Associations of step counts and aerobic stepping cadence with arterial stiffness in older adults

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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ACRONYMS

AS: Arterial Stiffness

CI: Confidence Interval

CVD: Cardiovascular Disease

HR: Hazard Ratio

MET: Metabolic Equivalents

MVPA: Moderate-Vigorous Intensity Physical Activity

PA: Physical Activity

PWV: Pulse Wave Velocity

cfPWV: Carotid-femoral Pulse Wave Velocity

OR: Odds Ratio

RR: Relative Risk

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ABSTRACT

Purpose: It is unclear if higher daily step counts are associated with lower arterial stiffness (AS) in older adults. Less is known about the effects of aerobic stepping cadence (steps/minute) independent of daily step counts on AS in older adults. We examined the independent and combined associations of objectively-measured step counts and aerobic stepping cadence withAS among older adults.

Methods: This cross-sectional study included 409 older adults aged ≥65 years (mean age = 72 ± 6, 59% female). AS was derived from carotid-femoral pulse wave velocity (cfPWV) using the SphygmoCor device (SphygmoCor system, AtCor Medical, Sydney, Australia). High AS, an established risk factor of cardiovascular diseases, was defined as cfPWV ≥10 m/s.. Step counts and aerobic stepping cadence averaged over 7 days were measured with accelerometer-based pedometers (Omron Alvita Optimized pedometer HJ-321, Kyoto, Japan). Odds ratios (ORs) and 95% confidence intervals (CIs) for high AS were calculated among quintiles (fifths) of step counts and five groups of aerobic stepping cadence (slow walkers with 0 aerobic steps and quartiles [fourths] of aerobic stepping cadence for fast walkers). Slow walkers included those participants who accumulated no aerobic stepping cadence data (cadence of >60 steps per minute for more than 10 consecutive minutes). The lowest step count quintile and the slow walkers were considered the reference groups in the corresponding analysis. Logistic regression was used to investigate the independent associations between step counts or aerobic stepping cadence with high AS. Participants were dichotomized as fast/slow walkers (obtaining any steps at >60 steps/minute or not) or active/inactive (≥5,000 steps/day or not) for a joint analysis. Covariates were sex, age, body mass index, smoking, heavy drinking, diabetes, hyperlipidemia,

hypertension medication, systolic blood pressure, and step counts or aerobic stepping cadence in respective analyses.

Results: Eighty-six (21%) older adults were identified as having high AS. Compared to the lowest quintile of step counts, ORs (95% CIs) of having high AS were 0.47 (0.23-0.99), 0.38 (0.18-0.82), 0.42 (0.19-0.93), 0.52 (0.22-1.11) for second, third, fourth and fifth quintile, respectively, after controlling for age and sex. After further adjustment for comorbidities, lifestyle factors, and aerobic stepping cadence, the second and third quintile of step counts still remained significant (OR, 0.38 [95% CI 0.16-0.91] and OR, 0.38 [95% CI 0.15-0.97]). Compared to those with 0 steps/min aerobic stepping cadence (slow walking), ORs (95% CIs) of having high AS were 0.50 (0.21-1.19), 0.47 (0.18-1.20), 0.46 (0.18-1.16), and 0.42 (0.16-1.09) for ascending groups of fast walking aerobic stepping cadence after adjustment for age, sex, comorbidities, lifestyle factors, and step counts. In the joint analysis, compared to Inactive & Slow walkers, ORs (95% CIs) of having high AS were 1.16 (0.31-4.34), 0.43 (0.20-0.95), and 0.48 (0.23-0.95) for Active & Slow walkers, Inactive & Fast walkers, and Active & Fast walkers, respectively.

Conclusion: Higher total daily step counts appeared to be associated with a lower prevalence of high AS status among older adults after adjusting for possible confounders including aerobic stepping cadence in logistic regression analyses. Although aerobic stepping cadence was not significant after adjusting for possible confounders including total daily step counts, this study also suggests a possible association between higher aerobic stepping cadence and lower prevalence of high AS with ORs of <1.00 in all fast walkers (≥1 aerobic steps) compared to slower walkers (0 aerobic steps).

CHAPTER 1: INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of premature mortality, representing 32% of global deaths (Chen et al., 2018). In the United States, over half of the prevalent cases of CVD and 80% of CVD deaths occur in adults aged 65 years and above, thus the rapidly aging population will be a challenge to our healthcare system in terms of morbidity and mortality due to CVD (Centers for Disease Control and Prevention, 2013; Mozaffarian et al., 2016; U.S. Census Bureau., 2008). Subclinical CVD, such as carotid atherosclerosis or arterial stiffness, is an asymptomatic portent towards clinical CVD that entails age-related changes in the structure and function of the cardiovascular system (Devereux & Alderman, 1993; Jakovljevic, 2018). The prevalence of any subclinical CVD is estimated to be 35% to 40% of community-dwelling older adults (Jakovljevic, 2018; Kuller et al., 1994; Wendell et al., 2017). Given that subclinical CVD has been associated with a higher risk of coronary heart disease among men and women 65 years or older, independent of other traditional risk factors (e.g. lipoprotein levels, glucose levels, inflammatory markers, body mass, and systolic and diastolic blood pressure), its assessment is relevant in cardiovascular health promotion and disease prevention (Kuller et al., 1994, 2006; Wendell et al., 2017).

Arterial stiffness (AS) is a predictor of CVD morbidity and mortality independent of traditional risk factors such as resting blood pressure in well-functioning older adults (Bonarjee, 2018; Sutton-Tyrrell et al., 2005; Vlachopoulos et al., 2010). It has been demonstrated that increased AS may predict cardiovascular events in asymptomatic individuals without overt CVD (Bonarjee, 2018; Roth et al., 2017). Therefore, AS is a plausible surrogate endpoint for CVD in

epidemiologic studies and a major reference point in cardiovascular risk management to evaluate the effects of preventive strategies among older adults.

Physical activity (PA) promotion is an evidence-based strategy for the prevention of CVD and cardiovascular mortality with proven benefits among older adults (Ahmed et al., 2012). Recently, the United States PA Guidelines Committee Scientific Report proposed the potential benefits of measuring PA in steps per day since it is easily understood by the community and may be useful for monitoring personalized PA goals in combination with wearable technologies, which are now widely accessible (Piercy et al., 2018). For older adults in particular, steps may be an especially useful PA measure since walking is the most popular form of PA among older adults (Bijnen et al., 1998; Valenti et al., 2016).

Previous evidence supports an inverse association between steps per day and a variety of health risk factors, morbidity, and mortality (Dwyer et al., 2007; Dwyer et al., 2015; Pal, Cheng, Egger, Binns, & Donovan, 2009). Cavero-Redondo *et al.*, suggested that greater steps per day has also been associated with less AS, particularly, among those who reach more than 10,000 steps per day (Cavero-Redondo et al., 2019). However, there is not a strong recommendation based on the optimal steps volume due to the lack of large scale epidemiologic and experimental studies supporting the relationship between steps per day and different health outcomes related to non-communicable diseases (Piercy et al., 2018). Furthermore, previous research studies have shown that the higher the intensity of an aerobic exercise program, regardless of the total amount of PA, the greater the benefit in improving AS measures (Ashor et al., 2014), yet few studies have examined the effects of stepping intensity, such as stepping cadence (steps/min), on CVD-related outcomes (Tudor-Locke et al., 2017). This knowledge might contribute to optimize step-

based PA recommendations that could be disseminated as a simple and actionable public health message for older adults.

The purpose of this study was to investigate the independent and combined associations of step counts (volume) and stepping cadence (intensity) with AS, among older adults who participated in the Physical Activity and Aging Study (PAAS), an ongoing prospective cohort study with over 700 participants in Iowa State University's Physical Activity Epidemiology Laboratory.

Specific aims

Aim 1: To determine the associations between step counts and AS, independent of aerobic stepping cadence, in older adults.

Null Hypothesis 1: Step counts would not be associated with AS, independent of aerobic stepping cadence, in older adults.

Alternative Hypothesis 1: Higher step counts would be associated with lower AS, independent of aerobic stepping cadence.

Aim 2: To determine the associations between aerobic stepping cadence and AS, independent of step counts, in older adults.

Null Hypothesis 2: Aerobic stepping cadence would not be associated with AS, independent of step counts.

Alternative Hypothesis 2: Higher aerobic stepping cadence would be associated with lower AS, independent of stepping cadence.

Aim 3: To determine the combined associations of steps counts and aerobic stepping cadence with AS in older adults.

Null Hypothesis 3: The combined association of step counts and aerobic stepping cadence with AS would not be stronger compared to either step counts or aerobic stepping cadence alone.

Alternative Hypothesis 3: The combined association of step counts and aerobic stepping cadence with AS would be stronger compared to either step counts or aerobic stepping cadence alone.

CHAPTER 2: REVIEW OF THE LITERATURE

This literature review will provide a synthesis of the concepts and epidemiologic evidence regarding 1) cardiovascular disease (CVD) in relation to arterial stiffness (AS), 2) the effect of physical activity (PA) on AS, and 3) the associations of different levels of step counts and stepping cadence with cardiovascular health.

Cardiovascular Disease and Arterial Stiffness

Cardiovascular disease (CVD) is the most prevalent chronic condition and the leading cause of death (Naghavi et al., 2017). Modifiable risk factors, such as hypertension, diabetes, cholesterol, overweight/obesity, tobacco use, and physical inactivity contribute to the development of CVD (Lee & Oh, 2010; de Rezende et al., 2017).

Arterial stiffness (AS) is a strong, independent predictor of CVD (Ben-Shlomo et al., 2014; Sťphane Laurent et al., 2001; Vlachopoulos et al., 2010). AS is the reduction in normal aortic compliance or elastic resistance to deformation that occurs with aging, reducing the capability of an artery to expand and contract in response to pressure changes (Cecelja & Chowienczyk, 2012; Safar et al., 2003). As a consequence, reduced compliance leads to an increased propagation velocity of the pulse pressure, called pulse wave velocity (PWV), along the arteries (Cecelja & Chowienczyk, 2012; Mitchell et al., 2004). A faster PWV may disrupt the natural timing of the cardiac cycle increasing the afterload of the left ventricle and decreasing coronary perfusion pressure (Ben-Shlomo et al., 2014). These changes may result in increased central pulse pressure and isolated systolic hypertension contributing to higher stress on the

cardiovascular system, specially on the left ventricle due to increase post-load and the myocardial tissue due to decreased mean coronary perfusion pressure, ultimately, leading to hypertrophy of the left ventricle, worsening of coronary ischaemia, increasing stress in the vascular walls, and increasing risk of adverse CVD events (Alvim et al., 2017). Thus, the slower PWV, the better for CVD prevention.

The predictive value of aortic AS for CVD endpoints has been traditionally measured using carotid-femoral PWV (cfPWV) in several epidemiologic studies since the carotid and femoral arteries are superficial, easy to access, and also cover the region of the aorta, which has the greatest age-related stiffening (Mitchell et al., 2004). PWV has been established as an independent predictor of CVD morbidity and mortality in elderly populations and among hypertensive, type 2 diabetes, and end-stage renal disease patients (Stephane Laurent et al., 2006). Furthermore, Consequently, PWV is recognized as a surrogate endpoint for CVD and a major reference point in cardiovascular risk management (Stephane Laurent et al., 2006; Obeid et al., 2017; Vlachopoulos et al., 2010).

The strength of these associations has been quantified throughout previous longitudinal studies. A systematic review and meta-analysis including 18 longitudinal studies and 15,877 participants found that individuals with high PWV had a pooled relative risk (RR) of 2.26 (95% CI 1.89 to 2.70) for total cardiovascular events compared to those without high aortic PWV. Furthermore, an increase in PWV by 1 m/s was associated with a 14% increased risk of cardiovascular events (RR, 1.14; 95% CI 1.09 - 1.20). Additionally, the comparison between high risk populations (e.g., subjects with coronary artery disease, renal disease, hypertension, and diabetes) and general population, presented a comparable increased RR for high PWV (RR, 2.44; 95% CI 2.01 - 2.97 vs. RR, 1.68; 95% CI 1.45 - 1.96, respectively; p= 0.003) (Vlachopoulos et

al., 2010). It is important to mention that there was not an established cutoff value to define high aortic PWV when this systematic review and meta-analysis was conducted; therefore, the authors implemented the cutoff values used by each study.

Furthermore, previous longitudinal studies have also shown that AS improves prediction of CVD beyond conventional risk factors such as sex, age, systolic blood pressure, serum cholesterol level, serum high density lipoprotein cholesterol (HDL-C) level, smoking, diabetes, and antihypertensive medications (Ben-Shlomo et al., 2014; Lee & Joo, 2019). In that regard, a meta-analysis by Ben-Shlomo *et al.* (2014) including 16 longitudinal studies and 17,635 subjects conclude that the inclusion of PWV into CVD predictive models increased the number of participants who were correctly classified, and improved the overall 10 year classification by 13%. (Ben-Shlomo et al., 2014).

In summary, there is compelling epidemiologic evidence supporting the predictive power of AS for further CVD independent of other traditional risk factors. Therefore, the measurement of AS may be an appropriate outcome to quantify the impact of preventative strategies for cardiovascular risk management among older adults.

Physical Activity and Arterial Stiffness

Aging is one of the strongest determinants of AS (Lee & Oh, 2010; de Rezende et al., 2017). A variety of cardiometabolic diseases such as obesity, diabetes, insulin resistance, dyslipidemia, and hypertension are major risk factors that have also been associated with an acceleration of the stiffening process of arteries along with aging (Sethi et al., 2014). Previous studies suggest that AS could possibly be reversible by lifestyle changes (Sacre & Kingwell,

2014; Vlachopoulos et al., 2006). Specifically, PA has been proven to affect structural and functional components of the arterial wall (Sacre et al., 2014). In that regard, Madden *et al*. (2009) showed that engaging in short-term aerobic exercise (3 months) significantly reduced AS among older adults aged >65 years old and diagnosed with diabetes, hypertension, and hyperlipidemia (Madden et al., 2009). Furthermore, Deiseroth *et al.* (2019) carried out an analysis within the EXAMIN Age study including adults between 50 and 80 years old. They estimated PA through objectively measured cardiorespiratory fitness (CRF) and compared active participants (mean CRF: 42.6 ± 8.2 ml/kg/min) with inactive participants (mean CRF: 29.7 ± 4.0 ml/kg/min) without cardiovascular risk factors. They found that cfPWV was 0.5 m/s lower in the active participants compared to their inactive counterparts and that each 10 ml/kg/min increase in maximal oxygen uptake (VO_{2max}) was associated with a decrease of 0.8 m/s in cfPWV (Deiseroth et al., 2019).

The effects of aerobic PA on AS are greater in the elastic arteries compared to the muscular ones, via increase in the elastin content in the arterial wall (Seals, 2003). Ashor *et al.* (2014) conducted a systematic review and studied the effects of aerobic exercise on PWV, revealing a significant reduction in PWV with exercise (Weighted Mean Difference: −0.63 m/s; 95% CI 0.90 - 0.35). Moreover, a significantly greater reduction in PWV was found after aerobic exercise interventions in participants with stiffer arteries, defined as PWV >8 m/s and trials lasting more than 10 weeks (Ashor et al., 2014).

Walking activities are major contributors of PA among older adults (Bijnen et al., 1998). However there is limited evidence on how many steps per day are needed for health as related to cardiovascular endpoints such as AS. Additionally, steps are walked at a determined cadence, but there is not enough evidence about how stepping cadence may affect AS independently or in

combination with step counts per day. Therefore, the current evidence related to step counts, stepping cadence, and cardiovascular health outcomes, including AS is addressed next.

Step Counts, Stepping Cadence, and Cardiovascular Health

Step counts

Walking is a rhythmic, dynamic, aerobic activity of large skeletal muscles that can provide a variety of health benefits with minimum adverse effects (Morris & Hardman, 1997). It is the most natural type of activity and the sustained dynamic aerobic exercise that is common to everyone except among seriously ill or disabled populations (Morris & Hardman, 1997; Murtagh, Murphy, & Boone-Heinonen, 2010).

Steps are the fundamental unit of human locomotor movement underlying all forms and purposes of ambulation. Objectively, they can be captured and summarized as step counts per day, as an indicator of walking volume, thanks to recently developed wearable technologies (Tudor-Locke et al., 2018). Before the incorporation of these technologies in research studies, some epidemiologic studies already described the health benefits of walking measured with validated PA questionnaires.

Large cohorts of adults have shown walking to be predictive of lower risk of coronary heart disease among men and women (Lee, Rexrode, Cook, Manson, & Buring, 2001; Sesso, Paffenbarger, & Lee, 2000). Specifically among older adult populations, Soares-Miranda *et al.* (2016) conducted an analysis among 4,207 US men and women whose mean age was 73 years old, and were free of CVD at baseline in the Cardiovascular Health Study. Usual walking habits including distance walked, were assessed with the validated Minnesota Leisure-Time Activities

questionnaire annually at each follow-up visit (Soares-Miranda et al., 2016). Compared to those who walked five or less blocks per week, the hazard ratio (HR) for total CVD were estimated in 0.70 (95% CI 0.58 - 0.83), 0.67 (95% CI 0.56 - 0.80), 0.56 (95% CI 0.46 - 0.68) and 0.53 (95% CI 0.44 - 0.65) among those who walked 6-12, 13-15, 26-48, and at least 49 blocks per week, respectively, indicating linearly decreased risks of total CVD by increasing walking steps per week (Soares-Miranda et al., 2016). A limitation from these results is that walking assessment was self-reported and its definition varied from study to study. Today, wearable technologies and more standardized tools for monitoring of daily ambulatory activity are accesible to optimize step counts assessment in research studies.

Pedometers are affordable, user-friendly tools that track step counts from the wearer and provide visual feedback on PA levels. These devices have the potential to promote daily living PA by stimulating a progressive increase in daily step counts (Gray et al., 2009; McKay et al., 2009; Pal et al., 2009). The implementation of pedometers as a research tool, but also as a public health strategy to promote walking and ultimately involvement in moderate PA have been described in previous studies among overweigh and obese women, community dwelling adults, and patients in primary care (Gray et al., 2009; McKay et al., 2009; Pal et al., 2009). Specifically among older adults, there have been studies that support the use of pedometers as a successful strategy to increase walking levels and achieve a total step counts goal per day and week (Croteau & Richeson, 2006; Harris et al., 2018; Rosenberg et al., 2012; Rosenberg et al., 2009). For example, Rosenberg *et al.* (2009) found that a brief multi-level place-based walking intervention led to 41% increase in average daily steps after two weeks among a continuing care retirement community of older adults (Rosenberg et al., 2009).

Pedometer-based step counts have been incorporated in research settings to better understand the effect of free-living patterns of mobility in a variety of health outcomes among different populations (Tudor-Locke & Rowe, 2012). The NAVIGATOR trial assessed the association between ambulatory activity and cardiovascular risk among individuals with impaired glucose tolerance and either existing CVD (e.g., if 50 years or older) or with at least one additional cardiovascular risk factor (e.g., if 55 years of age or older). Ambulatory activity was measured as average number of steps per day with a pedometer that participants were instructed to wear for 7 days and steps data were collected at baseline and 12-months. Among their main findings, at baseline each 2,000 step per day increment in ambulatory activity was associated with a 10% lower cardiovascular event rate. Furthermore, each 2,000 step increase or decrease in daily ambulatory activity from baseline to 12-months was associated with an additional 8% lower or higher risk of cardiovascular event, respectively. Results remained similar after adjustment for body mass index (BMI), change in estimated glomerular filtration rate, and the occurrence of unstable angina along the 12-months (Yates et al., 2014).

Regarding AS, a recent systematic review and meta-analysis led by Cavero-Redondo *et al.* (2019), synthetized the most updated evidence supporting an inverse association between pedometer- or accelerometer-determined step counts per day and PWV (Cavero-Redondo et al., 2019). Their findings quantified a reduction in PWV of 0.18 m/s per each 1,000 steps per day increase, which is the amount of steps equivalent to nearly 50% of PWV reduction observed in supervised exercise programs (Ashor et al., 2014; Cavero-Redondo et al., 2019). This is comparable to the minimum reduction of PWV that is expected to reduce the risk of vascular events and mortality by 3%, as has been estimated before (Vlachopoulos et al., 2015). The association found between PA behaviors based on steps per day cutoff values support that some

PA is better than none, but also more is better than less. Interestingly, these significant risk reductions were obtained with fairly achievable steps per day among adults and older adults (Cavero-Redondo et al., 2019; Tudor-Locke, Craig, Aoyagi, et al., 2011). However, no data were found in terms of stepping cadence and PWV.

In summary, walking is a popular and beneficial form of PA, particularly among older adults. Self-reported walking volume has been associated with reduced CVD and improved cardiovascular health. Similar results have been found for cardiovascular outcomes including AS with objective assessment of walking volume through step counts. However, the lack of studies accounting for stepping cadence is a limitation to consider, in order to better understand the association between step counts and AS independent of stepping cadence.

Stepping cadence

Cadence is a marker of walking intensity. Cadence, measured as steps per minute, has been strongly linked to objectively measured speed ($r= 0.97$) and intensity ($r= 0.94$) under controlled laboratory conditions, and it has the potential to represent behavioral patterns of ambulatory activity (Tudor-Locke, Craig, Brown, et al., 2011; Tudor-Locke & Rowe, 2012).

Higher natural stepping cadence has been associated with better health outcomes. A study evaluating the association between step-based PA metrics and cardiometabolic risk measured stepping cadence as peak 30-minute cadence. This variable captures the average steps/min recorded for the highest 30 minutes (not necessarily consecutive) in a day, reflecting the “natural best effort” in a day. They found that increasing quintiles of peak 30-minute cadence were inversely associated with waist circumference, weight, body mass index, and insulin for both men and women (Tudor-Locke et al., 2017).

More recently, Lee *et al.* (2019) analyzed stepping volume and intensity with all-cause mortality among 39,876 older women from the Women's Health Study. They analyzed stepping intensity via quartiles of several measures that reflected a person's best natural effort in a free-living environment. Interestingly, associations between stepping intensity and all-cause mortality were attenuated, and most of them were no longer significant after adjusting for steps per day, suggesting that step volume rather than stepping intensity may be more important for older adult women (Lee et al., 2019). Although this work did not study specifically a CVD outcome, its approach to analyze walking intensity through stepping cadence is a unique contribution to the objectively measured walking intensity.

Compelling evidence supports that the risk of CVD declines with increasing walking intensity measured as walking pace (Manson et al., 2002; Tanasescu et al., 2002). Stamatakis *et al*. (2018) conducted a pooled analysis of 50,225 walkers from 11 British cohorts and examined the association between self-reported walking pace with all-cause and CVD mortality. Walking was assessed using a question on number of days walked in the last four weeks, the average amount of time spent walking on each day and the usual walking pace ('slow pace, average pace, fairly brisk pace, fast pace—at least 4 mph'). Regarding all-cause mortality, they found a 20% (HR, 0.80; 95% CI 0.72 – 0.88) risk reduction for those walking at an average pace and 24% (HR, 76; 95% CI 0.67 – 0.87) risk reduction for those walking at a brisk/fast pace compared to those walking at a slow pace, after adjustment for total (non-walking) PA volume (MET-hours/week), walking volume (MET-hours/week), and highest (non-walking) PA intensity reached. Regarding CVD mortality, the same fully adjusted models estimated that walking at an average pace was associated with 24% (HR, 0.76; 95% CI 0.64 – 0.91) risk reduction and walking at a brisk/fast pace was associated with 21% (HR, 0.79; 95% CI 0.62 – 0.99) risk

reduction compared with those walking at a slow pace (Stamatakis et al., 2018). In fact, these results support that greater walking pace, either average or brisk/fast walking compared to slow walking, was associated with a reduced risk of all-cause and CVD mortality and may have implications for public health messaging. Likely, health benefits might be more feasible to obtain through higher walking intensity rather than volume or duration, as has been emphasized. However, further research will need to be undertaken to confirm if higher walking intensity might impact different health outcomes such as AS (PWV), independently of walking volume.

It is important to highlight that even though walking pace and stepping cadence (steps/min) can measure walking intensity, they are not interchangeable measures; walking pace measures intensity when walking purposefully (e.g., for exercise or transportation), and stepping cadence assesses overall best natural effort to step as fast as feasible in free-living conditions (Tudor-Locke et al., 2018).

According to previous studies, a consistent finding has been the reduced risk of cardiovascular adverse outcomes with increased walking intensity regardless of how intensity was measured (walking pace or stepping rate); however, most of these results were obtained via self-reporting, which is a limitation. Nevertheless, to confirm the beneficial effects of walking in cardiovascular health among older adults, objectively measured walking intensity is an aspect that needs further research. In that sense, wearable technologies that provide cadence indicators such as stepping rate are needed.

The evidence about the effect of stepping cadence on AS among older adults is still scarce; however, the current studies show promising results that need to be confirmed in larger

scale cohorts and clinical trials to better understand the intensity that should be added to the walking recommendation for older adults.

Conclusion

AS is a strong, independent predictor of CVD morbidity and mortality (Bonarjee, 2018; Vlachopoulos et al., 2010). Among older adults, there is a linear association between step counts and lower AS (Cavero-Redondo et al., 2019; Lee & Buchner, 2008; Murtagh et al., 2010). These findings support the benefits of increased step volume for CVD risk management through AS reduction.

However, step counts have been criticized for not capturing the intensity of PA, which is an important consideration for cardiovascular outcomes in particular (Garber & Blissmer, 2011; Stamatakis et al., 2018; Tudor-Locke et al., 2017). Very few studies have examined the association between objectively measured walking intensity as stepping cadence and AS, specifically among older adults. Some early data suggest cardiovascular benefits (e.g. reduced risk of CVD mortality and cardiometabolic risk) of higher stepping cadence, but further research is needed to better understand its effect on AS, independent of and combined with total walking volume, particularly among older adults (Cavero-Redondo et al., 2019; Tudor-Locke & Rowe, 2012).

Given that walking volume and intensity are related, the study of both as independent and joint exposures, may help to disentangle their independent effects on AS among older adults. Examining the independent and combined associations between step count and stepping cadence with AS could provide a unique contribution to refine step-based PA recommendations and influence how PA should be prescribed for older adults in future PA guidelines.

CHAPTER 3: METHODS

Study Population

The Physical Activity and Aging Study (PAAS) is an ongoing longitudinal cohort of over 700 older adults in the Ames, Iowa area. Inclusion criteria include 65 years of age or older, with no plans to move permanently out of Iowa. Exclusion criteria for this specific study included history of arrhythmia, cancer, myocardial infarction, or stroke in the past five years to minimize potential confounding effects in AS readings and interpretation of results. We excluded participants with missing data in PWV, which is the primary outcome.

The PAAS was approved by the Institutional Review Board of Iowa State University. All participants were required to sign an Informed Consent document prior to the beginning of their participation in the study.

Data Collection Procedures

Participants were invited to a comprehensive health and physical function assessment, which consisted of two visits over the course of one week. During the first visit, the participants were asked to complete a medical history questionnaire along with physical fitness, function, and strength assessments. At the end of the first visit, participants were given wearable devices including an accelerometer-based pedometer (Omron Alvita Optimized pedometer HJ-321, Kyoto, Japan) to track their routine PA for 7 days. A week later, participants returned for a second visit in which they completed fasted assessments including body composition, blood pressure, central hemodynamics including AS, and a blood draw.

Arterial Stiffness

AS was derived from carotid-femoral pulse wave velocity (cfPWV) using the SphygmoCor device (SphygmoCor system, AtCor Medical, Sydney, Australia). Participant's were indicated to abstain from engaging in vigorous-intensity PA during the 24 hours previous to the morning assessment and refrain from all forms of smoking and nicotine consumption for at least 4 hours prior to the morning assessment (Appendix. Supplementary Material). Participants arrived fasting (no food, alcohol, or caffeine for 12 hours prior to assessment) and performed this assessment in a room with centrally-controlled temperature and within the same time frame. A femoral cuff was placed around their upper left thigh to obtain a volumetric displacement waveform while applanation tonometry is used simultaneously on the neck to obtain the carotid pulse, allowing the device to estimate the pulse transit time of pressure waveforms (Butlin & Qasem, 2016). cfPWV is automatically calculated from measurements of the pulse transit time and the distance between the two recording sites, carotid and femoral [PWV=distance in meters (m)/transit time in seconds (s)] (Doonan et al., 2011; Stephane Laurent et al., 2006; Townsend et al., 2015). The transit time was measured from the foot of the carotid waveform to that of the femoral waveform (foot-to-foot method). In all participants, the total distance was defined as the distance from the suprasternal notch to femoral artery minus the distance from carotid artery to the suprasternal notch, and was measured directly with a caliper and a measuring tape (Doonan et al., 2011; Stephane Laurent et al., 2006; Townsend et al., 2015). This distance measurement method is termed the subtraction method and is recommended by the American Heart Association (Townsend et al., 2015).

cfPWV was measured in duplicate and values were averaged. However, when the difference between the two measurements was greater than 0.5 m/s, a third measurement was

taken and the median of all 3 measures was computed as recommended (Van Bortel et al., 2012). All of the assessments were conducted by the trained research staff following the standardized protocol in the lab.

In our analysis, high AS was defined as cfPWV $\geq$10 m/s, following the recommendation from the European Society of Hypertension Working Group on Vascular Structure and Function and the European Network for Noninvasive Investigation of Large Arteries, as the threshold for increased cardiovascular risk (Van Bortel et al., 2012).

Step Counts and Aerobic Stepping Cadence

Average step counts per day and aerobic stepping cadence over 7 days were measured with Omron accelerometer-based pedometers (Omron Alvita Optimized pedometer HJ-321, Kyoto, Japan). Participants were asked to wear the pedometer attached to the hip or inside the pocket during 7 days, removing it only during water-based activities. Participants self-reported their wear time each day (See Wear Time Log in the Appendix Section. Supplementary Material). Valid data was defined as wearing the device for at least 10 hours per day on 4 or more days, following the convention for compliant wear (Tudor-Locke et al., 2012). Mean step counts per day was calculated by summing the steps from all valid days and dividing this by the number of valid days.

Aerobic stepping cadence was derived from the average number of aerobic steps per minute, for that reason it will be referred as aerobic stepping cadence from here and ahead. Aerobic steps per minute were recorded by the pedometer when a participant has walked at a cadence of >60 steps per minute for more than 10 consecutive minutes, as determined by the manufacturer's algorithm. This threshold has been associated with a better representation of

more rhythmic walking that is different to incidental or sporadic movements and stepping indicative of purposeful ambulatory behavior, but not necessarily walking (Tudor-Locke & Rowe, 2012). Aerobic stepping cadence values that occured at equal or less than 60 steps per minute and/or for equal or less than 10 consecutive minutes were not recorded by the pedometer. Thus, participants who did not reach this threshold had values of zero steps per minute for their aerobic stepping cadence and were grouped in the reference category to test hypothesis 2, regarding aerobic stepping cadence and AS (PWV). Participants with aerobic steps (no zero aerobic stepping cadences) were split into quartiles to compare to the reference considering sample size and common epidemiological approach when there is no consensus for clinical cut-points. Aerobic stepping cadence was averaged from all valid days for the analysis (Omron Healthcare, 2012; Rider et al., 2014).

Self-reported PA time was also collected through questionnaires. Participants were asked about the amount of sessions per week and time per session of moderate- and vigorous-intensity PA in the past three months. Moderate-vigorous PA (MVPA) was computed based on the previous self-reported data, taking into account that one minute of self-reported moderate-intensity PA was equivalent to two minutes of self-reported vigorous-intensity PA (Piercy et al., 2018). This data was correlated with the pedometer-assessed step counts and aerobic stepping cadence.

Covariates

Sociodemographic covariates were sex and age (years) assessed through medical history questionnaire. Traditional modifiable risk factors associated to CVD were considered as covariates: smoking (current smoker yes/no), heavy drinking (>7 drinks per week for women,

>14 drinks per week for men), body mass index (BMI in kg/m^2), diabetes (yes/no), and hyperlipidemia (yes/no) (Vlachopoulos et al., 2015). In this analysis diabetes cases included self-reported diabetes, medication use specific to type II diabetes (e.g. biguanide, and insulin), and fasting glucose ≥126 mg/dL; and cases of hypercholesterolemia included self-reported hypercholesterolemia, low-density lipoprotein (LDL) ≥160mg/dL and lipid lowering medication use.

Relatedly, prescription medications for hypertension were considered as a single dichotomous variable (yes or no for any medication used related to hypertension), as has been done in previous studies assessing step counts and changes in AS (Dasgupta et al., 2017).

Systolic blood pressure was also considered as a covariate since structural changes occurring in the development of AS, lead to major increases in systolic blood pressure along with PWV and should be considered in PWV analyses (Lee & Oh, 2010). This parameter was obtained from the peripheral blood pressure assessment that participants did when they arrived fasting (no food, alcohol, or caffeine for 12 hours prior to assessment). It was performed in sitting position after 10 minutes rest, using a standard automatic peripheral blood pressure machine and the appropriately-sized blood pressure cuff in the left upper arm. Three readings were obtained and then averaged for the analysis following the guidelines (Whelton et al., 2018).

Step counts and aerobic stepping cadence are closely related and participants accruing more step counts per day are also more likely to be stepping at higher intensity. Consequently, aerobic stepping cadence was added as a covariate in the last model to test hypothesis 1 in which step counts is the main exposure. In a similar manner, step counts per day was added as a

covariate in the last model to test hypothesis 2 in which aerobic stepping cadence was the main exposure.

Multicollinearity between step counts and aerobic stepping cadence was assessed through a correlation matrix and the estimation of variance of inflation factor and tolerance in the regression model. Correlation coefficients ≥ 0.6, variance of inflation factor ≥ 5 and tolerance <0.1 were the criteria to indicate the presence of multicollinearity and the need of further methods of analysis (Schreiber-Gregory & Jackson, 2017). In the cases where concerning multicollinearity was found, the residual method was performed to adjust for step counts per day or aerobic stepping cadence, according to the corresponding model, following a previous approach (Lee et al., 2019; Willett & Stampfer, 1986).

Given that age is the strongest predictor of AS, a stratified analysis by age was conducted using 75 years old as the cut-point to define each strata. In addition, partial correlation coefficients were estimated between self-reported PA variables (moderate-intensity PA, vigorous-intensity PA, and moderate-vigorous PA) and objectively-measured step counts and aerobic stepping cadence in models adjusted for age and sex.

Data Analysis Procedures

Participants were split into five groups of step counts per day and aerobic stepping cadence following a previous approach in epidemiologic research upon this project is built on, to explore a greater range of volume- and cadence-based behaviors that may be more common in older adult samples (Tudor-Locke et al., 2017). The exposures categorized as five groups were used in the analysis to test the hypothesis 1 and 2.

To test hypothesis 1, five groups of steps per day were generated through quintiles having the lowest quintile as the reference category, given that five groups as quintiles have been commonly used in the literature (Tudor-Locke, Schuna, et al., 2013; Tudor-Locke et al., 2017).

To test hypothesis 2, five groups of aerobic stepping cadence were generated through quartiles of participants stepping at no-zero steps per minute compared to those stepping at zero steps per minute, the reference category.

Further, participants were categorized as active/inactive and fast /slow walkers, based on dichotomized values of step counts and aerobic stepping cadence, respectively, for a joint analysis of step counts and aerobic stepping cadence with high AS, to test the hypothesis of aim 3. Active participants were those walking $\geq$5,000 steps per day and inactive participants those walking <5,000 steps per day, following the step-defined sedentary lifestyle index definition, which established 5,000 steps per day as the cutoff point to distinguish sedentary lifestyle (<5,000 steps/day) from low active lifestyle (5,000 – 7499 steps/day) and physically active lifestyle ($\geq$7,500 steps/day) (Tudor-Locke et al., 2013). Fast walkers included those participants who had aerobic stepping cadence values >0 (i.e., they walked more than 60 steps per minute for more than 10 consecutive minutes, as this is the cutoff walking rate previously established by the pedometer's algorithm to detect aerobic stepping cadence data) (Omron Healthcare, 2012; Tudor-Locke et al., 2018). Slow walkers included those participants who accumulated no aerobic stepping cadence data (i.e., 0 aerobic steps per minute) due to not meeting the pedometer's algorithm over the 7 days.

Statistical Analysis Procedures

Descriptive Statistics

Descriptive statistics were calculated for each variable across the five groups of step counts per day and aerobic stepping cadence, using $\chi 2$ (categorical) and ANOVA (continuous). The assumptions of regression (normality and homogeneity of variance) were examined for PWV. Normality was assessed with Shapiro-Wilk test in the SAS software and Levene's test for homogeneity of variance.

PWV did not meet the regression criteria due to the lack of normality as assessed with the Shapiro-Wilk test (statistic=0.96; $p<0.001$). Consequently, to meet the assumptions of regression, PWV was log-transformed before performing statistical analysis.

Aim 1

To test hypothesis 1 that higher mean step counts per day were associated with lower AS, independent of aerobic stepping cadence, a multivariable logistic regression analysis was performed. Odds ratios (ORs) and 95% confidence intervals (CIs) of having high AS status were calculated across the four upper quintile categories of mean step counts per day. The reference category was the lowest quintile of mean step counts per day. The analysis models were progressively adjusted as follows:

Model 1: Sex and age

Model 2: Model 1 and heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure.

Model 3: Model 2 and five groups of aerobic stepping cadence

In addition, a multivariable linear regression analysis was conducted to determine the regression coefficient, with step counts per 1,000 increments as independent variable and AS measured as PWV, as dependent variable with further adjustment by each of the outlined models.

Aim 2

To test hypothesis 2, that higher aerobic stepping cadence was associated with lower AS, independent of step counts, a multivariable logistic regression analysis was performed. ORs and 95% CIs of having high AS status was calculated across categories of aerobic stepping cadence and compared to the reference. The reference category was defined as 0 aerobic steps per minute, including all participants who did not step for at least 60 steps per minute for at least 10 consecutive minutes over the 7 days.

The analysis models were progressively adjusted as follows:

Model 1: Sex and age

Model 2: Model 1 and heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure.

Model 3: Model 2 and quintiles of step counts

In addition, a multivariable linear regression analysis was conducted to determine the regression coefficient, with groups of aerobic stepping cadence per 10 steps/min increments as independent variable and AS measured as PWV, as dependent variable with further adjustment by each of the outlined models.

Aim 3

To test the hypothesis 3 that the combined association of step counts and aerobic stepping cadence with AS was stronger compared to either step counts or aerobic stepping cadence alone, a multivariable logistic regression was performed. ORs and 95% CI of presenting high AS status was calculated across combined categories of mean step counts per day and aerobic stepping cadence. To perform the joint analysis, participants were dichotomized keeping sufficient numbers in each category and to make the complicated joint analysis simple and easy to interpret from a public health perspective. As previously described, four categories combined steps per day and aerobic stepping cadence as the exposure for high AS (e.g., Inactive & Slow walkers, Active & Slow walkers, Inactive & Fast walkers, Active & Fast walkers). The reference group was the category containing Inactive & Slow walkers. The fully adjusted model included sex, age, heavy drinking, current smoking status, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure similar to Aims 1 and 2.

In addition, we compared specific combined categories to better understand which group was obtaining the greater benefits. Thus, we compared Active & Slow walkers vs. Active & Fast walkers; Inactive & Fast walkers vs. Active & Fast walkers, and Active & Slow walkers vs. Inactive & Fast walkers.

Sensitivity analysis

We conducted a sensitivity joint analysis by combined categories of step counts and three groups of aerobic stepping cadence, labeled as slow walkers, medium walkers, and fast walkers. Slow walkers included those participants who accumulated no aerobic stepping cadence data (i.e., 0 aerobic steps per minute) due to not meeting the pedometer's algorithm over the 7 days.

Medium walkers grouped those participants at the first and second quartiles with valid aerobic stepping cadence data, and fast walkers grouped those participants at the third and fourth quartiles with valid aerobic stepping cadence data. For this analysis, six categories were created combining steps per day and aerobic stepping cadence as exposure for AS status (e.g., Inactive & Slow walkers, Active & Slow walkers, Inactive & Medium walkers, Active & Medium walkers, Inactive & Fast walkers, and Active & Fast walkers).

The joint analysis was repeated with other relevant step counts cut-points previously referenced in the literature, such as: ≥7,500 steps/day to define meeting PA recommendations (Tudor-Locke, Craig, et al., 2013), and ≥4,400 steps/day which has been recently described as the threshold to reduce mortality risk among older women (Lee et al., 2019).

Exploratory analysis

A special feature in the pedometer was the assessment of total aerobic steps/day when walking at >60 steps/min during more than 10 minutes succesively. In an exploratory analysis, we assessed the independent effect of aerobic step counts on AS.

Multicollinearity between aerobic step counts and aerobic stepping cadence was also analyzed using the criterion previously outlined. Due to the presence of concerning multicollinearity, an independent measure of aerobic stepping cadence was included to tackle concerns of multicollinearity with aerobic step counts. The “counts-adjusted” value of aerobic stepping cadence was used in the multivariable logistic regression. This was computed as the residuals from the regression model with groups of aerobic step counts as the independent variable and groups of aerobic stepping cadence as the dependent variable. An analogue procedure was conducted due to concerning multicollinearity between aerobic step counts and step counts.

The analysis models were progressively adjusted as follows:

Model 1: Sex and age

Model 2: Model 1 and heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure.

Model 3: Model 2 and aerobic counts-adjusted aerobic stepping cadence

Model 4: Model 2 and aerobic counts-adjusted total step counts

Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC), with $p \leq 0.05$ considered significant.

CHAPTER 4: RESULTS

The analysis included 409 eligible older adults ≥ 65 years old, with a mean (SD) age of 72 (6) years old. The mean step counts was 5,741 steps/day (5,100 median, steps/day). The mean aerobic stepping cadence was 76 steps/min (median, 104 steps/min).

Five groups of analysis were created for both step counts and aerobic stepping cadence, and mean values and cut-points are presented in Table 1. Characteristics of the study participants are described per quintiles of step counts and groups of aerobic stepping cadence in Table 2a and Table 2b, respectively. Participants with higher step counts were more likely to be younger, more physically active in all self-reported PA intensities, with lower BMI, and PWV (Table 2a). Participants with higher aerobic stepping cadence were more likely to be female, more physically active in all intensities, with lower BMI and PWV (Table 2b).

Table 1. Cut-points for Step Counts and Aerobic Stepping Cadence Groups

	1 (Lowest)	2	3	4	5 (Highest)
Step Count (steps per day)[a]	<3282	3282-4419	4458-5869	5873-7953	≥7961
Aerobic Stepping Cadence (steps per minute)[b]	0	<103	103-109	110-117	≥118

[a]Averaged across valid days with ≥10 hours of wear time. The number of individuals in the quintiles from 1 to 5 was 81, 82, 82, 82, and 82, respectively.

[b]Recorded by pedometer when walking at >60 steps/min for >10 consecutive minutes. The number of individuals in the groups from 1 to 5 was 124, 71, 71, 72, and 71, respectively.

Table 2a. Characteristics of Study Participants by Quintiles of Step Counts per Day

Characteristics	Total	Quintiles of Mean Step Counts per Day					*p*-value
		1(Least active)	2	3	4	5 (Most active)	
n	409	81	82	82	82	82	
Age, y	72 (6)	74 (7)	72 (5)	72 (5)	70 (4)	70 (5)	**<.001**
Female, n(%)	240 (59)	52 (64)	50 (61)	51 (62)	45 (55)	42 (51)	0.408
Body mass index, kg/m^2	28 (5)	30 (5)	28 (4)	28 (4)	27 (4)	26 (4)	**<.001**
Heavy drinking, n(%)[a]	30 (7)	6 (7)	5 (6)	6 (7)	9 (11)	4 (5)	0.642
Smokers, n(%)	4 (1)	1 (1)	2 (2)	1 (1)	0 (0)	0 (0)	0.473
Chronic conditions							
Hypertension medication, n(%)	117 (29)	29 (36)	21 (26)	20 (24)	25 (30)	22 (27)	0.494
Hypercholesterolemia, n(%)[b]	214 (52)	48 (59)	42 (51)	38 (46)	48 (59)	38 (46)	0.265
Diabetes, n(%)[c]	41 (10)	17 (21)	6 (7)	7 (9)	4 (5)	7 (9)	**0.007**
Central hemodynamic parameters							
Pulse wave velocity, m/s	8.6 (1.7)	9.4 (1.8)	8.8 (1.7)	8.4 (1.7)	8.3 (1.7)	8.3 (1.5)	**<.001**
High arterial stiffness, n(%)[d]	86 (21)	29 (36)	16 (20)	13 (16)	13 (16)	15 (18)	**0.008**
Peripheral hemodynamic parameters							
Systolic blood pressure, mmHg	130 (17)	129 (15)	131 (16)	128 (18)	130 (18)	130 (16)	0.837
Diastolic blood pressure, mmHg	75 (10)	75 (9)	76 (10)	74 (10)	76 (11)	76 (8)	0.715
Physical activity							
Step counts per day, median (IQR)[e]	5100 (3624-7406)	2691 (1970-2993)	3931 (3622-4192)	5089 (4766-5428)	6762 (6399-7406)	9543 (8653-11110)	**<.001**
Aerobic stepping cadence, steps per min[f]	110 (13)	108 (11)	104 (12)	108 (14)	110 (12)	115 (13)	**<.001**
Moderate-intensity PA per day, min[g]	29 (36)	21 (30)	24 (27)	24 (26)	32 (26)	43 (57)	**<.001**
Vigorous-intensity PA per day, min[h]	10 (16)	5 (9)	7 (11)	10 (17)	11 (14)	15 (24)	**0.002**
Moderate-vigorous intensity PA per day, min[i]	48 (48)	32 (37)	38 (33)	44 (45)	53 (35)	73 (70)	**<.001**
Slow walkers, n (%)[j]	124 (30)	52 (64)	35 (43)	28 (34)	5 (6)	4 (5)	**<.001**

Unless otherwise indicated, values are presented as mean (SD).

[a] > 14 drinks/week for men, >7 drinks/week for women; [b]self-reported, taking medication, and/or hypercholesterolemia status based on ≥160 low-density lipoprotein levels-LDL; [c]self-reported diagnosed diabetes, taking medication specific to type II diabetes, including biguanide or insulin, and/or fasting glucose ≥126 mg/dL; [d]defined by pulse wave velocity ≥10 m/s; [e]average step counts per day from valid days; [f] aerobic stepping cadence estimates exclude all slow walkers. n=285, Q1=29, Q2=47, Q3=54, Q4=77 , Q5=78; [g,h]self-reported minutes of moderate- and vigorous-intensity physical activity per day in the past 3 months; [i]estimated moderate-vigorous physical activity per day based on self-reported vigorous and moderate intensity physical activity in the past 3 months; [j]slow walkers are participants with an average cadence of 0 steps per minute.

Quintile (Q) ranges: Q1 = <3282 steps/day, Q2 = 3282-4419 steps/day, Q3 = 4458-5869 steps/day, Q4 = 5873-7953 steps/day, Q5 = ≥7961 steps/day.

p-values were calculated with $\chi 2$ test for categorical variables and ANOVA for continuous variables.
Abbreviations: IQR, interquartile range; PA, physical activity

Table 2b. Characteristics of Study Participants by Five Groups of Aerobic Stepping Cadence

Characteristics	Total	Aerobic Stepping Cadence[a]					*p*-value
		1 (0 aerobic steps)	2 (Slowest)	3	4	5 (Fastest)	
n	409	124	71	71	72	71	
Age, y	72 (6)	72 (6)	73 (6)	71 (6)	71 (5)	71 (5)	0.316
Female, n(%)	240 (59)	81 (65)	28 (39)	47 (66)	41 (57)	43 (61)	**0.005**
Body mass index, kg/m^2	28 (5)	29 (5)	27 (4)	27 (5)	27 (5)	27 (5)	**0.003**
Heavy drinking, n(%)[b]	30 (7)	7 (6)	8 (11)	4 (6)	4 (6)	7 (10)	0.487
Smokers, n(%)	4 (1)	2 (2)	0 (0)	2 (3)	0 (0)	0 (0)	0.276
Chronic conditions							
Hypertension medication, n(%)	117 (29)	36 (29)	16 (23)	24 (34)	24 (34)	17 (24)	0.437
Hypercholesterolemia, n(%)[c]	214 (52)	62 (50)	37 (52)	39 (55)	39 (54)	37 (52)	0.967
Diabetes, n(%)[d]	41 (10)	19 (15)	5 (7)	5 (7)	7 (10)	5 (7)	0.202
Central hemodynamic parameters							
Pulse wave velocity, m/s	8.6 (1.7)	8.9 (1.8)	9.0 (1.7)	8.3 (1.5)	8.8 (1.7)	8.0 (1.6)	**<.001**
High arterial stiffness, n(%)[e]	86 (21)	35 (28)	15 (21)	11 (15)	14 (19)	11 (15)	0.159
Peripheral hemodynamic parameters							
Systolic blood pressure, mmHg	130 (17)	129 (15)	130 (16)	128 (17)	133 (20)	129 (16)	0.478
Diastolic blood pressure, mmHg	75 (10)	75 (9)	78 (9)	73 (10)	76 (11)	76 (9)	0.091
Physical activity							
Step counts per day, median (IQR)[f]	5100 (3624-7406)	3593 (2719-4629)	4861 (3919-6568)	5935 (4346-8113)	6634 (4543-8575)	7448 (5485-10598)	**<.001**
Aerobic stepping cadence, steps/min[g]	110 (13)	0 (0)	93 (7)	106 (2)	113 (2)	126 (8)	**<.001**
Moderate-intensity PA per day, min[h]	29 (36)	25 (35)	24 (19)	28 (22)	30 (21)	40 (63)	**0.043**
Vigorous-intensity PA per day, min[i]	10 (16)	7 (15)	13 (16)	7 (11)	9 (11)	15 (24)	**0.005**
Moderate-vigorous intensity PA per day, min[j]	48 (48)	39 (47)	49 (40)	42 (30)	48 (30)	70 (74)	**<.001**

Unless otherwise indicated, values are presented as mean (SD).

[a]The five groups of aerobic stepping cadence were generated through quartiles of participants stepping at no-zero steps per minute (groups 2, 3, 4, and 5) compared to those stepping at zero steps per minute (group 1).
[b]> 14 drinks/week for men, >7 drinks/week for women; [c]self-reported, medication and/or hypercholesterolemia status based on ≥160 low-density lipoprotein levels-LDL; [d]self-reported, diagnosed diabetes and/or taking medication specific to type II diabetes, including biguanide or insulin and/or fasting glucose ≥126 mg/dL; [e]defined by pulse wave velocity ≥10 m/s; [f]average step counts per day from valid days; [g]aerobic stepping cadence estimates include valid cadence data among fast walkers. All slow walkers are grouped in Q1; [h,i]self-reported minutes of moderate- and vigorous-intensity physical activity per day in the past 3 months; [j]estimated moderate-vigorous physical activity per day based on self-reported vigorous and moderate intensity physical activity in the past 3 months.
Group ranges: 1 = 0 steps/min (all slow walkers); quartiles of fast walkers, 2 = <103 steps/min, 3 = 103-109 steps/min, 4 = 110-117 steps/min, 5 = ≥118 steps/min.
p-values were calculated with $\chi 2$ test for categorical variables and ANOVA for continuous variables.

Abbreviations: IQR, interquartile range; PA, physical activity

Logistic and linear regression were used to investigate the risks of high AS status across step counts quintiles. Table 3 describes the independent associations of step counts with AS status and PWV. Compared to the lowest step counts quintile the OR (95% CI) of high AS for older adults in the second, third, and fourth quintiles of step counts were 0.35 (0.15-0.83), 0.32 (0.13-0.81), and 0.31 (0.12-0.81), respectively, after controlling for age, sex, heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure. After further adjustment for aerobic stepping cadence, the reduced odds of step counts were still significant in the second and third quintiles, (OR, 0.38 [95% CI 0.16-0.91] and 0.38 [95% CI 0.15-0.97], respectively) who walked on average between 3,282 and 5,869 steps/day (Table 3). No significant linear trend was found after adjusting for covariates. Multivariable linear regression analysis indicated that there was a significant inverse relationship between step counts and AS (as step counts per day increased, PWV decreased), when controlling for covariates, but not when controlling for aerobic stepping cadence (Table 3).

Table 3. Odds Ratios of High Arterial Stiffness by Step Counts Quintiles.

	n (Case)	Unadjusted	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
1(Least active)	81 (29)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
2	82 (16)	**0.43 (0.21-0.88)**	**0.47 (0.23-0.99)**	**0.35 (0.15-0.83)**	**0.38 (0.16-0.91)**
3	82 (13)	**0.34 (0.16-0.71)**	**0.38 (0.18-0.82)**	**0.32 (0.13-0.81)**	**0.38 (0.15-0.97)**
4	82 (13)	**0.33 (0.16-0.71)**	**0.42 (0.19-0.93)**	**0.31 (0.12-0.81)**	0.40 (0.15-1.10)
5 (Most active)	82 (15)	**0.40 (0.20-0.83)**	0.52 (0.22-1.11)	0.50 (0.20-1.27)	0.75 (0.27-2.09)
p-trend		**0.008**	0.074	0.143	0.579
Multivariable linear regression[a]	409 (86)	**-3.05 (<.001)**	**-2.46 (<.001)**	**-0.85 (0.002)**	-0.50 (0.100)

Data are odds ratio (OR) (95% confidence intervals [CI]) of high arterial stiffness (pulse wave velocity $\geq$ 10 m/) by quintiles of step counts.

Model 1: adjusted for age and sex

Model 2: model 1 and further adjusted for heavy drinking, smoker, body mass index, diabetes, hypercholesterolemia, hypertension medication , and systolic blood pressure

Model 3: model 2 and further adjusted for five groups of aerobic stepping cadence

Quintile (Q) ranges: 1 = <3282 steps/day, 2 = 3282-4419 steps/day, 3 = 4458-5869 steps/day, 4 = 5873-7953 steps/day, 5 = $\geq$7961 steps/day.

Logistic and linear regression were used to investigate the risks of high AS across groups of aerobic stepping cadence. Table 4 describes the independent associations of aerobic stepping cadence with high AS and PWV. Compared to the reference group (i.e., all the slow walkers with 0 aerobic step), the OR (95% CI) in the fastest group was 0.39 (0.16-0.96) after controlling for age, sex, heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure. After further adjustment for step counts, the association was no longer significant (0.42 [0.16-1.09]). A significant linear trend was found after adjusting for covariates excluding step counts. Multivariable linear regression analysis indicated there was not a significant inverse relationship between aerobic stepping cadence and AS (as aerobic stepping cadence increased, PWV decreased), when controlling for covariates including step counts (Table 4).

Table 4. Odds Ratios of High Arterial Stiffness by Five Groups of Aerobic Stepping Cadence

	n (Case)	Unadjusted	Model 1	Model 2	Model 3
			OR (95% CI)	OR (95% CI)	OR (95% CI)
1 (0 aerobic steps)	124 (35)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
2 (Slowest)	71 (15)	0.68 (0.34-1.36)	0.51 (0.24-1.07)	0.48 (0.21-1.11)	0.50 (0.21-1.19)
3	71 (11)	**0.47 (0.22-0.99)**	0.48 (0.22-1.05)	0.45 (0.19-1.11)	0.47 (0.18-1.20)
4	72 (14)	0.61 (0.30-1.24)	0.62 (0.30-1.29)	0.44 (0.18-1.03)	0.46 (0.18-1.16)
5 (Fastest)	71 (11)	**0.47 (0.22-0.99)**	0.48 (0.22-1.05)	**0.39 (0.16-0.96)**	0.42 (0.16-1.09)
p-trend		**0.028**	0.064	**0.023**	0.066
Multivariable linear regression[a]	285 (51)	**-3.03 (0.002)**	**-1.99 (0.033)**	-0.36 (0.188)	0.03 (0.924)

Data are odds ratio (OR) (95% confidence intervals [CI]) of high arterial stiffness (pulse wave velocity $\geq$ 10 m/s) by groups of Aerobic Stepping Cadence.

Model 1: adjusted for age and sex

Model 2: model 1 and further adjusted for heavy drinking, smoker, body mass index, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure

Model 3: model 2 and further adjusted for quintiles of step counts

Group ranges: 1 = 0 steps/min (all slow-walkers); quartiles of fast-walkers, 2 = <103 steps/min, 3 = 103-109 steps/min, 4 = 110-117 steps/min, 5 = $\geq$118 steps/min.

[a]Multivariable linear regression results are regression coefficients (β) and p-value of aerobic stepping cadence groups. Arterial stiffness as pulse wave velocity (PWV) was log-transformed, therefore PWV outcomes are expressed as percentage difference. Regression coefficients were back-transformed using 100*{ [exp(β)]-1} to calculate the percentage difference of PWV for each variable. How to interpret: for every 10 steps/min increase in aerobic stepping cadence, the mean pulse wave velocity significantly decreases by 3.03%. The analysis was conducted excluding those participants stepping at 0 steps/min due to the change between 0 and the next stepping cadence value (71 steps/min) for a better analysis of a linear trend, thus analytical sample size was n=285.

Table 5 presents the odds of having high AS across step counts quintiles and groups of aerobic stepping cadence, stratified by age using 75 years as a cutpoint to have enough numbers of participants and cases in both age groups (n=297 participants, 47 cases under 75 years old; n=112 participants, 39 cases in 75 years old or over).

In general, the main findings of reduced high AS among groups with higher step counts and higher aerobic stepping cadence were similar in both age groups (i.e., all ORs < 1.0), although many findings were no longer significant due to the smaller sample sizes. Among step counts quintiles, the results somewhat differed between strata with greater step counts being

more beneficial for those below 75 years old. Althought not significant, similar trends were observed between aerobic stepping cadence and AS.

Table 5. Odds Ratios of High Arterial Stiffness by Groups of Step Counts and Aerobic Stepping Cadence, Stratified by Age.

	Step Counts			Aerobic Stepping Cadence		
		n (Case)	OR (95% CI)		n (Case)	OR (95% CI)
<75 years old						
	1 (Least active)	43 (14)	1.00 (Reference)	**1 (0 aerobic steps)**	85 (20)	1.00 (Reference)
	2	55 (7)	**0.24 (0.07-0.82)**	**2 (Slowest)**	45 (6)	0.33 (0.10-1.13)
	3	61 (7)	**0.26 (0.07-0.91)**	**3**	55 (7)	0.38 (0.12-1.23)
	4	67 (7)	0.33 (0.09-1.22)	**4**	55 (8)	0.30 (0.09-1.02)
	5 (Most active)	71 (12)	0.88 (0.24-3.28)	**5 (Fastest)**	57 (6)	0.31 (0.09-1.12)
		p-trend	0.956		p-trend	0.062
≥75 years old						
	1 (Least active)	38 (15)	1.00 (Reference)	**1 (0 aerobic steps)**	39 (15)	1.00 (Reference)
	2	27 (9)	0.72 (0.20-2.64)	**2 (Slowest)**	26 (9)	0.78 (0.20-3.03)
	3	21 (6)	0.65 (0.14-2.92)	**3**	16 (4)	0.76 (0.14-4.14)
	4	15 (6)	0.51 (0.10-2.56)	**4**	17 (6)	0.75 (0.16-3.45)
	5 (Most active)	11 (3)	0.47 (0.08-2.91)	**5 (Fastest)**	14 (5)	0.67 (0.14-3.15)
		p-trend	0.322		p-trend	0.615

Data are odds ratio (OR) (95% confidence intervals [CI]) of high arterial stiffness (pulse wave velocity ≥10 m/s) by step counts and aerobic stepping cadence.

Adjusted for sex, heavy drinking, smoker, body mass index, diabetes, hypercholesterolemia, hypertension medication, systolic blood pressure, and quintiles of step counts or groups of aerobic stepping cadence for each other.

Quintile (Q) ranges of step counts: 1 = <3282 steps/day, 2 = 3282-4419 steps/day, 3 = 4458-5869 steps/day, 4 = 5873-7953 steps/day, 5 = ≥7961 steps/day.

Group ranges of aerobic stepping cadence: 1 = 0 steps/min (all slow walkers); quartiles of fast walkers, 2 = <103 steps/min, 3 = 103-109 steps/min, 4 = 110-117 steps/min, 5 = ≥118 steps/min.

Joint analysis was used to investigate the combined effects of step counts and aerobic stepping cadence on AS. Compared to the Inactive & Slow walkers, Inactive & Fast walkers and Active & Fast walkers had reduced OR (95% CI) of high AS with 0.43 (0.20-0.95), and 0.48 (0.23-0.95), respectively (Table 6). This result indicates that fast walking is associated with lower prevalence of high AS regardless of total daily steps (total activity levels) in older adults.

When performing specific comparisons among the combined categories in additional analyses, estimated OR (95% CI) were 0.33 (0.08-1.43) among Active & Fast walkers compared to Active & Slow walkers, 0.36 (0.09-1.48) among Inactive & Fast walkers compared to Active & Slow walkers, and 1.19 (0.56-2.55) among Active & Fast walkers compared to Inactive & Fast walkers.

When three categories of aerobic stepping cadence were used (Slow, Medium, and Fast walkers), we found similar results with significantly lower odds of having high AS (range of ORs: 0.40-0.60) among Medium & Fast walkers compared to slow walkers regardless of step counts, inactive or active.

Sensitivity joint analyses with other cut-points for step counts were conducted. When 4,400 steps/day was the cut-point to define the active and inactive participants based on Lee *et al.*, recent study, there were significantly reduced odds of high AS among the Fast walker & Active, and Fast walker & Inactive groups compared to the Slow walker & Inactive group. Similar results were observed when using 7,500 steps/day as a cut-point (data not shown), since this has been proposed as a possible threshold to define who meets the PA recommendations (Tudor-Locke et al., 2013).

Table 6. Odds Ratio for High Arterial Stiffness status in joint analysis by combined categories of Step Counts and Aerobic Stepping Cadence

Steps Counts	OR (95% CI)[a]	
	Aerobic Stepping Cadence	
	Slow walker (0 aerobic step)	Fast walker (>0 aerobic steps/min)
Inactive (<5,000 Steps/day)	1.00 (Reference)	**0.43 (0.20-0.95)**
Active (≥5,000 Steps/day)	1.16 (0.31-4.34)	**0.48 (0.23-0.95)**

[a]Data are odds ratio (95% CI) of high arterial stiffness (a pulse wave velocity ≥10 m/s) by step counts and aerobic stepping cadence joint analysis. The number of individuals (cases of higher arterial stiffness) in the Inactive & Slow Walker, Inactive & Fast Walker, Active & Slow Walker, and Active & Fast Walker groups were 103 (30), 98 (20), 21 (5) and, 187 (31), respectively.

The model was adjusted for age, sex, heavy drinking, smoker, body mass index, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure.

Table 7 presents the partial correlation coefficients between objectively-measured step counts, aerobic step counts, aerobic stepping cadence, and self-reported PA after adjustment for age and sex. The strongest correlations were found between step counts and aerobic step counts, followed by aerobic step counts and aerobic stepping cadence. Overall, self-reported moderate-intensity PA was weakly correlated to step counts while vigorous-intensity PA was weakly correlated to aerobic step counts.

Table 7. Partial correlation between objectively measured step counts, aerobic step counts, aerobic stepping cadence, and self-reported physical activity.

	r (p-value)[a]		
	Step counts, steps/day	Aerobic steps per day, steps/day	Aerobic stepping cadence, steps/min
Step counts, steps/day	1.00	0.62 (<.001)	0.31 (<.001)
Aerobic steps per day, steps/day	0.62 (<.001)	1.00	0.42 (<0.001)
Aerobic stepping cadence, steps/min	0.31 (<.001)	0.42 (<.001)	1.00
Moderate-intensity PA	0.32 (<.001)	0.18 (0.002)	0.11 (0.070)
Vigorous PA	0.14 (0.020)	0.22 (<.001)	0.13 (0.032)
Moderate-vigorous PA	0.34 (<.001)	0.29 (<.001)	0.17 (0.005)

[a]Data are Pearson's partial correlation coefficients (r) and p-values estimated in a model adjusted for age and sex.

Abbreviations, PA= Physical Activity

The exploratory analysis assessed the independent associations of aerobic step counts on high AS (Appendix Table 8). Compared to the group who did not have any aerobic steps, the OR (95% CI) for older adults in the most active group was 0.35 (0.14-0.88) after controlling for age, sex, heavy drinking, smoking, BMI, diabetes, hypercholesterolemia, hypertension medication, systolic blood pressure, and step counts with a significant linear trend (P=0.023). When the model was adjusted for aerobic stepping cadence instead of step counts the results were comparable (0.35 [95% CI 0.14-0.89]). However, according to linear regression analysis aerobic step counts was not a significant predictor of PWV in the fully adjusted models with either step counts or aerobic stepping cadence.

CHAPTER 5: DISCUSSION

Independent Associations of Step Counts and Aerobic Stepping Cadence on Arterial Stiffness among Older Adults

There were three major findings in this study: First, based on linear regression analysis, step counts was a significant predictor of PWV, independent of age, sex, lifestyle factors, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure, but not independent of aerobic stepping cadence. Moreover, based on multivariable logistic regression second, third, and fourth quintiles of step counts were associated with significantly reduced odds of having high AS, independent of age, sex, lifestyle factors, BMI, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure. After further adjustment for aerobic stepping cadence, the second and third quintiles of step counts still had significantly reduced odds of high AS. Second, based on linear regression analysis, aerobic stepping cadence was not a significant predictor of PWV after adjusting for confounders. However, based on logistic regression analysis the fastest group of aerobic stepping cadence was associated with significantly reduced odds of having high AS independent of confounders, but not step counts, compared with the slow walking reference group although all ORs in fasting walking groups (≥1 aerobic steps) in all models were still <1.00. Lastly, the joint analysis indicated that fast walking, regardless of being active or inactive (steps/day), was significantly associated with reduced odds of having high AS, compared to Inactive & Slow walker group.

Evaluation of step counts in relation to health is a recent development in the scientific literature. A meta-analysis including 10 cross-sectional studies found a significantly inverse association between objectively-measured step counts and PWV, regardless of how they were

measured although they did not adjust for stepping cadence (walking intensity) (Cavero-Redondo et al., 2019). Specifically, adults and older adults achieving ≥7,500 steps per day showed the greatest reductions in AS measured by cfPWV (cfPWV=8.77 m/s among those taking 7,500-9,999 steps/day vs. cfPWV=10.02 m/s among those taking 5,000-7,499 steps/day). Our results are consistent with this previously established relationship; as step counts per day increased, PWV decreased significantly after adjusting for confounders ($\beta = -0.85$, $p = 0.002$), although not significant after adjusting for aerobic stepping cadence ($\beta = -0.50$, $p = 0.100$) (Table 3). These results support the beneficial effects of step counts on AS, as previous cross-sectional epidemiological studies have found, but does not support that step counts have an effect independent of aerobic stepping cadence, which suggests that the effect of higher step counts are partially due to faster walking.

In the current study, when aerobic stepping cadence were added to the logistic model, step counts was significantly beneficial only in small volume compared to the lowest quintile, meaning that taking between 3,282-5,869 steps/day was enough to have reduced odds of high AS regardless of aerobic stepping cadence (Table 3). This result slightly deviates from Lee and colleagues' finding of 4,400 steps/day as the threshold to have significantly reduced mortality risk independent of stepping intensity (steps/min) among older age women, given that our data found significant health benefits with fewer steps per day (Lee et al., 2019). In Lee's analysis, any step counts greater than 4,400 steps/day were significantly associated with reduced risk of all-cause mortality after adjustment for stepping intensity, which supports any step volume above this threshold to be more beneficial for all-cause mortality. In the present study, taking between 3,282-5,869 steps/day was enough to significantly reduce odds of high AS, independent of aerobic stepping cadence. This suggests that the dose-response associations of step counts would

be different depending on different health outcomes (mortality vs. arterial stiffness). Moreover, since the association of greater step counts and reduced AS appeared to be consistent, further studies are needed to confirm if there is an upper limit of step counts (i.e., more than 5,869 steps/day) that provides no further significant effect on the association with AS in older adults.

Also, it is important to analyze how the characteristics of the participants differed among quintiles of step counts. Overall, participants in the reference group (i.e., lowest quintile of step counts) had a greater percentage of slow walkers (64%) compared to the second and third quintiles with 43% and 34%, respectively. These characteristics could explain why after controlling for aerobic stepping cadence, the associations between step counts and arterial stinffes got weaker, suggesting that the lower odds of having higher AS in higher step counts groups were due to higher stepping cadence. On the other hand, the categorization of aerobic stepping cadence was not as uniform as for step counts in order to have a clear division between slow walkers with 0 aerobic step and groups of fast walkers with ≥1 aerobic steps. This could also explain why the gradient between the reference group (slow walkers) and the participants among the lower aerobic stepping cadence was not observed, given that 30% of the total analytical sample was grouped in the slow walker reference category. The significant finding particularly among the second quintile of step counts compared to the reference group, is also comparable with previous PA research that supports that any PA is better than none (Piercy et al., 2018). Therefore, these results support encouraging older adults to take at least some step counts as opposed to none.

We lack an assessment of cadence among slow walkers (no stepping cadence data in slow walkers), instead our cadence assessment is accounting for cadence values that are already above a threshold of >60 steps/min during more than 10 consecutive minutes to define aerobic stepping

(fast walking). This can potentially impact the interpretation of the results in the sense that we should not generalize, e.g., that cadence is overall more beneficial than volume, given that our cadence measures do not necessarily reflect a whole range of stepping cadence values among older adults. The participants with valid aerobic stepping cadence data are already walking at a higher intensity (e.g., aerobic walking). Two thirds of our sample had valid aerobic stepping cadence data (vs. about 30% slow walkers in total), and those able to achieve greater aerobic stepping cadences were more physically active, younger, and healthier. In this regard, the reference group with the slow walkers had a greater sample size compared to the other groups. Thus, these baseline characteristics may be reflected in more favorable AS outcome.

On the other hand, greater stepping cadence is related to increased relative exercise intensity; therefore, the fact that the majority of participants were walking at aerobic intensity-cadence may weaken the effect of step counts on the outcome assessed. This could be further explained by a greater stimulus from higher stepping intensities to induce physiologic adaptations in pathways associated specifically to CVD mortality (Eijsvogels et al., 2016). These mechanisms lead to an improved CVD risk profile by reducing triglyerides and increasing high-density lipoprotein cholesterol, lowering blood pressure, improving glucose metabolism and insulin sensitivity, reducing body weight, and reducing inflammatory markers. Altogether, this improvement has been associated to 59% of CVD reduction (Mora et al., 2007). Other adaptations, such as lower heart rates due to increased vagal tone, improved endothelial function, vascular remodeling, and increased nitric oxide biolavailability appeared to account for about 41% of CVD reduction (Eijsvogels et al., 2016). Therefore, the impact of this mechanisms through different CVD-related pathways enhanced the relevance of aerobic intensity walking benefits on CVD-related outcomes.

Lack of detail about slow walker's stepping cadence limited our analysis to truly appreciate a dose-response association between stepping cadence (i.e., aerobic or not) and AS, because those stepping at ≤60 steps/min are grouped all together as 0 aerobic step (all as slow walkers). Thus, the independent effect of the lower spectrum of cadences on AS among older aduls is unknown.

Also, it is likely that the effect of step counts independent of aerobic stepping cadence vary depending on the outcome of interest (e.g., all-cause mortality vs. high AS). A CVD-mortality related outcome, such as AS, can be greatly impacted by aerobic exercise in a more concise physiological pathway compared to the mechanisms associated to all-cause mortality. These mechanisms may include the physiological adaptations from the coronary microcirculation in response to aerobic PA, involving endothelium-dependent vasodilation pathways and increased nitric oxide production, that have been proven to improve arterial health (Ashor et al., 2014). Furthermore, these mechanisms may also be linked to the research showing that aerobic exercise has a greater benefit on central arteries (i.e., aorta) compared to peripheral arteries (Shibata et al., 2018). Our assessment method of AS uses cfPWV and provides a non-invasive AS evaluation focused on central arteries and its branches, which is the region that contains the greatest age-related arterial stiffening and that benefits the most from aerobic exercise (Obeid et al., 2017; Vlachopoulos et al., 2010). This may support that aerobic exercise through increased step counts is a feasible strategy to impact arterial health.

Another study assessed the effect of step counts and stepping intensity on all-cause mortality and CVD mortality in a large representative cohort of US adults using accelerometers worn during 7 days (Saint-Maurice et al., 2020). Similarly, they found reduced risk of all-cause and CVD mortality with greater step counts. Among adults aged at least 40, they found that those

who took 8,000 steps/day had significantly lower CVD mortality risk, compared to those who took 4,000 steps/day (0.49 [95% CI=0.40-0.60]) (Saint-Maurice et al., 2020). On the other hand, there were not significant effects from stepping cadence after adjusting for step counts. Although these findings were obtained in a sample with different demographics to ours, it also supports that increased PA as greater step counts are impactful on CVD related health outcomes.

This is meaningful in the sense that other step count studies with mortality conducted among healthy adults, patient populations, and older adults have consistently shown that a greater number of steps per day is associated with lower mortality; however, they did not account for the potential confounding effect of stepping cadence in the outcome (Dwyer et al., 2015; Izawa et al., 2013; Jefferis et al., 2019). Thus, this research accounting for an objective measure of aerobic stepping cadence may provide other insights to the discussion.

There is limited research on the relationship between stepping cadence and AS. In the present study (Table 4), aerobic stepping cadence was not a significant predictor of PWV in linear regression, although these results might be interpreted cautiously under the previously outlined considerations of our stepping cadence assessment method (no stepping data in slow walkers). On the other hand, in logistic regresson, aerobic stepping cadence was significantly associated with reduced odds of having high AS, but it was not independent of step counts. Similarly, our findings support a significant linear trend between aerobic stepping cadence and reduced PWV that was not independent of step counts. The only significant results were found among the participants in the fastest walking group, who reached at least 118 steps/min (Table 1), a cadence that has been previously associated with brisk walking in adults (Tudor-Locke, Camhi, Leonardi, et al., 2011). These results support the influence of aerobic intensity walking on arterial health as other physiological mechanisms have explained at molecular levels,

including increased anti-inflammatory cytokines, reduced pro-inflammatory cytokines, and increased nitric oxide production (Ashor et al., 2014; Kojda & Hambrecht, 2005; Madden et al., 2009; Shibata et al., 2018). In addition, greater aerobic stepping cadence could also reflect the fitness levels of the sample. Consequently, it is also possible that the significantly reduced odds of high AS among those walking at the fastest aerobic stepping cadence is associated to their cardiorespiratory fitness levels, given that previous research on longitudinal studies has shown greater levels of cardiorespiratory fitness associated to reduced CVD-related mortality risk (Lee et al., 2010). In this regard, the fact that only the fastest walkers had significantly reduced odds of high AS, could possibly be attributed to their baseline fitness levels. This feature may also explain why a significant linear relationship was found after adjusting for confounders excluding step counts. The effects of baseline fitness levels should also be taken into account when interpreting the aerobic stepping cadence results since walking speed is related to their finess levels, especially in older adults.

Nevertheless, as the 100 steps/min cut-point is based on studies conducted among adults, it is likely that lower values are already the faster forms of locomotion among older adults (Tudor-Locke et al., 2018). In any case, it is interesting to highlight that some older adults in our sample were able to reach brisk walking intensity based on adult sample studies. These results are comparable to previous research in which walking intensity, measured as self-reported walking pace, was associated with reduced risk of cardiovascular mortality among those reporting walking at the greater pace, compared to those walking at the slowest pace (Stamatakis et al., 2018). Other epidemiological studies accounting for objectively measured stepping volume and intensiy have also found similar results on mortality-related outcomes. Lee *et al*. (2019), found that the number of steps, rather than the stepping intensity, was the step-metric

consistently associated with significantly lower all-cause mortality rates, independent of stepping cadence. Moreover, Saint-Maurice *et al.* (2020), also found that the effect of stepping cadence was not independent of step counts on either all-cause or CVD mortality. Therefore, step counts could be an appropriate step-metric to measure total PA and its effects on CVD related outcomes.

In the stratified analysis, although not statistically significant, we observed stronger potential associations of step counts and aerobic stepping cadence with AS among those younger than 75 years old, although these estimates were not significant. This can be contrasted with findings from Stamatakis (2018) who evaluated self-reported walking pace on CVD mortality satrtified by age. In an overall younger cohort compared to our participant, Stamatakis and colleagues found that greater walking pace was associated with reduced risk of CVD mortality only among those aged at least 50 years old. Nevertheless, it should be highlighted that the present study performed objective assessment and the results from self-reported walking pace may be prone to inaccuracies due to recall bias or a distorted perception of the real walking pace effort. In this sense, it is important to outline that our pedometer provides a measure of absolute intensity rather than relative intensity of walking, therefore, fitness levels are not considered and this may have implications on an accurate walking assessment relative to the effort of older adults.

Another issue that merits discussion is the validity of step counts and aerobic stepping cadence. There are no official cut-points to define how many steps are enough or how fast is enough to obtain health benefits among older adult populations, thus most of our cut-points are sample-based or referenced on adult age population studies following previous approach (Cavero-Redondo et al., 2019). Hence, we performed correlation analysis to validate the

objectively measured step parameters with each other and the self-reported PA data. As a result, in this sample, daily overall step counts was more strongly correlated with moderate-intensity PA ($r=0.32$; $p<0.001$) and aerobic stepping cadence was more strongly correlated with virogous-intensity PA ($r=0.13$; $p=0.032$). Consequently, it is likely that our aerobic stepping cadence compared to daily step counts is more likely to be associated with higher intensity PA, which reduced the strength of the associations between step counts and AS (weakened ORs after further adjusting for aerobic stepping cadence).

Aerobic PA such as walking has been associated to reduced AS (Madden et al., 2009). Its beneficial effects on hemodynamics can be explained through several mechanisms. There is evidence that supports the reduction of systemic oxidative stress as a mechanism, potentially through up-regulation of superoxide dismutases (SOD1, SOD3) and down-regulation of NAD(P)H oxidase (Ashor et al., 2014). In addition, clinical research have described the antiinflammatory effect of regular PA by increasing the anti-inflammatory cytokines (interleukin 4, 10) and reducing proinflammatory cytokines (interleukin 6 and tumour necrosis factor alpha) (Kojda & Hambrecht, 2005). Other mechanisms involve enhance endothelium-dependent vasodilation throughout the coronary microcirculation due to increasing production of nitric oxide, as a result of increased expression of nitric oxide synthase (Lavie et al., 2015; Tanaka et al., 2000). Lastly, there is evidence that aerobic exercise reduces the concentrations of vasoconstrictor agents such as endothelin I and angiotensin II (Higashi & Yoshizumi, 2004). Overall, these mechanisms can also counteract the negative influences of other cardiovascular risk factors such as hypertension, diabetes, and BMI on AS, making AS a potentially modifiable risk marker for occurrence of further CVD events (Cavero-Redondo et al., 2018; Mandini et al.,

2018). Altogether, these physiological explanations support the biological plausibility of improved AS through increased aerobic PA by walking.

Combined Associations of Step Counts and Arterial Stiffness among Older Adults

Although step counts and aerobic stepping cadence were moderate-to-weak correlated in this study ($r=0.31$; $p<.001$), higher accumulations of step counts have been associated with a greater amount of time spent in higher-intensity PA (Tudor-Locke, Leonardi, Johnson, et al., 2011). Therefore, investigating the relative importance between step counts and aerobic stepping cadence is relevant to better understand what step-based metric is more beneficial for AS and contribute to a better design of step-based PA recommendations for older adults. In the joint analysis (Table 6), the Fast walking regardless of step counts (Active or Inactive) was significantly associated with reduced odds of high AS compared to the Slow walker & Inactive reference group.

The Slow walker & Active group presented a non-significant OR (1.16) in the opposite direction with a large confidence interval that could be explained due to the small number of cases (n=5) in the category. Based on the results of the joint analysis, the contribution of greater aerobic stepping cadence seemed to be stronger than the contribution of step counts to have reduced odds of high AS although further studies with larger sample sizes and higher case counts are required. Comparable results were found in sensitivity analysis, in which participants within the Fast walker group had significant reduced odds of high AS, regardless of the step counts classification, when it was defined by either 4,400 or 7,500 steps/day as cut-points based on other studies. In conclusion, the joint analyses suggest that the possible effects on AS may be associated with engaging in fast walking regardless of the overall step counts in older adults.

Limitations

Some limitations of this study should be outlined. A major limitation is the lack of diversity in this cohort of older adults who were predominantly white, highly educated, healthy, and living independently. Results may be affected by unmeasured confounding (e.g., baseline fitness levels, usual gait speed, functional limitations, built environment, time spent in zero-aerobic stepping cadence, slow walkers' cadence) in this cohort. Also, even though an objective assessment of step counts and aerobic stepping cadence was performed, the lack of a validity study of the Omron HJ-321 pedometer in older adults should be considered when interpreting the results, taking into account that the Omron brand has shown inconsistent accuracy and a tendency to underestimate step counts (±37% of actual steps taken, 95% of the time), particularly at slower cadences (Crouter et al., 2003; Schneider et al., 2003). This is a result from the fact that this pedometer is wore close to the waist (i.e., belt or pocket) and vertical accelerations of the waist are less pronounced at slow walking speeds, so, it is less likely to exceed the threshold value to record a step (Crouter et al., 2003).

The aerobic stepping cadence (steps/min) estimates used in this study should be interpreted cautiously. The recorded values reflect the cadence when walking at >60 steps/min during >10 consecutive minutes, as established by the pedometer's fixed algorithm, therefore they do not reflect the cadence walked below this pre-defined threshold. Consequently, this device may underestimate the activity in free-living conditions (e.g., no aerobic step if it was performed less than 10 consecutive minutes), and therefore generalizability may be limited and not applicable to all real-world conditions.

Factors such as eating, hydration status, and room temperature during AS assessment should also be considered, since they may interfere with an accurate cfPWV measure that can be compared between different individuals. However, preparation guidelines were provided prior to the assessments to have participants fasting for 12-hours before the assessment, encouraging them to continue drinking water while fasting, and during the assessment morning the research team ensured that the measurements were completed within the same time slot for all participants. Room temperature was centrally controlled at a similar level throughout the study. In any case, the potential confounding effect of these factors are expected to be minimal when comparing PWV between subjects, due to our large sample size. Lastly, since our analysis is a cross-sectional analysis, causation cannot be established, thus we cannot support that greater step counts or aerobic stepping cadence are the cause of reduced AS or that reduced AS is the cause of greater step counts or aerobic stepping cadence accumulation. Nevertheless, this study sets the rationale for further longitudinal and experimental studies of step counts and stepping cadence with AS among older adults.

On the other hand, a major strength of this study is that it objectively assesses daily living ambulatory behavior among healthy older adults and a CVD-related outcome such as AS, which was measured with the gold-standard technique. AS as an outcome may also help to understand the impact of ambulatory behavior and step-defined PA in the risk of several health outcomes like CVD progression and mortality, among older adults.

Conclusion

Higher total daily step counts were associated with a lower prevalence of high AS status among older adults after adjusting for possible confounders including aerobic stepping cadence in logistic regression analyses. Although it was not significant after adjusting for possible confounders including total daily step counts, this study also suggests a possible association between higher aerobic stepping cadence and lower prevalence of high AS with ORs of <1.00 in all fast walkers (≥1 aerobic steps) compared to slow walkers (0 aerobic steps). The large sample size in older adults, an objective assessment of step counts and aerobic stepping cadence, the gold-standard method to assess AS, and unique combined analysis of step counts and aerobic stepping cadence strengthen our findings.

Although this study does not include CVD related variables, based on earlier studies on PWV and CVD, it is possible that improving AS by increasing regular walking may lower the risk of CVD. However, prospective studies are clearly warranted. Nevertheless, our findings provide evidence with clinical, public health, and scientific implications. From a clinical perspective, these findings may encourage health practitioners to promote the beneficial effects of walking for cardiovascular risk management among older adults. From a public health perspective, it may motivate the need to include an evidence-based step metric to deliver an optimal PA recommendation for older adults. Lastly, from a scientific perspective, we provide insight on future studies that could help to refine step-based PA recommendations for older adults.

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APPENDIX A. PEDOMETER WEAR TIME AND MUSCLE STRENGTHENING ACTIVITIES LOG

My FULL name is: _____ My PAAS ID# is _____

Pedometer Wear Time and
Muscle Strengthening Activities

Please record the times you PUT ON and TAKE OFF your pedometer for EACH day during the testing week (use the 24-hour clock format, otherwise known as 'MILTARY TIME').

How to write 12-hour times in 'MILITARY TIME':

12-hour	MILITARY		12-hour	MILITARY
Midnight (12am)	00:00		Noon (12pm)	12:00
1am	01:00		1pm	13:00
2am	02:00		2pm	14:00
3am	03:00		3pm	15:00
4am	04:00		4pm	16:00
5am	05:00		5pm	17:00
6am	06:00		6pm	18:00
7am	07:00		7pm	19:00
8am	08:00		8pm	20:00
9am	09:00		9pm	21:00
10am	10:00		10pm	22:00
11am	11:00		11pm	23:00

DAY OF WEEK	TIME I PUT ON MY PEDOMETER: (remember to write this in military time)	TIME I TOOK OFF MY PEDOMETER: (remember to write this in military time)	Minutes per day of any Muscle-Strengthening Activities (e.g. weight lifting, push-ups, sit-ups, squats, chopping wood)
Tuesday (1)			**: _____** minutes
Wednesday (2)			**: _____** minutes
Thursday (3)			**: _____** minutes
Friday (4)			**: _____** minutes

Saturday (5)			**: _____** minutes
Sunday (6)			**: _____** minutes
Monday (7)			**: _____** minutes

Reminders:

Friday Morning _____ (Month) / _____ (Day)

- Charge your FitBit using the USB wall-charger and charging cable for approximately 30 mins.

Tuesday Morning _____ (Month) / _____ (Day)

- Your appointment is at _____:_____ **AM** (please meet in Forker Building Room 0189).

- Please arrive to your Tuesday morning assessment after fasting for 12 hours.
    - This includes no FOOD, NO caffeine, and NO alcohol.

- Please do not engage in vigorous-intensity physical activity during the 24 hours leading up to Tuesday morning visit.

- Please refrain from ALL forms of smoking and nicotine consumption (cigarettes, cigars, e-cigs, chewing tobacco, nicotine patches etc.) for AT LEAST 4 hours before your Tuesday morning assessment.

- Bring your pedometer, Fitbit, Fitbit charger, and ‘pedometer wear-time’ sheet to your Tuesday morning visit.

APPENDIX B. STATISTICAL RESULTS

Table 8A. Odds Ratios of High Arterial Stiffness by Aerobic Step Counts Groups.

	n (Case)	Unadjusted	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
1 (0 aerobic steps)	124 (35)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
2 (Least active)	71 (14)	0.63 (0.31-1.26)	0.50 (0.24-1.05)	**0.41 (0.18-0.96)**	0.43 (0.17-1.10)	**0.41 (0.18-0.95)**
3	71 (13)	0.57 (0.28-1.17)	0.51 (0.24-1.09)	0.58 (0.25-1.36)	0.60 (0.25-1.46)	0.58 (0.25-1.35)
4	72 (14)	0.61 (0.30-1.24)	0.62 (0.30-1.29)	0.44 (0.19-1.05)	0.45 (0.19-1.06)	0.44 (0.18-1.05)
5 (Most active)	71 (10)	**0.42 (0.19-0.90)**	0.46 (0.20-1.02)	**0.35 (0.14-0.88)**	**0.35 (0.14-0.89)**	**0.35 (0.14-0.88)**
p-trend		**0.023**	0.055	**0.023**	**0.022**	**0.023**
Multivariable linear regression[a]	**409 (86)**	**-1.23 (0.009)**	-0.80 (0.071)	**-0.77 (0.044)**	-0.41 (0.318)	-0.46 (0.277)

Data are odds ratio (OR) (95% confidence intervals [CI]) of high arterial stiffness (pulse wave velocity ≥10 m/s) by aerobic step counts quintiles.
Model 1: adjusted for age and sex

Model 2: model 1 and further adjusted for heavy drinking, smoker, body mass index, diabetes, hypercholesterolemia, hypertension medication, and systolic blood pressure

Model 3: model 2 and further adjusted for groups of aerobic stepping cadence. This model was conducted using the residuals of aerobic stepping cadence method explained in the methods section of the manuscript due to concerning multicollinearity between quintiles of aerobic steps and groups of aerobic stepping cadence.
Model 4: model 2 and further adjusted for step counts quintiles. This model was conducted using the residuals of step counts method explained in the methods section of the manuscript due to concerning multicollinearity between quintiles of aerobic steps and quintiles of step counts.
Group ranges: 1 = 0 aerobic steps/day; quartiles of aerobic step counts, 2 = <2169 aerobic steps/day, Q3 = 2171-3375 aerobic steps/day, 4 = 3416-4634 aerobic steps/day, 5 = ≥4672 aerobic steps/day.

[a]Multivariable linear regression results are regression coefficients (β) and p-value of aerobic step counts groups. Arterial stiffness as pulse wave velocity (PWV) was log-transformed, therefore PWV outcomes are expressed as percentage difference. Regression coefficients were back-transformed using 100*{ [exp(β)]-1} to calculate the percentage difference of PWV for each variable. How to interpret: for every 1,000 increase in aerobic step counts, the mean pulse wave velocity significantly decreases by 0.77%.