so for at least five minutes and then completed at least three full laps during which oxygen consumption

rate was recorded. *GCoW* (in J $kg^{-1}m^{-1}$) was calculated from the oxygen consumption rate using Lusk's equation (Lusk, 1923) and dividing by *PWS*:

$$GCoW = (15962 + 5155 \cdot RER) \cdot \dot{V}O_2 \cdot PWS^{-1} \quad (2)$$

where $(15962 + 5155 \cdot RER)$ is the Energetic equivalent of oxygen (in J l^{-1}), *RER* is the Respiratory Exchange Ratio (dimensionless), $\dot{V}O_2$ is oxygen consumption rate (in l $kg^{-1}s^{-1}$), and *PWS* was the average walking speed during the last three laps (in $m \cdot s^{-1}$). In order to establish *PWS* participants were asked in the morning overground trial to walk at the speed they would normally walk while going from their home to a supermarket. *NCoW* was obtained by inserting net $\dot{V}O_2$, i.e., the difference in $\dot{V}O_2$ between walking and sitting.

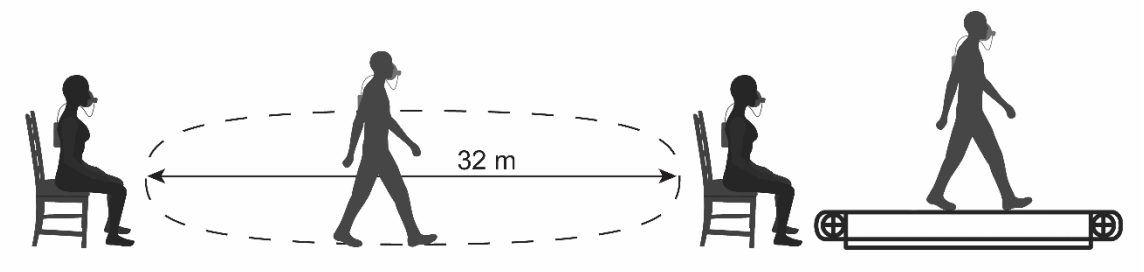

Figure 2. Schematic overview of the energy expenditure measurements during one session. Resting Metabolic Rate was measured while the participants were seated for 5 minutes on a chair as shown above, both before the overground walking trial and before the treadmill trial. Metabolic Cost of Walking was measured for both young and elderly adults in two separate sessions, each involving an overground trial and a treadmill trial. During the overground trial participants walked continuously along the schematically drawn oval track with 32 m straights interconnected by two half-circles of 4 m radius. During the treadmill trial participants walked on a treadmill at their imposed overground *PWS*.

Following the overground trial, all participants walked for 15 minutes on a dual-belt motorized treadmill (ForceLink BV, Culemborg, the Netherlands) while oxygen consumption rate was measured (Fig. 2). Treadmill speed was gradually increased over the first 2-3 min to the individual's overground *PWS*, and then kept constant. At the end of the trial, the treadmill speed was gradually reduced to zero and then oxygen consumption rate measurement was stopped. The last 30 seconds of data were always

discarded and the oxygen consumption rate of the preceding last 3 full minutes was used to calculate *MCoW* for all our participants and all the treadmill trials.

In the afternoon session, we first instrumented the participants with kinematic markers and *EMG*-electrodes, and then repeated the overground walking and treadmill trials while we measured not only oxygen consumption rate, but also kinematics, *EMG* and ground reaction forces. Note, however, that in this paper we only focus and report on *MCoW*.

Statistical tests

Before analysing differences between *OA* and *YA* in anthropometric parameters and *PWS*, we first used a Shapiro-Wilk test to check for normality. We then used a Levene's test to check for the equality of variances. Additionally, Quantile-Quantile plots, boxplots and violin plots were used to visually check for normality. To analyse between-group differences in anthropometric parameters and *PWS* we used the parametric two-sided independent samples Student's t-test.

Before embarking on a full-blown statistical analysis of results of *NCoW*, we averaged *GCoW* and *NCoW* time histories over participants and graphically displayed means and Standard Error of the Mean (*SEM*) as a function of time for the last 4 min of the trials. From these graphs, it can already be deduced which differences among groups are not statistically significant and which differences might be significant. We then performed an *ANCOVA* of *NCoW* normalized for distance only with body mass of the participants to check whether the outcome of our study could have been inadvertently affected by mass normalization (Packard & Boardman, 1999). To analyse both between-group and within-subject differences in *NCoW* between our *YA* and *OA* participants, we performed a Mixed *ANOVA* analysis with three levels: group (*OA* versus *YA*), 'surface' (overground versus treadmill) and session (morning versus afternoon). We further

analysed statistically significant main effects and interaction effects using Post hoc tests with Tukey's correction for non-repeated measures tests and Bonferroni's correction for repeated measures tests. As will be explained in the discussion, we also tested a few differences using simple t-tests without correction to see whether false negatives had occurred.

The effect sizes (Hedge's G) for the comparisons between age, anthropometric parameters and *PWS* were calculated as the mean values for *YA* subtracted from the mean values for *OA* divided by the pooled standard deviation of both the groups. The effect sizes for both the within-subject and between-group comparisons on *NCoW* from the Mixed *ANOVA* analysis will be reported as partial eta-squared (η^2_p). Effect sizes of the post-hoc comparisons and those of the separate unpaired and paired Student's t-tests as mentioned in the Discussion section are reported as Cohen's d. The open-source software JASP (version 0.13.0.0) was used for all the statistical tests. The default value of α=0.05 was chosen as the level of statistical significance.

2.3 Results

We found no deviations from normality or equality of variances for any of the anthropometric parameters or *PWS*, with one exception: for Body Mass Index (*BMI*) the assumption of equality of variances was violated. Hence, for testing differences in *BMI* we resorted to the parametric two-sided independent samples Welch's t-test.

Age, Walking speed and Anthropometrics

Table 1 lists the age, anthropometric parameters and *PWS* of the participants as mean ± *SD* (Standard Deviation). Other than the obvious difference in age, there were no statistically significant differences between *YA* and *OA* in anthropometric parameters or *PWS* ($p>0.05$); *PWS* was 1.27 $m \cdot s^{-1}$ both in *OA* and *YA*.

Metabolic Cost of Walking

Figure 3 provides a helicopter view of means and *SEM* (standard error of mean, gray area) of *GCoW* and *NCoW* for *YA* and *OA* for all trials. The long horizontal lines in the top diagrams (*OA*) and bottom diagrams (*YA*) are for easy reference; they are plotted at the values attained by *OA* at the end of the first overground walking trial. Just as a first indication, for a difference between means in *OA* and *YA* to be statistically significant, it should be bigger than about twice the pooled *SEM*. In *YA*, *GCoW* and *NCoW* seemed to reach a steady state value that was the same on treadmill and overground. In *OA*, however, *GCoW* and *NCoW* tended to increase during the treadmill trials (even after 10 min in the morning treadmill trial) and seemed to reach values that were higher on the treadmill than overground. This increase in *GCoW* and *NCoW* after 10 min in the morning treadmill trial occurred specifically in three *OA*, which was due to a slow increase in the rate of oxygen consumption for all of them and additionally by an increase in *RER* in one of these three *OA*. The slow increase in the rate of oxygen consumption could indicate that less efficient muscle fibers are recruited and it could be a sign of fatigue (Barclay, 1996; Jones et al., 2011; Woledge, 1998). Additionally, it could also be that fatigue led these *OA* to walk with a different gait pattern, perhaps with more muscle coactivation, which in turn led to the increase in *MCoW*. As a matter of fact, indeed two out of these three *OA* felt it quite hard to complete the 15 min treadmill trial and reported feeling fatigued after the trial. All these three *OA* were also at least 80 years old or above, possibly indicating that such a manifestation of fatigue was seen only in our octogenarians.

As our *YA* and *OA* groups were anthropometrically matched (see: Table 1), it was unlikely that differences in anthropometrics substantially influenced our outcomes. The results of the *ANCOVA* confirmed this; there was no statistical effect of body mass normalization.

Our Mixed *ANOVA* detected no problems with data homogeneity. It yielded only two statistically significant effects, a main effect of 'surface' ($p=0.04$, $\eta^2_p=0.21$) and a

Table 1: Age, anthropometric parameters and *PWS* of *YA* and *OA*

	YA	***OA***	**Effect Size**
Age (years)	25.5 ± 3.4	75.3 ± 6.3	9.4
Body Mass (kg)	62.4 ± 12.5	63.3 ± 7.3	0.1
Height (m)	1.63 ± 0.10	1.65 ± 0.09	0.2
BMI (kg/m^2)	23.6 ± 4.5	23.2 ± 2.3	-0.1
BAI (%)	28.7 ± 5.9	28.0 ± 4.2	-0.1
W/H Ratio	0.5 ± 0.1	0.5 ± 0.1	0
Lower Limb Length up to Malleolus (m)	0.79 ± 0.05	0.80 ± 0.05	0.2
Lower Limb Length up to Foot (m)	0.84 ± 0.06	0.86 ± 0.06	0.3
Overground PWS (m·s^{-1})	1.3 ± 0.2	1.3 ± 0.1	0

BMI = Body Mass Index, BAI=Body Adiposity Index, W/H=Waist-to-Height

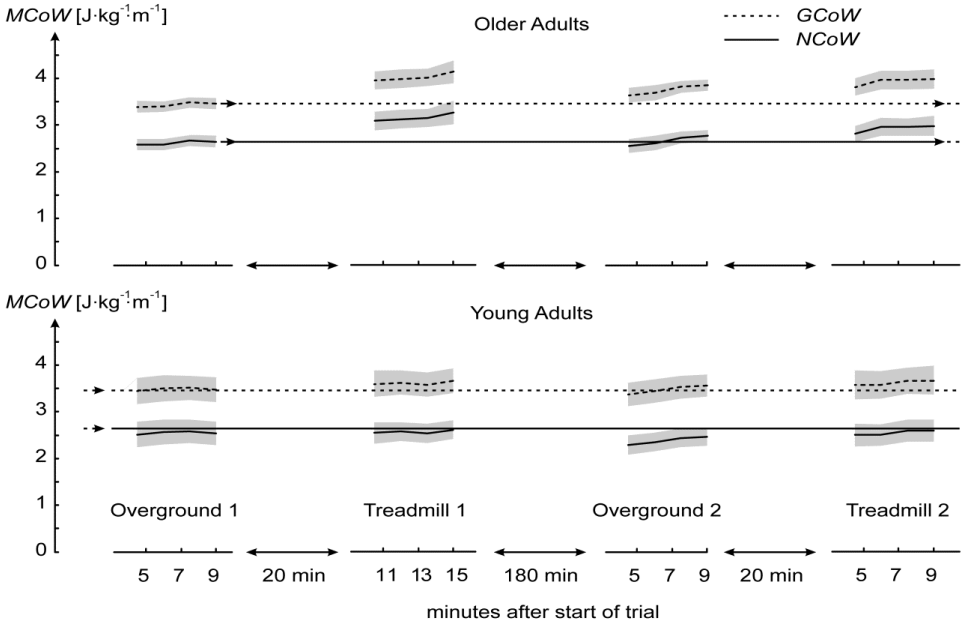

Figure 3. Overview of metabolic cost results. Means and *SEM* (standard error of mean, gray area) of Gross and Net Cost of Walking (*GCoW* and *NCoW*, respectively) have been plotted for all trials. Just for easy reference, long horizontal lines in the top diagrams and bottom diagrams are plotted at the values attained by older adults at the end of the first overground walking trial.

combined interaction effect of group*surface*session ($p=0.02$, $\eta^2_p=0.27$). Overall there were no group effects (*YA* vs *OA*, $p=0.16$, $\eta^2_p=0.11$); there were no violations of data homogeneity.

Our first question was whether *OA* had a higher *NCoW* than *YA* during overground walking. During the morning overground trial, *NCoW* was 2.64 ± 0.38 J $kg^{-1}m^{-1}$ in *OA* and 2.56 ± 0.79 J $kg^{-1}m^{-1}$ in *YA*, a difference of only 0.08 J $kg^{-1}m^{-1}$. During the afternoon overground trial the difference was 0.3 J $kg^{-1}m^{-1}$. According to the Mixed *ANOVA* results, these differences were not statistically significant ($p>0.05$) after post-hoc corrections.

Our second question was whether *NCoW* in treadmill walking was higher than in overground walking in *OA* and in *YA.* Post hoc tests revealed that *NCoW* was overall higher on the treadmill than overground by 0.24 J $kg^{-1}m^{-1}$ ($p=0.04$) with a medium effect size (Cohen's $d=0.48$). Specifically, *NCoW* was higher on the treadmill than overground in *OA* in the morning session by 0.61 J $kg^{-1}m^{-1}$ ($p=0.03$). This was already the case after 5 minutes of treadmill walking, when none of the *OA* reported any fatigue.

Our third question was whether *OA* had a higher *NCoW* than *YA* during treadmill walking. During the last 3 full minutes of the morning treadmill trial, *NCoW* in *OA* was 3.25 ± 0.68 J $kg^{-1}m^{-1}$ and 2.59 ± 0.6 J $kg^{-1}m^{-1}$ in *YA*, a difference of 0.66 J $kg^{-1}m^{-1}$. During the afternoon treadmill trial the difference was smaller, 0.36 J $kg^{-1}m^{-1}$. According to the Mixed *ANOVA*, there was no group effect and these differences were also not statistically significant ($p>0.05$) after post-hoc corrections. Note, however, that the afternoon treadmill trial was shorter than that in the morning; *NCoW* continued to increase until the end of the morning treadmill trial, and might have increased further in the afternoon trial if that trial had lasted longer.

In Fig. 3 it can be seen that the curves of *GCoW* and *NCoW* in the afternoon are further apart than those in the morning. This is mostly because *RMR* was higher in the afternoon by 7.3 % in *YA* and by as much as 25 % in *OA*. For *YA*, *RMR* was 77 ± 19.4 J kg^{-1} min^{-1} in the morning session and 82.6 ± 14.7 J kg^{-1} min^{-1} in the afternoon session. For *OA*, *RMR* was 64.8 ± 11 J kg^{-1} min^{-1} in the morning session and 81 ± 15 J kg^{-1} min^{-1} in the afternoon session. For both the morning and the afternoon session, *RMR* values were averaged for the *RMR* values obtained before each overground and the treadmill walking trial. Although we measured oxygen consumption rate in the afternoon session at least two hours after finishing lunch, we cannot rule out some remaining thermic effects of food leading to the elevation in the resting oxygen consumption rate. Thus, *RMR* is a potential source for variability of *GCoW*, and this strengthens our choice to focus on *NCoW*. The question may be raised whether our conclusions with respect to *NCoW* would have been different if we had measured *RMR* during standing rather than sitting. It is possible that the metabolic cost of standing is higher in *OA* than in *YA* due to balance being impaired in *OA*. In that case, if we had subtracted *RMR* in standing rather than sitting from the gross metabolic rate of walking, the difference in *NCoW* between *OA* and *YA* would have been even smaller.

2.4 Discussion

When summarizing published data on differences between *YA* and *OA* for *MCoW*, we found *OA* have a statistically significantly elevated *MCoW* compared to *YA* (Das Gupta et al., 2019). However, we could not determine whether this elevation was caused directly by age or was due to confounders and in particular due to walking on a treadmill. The purpose of the present study was to determine to which extent the elevated *MCoW* reported in the literature for *OA* is due to the effect of age *per se*. We solicited matched, healthy, active and fit *OA* and *YA*, determined their *PWS*, and

measured their *MCoW* at that speed both overground and on a treadmill. We found both *OA* and *YA* to have a *PWS* of 1.27 $m \cdot s^{-1}$. Only Malatesta, Canepa, & Menendez Fernandez, (2017) found a similar *PWS* in *OA* and *YA*; and in some previous studies, *PWS* was lower in *OA* than in *YA* (e.g., (Jones et al., 2009; Martin et al., 1992; Waters et al., 1983, 1988)). Note, however, that all but the studies from (Waters et al., 1983, 1988) measured *PWS* on a treadmill and we cannot rule out that treadmill walking influences *PWS*. In any case, in our study, differences in anthropometrics and *PWS* were not present and could be ruled out as confounders, so that we could purely focus on answering the following three questions: (1) Is *NCoW* in overground walking higher in *OA* than in *YA*? (2) Is *NCoW* in treadmill walking higher than in overground walking in *OA* and in *YA*? (3) Is *NCoW* in treadmill walking higher in *OA* than in *YA*? Below, we will answer each of these questions, but not before discussing the statistical power of our study.

The question of whether *NCoW* was higher in overground walking in *OA* than in *YA* is obviously the most ecologically relevant question. After all, participating in society requires overground walking, not treadmill walking. Our Mixed *ANOVA* detected no main effect of group on *NCoW*, which implies that *NCoW* was not higher in overground walking in *OA* than in *YA*. In principle, this could be a false negative outcome. After all, we had only 10 participants in each group, and the *ANOVA* formally required no less than 28 family-wise post-hoc corrections, which may mask relevant differences among groups. In our defence of group sizes: most previous studies reporting an elevated *MCoW* in *OA* compared to *YA* have been performed with similarly sized *OA* and *YA* groups, we repeated all measurements in the second session, and we focused on *NCoW*; *NCoW* has smaller variability and greater reported effect size than *GCoW*, probably due to a highly variable *RMR* (see also (Das Gupta et al., 2019)). With

respect to using a Mixed *ANOVA*: while this is the formal approach, we can also do simple unpaired Student's t-test on the difference in *NCoW* in the morning overground trial, to minimize the chance of a false negative outcome. Note that this statistical test would be standard for studies investigating the *MCoW* in *OA* and *YA* in a single overground trial (as for example done in (Waters et al., 1983, 1988)). In our morning overground walking trial, the difference in *NCoW* between *OA* and *YA* was only 0.08 J $kg^{-1}m^{-1}$ and not statistically significant (2.64 ± 0.38 J $kg^{-1}m^{-1}$ in *OA* and 2.56 ± 0.79 J $kg^{-1}m^{-1}$ in *YA*, p=1). In fact, the difference was negligible and no realistic number of participants would have rendered it statistically significant. In the afternoon trial the difference was 0.3 J $kg^{-1}m^{-1}$; this value is close to the difference between *OA* and *YA* emanating from our meta-analysis of studies in the literature (~0.4 J $kg^{-1}m^{-1}$), but even in a simple unpaired Student's t-test it was not statistically significant (p=0.18, Cohen's d=0.6). Even if there had been a statistically significant difference in the afternoon overground trial, it would be difficult to attribute it to an effect of age *per se*. Merely looking at trends in the time histories of *NCoW* in Fig. 3, we see that the difference between *OA* and *YA* in *NCoW* during overground walking had grown in the afternoon relative to the morning session because *NCoW* of *YA* had dropped, not because *NCoW* of *OA* had increased. Interestingly, *GCoW* in *YA* had not changed; the drop in *NCoW* was due to an unexpected increase in *RMR* in *YA*. All in all, we feel safe to conclude that there is no direct effect of age on *NCoW* at *PWS* in overground walking.

Even though we did not find a direct effect of age on *MCoW*, it seemed that walking at *PWS* was more challenging for *OA* than for *YA*. After all, none of our *YA* felt any fatigue at the end of the day of measurements but, as we already mentioned, some of our *OA* felt fatigued at the end of the afternoon treadmill trial, even after walking 800 m. We can think of several possible reasons. One is that the rest period we had included

between the morning and afternoon sessions and/or that between the overground trial and treadmill trial within a session, was sufficient for *YA* but not for *OA*. A second possible reason is that *OA* overestimated their *PWS*, wanting to show in the morning overground trial that they were fit. A third possible reason is that *OA* had slower oxygen uptake kinetics and a lower aerobic capacity than *YA*. It has been shown in the literature that with age there is a marked reduction in lactate threshold, critical power and the maximal rate of oxygen uptake (Conley et al., 2000; Hawkins & Wiswell, 2003; Wolthuis, Froelicher, Fischer and Triebwasser, 1977, Rossiter, 2011). Slower oxygen uptake kinetics could be related to a lowering of critical power, leading to a continued increase in oxygen consumption during treadmill walking and earlier fatigue (Pooles, Ward, Gardner, & Whipp, 1988; Rossiter, 2011). Furthermore, we have not measured maximal rate of oxygen uptake in our subjects, but it could well be that having the same *NCoW* as *YA* presented a relatively higher exercise intensity for *OA* than *YA* in our study. A final reason could also be that *OA* started walking with more efficient, smaller muscle fibers, but over time these became fatigued and then they recruited less efficient larger muscle fibers (Barclay, 1996; Jones et al., 2011; Woledge, 1998) during the treadmill walking sessions.

We believe that the results obtained in our *OA* participants may be generalized to healthy and fit elderly in general. This is because both our *YA* and *OA* were normal community-dwelling humans able to carry out their daily chores independently. None of them were involved in any kind of specific exercise programs, or strength and endurance training. Our results differ from those of the only two previous studies in which *NCoW* in *YA* and *OA* was measured during overground walking (Waters et al., 1983, 1988) and *NCoW* was found to be higher in *OA* than in *YA* by 0.29 and 0.26 J $kg^{-1}m^{-1}$, respectively. However, in those studies, *OA* were walking at a significantly lower *PWS* than *YA*,

which we marked as our first confounder. It is known from the literature that *MCoW* depends on walking speed (Ralston, 1958; Zarrugh et al., 1974) and the U-shaped relationship between *MCoW* and walking speed is of similar shape in *YA* and *OA* (Malatesta et al., 2003; Martin et al., 1992; Mian, Thom, Ardigò, Narici, & Minetti, 2006; Sanseverino et al., 2018). Furthermore, as we also know from the literature that *YA* select a *PWS* near the minimum *MCoW* (e.g.,(Ralston, 1958), it may well be that the measured increase in the reported *NCoW* in *OA* in the studies of Waters et al. (Waters et al., 1983, 1988) was due to *OA* selecting a lower *PWS* (a scenario explained schematically in Fig. 1C), as acknowledged by Waters et al. themselves (Waters et al., 1983, 1988).

We asked our second question of whether *NCoW* in treadmill walking is higher than in overground walking in *OA* and in *YA* because, except for the two studies of (Waters et al., 1983, 1988), all studies on the difference in *MCoW* between *OA* and *YA* in the literature were on treadmill walking. It has been shown previously that *OA* have a 23% higher metabolic energy requirement during treadmill walking than during overground walking at *PWS* (Parvataneni et al., 2009). It may be that *OA* react differently to treadmill walking (Schellenbach et al., 2010; Wass et al., 2005), causing the use of a treadmill to be a confounder. Our findings support this idea. In *YA*, no differences in *NCoW* were found between treadmill and overground. In *OA*, however, *NCoW* was higher on the treadmill than overground (Fig.3, columns 1 and 2). This was already the case after 5 minutes of treadmill walking, when none of the *OA* reported any fatigue. Also, in the morning treadmill trial, *NCoW* seemed to increase until the end in *OA* (Fig. 3), while we had expected it to reach a steady state just like in *YA.* In *OA*, during the last 3 minutes of the morning treadmill trial, *NCoW* was substantially elevated by 0.6 J $kg^{-1}m^{-1}$compared to *NCoW* in the morning overground trial. The trend

for *NCoW* to increase could perhaps be related to fatigue. As a matter of fact, two participants in the *OA* group specifically indicated that they became fatigued during the trial, and similar accounts during pilots were the reason for making the afternoon treadmill trial shorter. Fatigue could cause participants to change to a different walking pattern requiring more mechanical and hence more metabolic energy. In the afternoon treadmill trial, *NCoW* in *OA* ended up somewhat lower than in the morning treadmill trial and the difference would have been statistically significant if we only had the two treadmill trials in *OA* and had used a paired Student's t-test ($p=0.02$, Cohen's $d=0.86$). Perhaps *OA* had become familiar with treadmill walking and were more relaxed (but note that *NCoW* was still higher than during the overground trials). Or perhaps *NCoW* in *OA* would have increased further if participants had kept on walking as long as they did in the morning treadmill trial. Be that as it may, in the morning session *YA* did not have a higher *NCoW* on the treadmill than overground, and *OA* clearly did have a higher *NCoW* on the treadmill. Hence, *YA* and *OA* did react differently to treadmill walking, and the treadmill may have acted as confounder in the literature on age effects on *MCoW*, especially when only one treadmill trial was included.

The finding that *OA* react differently to treadmill walking than *YA* brings us to the third question of whether *NCoW* in treadmill walking is higher in *OA* than in *YA*. This comparison is between groups, and due to the inter-individual variation the chance of a false negative outcome is larger than in the within-subject comparison made for question 2. In the morning session, *NCoW* was higher in *OA* than in *YA* by 0.66 J $kg^{-1}m^{-1}$. The Mixed *ANOVA* did not detect this group effect. However, studies on the effects of age on *MCoW* tend to have only one treadmill trial. Such studies rely on an unpaired Student's t-test on the difference in *NCoW* between *OA* and *YA;* when we performed this test on the 0.66 J $kg^{-1}m^{-1}$ difference in treadmill *NCoW* between *OA* and

YA in the morning session, it was statistically significant (p=0.03, Cohen's d=1.03). Our data thus suggests that the elevated metabolic cost of walking in *OA* compared to *YA,* which is reported in the literature, is not an effect of age *per se*, but due to an elevated metabolic cost of treadmill walking compared to overground walking in *OA* but not in *YA.* In the afternoon session, the difference in *NCoW* between *OA* and *YA* on the treadmill was 0.36 J $kg^{-1}m^{-1}$. This is a smaller difference than in the morning session, yet still close to the difference between *OA* and *YA* typically reported in the literature (Das Gupta et al., 2019). As mentioned before, it cannot be ruled out that this difference might have been increased if the afternoon treadmill trial had been longer. In any case, we found that even after our second treadmill session –thus after at least 24 minutes of treadmill walking– *NCoW* was not fully restored to values measured overground. In the literature, treadmill trials range from 1.2 minutes (Pincheira et al., 2017) to 10 minutes (Ortega & Farley, 2015) per walking condition. This may thus well be too short to arrive at values of *NCoW* that are comparable to those measured overground. Our results indicate that treadmills should be used carefully when studying differences in locomotion between *OA* and *YA.* It remains to be established if *OA* familiarize to treadmill walking after a longer time than used in our study; perhaps they do not familiarize at all.

It would be interesting to understand why *OA* have a higher metabolic cost at *PWS* in treadmill walking than in overground walking, while *YA* do not. Considering that the difference is already present after a few minutes of treadmill walking, it is unlikely to be due to fatigue. During the experiments, we noted that several of the participants in the *OA* group had difficulty with the task of treadmill walking. Also, some of them spontaneously reported feeling anxious. In this context it is relevant to note that the walking surface of the treadmill that we used was about 60 cm above the

floor. Even though there was a platform around the treadmill belt, it is possible that anxiety caused *OA* to walk with more co-contraction on the treadmill than overground, which in turn caused *NCoW* to be higher during treadmill walking, as already reported in previous studies (Mian et al., 2006; Peterson & Martin, 2010; Pincheira et al., 2017).

2.5 Conclusion

In this study we compared the metabolic cost of walking in healthy, active and anthropometrically matched *OA* and *YA* overground and on a treadmill in a morning session and in an afternoon session. *PWS* was the same in the two groups. Considering that there was only a negligible difference in *NCoW* between *OA* and *YA* in the morning overground trial, it seems safe to conclude that there is no effect of age *per se* on *NCoW* at *PWS*. While *NCoW* in *YA* seemed to be the same on a treadmill and overground, it was elevated on the treadmill in *OA*, and statistically significantly so in the morning session. In the literature, a higher metabolic cost of walking is reported for *OA* than for *YA* at the same walking speed on a treadmill. Considering the results of our study, this is likely to be due to a differential reaction of *OA* and *YA* to treadmill walking, and not to an effect of age *per se* on the cost of walking at a given speed.

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Chapter 3

The elevated Metabolic Cost of Walking at preferred speeds of healthy elderly on treadmills compared to overground is not related to increased self-reported anxiety

(Adapted from) Sauvik Das Gupta, Herre Faber, Dinant Kistemaker and Maarten Bobbert. The elevated metabolic cost of walking at preferred speeds of healthy elderly on treadmills compared to overground is not related to increased self-reported anxiety. *European Journal of Applied Physiology* (2023). https://doi.org/10.1007/s00421-023-05138-y

The purpose of this study was to investigate whether the elevation in Metabolic Cost of Walking on treadmills compared to overground for healthy elderly is related to self-reported anxiety and if changes in self-reported anxiety are related to changes in heart rate. We measured overground preferred walking speed, oxygen consumption rate and heart rates during rest and walking, and self-reported anxiety in 10 elderly (mean age 69.5 ± 3.1 years, 8 males and 2 females). At their preferred speed, the participants first walked overground, then on a high treadmill, and then on a low treadmill. Gross and Net metabolic costs of walking were calculated from the rates of oxygen consumption. Gross and Net Metabolic Cost of Walking were higher ($p<0.05$) on high treadmill (Net cost: 2.64 J $kg^{-1}m^{-1}$) and low treadmill (Net cost: 2.68 J $kg^{-1}m^{-1}$) compared to overground (Net cost: 2.44 J $kg^{-1}m^{-1}$), and the same was true for heart rate. There were no significant differences ($p>0.05$) in metabolic costs and heart rates between the two treadmill conditions. Self-reported anxiety was higher on the high treadmill compared to overground ($p=0.004$) and compared to low treadmill ($p=0.02$). We found no significant difference ($p>0.05$) for self-reported anxiety between overground and the low treadmill. These results show that treadmill walking cannot be adequately generalized to overground walking. The differences found in metabolic cost on treadmills compared to overground were not related to differences in self-reported anxiety. Furthermore, the changes in heart rate are not related to changes in self-reported anxiety.

List of Abbreviations

d	Cohen's d
GCoW	Gross Cost of Walking
MCoW	Metabolic Cost of Walking
NCoW	Net Cost of Walking
PWS	Preferred Walking Speed
RER	Respiratory Exchange Ratio
RM-ANOVA	Repeated Measures-Analysis Of Variance
SD	Standard Deviation
SEM	Standard Error of Mean
$\dot{V}O_2$	Oxygen consumption rate
$\dot{V}CO_2$	Carbon dioxide production rate
VAS	Visual Analogue Scale

3.1 Introduction

Metabolic energy expenditure during walking is an important factor that determines whether a person is able to satisfactorily perform activities of daily life. It has been previously shown in the literature that Metabolic Cost of Walking (*MCoW*), defined as the metabolic energy expended per kilogram of body mass per meter travelled, is about 15% higher in elderly compared to young (Das Gupta, Bobbert, & Kistemaker, 2019). When *MCoW* is expressed as Gross Cost of Walking (*GCoW*) the elevation is in the order of ~ 0.3 J $kg^{-1}m^{-1}$ (d = 0.65), and when *MCoW* is expressed in terms of Net Cost of Walking (*NCoW*), the elevation is in the order of ~ 0.4 J $kg^{-1}m^{-1}$ (d = 1.00) (Das Gupta et al., 2019). Most of the studies on *MCoW* have been conducted on treadmills (e.g.,Gaesser, Tucker, Sawyer, Bhammar, & Angadi, 2018; Malatesta et al., 2004, 2003; Mian, Thom, Ardigò, Narici, & Minetti, 2006; Peterson & Martin, 2010). Previously, we compared *MCoW* in healthy young and elderly during both overground and treadmill walking (Das Gupta, Bobbert, Faber, & Kistemaker, 2021). We found no effects of age *per se* on *MCoW* during overground walking. However, *NCoW* during treadmill walking was increased by 0.6 J $kg^{-1}m^{-1}$ compared to overground walking in elderly; in young adults, there was no elevation in *NCoW* on the treadmill compared to overground walking. Previously, (Parvataneni et al., 2009) measured *MCoW* in elderly and showed that there was an excess metabolic energy consumption of 23% on a treadmill compared to overground, accompanied by a 6% increase in heart rate on the treadmill compared to overground. This suggests that the elevated *MCoW* of elderly compared to young reported in the literature may be caused by elderly using a different gait on treadmill compared to overground. For example, elderly could walk on a treadmill with more co-contraction of hip, knee, and ankle muscles (Tudor-Locke et al., 2021) to increase stability. Obviously, co-contraction causes increased *MCoW*, as shown

previously (Peterson & Martin, 2010; Hortobágyi, Finch, Solnik, Rider, & De Vita, 2011; Ortega & Farley, 2015; Mian, Thom, Ardigò, Narici, & Minetti, 2006; Tudor-Locke et al., 2021; Piche et al., 2021). This then prompts the question what the trigger is for elderly to use a different gait on treadmill compared to overground.

It has been proposed that anxiety could be a trigger for elderly to walk differently on treadmill than overground (Parvataneni et al., 2009), especially if elderly are unfamiliar with treadmill walking. The anxiety, in turn, could be caused by the fact that visual flow is unnatural on a treadmill (Dal, Erdogan, Resitoglu, & Beydagi, 2010; Martin & Li, 2017; Murray, Spurr, Sepic, Gardner, & Mollinger, 1985); after all on a treadmill, there is a discrepancy between visual information and vestibular and other proprioceptive information. On the other hand, anxiety could also be caused by the treadmill's height above the floor level. In the literature, it has been observed in elderly that walking heart rate relative to rest is higher on treadmill than overground (Greig, Butler, Skelton, & Mahmud, 1993; Parvataneni et al., 2009) and the suggestion that anxiety is increased on a treadmill in elderly is based on this observation. However, rather than reflecting increased anxiety, increased heart rate might be directly related to increased *MCoW*. After all, it is known that in healthy individuals, metabolic rate and cardiac output are closely correlated (e.g., Guyton et al., 1973). Therefore, the theory proposed in the literature that increased *MCoW* on a treadmill is due to changes in gait pattern, and/or increased muscle co-contraction, which are triggered by anxiety, may not be correct. It could also be that elderly have higher *MCoW* on a treadmill due to gait changes arising from (unknown) biomechanical or/and neuromuscular issues that are not related to increased anxiety. This would elevate their heart rate simply because higher *MCoW* requires higher cardiac output.

The aim of the present study was to determine whether increased *MCoW* of elderly on a treadmill compared to overground is related to self-reported anxiety. For this aim, we first set out to replicate our result from the previous study (Das Gupta, Bobbert, Faber, & Kistemaker, 2021), i.e., that *MCoW* of elderly was increased on our treadmill compared to overground. Additionally, we asked our participants to indicate their anxiety level on a Visual Analogue Scale (*VAS*) and examined whether the increased *MCoW* on our elevated treadmill compared to overground was accompanied by increased self-reported anxiety. Second, we studied whether self-reported anxiety was reduced when our elderly walked on a floor-level treadmill compared to our elevated treadmill and was in the same range as that of overground. Furthermore, we checked whether *MCoW* on this floor-level treadmill differed, compared to the *MCoW* on the elevated treadmill and to overground. We did so, because in our previous study of *MCoW* in elderly and young adults on treadmill and overground, we used a treadmill that was elevated by 60 cm (Das Gupta et al., 2021), while other authors of comparable studies generally used a treadmill at floor level. It could be that in our previous study, the extra height of the treadmill was a cause for increased anxiety and thereby triggered gait changes resulting in increased *MCoW* in our elderly. During all experiments, we also measured heart rates and examined whether heart rates were related to self-reported anxiety.

3.2 Methods

Characteristics of Participants and Ethics Statement

We recruited 10 healthy elderly (mean age 69.5 ± 3.1 years, 8 males and 2 females) and measured their anthropometrics and overground Preferred Walking Speed (*PWS*; see Table 1 in Results). All the participants were physically active and carried out their normal day-to-day activities without any assistance. None of the participants were

actively involved in any kind of special strength or endurance training. They had at least once walked previously on a treadmill in their life, but never on a high (from ground level) treadmill. We excluded participants if they had any pathological condition (like chronic heart disease, diabetes, past surgeries or prosthesis in the lower limbs and feet, neuromuscular disabilities), were on medication (including sleeping aids or antidepressants which may impact mobility) or had experienced a fall (we used the general definition of a fall: losing one's balance and collapsing to the ground) in the past 6 months. We excluded all these conditions or factors as they might have an influence on the *MCoW*. Written informed consent was obtained from each participant. Before the start of the experimental protocol, body mass and height were measured. Lower limb lengths from the greater trochanter to the malleolus and from the greater trochanter to the foot were also measured to obtain the leg lengths. The ethical review committee of the Faculty of Behavioural and Movement Sciences of the Vrije Universiteit Amsterdam approved the experimental protocol.

Protocol

Pre-experimental factors

To ensure that diet did not have an effect on *MCoW* prior to the experiments, participants were told to have only a light breakfast (i.e., moderate intake of carbohydrates and fats, minimalize intake of protein and fibre-rich foods as much as possible; food intake was listed). They were also asked to not consume any caffeine or alcohol-containing products and to refrain from smoking tobacco until the experiments were finished. We checked and ensured that the participants adhered to both these requests from us. The participants walked overground along an oval track with 32 m straights, interconnected by two half-circles of 4 m radius inside a laboratory (see Fig.1). After walking for 5 minutes, participants completed three full laps. To establish *PWS*

participants were asked to walk at the speed, they would normally walk while going from their home to a supermarket. *PWS* was calculated as the average walking speed during the last three laps. Subsequently, *PWS* was imposed on a treadmill. Two treadmills were used for the study: one treadmill (from ForceLink BV, Culemborg, The Netherlands) placed at a height of 60 cm above the ground (henceforth referred to as high treadmill) and one standard treadmill (from Motek Medical BV, Houten, The Netherlands) with the walking surface at 15 cm above the ground (henceforth referred to as low treadmill). Handrails were not provided to allow the participants to have a natural arm-swing. The participants were secured to an overhead rail with a chest-harness while walking on the treadmills. To give adequate rest to the participants, we ensured that there were at least 20 minutes between two consecutive trials. We had a fixed order of our walking trials overground and on the treadmills, which we will discuss in the following section.

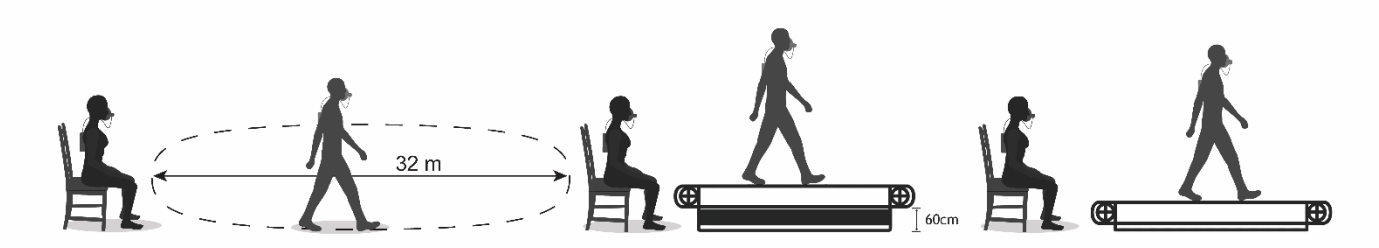

Figure 1. Schematic overview of the energy expenditure measurements during the experimental protocol. Resting Metabolic Rate (RMR) was measured, while the participants were seated for 5 minutes on a chair preceding each of the overground and the treadmill walking trials. During the overground trial, participants walked continuously along the schematically drawn oval track with 32 m straights interconnected by two half-circles of 4 m radius. During the treadmill trials, the participants first walked on the high treadmill (placed at a height of 60cm from the ground) and then on a normal floor-level treadmill at their imposed overground preferred walking speeds

Measurement of self-reported anxiety, heart rate, and Metabolic Cost of Walking

To test the idea that anxiety is leading to a higher *MCoW* while walking on a treadmill, we measured both heart rate as well as self-reported anxiety using a Visual Analog Scale (*VAS*). We measured heart rate using a Polar heart rate chest sensor (from Polar, Kempele, Finland) to test the suggestion done in the literature that the increased

heart rate when walking on a treadmill indicates an increased anxiety, which is then suggested to lead to an increase in *MCoW*. However, heart rate is a main determinant of the cardiac output and as such is also directly related to physical exertion. Since the main goal of this study was to investigate if found differences in *MCoW* (indicating differences in physical exertion) could be explained by anxiety, we also used an anxiety measurement that is not (e.g., like sweat production and body temperature) confounded by physical exertion: the "anxiety thermometer" (Houtman & Bakker, 1989) which is basically a *VAS*. This anxiety thermometer has been extensively validated to be a measure of a person's state and trait anxiety (Almazrouei, Morgan, & Dror, 2022; Kantor, Endler, Heslegrave, & Kocovski, 2001; Malian et al., 2021; Thibodeau, Gómez-Pérez, & Asmundson, 2012; Williams, Morlock, & Feltner, 2010; Bourne & Vladeanu, 2011; Elwood, Wolitzky-Taylor, & Olatunji, 2012; Ertuğ, Ulusoylu, Bal, & Özgür, 2017; Hulsman et al., 2010; Arora et al., 2010) and has been used in several studies to measure self-reported anxiety (Englert & Seiler, 2020; Harris, Purcell, & Wilmut, 2022; Linssen, Landman, van Baardewijk, Bottenheft, & Binsch, 2022; Payne, Wilson, & Vine, 2019) including in studies that involved various locomotor tasks (Cañal-Bruland, Pijpers, & Oudejans, 2010; Nibbeling, Daanen, Gerritsma, Hofland, & Oudejans, 2012; Nieuwenhuys & Oudejans, 2010; Pijpers, Oudejans, & Bakker, 2005; Pijpers, Oudejans, Bakker, & Beek, 2006; Pijpers, Oudejans, Holsheimer, & Bakker, 2003).

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production rates ($\dot{V}CO_2$) were measured using a portable Cosmed K4b2 (from Cosmed, Rome, Italy) breath-by-breath indirect calorimetry device and a face-mask. The Cosmed K4b2 was always calibrated according to the manufacturer's guidelines before collecting any data. The participants sat in a chair for 5 minutes in a relaxed state, and the resting oxygen consumption (Resting Metabolic Rate, *RMR*) and resting heart rate level were measured (see Fig.1).

For reasons provided elsewhere (Weyand, Smith, & Sandell, 2009), we chose to measure their resting values in a seated posture (see also: Das Gupta et al., 2021). The *RMR* measured immediately before a walking trial was used to calculate the *NCoW* for the corresponding walking trial. We chose to include the *GCoW* measurements alongside the standard *NCoW* measurements to account for any variations in the baseline metabolic rate that might occur during the walking protocols (see: (Courter, Alvarez, Enoka, 2022)). *GCoW* (in J $kg^{-1}m^{-1}$) was calculated from the rate of oxygen consumption using Lusk's equation (Lusk, 1923) and *PWS* as follows:

$$GCoW = (15962 + 5155 \cdot RER) \cdot \dot{V}O_2 \cdot PWS^{-1} \quad (1)$$

where $(15962 + 5155 \cdot RER)$ is the energetic equivalent of oxygen (in J l^{-1}), *RER* is the Respiratory Exchange Ratio (dimensionless), $\dot{V}O_2$ is the oxygen consumption rate (in l $kg^{-1}s^{-1}$), and *PWS* is the average walking speed during the last three overground laps (in $m \cdot s^{-1}$). *NCoW* (in J $kg^{-1}m^{-1}$) was obtained by inserting net$\dot{V}O_2$, i.e., the difference in $\dot{V}O_2$ between walking and sitting. The gross heart rate values were reported from the Polar heart rate monitor directly in beats per minute (bpm).

Preparation

Before any walking exercise, we explained the anxiety thermometer to our elderly participants. We explained them that '0' on the scale means no anxiety and a '10' on the scale means extreme anxiety. Then, we told our participants to mark a 'X' with a pen on the thermometer corresponding to how anxious they felt after they completed the overground or the treadmill walking tasks. All the instructions were given in the participants native language (i.e., Dutch). Then, the particpants were fitted with the portable Cosmed device and a face-mask, and with the heart rate sensor.

Experimental protocol

After fitting of the equipment, the participants first completed the overground walking protocol and then walked for 15 minutes on the high treadmill and then for another 15 minutes on the low treadmill at their overground *PWS*, while we measured their oxygen consumption and heart rate levels (see Fig.1). We kept the walking protocol identical to our previous study, because we wanted to replicate our own results of *MCoW* overground and on a treadmill (Das Gupta et al., 2021). Just like in that study, on both the treadmills, the speed was gradually increased over the first 2 minutes until it matched the participants' overground *PWS* and then kept constant. At the end of the trial (i.e., after 15 minutes of walking), the speed was slowly reduced to zero, and then, the measurement of oxygen consumption and heart rates were stopped. Before each of the walking trials, we repeated the (seated) resting oxygen consumption and heart rate measurements.

From the overground oxygen consumption rate and heart rate data, the last 30 seconds of data were always discarded, and then, the remaining last 2 complete minutes of data were used to calculate *MCoW* and heart rate levels. A similar duration of oxygen consumption and heart rate data from the treadmills, exactly corresponding to the minutes of the overground trials, were used to calculate the *MCoW* and heart rate levels on the treadmills. Immediately after each of the walking sessions, the participants filled in their anxiety level on the anxiety thermometer (0-10 with 0.1 point increment) (Houtman & Bakker, 1989).

Statistical tests

For *GCoW*, *NCoW*, heart rate, and self-reported anxiety level, statistical comparisons were performed between the three walking conditions (overground, high treadmill, and low treadmill). Since the purpose of the study was to explain differences in *MCoW* between treadmill and overground walking with differences in self-reported

anxiety between treadmill and overground walking, we chose to look at intra-individual (within-subjects) differences in *MCoW,* heart rates, and self-reported anxiety levels. As all the comparisons were within-subjects, the assumption for the equality of variances was always met. Furthermore, we also averaged the time histories of gross metabolic power over participants and graphically displayed means and Standard Error of the Mean (*SEM*) as a function of time for overground, high treadmill, and low treadmill walking conditions before doing a full-blown statistical analysis. We then conducted a non-parametric Friedman *RM-ANOVA* test, and in case of statistically significant results ($p<0.05$), post hoc Durbin-Conover pairwise comparisons were conducted to check which two walking conditions differed significantly from one another. The open-source software *JAMOVI* (version 1.8.2.0) was used for all the statistical tests and the default value of $\alpha=0.05$ was chosen as the level of statistical significance.

3.3 Results

Age and Anthropometric measurements

In Table 1, we have listed the mean values and standard deviations (mean ± *SD*) of age, anthropometrics, and overground *PWS* (on average 1.4 ± 0.1 $m \cdot s^{-1}$) that was imposed on the treadmills. Furthermore, none of our older adults encountered a fall during the course of the experiments.

Table 1: Age, anthropometric parameters, and *PWS* of healthy elderly

	Healthy elderly
Age (years)	69.5 ± 3.1
Body Mass (kg)	75.6 ± 14.2
Height (m)	1.72 ± 0.10
Lower limb length up to malleolus (m)	0.83 ± 0.07
Lower limb length up to foot (m)	0.90 ± 0.07
Overground PWS ($m \cdot s^{-1}$)	1.4 ± 0.1

Metabolic Cost of Walking, Heart rate, and Anxiety levels

Figure 2 provides an overview of means and standard error of the mean (*SEM*, grey area) of gross metabolic power for the elderly subjects for all the three walking conditions of overground, high treadmill, and low treadmill. Just as a first indication, for the difference between the means in the gross metabolic power to be statistically significant between the three walking conditions, it should be bigger than about twice the pooled *SEM*. Before doing any further statistical analysis, this figure shows clear differences between overground walking and the two treadmills, but not between the two treadmill conditions.

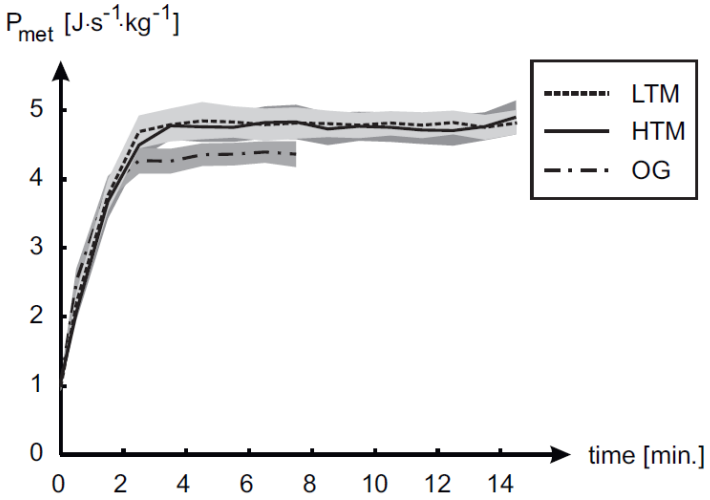

Figure 2. Overview of gross metabolic power (P_{met}) results. Means and *SEM* (standard error of mean, grey area) of gross metabolic power over time (in minutes) have been plotted for the overground and the two treadmill trials. *OG* represents overground, *HTM* high treadmill and *LTM* low treadmill

Figure 3 provides the boxplot showing the median values and the interquartile range, along with the outliers and statistically significant results for the three walking conditions of overground, high treadmill, and low treadmill for *MCoW*, heart rates, and self-reported anxiety levels. These results are further discussed in detail in the following section.

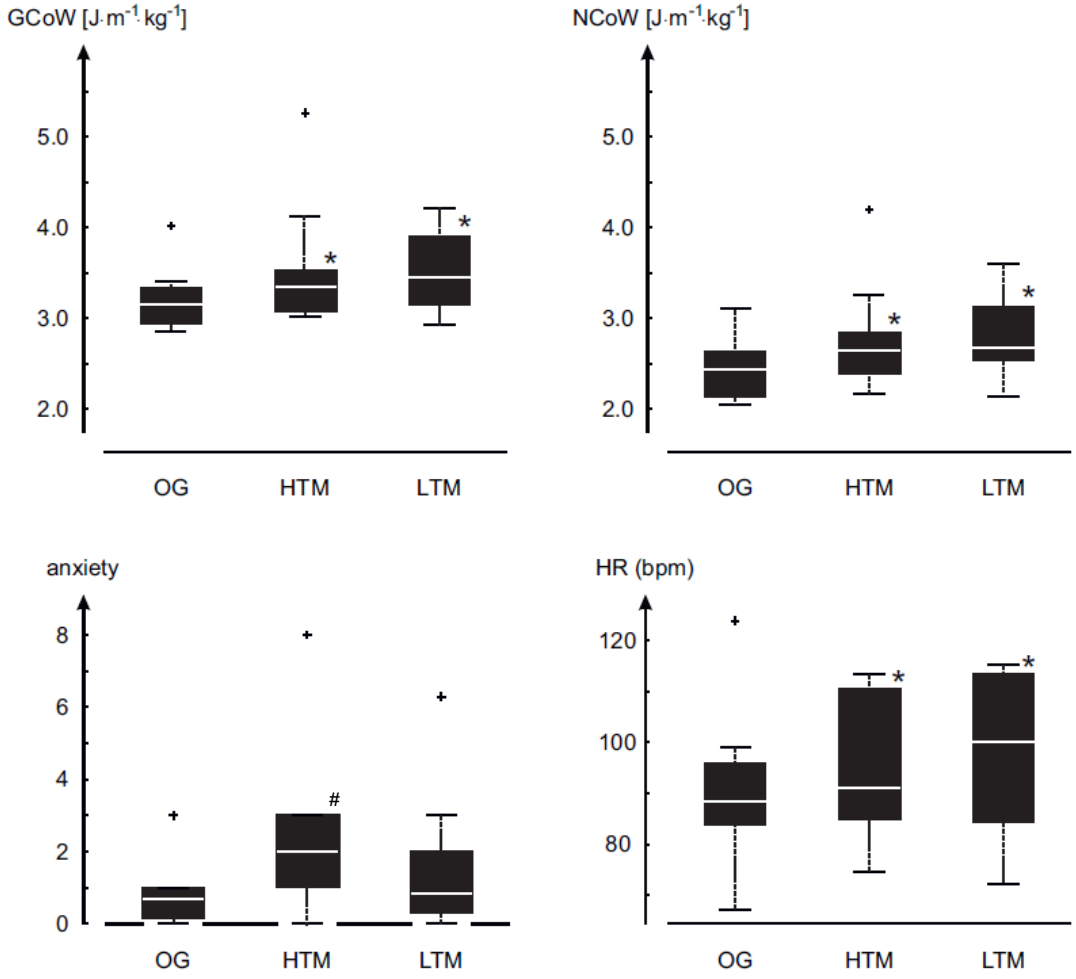

Figure 3. Boxplots of Gross Cost of Walking (*GCoW*), Net Cost of Walking (*NCoW*), self-reported anxiety, and Heart Rates (*HR*) are shown for overground (*OG*), high treadmill (*HTM*), and low treadmill (*LTM*) walking conditions. The central white horizontal lines in the boxplots represent the median and the black edges of the boxes represent the 25th and the 75th percentiles. The black whiskers of the boxplots extend up to the values that are not considered as outliers and the outliers are depicted by the black '+' symbols. The '*' symbols signify statistically significant elevation ($p<0.05$) compared to overground values. For the self-reported anxiety plot, the '#' symbol signify statistically significant elevation ($p<0.05$) compared to overground and *LTM* values

Statistical analyses

We found statistically significant differences in *GCoW* ($p=0.02$) and *NCoW* ($p=0.002$) between the three walking conditions (overground, high treadmill, and low treadmill). Post hoc analyses showed statistically significantly increased *GCoW* for high treadmill walking (6.03%, $p=0.03$) compared to overground and for low treadmill walking (9.5%, $p=0.004$) compared to overground. For *NCoW*, we again found a statistically significant increase for high treadmill walking (8.2%, $p<0.001$) compared to overground and also for low treadmill walking (9.8%, $p<0.001$) compared to

overground. There were no statistically significant differences ($p>0.05$) between the two treadmills for either *GCoW* or *NCoW*. This means that for elderly, there is an increased *MCoW* on treadmills compared to overground, irrespective of whether the treadmill is at a height or not.

In addition to these differences, statistically significant differences were found in self-reported anxiety levels ($p=0.02$) between the three walking conditions. Post hoc analysis showed statistically significantly elevated anxiety for high treadmill walking ($p=0.004$) compared to overground and for high treadmill walking compared to low treadmill walking ($p=0.02$). There were no statistically significant differences ($p>0.05$) in self-reported anxiety level between overground and low treadmill walking. In absolute numbers, self-reported anxiety had a median value of 2.0 on the high treadmill, 0.85 on the low treadmill, and 0.7 overground.

We also found statistically significant differences in heart rates ($p=0.03$) between the three walking conditions. Post hoc analyses showed statistically significantly increased heart rates for high treadmill walking (2.7%, $p=0.009$) compared to overground and for low treadmill walking (13.5%, $p=0.02$) compared to overground. No statistically significant differences were found for heart rate values ($p>0.05$) between high treadmill and low treadmill walking. These findings go against the subjectively measured higher anxiety on the high treadmill than the low treadmill, as stated earlier. To summarise, we see that there is a higher *MCoW* on the low treadmill (like the high treadmill), but no higher self-reported anxiety level compared to overground. Also, we see that higher anxiety on the high treadmill is not accompanied by higher *MCoW*, compared to low treadmill.

3.4 Discussion

In this study, we recruited healthy, physically fit and active elderly, measured their *PWS* overground, and then imposed that speed on two treadmills – namely high treadmill placed at a height of 60 cm from the ground and low treadmill placed at 15 cm from the ground. We found an increased *MCoW* on both the treadmills compared to overground, thereby replicating our previous study (Das Gupta et al., 2021). Additionally, we found elevated self-reported anxiety on the high treadmill compared to overground, and on the high treadmill compared to the low treadmill, but not on the low treadmill compared to overground. This indicates that self-reported anxiety does not explain the increased *MCoW* on a treadmill for elderly observed in our current and previous study (Das Gupta et al., 2021). Differences in heart rate followed the results of *MCoW*: heart rate was increased on both treadmills and no significant differences were observed between the two treadmills. Below, we will discuss our results in detail and argue that it must be something other than self-reported anxiety that triggers the supposed gait changes leading to increased *MCoW* for elderly on a treadmill.

In the present study, we not only replicated our previous finding of a higher *MCoW* for elderly on high treadmill compared to overground (Das Gupta et al., 2021), but also showed that elderly have an increased *MCoW* on a low treadmill compared to overground. If we look at the literature, there are suggestions that anxiety could be a trigger for elderly to walk differently on a treadmill than overground (Parvataneni et al., 2009; Martin & Li, 2017). This anxiety could be caused by unnatural visual flow on a treadmill (Dal et al., 2010; Martin & Li, 2017; Murray et al., 1985) as there is a discrepancy between visual, vestibular, and other proprioceptive information. Increased anxiety can then lead to gait changes resulting in, for example, excessive muscle co-contraction which leads to an increased *MCoW*. In our previous study, we used a high

treadmill that was elevated by 60 cm and this height could also (further) induce anxiety. We indeed observed that self-reported anxiety was higher for the high treadmill condition compared to the low treadmill. We did not observe elevation in self-reported anxiety in low treadmill walking compared to overground. Yet, *MCoW* did not differ between high treadmill and low treadmill, and were both elevated compared to overground. As such, we conclude that self-reported anxiety is not the trigger for gait changes that are responsible for the increased *MCoW* on a treadmill for elderly, but that apparently the treadmill itself is the trigger.

In this study, to be able to directly replicate our previous findings (Das Gupta et al., 2021) , we did not randomize our conditions: all our participants first walked overground, then on the high, and then on the low treadmill. Therefore, it is possible that fatigue and familiarization influenced our results on *MCoW* and self-reported anxiety. To prevent fatigue, we provided at least 20 minutes rest before the start of a walking trial. Furthermore, it is likely that a (difference in) fatigue would have shown up in our *RMR* measurement directly prior to each condition, but no such difference was observed. In addition, none of the participants reported any physical fatigue after completion of a walking trial. Finally, if physical fatigue would have played a role, it would have been most pronounced in the last condition (low treadmill), which we did not observe. All in all, we are confident that the order of our conditions did not influence our *MCoW* results. It may well be, however, that familiarization during high treadmill walking caused a lower self-reported anxiety scores on the low treadmill and may be part of the reason of the differences in self-reported anxiety found between these two conditions. Be that as it may, we did not find a difference in *MCoW* between these two conditions, and thus, we feel it is safe to conclude that this difference in *MCoW* is not related to self-reported anxiety.

In the literature, it has been suggested that that the increased *MCoW* on a treadmill is due to anxiety, and this suggestion was based on observed changes in heart rate (Parvataneni et al., 2009). Here, we found that heart rates during walking on the two treadmills are comparable and that both are significantly higher than during overground walking. This is in line with our findings on *MCoW*: we observed no differences in *MCoW* between high treadmill and low treadmill, and we observed that *MCoW* was significantly higher on both treadmills than overground. Differences in heart rate were not in line with self-reported anxiety: we observed an increase in self-reported anxiety on high treadmill compared to low treadmill and on high treadmill compared to overground but, importantly, not on low treadmill compared to overground. Therefore, from these results, we conclude that heart rates reflect metabolic rate and not anxiety.

This study shows that the increase in *NCoW* for elderly on the high treadmill that we found in our previous study (Das Gupta et al., 2021) was not related to self-reported anxiety. At this point, we do not know what triggered the gait changes in treadmill walking in elderly that caused elevated *MCoW*. Nevertheless, here, we do show that *MCoW* measured during treadmill walking cannot be readily generalized to and directly compared with overground values for elderly, even when walking at the same walking speed. Since overground walking is ecologically more valid than treadmill walking, it follows that while studying human walking energetics, especially in elderly, the usage of a treadmill can be considered as a methodological confounder, as suggested in our previous study (Das Gupta et al., 2021). In our previous study (Das Gupta et al., 2021), we showed that for young adults, there were no differences between treadmill and overground *MCoW*. Due to this differential reaction of young and elderly to a treadmill, we recommend that for future research, effects of age on *MCoW* should not be studied on treadmills.

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Chapter 4

Summary and General Discussion

The Metabolic Cost of Walking (*MCoW*) is typically expressed either in terms of Gross Cost of Walking (*GCoW*) or Net Cost of Walking (*NCoW*). *MCoW* is defined as the metabolic energy expended per meter travelled and is an important variable in daily life. From the literature we can see that increased *MCoW* for elderly compared to young might lead to elderly becoming fatigued and sedentary (Das Gupta, Bobbert, & Kistemaker, 2019; Hortobágyi, Finch, Solnik, Rider, & De Vita, 2011; Mian, Thom, Ardigò, Narici, & Minetti, 2006). Increased fatigue and sedentarism limits their functional possibilities, thereby affecting the overall quality of life. The motivation for this doctoral project was that we wanted to know the reasons why elderly have a higher *MCoW* compared to young, especially during overground walking. We were interested in overground walking since it is the ecologically relevant mode of walking. However, before trying to know why elderly have a higher *MCoW* than young we wanted to see whether this finding of elevated *MCoW* for elderly was valid in the first place and if so, specifically for overground walking.

4.1 Summary and general implications of the findings

In order to answer why elderly have a higher *MCoW* compared to young overground and to see whether the commonly published elevation of *MCoW* for elderly over young was valid, we started the doctoral project with a systematic review and meta-analysis and have reported on this study in Chapter 1. We conducted this study aiming to quantify the elevation in *MCoW* for healthy elderly over healthy young adults, which is normally seen in the literature. There are several problems in the literature which make it difficult to quantify the elevation in *MCoW* with aging and calculate a raw and standardized effect size for the difference in *MCoW* between Young Adults (*YA*) and Older Adults (*OA*). For example, it is not straightforward to compare values across studies due to differences in the way *MCoW* was expressed (*GCoW* or *NCoW*), the units

in which it was reported (for example, J $kg^{-1}m^{-1}$, ml $kg^{-1}m^{-1}$ or simply in units of metabolic rates like W kg^{-1}, J $kg^{-1}min^{-1}$, ml $kg^{-1}s^{-1}$ or ml $kg^{-1}min^{-1}$) and the walking speed at which it was measured. In order to overcome these problems, we undertook several steps in conducting our study. We conducted two separate meta-analyses for *GCoW* and *NCoW*, converted all units in which *MCoW* was reported in the literature to the Standard International unit of J $kg^{-1}m^{-1}$ and if studies reported *MCoW* measured at multiple speeds, selected those values for healthy *YA* and *OA* at which *MCoW* was minimal. In this review, we quantified the elevation in *MCoW* for *OA* over *YA* and calculated the effect size (both raw and standardized). For the elevation in *GCoW*, the standardized effect size (d) was 0.65 (medium to large) and for the elevation in *NCoW* the standardized effect size (d) was 1 (large). As both these effect sizes were in the range from medium to large, they are deemed to be clinically relevant.

Though we quantified the elevation in terms of effect sizes, unfortunately as it turned out, no clear explanations were given in the literature on the reasons for this elevation. In the studies that we reviewed, we saw that apart from the obvious difference in age between *YA* and *OA* there were also some other differences between *YA* and *OA*. For example, there were differences in anthropometrics, physical activity and fitness levels between *YA* and *OA* and interaction between age and treadmill walking. Due to an interaction between age and walking on a treadmill, the elevation in *MCoW* for *OA* could be mistaken for an effect due to aging. The results of this systematic review and meta-analysis are ambiguous when comparing *MCoW* between *YA* and *OA* overground. This is because we saw from the literature that there were very few overground studies and across all the studies there were differences in anthropometrics, physical fitness and activity status. We also thought that the interaction effect of age and treadmill walking was affecting *MCoW*. After completing this study, we wanted to investigate two further

questions. Firstly, we wanted to recruit a cohort of *YA* and *OA* who are similar in anthropometrics, physical fitness and activity status, and if we succeed in the recruitment, whether we can find a difference in their overground Preferred Walking Speeds (*PWS*) and *MCoW*. Secondly, we wanted to find out whether there were any interaction effects between age and treadmill walking. In order to answer these two research questions, we designed our first experimental study.

We designed a study involving both healthy *YA* and *OA*. In this study, reported in Chapter 2, we looked at *MCoW* at *PWS* both overground and on a treadmill after sufficient familiarization for all our participants. We wanted to have age as the only differentiating factor between our participants that could affect *MCoW* and so we recruited *YA* and *OA* who were similar in anthropometrics, physical activity and fitness levels. We found the overground *PWS* for all our *YA* and *OA* in a laboratory as discussed and shown in Chapter 2. Then we imposed this same overground *PWS* on the treadmill for all our participants. There were no statistically significant differences in *PWS* between our *YA* and *OA*. Two important results from this chapter that we focus on are that: 1) we did not find a statistically significant elevation in *NCoW* between our *YA* and *OA* (difference of 0.08 J $kg^{-1}m^{-1}$), especially during the ecologically relevant overground walking and 2) we found a statistically significant elevation in *NCoW* on the treadmill compared to overground for our *OA* by 0.61 J $kg^{-1}m^{-1}$ (in the morning session in the study reported in Chapter 2). Interestingly there was no elevation in treadmill *NCoW* compared to overground in our *YA*, suggesting that there was an interaction between age and treadmill walking affecting *MCoW*. However, from this study we could not understand the reasons for a higher *NCoW* on treadmill compared to overground for our *OA* as we were unclear on the nature and the reasons behind the interaction effect.

Regarding the elevation of treadmill *NCoW* compared to overground *NCoW* for our *OA*, we hypothesized that this could be due to anxiety of walking on a treadmill, especially as our *OA* had limited to no experience of walking on a treadmill before. This anxiety could also arise from the fact that walking on a treadmill is accompanied by unnatural visual flow (Dal, Erdogan, Resitoglu, & Beydagi, 2010; Martin & Li, 2017; Murray, Spurr, Sepic, Gardner, & Mollinger, 1985). Since in the study reported in Chapter 2, there were no effects of anxiety on *MCoW* for *YA* on a treadmill, we predicted that anxiety could cause *OA* to change their gait on a treadmill due to the interaction between age and treadmill walking. As there were no differences between overground and treadmill *MCoW* for *YA*, we predicted that anxiety would not cause any gait changes in *YA.* The gait changes in *OA* could lead to simultaneous muscle co-activation of agonists and antagonists around a joint (for example hip, knee, ankle) which would then lead to an increase in *MCoW* on the treadmill. Additionally, the treadmill that we used in this study in Chapter 2 was placed at a height of 60 cm from the ground. Fear of height *per se* could also induce anxiety in our *OA* participants that could then lead to a higher *MCoW* on the treadmill. Furthermore, if we look at the literature, it has been reported that walking heart rate (compared to rest) on a treadmill for *OA* is higher than that of overground and authors of other studies could have taken this as an indication that anxiety is also higher on a treadmill (Parvataneni, Ploeg, Olney, & Brouwer, 2009). However, from literature we also know that heart rates and metabolic rates are related (Guyton et al., 1973) and higher metabolic rates require higher cardiac output and hence result in higher heart rates. So it is important to disentangle whether heart rates are related to anxiety in addition to metabolic rates. At the same time it is important to know whether elevated heart rates also signify increased anxiety while metabolic energy expenditure is present (like in walking). In order to find out whether

anxiety in *OA* while walking on a treadmill is leading to the interaction effect between age and treadmill walking, we designed a follow-up experimental study.

We reported on this experimental study in Chapter 3. We introduced a treadmill placed at the floor level in addition to our high treadmill as reported in Chapter 2. Our walking protocol consisted of overground walking, followed by walking on the elevated treadmill as used in Chapter 2, followed by walking on the floor level treadmill. For this study, we recruited *OA* who had prior treadmill walking experience and recorded their overground *PWS* which was then imposed on both the treadmills. As we also wanted to replicate the results for *MCoW* of our *OA* from the study in Chapter 2, we used a fixed order of experiments in this study. We found a statistically significant elevation for *GCoW* (by 6%) on the elevated treadmill compared to overground and by 9.5% on the floor level treadmill compared to overground. For *NCoW* there was a statistically significant elevation by 8% on the elevated treadmill compared to overground and by 9.8% on the floor level treadmill compared to overground. For heart rates, there was a 2.7% elevation (statistically significant) on the elevated treadmill compared to overground and a 13.5% elevation (statistically significant) on the floor level treadmill compared to overground. There were no statistically significant differences between the two treadmills for *GCoW, NCoW* and heart rates. However, for self-reported anxiety levels there were statistically significant elevations on the elevated treadmill compared to overground ($p=0.004$) and on the elevated treadmill compared to the floor level treadmill ($p=0.02$). There were no statistically significant differences in self-reported anxiety between the floor level treadmill and overground walking.

With these results in Chapter 3 we showed that self-reported anxiety did not play a causal role in the elevation of treadmill *MCoW* compared to overground for our *OA,* because irrespective of changes in self-reported anxiety, *MCoW* was higher on both the

treadmills compared to overground. Additionally, we showed that in situations where there is metabolic energy expenditure (like in walking), heart rates are not related to anxiety, but are related to metabolic rates. This is due to the fact that cardiac output associated to metabolic rates has a dominant effect on heart rates, compared to the effect of anxiety on heart rates. Furthermore, we showed that anxiety was specifically induced by the height of the elevated treadmill (used in both Chapters 2 and 3) for our *OA* participants. After showing in the study reported in Chapter 2 that there are no statistically significant differences in overground *MCoW* between *YA* and *OA* (difference of 0.08 J $kg^{-1}m^{-1}$), the single most important finding that we proved from both our studies in Chapters 2 and 3, is that *MCoW* is different (about 6-20% higher) for healthy *OA* on treadmills compared to overground. This is true for *OA*, even after walking for 15-30 minutes on treadmills at their overground *PWS*. This shows that in order to study if there is an elevation in *MCoW* for *OA* compared to *YA*, as commonly reported in the published literature, future researchers should not conduct studies on a treadmill but conduct them overground. Even if researchers have to conduct studies on a treadmill (say, due to methodological reasons of non-availability of large overground walking surfaces) and report an elevation in *MCoW* for *OA* over *YA*, they should report that their results could be affected by the interaction between age and treadmill walking.

Thus to summarise, from the studies conducted in this thesis, we have shown that the commonly reported elevation in *MCoW* for *OA* over *YA* is not due to age *per se* but is probably due to the differential reaction to treadmills by healthy *YA* and *OA*. In Chapters 2 and 3 we have shown that *OA* react differently to overground and treadmill walking (elevated *MCoW* on treadmills compared to overground) at their overground *PWS*, which we directly imposed on the treadmills. *YA* do not react differently to overground and treadmill walking at the same speed as they have similar *MCoW*. Due to

this differential reaction to overground and treadmill walking by *YA* and *OA* walking energetics are different between the two groups. However, there are some limitations of this thesis, on which we will elaborate in the following section.

4.2 Limitations of the thesis

Firstly, all our experiments have been conducted on healthy *YA* and *OA* and though we specifically wanted to see the differences in *MCoW* between healthy young and elderly, healthy *OA* are not a very good representative sample of all *OA.* Not all *OA* are as fit and healthy like the *OA* participants in our studies and hence our *OA* do not represent the complete elderly population. Secondly, in our studies there is the possibility of a so-called 'lab-coat' scenario, where the participants possibly tried to perform their best during the walking trials inside the laboratory. For example, it could be that our participants wanted to perform to the best of their abilities and show they are extremely fit in order to impress us, the researchers. This could then result in a possible scenario where we would observe a higher than normal *PWS* (average *PWS* is in the range of 1.2 to 1.3 ms^{-1} for humans) which would not be representative of their daily life *PWS.* If the *PWS* that we found in the laboratory was higher than the average for our participants, then metabolic power would also be higher. However, since we calculate *MCoW* for all our *YA* and *OA*, where we divide metabolic power by *PWS* for all our participants, we do not think this limitation has affected our study results and the conclusions that we draw from them.

Finally, it has to be acknowledged that the sample sizes are on the lower side (10 individuals per group in Chapter 2 and again 10 individuals in Chapter 3) in the experimental studies. In general, this might lead to (potential) lowering of statistical power while doing statistical comparisons, thereby resulting in Type-2 errors (false negative outcomes) or/and inflated effect sizes. In the study reported in Chapter 2, we

conduct between-group comparisons for *MCoW* (*YA* vs *OA*) and report no statistical differences in *MCoW* between the cohorts. This result can be a possible Type-2 error. In our defence, we mainly looked at *NCoW* which has smaller variability and higher effect size than *GCoW* (as seen from the meta-analysis results in Chapter 1) and since the average overground *NCoW* was almost similar (difference of 0.08 J $kg^{-1}m^{-1}$) between our *YA* and *OA*, increasing the sample size would not have made this result statistically significant. For the other within-group (paired design) comparisons in the study reported in Chapter 2, we also repeated our walking experiments (morning and afternoon sessions). All in all, these limitations do not affect the main finding of the studies in the chapters (i.e., there are no differences in *PWS* and overground *NCoW* between our *YA* and *OA*) and as such do not affect the conclusions that we draw after interpreting the data from the empirical studies.

4.3 Proposed future work

We can identify potentially four directions of future work arising from the work of this thesis. Firstly, in order to find out whether there are differences in *MCoW* between *YA* and *OA*, it is important to conduct studies in even higher-age groups of elderly to see if we can reproduce our results for this group of elderly. It would be important to see whether octogenarians and older exhibit similar levels of overground *MCoW* like young people. This is because people over 80 years old could get more tired while walking (as reported by a couple of our participants in our study in Chapter 2 who were above 80 years old), have an altered gait pattern and have a higher *MCoW* compared to relatively younger elderly (between 65 to 80 years old) and *YA*. For example, in the study of (Gaesser, Tucker, Sawyer, Bhammar, & Angadi, 2018) there were statistically significant elevation in *NCoW* only in the highest age group (>= 70 years old). If in future studies involving octogenarians and older we see that their

overground *MCoW* is higher than relatively younger elderly and *YA*, this could mean that people of 80 years of age and older will become more easily fatigued than others. Future researchers should then treat the group of octogenarians and older separately from all other relatively younger elderly and devise specific training interventions to try and reduce their *MCoW* so that they do not get tired and become more sedentary. This can be achieved through interventions aiming to increase energy capacity by improving overall fitness and strength or/and to increase walking efficiency through motor skill training (VanSwearingen & Studenski, 2014).

Secondly, these experiments should also be done with comparatively less fit Dutch elderly and those from other ethnic groups. In this thesis, we had conducted experiments only on those Dutch elderly who were generally fit, active and living a healthy life. It is generally known that on an average, Dutch people bicycle regularly right from their early childhood. Almost all our participants for the experimental studies were still bicycling regularly in their daily life and some of them even bicycled to our laboratory on the day of the experiments. There are some evidences in the literature showing that bicycling for exercise (compared to walking for exercise) helped elderly to maintain a *MCoW* similar to *YA* (Aslan, Collette, & Ortega, 2017). The greater aerobic intensity of bicycling compared to walking could mean that in general, Dutch elderly become aerobically fitter and have similar levels of *MCoW* like *YA*, something which we might have seen in the results of our experiments. Future researchers interested in learning about differences in *MCoW* between *YA* and *OA* need to conduct studies like ours with less fit Dutch elderly (for example, who do not bicycle regularly) and elderly people from other countries who do not have a habit of regular bicycling or other similar aerobic exercises from their childhood. There can be several implications for conducting such studies. It could be that less fit Dutch elderly and elderly from these other countries

have a higher *MCoW* than *YA*. If so, one of the prime advices to help people to have a lower *MCoW* and not get tired and become sedentary too quickly when they grow old would be to develop a habit of bicycling and other aerobic exercises from an early age. Additionally, those studies should also be included in a future review and meta-analysis by researchers so that we can constantly have a quantified effect size of the difference in *MCoW* between *YA* and *OA*.

Thirdly, our experimental studies in Chapters 2 and 3 should be performed outside of a laboratory, preferably in a setting which mimics the daily life settings of humans. If inside a laboratory, people tend to show 'lab-coat effects' as explained previously in the limitations section, it could lead to changes in their gait. For example, people can walk at a speed which is higher than their daily life overground *PWS,* which could also mean having a less than optimal *MCoW* due to the hyperbolic nature (see: Figure 1 of Chapter 2 of this thesis) of the relationship between *MCoW* and gait speed (Ralston, 1958). In that case we would not be studying the ecologically relevant daily life gait and would be instead studying an 'artificial' laboratory gait. With the aid of wearable sensors and portable gas analysers, these experiments can easily be reproduced outside of the laboratories, where people might feel more at ease and comfortable and not show the 'lab-coat effects'. Our experiments in the study in Chapter 2 were quite long (one full day with a morning and afternoon session), involved both overground and treadmill walking and this was often tiring and extremely time-consuming for our *OA* subjects (as mentioned in Chapter 2). As a result, we could not perform the same experiments again outside of the laboratory with the same cohort, in particular, involving the same *OA*.

Finally, as we were unable to find the reasons for a higher *MCoW* on treadmills compared to overground for our elderly (in Chapters 2 and 3), it is important that we

conduct follow-up studies to search for the reasons. The higher *MCoW* on treadmills compared to overground for *OA* (which we do not see in our *YA*) indicate that there is an interaction between age and treadmill walking and we need to know why this interaction is not there for *YA*. After the experiments in Chapter 3 we proved that self-reported anxiety did not solely trigger gait changes that lead to an elevation in *MCoW* for elderly on treadmills compared to overground; so there must be something else which is causing the elevation in *MCoW*. Future researchers should conduct simultaneous gait analysis involving kinematics and kinetics, muscle activation through Electromyography (*EMG*) and oxygen consumption so as to find the reasons for the elevation in *MCoW*, which must be due to changes in gait patterns and parameters on treadmills compared to overground for elderly. Unfortunately, we have neither the kinematic, kinetic, *EMG* data of our *YA* nor do we have of our *OA* in the morning session in the study reported in Chapter 2, the session where we see the statistically significant elevation in treadmill *NCoW* compared to overground for our *OA*. If we would have had the data, we would have looked for (possible) differences in kinematics, kinetics and *EMG* between the overground and treadmill values for our *OA*, which could have explained why there was an elevation in the treadmill *NCoW* compared to overground in that session. In the study in Chapter 2, we chose not to measure kinematics, kinetics and *EMG* in the morning session. We did so mainly due to two reasons. Firstly, we were interested in seeing whether there were differences in *MCoW* when *OA* and *YA* are similar in anthropometrics, physical activity and fitness levels and walked at the same *PWS*, especially during the ecologically relevant overground walking. Secondly, we wanted to keep the length of the study feasible for our participants as we already had a morning and afternoon session of experiments. In hindsight, we should have included those

measurements and we recommend that future researchers do so (if possible) even if they have multiple experimental sessions like ours.

4.4 Conclusion

In conclusion, through this thesis we have shown how to have age as the only differentiating factor between *YA* and *OA* while trying to learn about the differences in *MCoW* between the cohorts. There were no differences in anthropometrics, physical activity and fitness levels and overground *PWS* between our *YA* and *OA*. Our main finding is that we have shown there are no differences in overground *MCoW* between them. As overground walking is the ecologically relevant mode of walking, overground *MCoW* should be considered important. Due to the differential reaction of *YA* and *OA* to treadmills, treadmills are not suitable for comparison of *MCoW* between the two populations. The results of similar overground *MCoW* of *YA* and *OA* could have some clinical implications. For example, these results could be interpreted in the way that if people adopt a healthy and active lifestyle from an early age, when they grow old, they can have similar levels of *PWS* and *MCoW* like a young person. Comparable levels of *PWS* would generally mean that mobility and daily life gait will not be affected and similar levels of *MCoW* will mean that elderly will expend the same amount of energy after walking the same distance as a young person. Overall this will imply that elderly will be able to lead a normal healthy lifestyle, not become fatigued and sedentary, and we can achieve the goals of healthy aging in our society.

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Acknowledgment

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Department of Human Movement Sciences, Vrije Universiteit Amsterdam

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I also want to thank my Assessment committee members – **Glenn**, **Veronique**, **Maria**, **Han** and **Hein** for their valuable suggestions and guidance to substantially

improve my thesis. Without your critical suggestions, I do not think this thesis would have culminated in its final form.

It's often called that a PhD is a journey and not really a degree course to be completed. So no journey is possible without the involvement and company of friends and colleagues. **Koen**, it goes without saying that I have gained you as a friend for lifetime through this journey. Our discussions on literally any topic, ranging from science to politics was something which I always cherish. That you and **Dirk** could make it to my wedding in India earlier this year was a testament to our friendship that has grown over the years. **Encar**, you are another of those close friends that I made during this journey. Our interactions during the various workshops and meetings, our tours across the length and breadth of India and the numerous parties in Amsterdam and The Hague are some memories that I still dream about till date. Hope we see each other soon someday. **Herre**¸ you have been a true colleague in all possible terms. From doing most of our experiments together, to recruitment of subjects, helping me with Dutch while interacting with our elderly participants, the list goes on and on! I have also learnt how to look at science and think about results that we see from our experiments from you in a critical way. Thanks for teaching me that!

I still consider MF A-625 as my room where most of the journey happened. It was a fantastic experience to work and share time with my various room-mates over there. **Mina** and **Nathalie**, though we met briefly in the first year of my PhD, it was great knowing you both and sharing good times. **Lotte, Dirk** and **Stephan**¸ I will never forget the various academic and fun discussions that we have had over the years. Due to departmental policy I also had many temporary colleagues in A-625. I may not remember all of you, but **Makkiko** and **Kazu** it was also great knowing you. I have a

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Life apart from academic colleagues

Being an Indian, it is probably no surprise that I had lots of Indian friends during my PhD life in Amsterdam. **Sudip, Partha, Subhronil, Suman da, Reshmi, Anwesha, Nitesh, Anandi, Ashish, Oindrila, Nikita, Rehan da, Ruhina, Arijit, Avratanu, Sayan da, Souloke, Madhubanti, Vasilica (I made you an Indian ;))** this list is also huge. All our dinners, trips all across Europe, crazy parties in Amsterdam and the numerous fun stuff were something that I always looked up to have some variation and chill in my non-academic life in Amsterdam! The various Indian parties and festivals like attending Durga puja, Saraswati puja, Poila boisakh, among others were truly memorable for me. However it was not only Indians over here too. My relationship with the International office in the University and later with the Indian Student Association (ISA) in Netherlands was also something from which I came to know many other people and became friends with. **Sandra and Reyka** you two have been long-time friends in that regard. From organizing Diwali and Holi parties to making the Global room a success, I had many fond memories. **Reshmi, Hardit, Harsh, Girish, Avijit, Sweta,**

Saachi all our times and interactions were awesome and fun in Amsterdam. **Arthur** and **Emma** our trip to Germany with **Suman da** is still etched clearly in my memory!

Of course Fitsurance was and is a part and parcel of my PhD journey since I consider that valorization should have an equally important role with research in a doctoral trajectory wherever possible. **Stef** and **Tommie** and later **Arun** and **Arnab** in India, you all have been both good friends and also great co-founders to work with over the past 3 years. Our critical discussions, friendly chats, agreements and disagreements regarding scientific and commercial aspects of a start-up business are something clearly etched in my memory and something that I look to carry with me. **Moritz** in the beginning and **Marcel** later, you both have been also great friends and colleagues to work with in Fitsurance. Also a special mention to **Enno, Jos, Melvyn, Davide, Art, Dorien, Brenda, Berno** among others at Human Movement Sciences, Demonstrator lab and Innovation Exchange Amsterdam (IXA) for helping me and my team with equipment, ideas, guidance and above all, time. I believe that all your friendly guidance helped us to slowly try to adapt and become good entrepreneurs from scientists. Academic entrepreneurship is real and here to stay, cheers to that!

Friends in India

Though I was in Amsterdam, I knew that a part of my life always remained in India. That's why I probably made at least twice a year journeys to India to meet my friends and hang out with them in Bengaluru, Mumbai, Chennai, Delhi and of course Serampore. **Sujoy, Raktim, Vicky, Nirupam, Arindam, Pratyay, Biswaroop, Abhishek Dutta, Kousan, Ipsita, Sayantani, Sraboni, Arkabrata, Sasanka, Akanksha, Abhishek Mallik, Subhodeep, Subrata, Aruni, Atanu, Pinja, Jayoti, Reshma** and many other senior and junior friends in Serampore, you all are there in a very special and beloved part of my brain and heart always. May our friendship last the

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Of course, it goes without saying that without the support of my family this would not have been possible. **Ma** and **Baba** it would not have been possible without your love, affection, motivation and support all through these years. You have taught me how to have a balanced outlook and attitude towards life and why having a sense of normalcy and urgency at the same time is necessary to face just about everything. My sister **Reshmi,** brother-in-law **Subham,** his mother **Sriparna kakima** and our dearie **Smiley** (a Golden Retriever) have always been a constant support and strong pillars of my life and whatever little success I have been able to achieve till date. Later on my extended family - **mother-in-law, father-in-law, uncle-in-law, aunt-in-law and all the brothers and sisters-in-law**, thank you all for being a constant motivation and support during this enriching but at times tough and tedious journey of being a doctoral researcher.

Finally, it has to be **Alka (**or should I say **Shin Aruca as the Japanese say here?),** my intelligent, loving, gorgeous and beautiful wife! Without you this would not have been possible, oh the age-old cliché ;) Our relationship have gone through all the ebbs and flows during this journey and also your Master's and doctoral journey simultaneously in Japan. We have grown stronger together, learnt to laugh and cry it out together, support and criticize (rightfully at times) each other and above all understand and trust each other. It's probably a fitting finale that both of us are almost going to finish our journeys simultaneously now. Let us keep on holding our hands and grow older together and imbibe all the learnings and knowledge from these doctoral journeys

of ours and use them in our lives to the fullest! Thanks to you and my whole family for being there!

N.B.:- The thesis cannot end without thanking and acknowledging the participants of my experiments. I cannot disclose their names due to privacy concerns but without them of course nothing of this would have been possible. I thank them from the bottom of my heart for having interest, time and patience to endure day-long experimental sessions and go through the pain of the protocol and diet restrictions! Thanks for everything.

Valorization

Personally, I believe that if possible, researchers should also dedicate more time and effort for valorization. This would enhance the chance of contributing more directly for the common people (or taxpayers) in the society as normally the public would not be reading our journal papers or attend our conference presentations. This belief led me to form an independent team of my own (with some fellow students from our department of Human Movement Sciences at Vrije Universiteit Amsterdam) to incubate and incorporate a preventative healthcare start-up named Fitsurance in 2018, which was indirectly inspired from the results of my doctoral research. In this short section I intend to acknowledge this belief and the efficacy of my overall doctoral training as a motivation for taking up this route of valorization.

To elaborate, after conducting my doctoral research, I believed that the human subjects whom I researched in my doctoral dissertation (especially the elderly) were extremely fit and active, compared to a generally older population. As a result they could preserve their youthful gait and Metabolic Cost of Walking (*MCoW*) and had similar levels of preferred walking speed and overground *MCoW* like our young subjects. Therefore, we believed that if we can promote the message of staying fit and

active right from a young age through Fitsurance we would be able to achieve the goals of healthy aging further in the Dutch society.

Disclaimer – As the author of this doctoral thesis, I want to state that the scientific content of the thesis do not directly support or provide any evidence for the approach or effectiveness of Fitsurance. At the same time, in no way was Fitsurance involved or influenced the study design, results and conclusions of my chapters. My only intention of briefly acknowledging this valorization activity is purely to show the diversification of my doctoral training and in no way to make a commercial statement or/and give any unwarranted impression of scientific support for Fitsurance obtained from this doctoral project.

List of Publications related to this thesis

Das Gupta, S., Faber, H., Kistemaker, DA., Bobbert, M. (2023). The elevated Metabolic Cost of Walking at preferred speeds of healthy elderly on treadmills compared to overground is not related to increased self-reported anxiety. *European Journal of Applied Physiology.* https://doi.org/10.1007/s00421-023-05138-y (Impact Factor - 3.35)

Das Gupta, S., Bobbert, M., Faber, H., Kistemaker, D. (2021). Metabolic cost in healthy fit older adults and young adults during overground and treadmill walking. *European Journal of Applied Physiology, 121(10), 2787-2797.* https://doi.org/10.1007/s00421-021-04740-2 (Impact Factor - 3.35)

Das Gupta, S., Bobbert, M.F., Kistemaker, D.A. (2019). The Metabolic Cost of Walking in healthy young and older adults – A Systematic Review and Meta Analysis, *Scientific Reports, 9, 1-10. [9956]*. https://doi.org/10.1038/s41598-019-45602-4, (Impact Factor – 5.00)

Das Gupta, S., Faber, H., Kistemaker, D., Bobbert, M. (2018). P 097 - Metabolic Cost of Transport in over-ground and treadmill walking of healthy elderly and effects of a treadmill familiarization protocol. *Gait & Posture, Volume 65, Supplement 1, 389-392.* https://doi.org/10.1016/j.gaitpost.2018.06.069 (Impact Factor – 2.75)

Selected list of Conference Proceedings related to this thesis

The Elevated Metabolic Cost Of Walking At Preferred Speeds Of Healthy Elderly On Treadmills Compared To Overground Is Not Related To Increased Anxiety, selected and presented as an oral presentation at the 9th World Congress of Biomechanics (WCB), Taipei, Taiwan - 2022

The Metabolic Cost of Walking in healthy young and older adults – A Systematic Review and Meta Analysis, selected and presented as an oral presentation at the 27th annual meeting of the International Society of Biomechanics/American Society of Biomechanics (ISB/ASB), Calgary, Canada – 2019

Metabolic cost of transport in over-ground and treadmill walking of healthy elderly and effects of a treadmill familiarization protocol, selected for publication as a Short Communication and presented as a poster at the 27th Annual Meeting of the European Society for Movement Analysis in Adults and Children (ESMAC), Prague, Czech Republic - 2018

The Metabolic Cost of Transport is not elevated in healthy elderly compared to young in overground and treadmill walking, selected and presented as an oral presentation at the 8th World Congress of Biomechanics (WCB), Dublin, Ireland - 2018

The Metabolic Cost of Walking in healthy young and older adults – A Systematic Review and Meta Analysis, presented as an oral presentation at the MOVE-AGE Annual Conference held at Manchester Metropolitan University, Manchester, UK - 2017

List of Grants/Subsidies related to the valorization part of this thesis

Eurostars program of the European Union (Proposed for funding), June and December, 2022

SME Innovation Stimulation Region and Top Sectors (MIT) Feasibility project; RVO, The Netherlands, (funded by Provincie Noord Holland, The Netherlands), April 2021

SME Innovation Stimulation Region and Top Sectors (MIT) Feasibility project; RVO, The Netherlands, May 2019

NWO-TTW Take-off (Phase 1), NWO, The Netherlands, December 2018

List of Courses and training activities taken as a part of doctoral training

Name	Date achieved	EC
External courses		
Academic English	29-01-2016	3.00
Research Ethics	27-05-2016	3.00
Personal Development	28-10-2016	2.00
Advanced Methodology	26-05-2017	6.00
Scientific Communication	30-06-2017	3.00
Entrepreneurship in Human Movement Sciences	25-05-2018	6.00
Research related		
Open-sim workshop	12-02-2016	2.26
Graduate School Colloquiums/Symposiums	28-09-2018	3.00
ESMAC, ISB, WCB, MOVE-AGE, AMS conferences	14-07-2022	2.00
International conferences - WCB, ISB and ESMAC	14-07-2022	1.00
Other Academic activities		
Organizing Science Transmission meeting on Bayesian Statistics	09-04-2018	1.00
Attending Science Transmission meetings	28-09-2018	1.00
AMS annual conferences	28-09-2018	1.00
Organizing VvBN conference at Vrije Universiteit Amsterdam	30-11-2018	1.00
Total number of ECTS credits		------ +
		35.26

Brief Biography



Sauvik Das Gupta was born in Serampore, West Bengal, India on the 8th., of December 1987. After completing his schooling in West Bengal, he pursued a Bachelor in Technology (B.Tech) in Instrumentation & Control Engineering from SRM University, Chennai, Tamil Nadu. His bachelor's thesis was in the field of Micro-Electro-Mechanical-Systems (MEMS) software modelling under the supervision of Prof. Dr. A Vimala Juliet. He graduated as a University level Bronze medallist in 2010. After his B.Tech, he travelled to Oklahoma in USA to do his Master of Science (MS) in Electrical Engineering from Oklahoma State University in Stillwater. During his master's he specialized in Robotics and embedded computing. Under the supervision of Dr. Weihua Sheng he completed his Master's dissertation in 2012. The topic of his dissertation was prediction of arm force and joint angles from surface EMG signals using a haptic device and Artificial Neural Networks (ANNs).

After completing his Master's, Sauvik came back to India and was involved in his first start-up venture named ESL in Kolkata, West Bengal. He stayed for 3 years (2012-2015) at ESL and was involved in academic training and research in the field of Embedded electronics, Image processing and Computer vision. In 2015, Sauvik was awarded the prestigious Erasmus Mundus Joint Doctoral scholarship from the EU to pursue his doctorate from the Vrije Universiteit Amsterdam and KU Leuven, Belgium.

Currently Sauvik is the Co-founder and Chief Technical Advisor of Fitsurance BV in The Netherlands and Co-founder and Data Science and R&D and Business Development Lead at Fitsurance India Pvt. Ltd. In India where he is mainly in charge of the R&D wing of his start-ups. He is facilitating the collaboration between the Dutch and Indian entities of Fitsurance and trying to promote cross-border research and valorization in the field of preventative healthcare between The Netherlands and India. Sauvik loves research and soon he aims to start in a postdoctoral research position in Japan where he can continue his research in the field of biomechanics and energetics and also be active in scientific knowledge valorization. He aims to be active with his collaborators and start-ups in The Netherlands and India too while pursuing research in Japan.