Associations of muscular strength and cardiorespiratory fitness with bone mineral density in older adults

Hyunsoo Kim
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

Part of the Kinesiology Commons

Recommended Citation
Kim, Hyunsoo, "Associations of muscular strength and cardiorespiratory fitness with bone mineral density in older adults" (2019). Graduate Theses and Dissertations. 17485.
https://lib.dr.iastate.edu/etd/17485

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Associations of muscular strength and cardiorespiratory fitness with bone mineral density in older adults

by

Hyunsoo Kim

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Major: Kinesiology

Program of Study Committee:
Duck-chul Lee, Major Professor
Rick L. Sharp
Timothy R. Derrick

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.

Iowa State University
Ames, Iowa
2019
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>NOMENCLATURE</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>CHAPTER 1: INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 2: REVIEW OF LITERATURE</td>
<td>6</td>
</tr>
<tr>
<td>Risk Factors of Bone Mineral Density</td>
<td>6</td>
</tr>
<tr>
<td>Associations between Muscular Strength and Bone Mineral Density</td>
<td>8</td>
</tr>
<tr>
<td>Associations between Cardiorespiratory Fitness and Bone Mineral Density</td>
<td>11</td>
</tr>
<tr>
<td>Conclusion</td>
<td>12</td>
</tr>
<tr>
<td>CHAPTER 3: METHODOLOGY</td>
<td>14</td>
</tr>
<tr>
<td>Participants</td>
<td>14</td>
</tr>
<tr>
<td>Study Procedure and Measurements</td>
<td>15</td>
</tr>
<tr>
<td>Statistical Analyses</td>
<td>19</td>
</tr>
<tr>
<td>CHAPTER 4: RESULTS</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER 5: DISCUSSION</td>
<td>36</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>42</td>
</tr>
<tr>
<td>APPENDIX: IRB APPROVAL</td>
<td>48</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Figure 1</td>
<td>Patient experience 1 year after hip fracture</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Vicious cycle of reduced physical activity, lower bone mass, and increased risk of fracture</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Age related alterations in bone mass in men and women</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Grip strength measurement</td>
</tr>
<tr>
<td>Figure 5</td>
<td>400-meter walk test</td>
</tr>
<tr>
<td>Figure 6</td>
<td>1-RM chest press and leg press measurements</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Odds Ratios of Low Bone Mineral Density by Combined Muscular Strength and Cardiorespiratory Fitness in Women (above) and Men (below)</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Means and cut-points for sex-specific muscular strength and cardiorespiratory fitness tertiles by body mass index 24
Table 2. Participant characteristics by muscular strength and cardiorespiratory fitness in women 25
Table 3. Participant characteristics by muscular strength and cardiorespiratory fitness in men 26
Table 4. Association of muscular strength with t-scores: multivariable linear regression 27
Table 5. Odds ratios of low bone mineral density by muscular strength 28
Table 6. Association of cardiorespiratory fitness with t-scores: multivariable linear regression 29
Table 7. Odds ratios of low bone mineral density by cardiorespiratory fitness 30
Table 8. Association of muscular strength and cardiorespiratory fitness with whole-body bone mineral density: multivariable linear regression 31
Table 9. Associations of whole-body and different regional (arm, pelvis, leg, and lumbar) bone mineral density with different muscular strength measurements (grip strength, 1-repetition maximum chest press, and 1-repetition maximum leg press), independent of cardiorespiratory fitness 34
## NOMENCLATURE

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory fitness</td>
</tr>
<tr>
<td>MS</td>
<td>Muscular strength</td>
</tr>
<tr>
<td>NWRC</td>
<td>The Nutrition and Wellness Research Center</td>
</tr>
<tr>
<td>PAAS</td>
<td>The Physical Activity and Aging Study</td>
</tr>
<tr>
<td>RM</td>
<td>Repetition maximum</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I sincerely thank my major professor Dr. Duck-chul Lee for his commitment to support my study with his time, effort, passion, and resources. Also, I sincerely thank my committee members Dr. Rick L. Sharp and Dr. Timothy R. Derrick for encouraging and inspiring me to think more creatively and conduct a more meaningful study for the public. In addition, I want to appreciate priceless support from Dr. Angelique Brellenthin and my fellow graduate students, Emma Albin, Markus Flynn, Heather Thompson, and Joseph Saavedra.

I also want to say thank you to Dr. Nathan Meier, the previous project manager of the Physical Activity and Aging Study (PAAS). Working with Nathan Meier and Emma Albin for the study was an honorable experience for me.
**Purpose:** To evaluate the independent and combined associations of muscular strength (MS) and cardiorespiratory fitness (CRF) with bone mineral density (BMD) in older adults.

**Methods:** This cross-sectional study included 353 older adults (189 women and 164 men) aged 65 years or older (mean ages 72 and 71 in women and men, respectively). MS was assessed by grip strength (average of the highest force values after three trials on each hand, Jamar+ digital hand dynamometer, Patterson Medical) and CRF was assessed by the 400-meter walk test (minutes to complete 400-meter walking). Whole-body BMD was measured by dual-energy X-ray absorptiometry (DXA, Hologic Horizon W, Hologic). Low BMD was defined as t-score below -1.0, the value that was calculated with BMD (g/cm$^2$). Linear regression was used to find the associations of MS and CRF with BMD in women and men, separately. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) of having low BMD in BMI-specific tertiles of MS and CRF in women and men, separately. To investigate the combined association of MS and CRF with low BMD, joint analyses were conducted after dichotomizing MS and CRF (upper two thirds and lower one third). Then, study participants were assigned to one of weak and unfit, weak and fit, strong and unfit, and strong and fit groups.

**Results:** In women, only MS was positively associated with t-score after adjusting for age from linear regression. In men, only MS was positively associated with t-score after adjusting for age, current smoking status, heavy alcohol consumption, physical inactivity, supplement/medication for bone health, body mass index, and CRF. In women, MS and CRF appeared to be inversely associated with the prevalence of low BMD, although not significant (all p-trends>0.05) from logistic regression. In men, only MS appeared to be inversely associated with the prevalence of low BMD, although not significant (all p-trends>0.05). In the joint analyses of MS and CRF with
low BMD, although not significant, being strong and fit appeared to be more strongly associated with lower prevalence of low BMD in women, but not in men possibly due to the very small number of cases of low BMD in each group (n=1-3).

**Conclusion:** Our study suggests that higher levels of MS and CRF may be associated with better bone health in older adults although most results did not reach statistical significance in this relatively healthy and fit population. Further prospective studies from large representative older adult populations are clearly warranted.
The prevalence of low bone mineral density (BMD) including osteopenia (a precursor to osteoporosis) and osteoporosis was about 53.6 million in older adults aged 50 years or older in 2010 in the United States (U.S.) (Wright. et al., 2014). According to the World Health Organization (WHO) definition, osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk (WHO, 1994). In scientific terms, osteopenia is defined as bone mass between 1.0 and 2.5 standard deviation (SD) below that of the mean level for a young-adult reference population (a t-score, the value calculated by dual-energy X-ray absorptiometry (DXA), between -1.0 and -2.5) and osteoporosis is defined as bone mass 2.5 SD or more below that of the mean level for a young-adult reference population (t-scores ≤ -2.5) at the femoral neck, total hip, or lumbar spine (WHO, 1991). By aging and increased frailty, fractures become prevalent in older adults. It was reported that almost three fourths of fractures in the hip and spine occur in adults aged 65 years or older (Sànchez-Riera & Wilson, 2017). In addition to pain and suffering, fractures are also an economic burden. It was reported that between 2008 and 2013, mean costs for fracture treatments of Medicare and commercially insured older people were $27,844 and $29,316, respectively, in the year following the fracture (Weaver et al., 2017).
Figure 1. Patient experience 1 year after hip fracture. From Cooper C. *Am J Med.* 1997.

Decreased physical fitness and BMD that occur with age increase the risk of falls and result in fractures (DeFina et al., 2016). As shown in Figure 1, fractures are particularly dangerous among older adults because they can lead to unhealthy conditions, such as loss of mobility, sarcopenia, or disability, and the mortality rate after a fracture increases with age (Castronuovo et al., 2011; Cummings & Melton, 2002; Curtis et al., 2015; Keene et al., 1993). Most critically, about 20-40% of patients lose their ability to walk, inducing a loss of independence and physical activity (Cummings & Melton, 2002; Cooper C., 1997). Reduced physical activity reduces bone density by decreased mechanical load to bones, thus leading to a vicious cycle of lower BMD and increased risk of falls and fractures (Figure 2) (Cummings & Melton, 2002; Curtis et al., 2015; Kerr et al., 2017; Novotny et al., 2015). To reduce the impact of low BMD on the economy and quality of life, finding and understanding factors that are more
strongly associated with BMD and developing more efficient and effective prevention strategies focusing on these factors is important, especially in older adults.

Figure 2. Vicious cycle of reduced physical activity, lower bone mass, and increased risk of fracture. From Kerr et al., *Osteoporosis Int.* 2017.

Several exercise intervention studies have shown that exercise training improves or maintains BMD in older adults (Beavers et al., 2017; Hinton et al., 2015; Huovinen et al., 2016; Kukuljan et al., 2011; Marques et al., 2013; Winters-Stone et al., 2011). However, most exercise training studies have some limitations such as small sample size, focus primarily on women, a short intervention period (e.g., 8-24 weeks), and/or no control group in their study design
(Hinton et al., 2015; Huovinen et al., 2016; Marques et al., 2013). Also, most large epidemiological studies used self-reported physical activity, which has a significant measurement error since most people over-report their physical activity levels because it is a socially desirable behavior (Adams et al., 2005). Physical fitness, such as muscular strength (MS) and cardiorespiratory fitness (CRF), is an objective marker of recent physical activity and exercise that has been shown to be associated with improved BMD (Boudenot et al., 2015; Chien et al., 2000). However, most studies have examined either MS or CRF on bone health, and the data on the independent and combined effects of MS and CRF on BMD is still limited.

The purpose of this study is to determine the independent and combined associations of MS and CRF with BMD in older adults (≥65 years old) using the data from the “Physical Activity and Aging Study (PAAS)”, which is a longitudinal cohort study. This study will provide important data to answer one of the most common questions in exercise science, “Which type or combination of exercise or fitness is most beneficial to improve BMD in older adults?” This study will contribute to the development of more effective strategies in osteoporosis prevention in older adults from both clinical and public health perspectives.

**Specific aims**

**Aim 1:** To determine the association between MS and BMD, independent of CRF, in older adults.

*Hypothesis:* MS will be positively associated with BMD independent of CRF.

**Aim 2:** To determine the association between CRF and BMD, independent of MS, in older adults.
Hypothesis: CRF will be positively associated with BMD independent of MS.

Aim 3: To determine the combined association of MS and CRF with BMD in older adults

Hypothesis: The combined association of MS and CRF with BMD will be stronger than either MS or CRF alone.
CHAPTER 2

REVIEW OF LITERATURE

This literature review will cover 1) risk factors of BMD, 2) the association between MS and BMD, and 3) the association between CRF and BMD.

Risk Factors of Bone Mineral Density

There are several important demographic and lifestyle factors that are either positively or negatively associated with BMD. Nevertheless, these factors were prevalently overlooked in many studies (Bayramoğlu et al., 2005; Beavers et al., 2017; Blain et al., 2001; DeFina et al., 2016; Hinton et al., 2015; Marques et al., 2013; Rhodes et al., 2000; Wainstein et al., 2016; Yingling et al., 2017; Zhou et al., 2013). The factors that are positively associated with BMD include younger age (birth until mid-20s), physical activity, body composition (muscle mass), diet/supplementation (e.g., calcium and vitamin D), and hormone therapy (Curtis et al., 2015; Dogan & Posaci, 2002; Office of the surgeon, 2004; Pluijm et al., 2001; Shapses & Sukumar, 2012). In contrast, the factors that are negatively associated with BMD include older age (mid-20s until death), female gender (experiencing menopause), physical inactivity, smoking, and heavy alcohol consumption (Curtis et al., 2015; Office of the surgeon, 2004; Pluijm et al., 2001; Sommer et al., 2013). These risk
factors are important covariates that will be considered in the analyses of the associations of MS and/or CRF with BMD as potential confounders. The following is more detailed information for each risk factor on BMD.

Bones grow until the mid-20’s in both men and women. After young adulthood, the balance between bone resorption and bone formation favors resorption, and this imbalance leads to a loss of BMD (Figure 3) (Dogan & Posaci, 2002). Female bone resorption is accelerated compared to male because women go through menopause, which results in changes in hormone secretion involved with bone and calcium metabolism (Khosla & Riggs, 2005). To mitigate the effects of menopause on bone loss in women, estrogen replacement is commonly employed as a hormone therapy since deficiency of estrogen contributes to osteoporosis in older women (Riggs, 2000). Body composition or body weight is positively associated with BMD (Curtis et al., 2015; Shapses & Sukumar, 2012). As mentioned above, bones grow by mechanical load, and this load can also be provided by body composition including lean mass and fat mass (Cummings & Melton, 2002; Curtis et al., 2015; Novotny et al., 2015). Diet and supplements are factors that are commonly promoted to the general population as ways to improve or maintain bone health. Calcium and vitamin D intake are closely related to bone density since bones are comprised of calcium, and vitamin D facilitates calcium absorption to the body. (Office of the Surgeon, 2004; Shea et al., 2002). In addition to body weight and dietary intake, other lifestyle factors can affect bone health. The effects of smoking on bone is not clear (Paganini-Hill et al., 1991); however, there is a general consensus that smoking negatively affects BMD, with some variation depending on the length of smoking period and regional bone tissue (Law & Hackshaw, 1997; Hollenbach et al., 1993; Rapuri et al., 2000). Small or moderate alcohol consumption (1-3 drinks/week) appears to have a protective effect on bone health (Hansen et al., 1991; Holbrook &
Barrett-Connor., 1993; Sommer et al., 2013); however, heavy alcohol consumption has adverse
effects on bone health, delaying bone formation and causing vitamin D deficiency (Curtis et al.,
2015; Moniz, 1994). Physical activity can stimulate bone remodeling through the mechanical
load. Bone growth has been observed as an outcome of a variety of activities including
housework, walking, jumping, and weight lifting (Beavers et al., 2017; Marques et al., 2013;
Muir et al., 2013; Shapses & Sukumar, 2012). Additional factors that directly or indirectly affect
bone health include genetics, hormone secretion, etc. (Khosla & Riggs, 2005).

**Association between Muscular Strength and Bone Mineral Density**

A major contributor to bone growth and remodeling throughout adulthood is physical
activity. According to the WHO, “physical activity is defined as any bodily movement produced
by skeletal muscles that requires energy expenditure.” (WHO, 2010). Thus, bodily movement is
created when skeletal muscles apply mechanical forces to bones via contractile pulling. In
addition to daily movement, these mechanical forces to bones can be amplified with resistance
training (RT) (Cummings & Melton, 2002; Curtis et al., 2015; Novotny et al., 2015). There have
been many studies examining the effects of RT on bone, and in general, they have found that RT
improves BMD. A 16-week RT intervention study with 37 older women reported that
participants increased their hip BMD by 6% (p<0.001) on average (Huovinen et al., 2016). Not
surprisingly, the women also increased their strength from 43-118% depending on the muscle
group (all p<0.001). Other than this finding, many studies have reported that RT improved BMD
in both men and women (Kukuljan et al., 2011; Marques et al., 2013).
Participation in RT contributes to the improvement of MS, alongside genetics, BMI, and other lifestyle factors, and MS is often considered an objective proxy for recent RT in large epidemiological studies, where over-reporting of physical activity is commonplace (Beavers et al., 2017; Blain et al., 2001; Hinton et al., 2015; Huovinen et al., 2016; Kukuljan et al., 2011; Marques et al., 2013; Winters-Stone et al., 2011; Zhou et al., 2013). Thus, in general, it is expected that MS should also be associated with greater BMD; however, there is not a large body of literature analyzing the association between MS and BMD in older adults. There are few studies that investigated the association, but these studies share some weaknesses, such as women mostly given attention, small sample size, and uncontrolled covariates related to BMD, such as aerobic physical activity (Bayramoğlu et al., 2005; Beavers et al., 2017; Blain et al., 2001; Hinton et al., 2015; Marques et al., 2013; Rhodes et al., 2000; Yingling et al., 2017; Zhou et al., 2013). Also, there is some inconsistency among previous findings on the magnitude (strength) of the association between RT and BMD.

Yingling and colleagues (2017) investigated the relationships between peak power and MS and tibia bone strength with 86 collegiate athletes using a vertical jump test, a relative grip strength (combined maximal force of left and right hand ÷ body weight) and a relative 1-RM leg press (1-RM leg press ÷ body weight). This study found that only less than 1% of variance was explained by a relative grip strength and 1-RM leg press. This study implied a necessity of investigations with older study population who are at higher risk of osteoporosis.

Contrary to the findings by Yingling and colleagues (2017), one cross-sectional study did find an association between MS and BMD in 293 postmenopausal women aged from 45 to 64 years, using isokinetic and isometric MS measurements with various protocol (angle/speed) and BMD measurements at different sites including total body, lumbar spine, and hip (Zhou et al.,
2013). Even though the correlations were weak or moderate, most of the MS values showed positive correlations with BMD after adjusting for age (most p<0.05). However, in the statistical analyses, only age and the number of years from menopause were included and other factors affecting BMD such as smoking status, hormone replacement, or physical activity were not included (Muir et al., 2013; Paganini-Hill et al., 1991; Riggs, 2000).

One study with 56 postmenopausal women that investigated the relationship between quadriceps strength and BMD of the femoral neck and lumbar spine reported different results (Blain et al., 2001). While femoral neck BMD was strongly associated with quadriceps strength, lumbar BMD was not associated with quadriceps strength. The study suggested site-specific effects of MS on BMD.

As suggested by Blain et al., (2001), one cross-sectional study assessed hip, trunk, and grip MS in 62 older women (Bayramoğlu et al., 2005). BMD was measured at the lumbar spine, femur, and distal radius. The study found weak or no correlations between MS and BMD in which regional MS measurements were analyzed along with regional BMD closely related to each MS measurement. No correlations were found between trunk MS and lumbar BMD as well as between grip strength and distal radius BMD. Only the correlation between hip abductor strength and femoral BMD was significant (r=0.33, p<0.01). Bayramoğlu pointed out a smaller sample size as the potential reason why the study showed different results with other studies.

As discussed, previous studies had some limitations so that strong and conclusive results were not found. Therefore, larger studies are needed, especially in older adults.
Association between Cardiorespiratory Fitness and Bone Mineral Density

As MS is generally improved by RT, CRF is also improved mostly by aerobic training (AT). Weight-bearing aerobic physical activity such as walking or jogging could improve BMD since these types of AT also impose mechanical forces on the bones (Boudenot et al., 2015; Chien et al., 2000). One study investigated the effects of AT in 101 postmenopausal women (Chien et al., 2000). After a 24-week AT intervention consisting of graded treadmill walking and stepping exercises, it was found that AT significantly improved not only CRF (VO_{2\text{max}}) (p<0.05), but also BMD by 6.8% on the femoral neck (p<0.05). However, in most observational studies, aerobic physical activity is generally over reported, thus CRF is often used as a more objective proxy for recent aerobic physical activity.

Previous observational studies on the associations of CRF and BMD also had the similar limitations in study designs as in the studies regarding MS. These include smaller sample sizes (less than 100), a focus primarily on women, samples that were younger or athletes, and less consideration of potential confounders (Bayramoğlu et al., 2005; Blain et al., 2001; DeFina et al., 2016; Wainstein et al., 2016; Yingling et al., 2017; Zhou et al., 2013). Even though the relationship between CRF and BMD has not been explored as thoroughly as the relationship between MS and BMD, earlier studies on CRF and BMD showed more consistent dose-response relationships.

One cross-sectional study with 2,569 men aged 50 to 95 years old measured the participants’ CRF via maximal treadmill tests, and BMD was assessed at the femoral neck (Wainstein et al., 2016). After CRF and BMD assessments, the participants were categorized into three groups (using 20^{th} and 60^{th} percentiles) based on their CRF levels to calculate odds ratios...
(ORs) and 95% confidence intervals (CIs) for the prevalence of osteoporosis. After adjusting for body weight, age, and days per week of RT, significantly lower ORs were found in the high (OR: 0.19, 95% CI: 0.09-0.42) and moderate (OR: 0.34, 95% CI: 0.16-0.74) CRF groups compared with the low CRF group as a reference.

Another cohort study with 1,720 menopausal women that measured CRF by maximal treadmill tests and BMD at the femoral neck found similar results suggesting that there were lower odds of having osteoporosis among the high (OR: 0.29, 95% CI: 0.12-0.71) and moderate (OR: 0.30, 95% CI: 0.11-0.80) CRF groups compared with the low CRF group after adjusting for age, body weight, and resistance activity level (DeFina et al., 2016).

In contrast to studies on the association between MS and BMD, the studies on the association of CRF and BMD showed consistent results. However, the studies above were conducted by the same research group, Cooper Center Longitudinal Study (CCLS) based in Dallas, Texas. Even though CCLS had a large cohort, the population was very homogeneous comprised of more than 90% of non-Hispanic whites; thereby making the results less generalizable to all races. Also, CCLS does not have an objective measurement for physical activity that could lead to the overestimation of physical activity. Therefore, future studies with various populations and objective measures of physical activity are needed to add to the body of knowledge.

**Conclusion**

There is still limited data on the associations of MS and CRF with BMD in older adults. Also, the results found from the studies were somewhat inconsistent (Bayramoğlu et al., 2005;
Blain et al., 2001; Yingling et al., 2017; Zhou et al., 2013). Further, most studies had some limitations. Several studies primarily focused on young adults or athletes, had a smaller sample size, and overlooked some covariates such as smoking, alcohol consumption, and physical activity (Bayramoğlu et al., 2005; Blain et al., 2001; DeFina et al., 2016; Wainstein et al., 2016; Yingling et al., 2017; Zhou et al., 2013). Also, most studies examined either MS or CRF alone with BMD.

To our knowledge, there were no other studies that examined independent and combined effects of MS and CRF on BMD, even if both are shown to be associated with BMD (Bayramoğlu et al., 2005; Blain et al., 2001; DeFina et al., 2016; Wainstein et al., 2016; Zhou et al., 2013). In the previous studies on the association between MS and BMD, a main focus was simply on the association between MS and BMD, not taking into account the effect of CRF on BMD. There was a lack of CRF data, thus the independent effect of MS on BMD controlling for CRF could not be investigated. In the studies above on the association between CRF and BMD, MS data were not considered, although resistance activity data were collected and controlled as categorical variables (resistance activity ≥ 2 days/week or < 2 days/week). Therefore, the effect of CRF on BMD independent of and combined with MS could not be investigated.

Since 2015, the PAAS study has collected both MS and CRF data in over 650 older adults. Therefore, this study investigated the independent associations of MS and CRF with BMD in older adults. Furthermore, combined effects of MS and CRF on BMD were investigated, which is the strength of the current study. These results will help better inform the appropriate mode or combination of physical activity and fitness to prevent osteopenia and osteoporosis in older adults.
CHAPTER 3
METHODOLOGY

Participants

Participants were from the “Physical Activity and Aging Study (PAAS)”, an ongoing cohort study of older adults launched in 2015 at Iowa State University Physical Activity Epidemiology laboratory. The Iowa State University Institutional Review Board approved this study (Appendix). All participants signed an Informed Consent document before beginning participation. The cohort consisted of both men and women who were 65 years of age or older. Participants with complete MS (grip strength), CRF (400-meter walk test), and BMD (DXA scan) data were included. Participants were excluded if they have had history of a cancer other than skin cancer over the last 5 years that may significantly affect physical activity and other lifestyles. We considered artificial joints on the associations of MS and CRF with BMD since it is known to artificially inflate t-scores (Di Monaco et al., 2011). From fall 2015 through spring 2017, the study has been conducted at the Nutrition and Wellness Research Center (NWRC) of Iowa State University and since fall 2017, the study has been conducted in Kinesiology Department Forker building. We found significant discrepancies in DXA results measured by two different DXA machines of the NWRC and the department. The DXA machine in the department building showed significantly higher t-scores. When comparing 131 older participants’ t-scores who came to both the NWRC and the department building for the study with a 1-3 year time interval (mean 2.4 years) as the follow-up visit, two things were found. First, of the 131 older adults, 126 people (96%) improved their t-score. Second, it was found that their t-score improved, on average, by 0.8 (160 %). Of the 131 older adults, 40% were
osteopenic or osteoporotic (defined as t-score below -1.0) in the sample at the NWRC. However, when they came back to the study in Forker building over 2.4 years on average and their bone health was measured by the department DXA machine, only 24% of them were osteopenic or osteoporotic, which is unexpected in older adults who were on the stage of bone loss due to aging. In conclusion, we found that the two DXA machines differently measure BMD, so we decided to include older adults who participated in the study from fall 2017 using only the department DXA machine for primary analyses. Data collected at the NWRC were analyzed for exploratory analyses involving 1-repetition maximum (RM) chest and leg press because these MS measurements were collected at the NWRC only.

**Study Procedure and Measurements**

Participants came to the lab for two study visits separated by one week, and they were invited on an annual basis.

1) **Visit 1: Visit 1 occurred in the evening, and included both MS and CRF fitness tests.**

   Each participant received an accelerometer-based pedometer and was asked to wear it for 1 week and return it in Visit 2.

   a. **Orientation & Paperwork**

      Study procedures and intended outcomes were explained to participants.

      Participants completed Informed Consent and a Medical History Questionnaire. The Medical History Questionnaire developed by our research team included questions about demographics, medication use, disease history, family disease history, alcohol consumption, smoking status, and physical activity (Sànchez-Riera et al., 2017).
b. Grip Strength

Grip strength (kg) has been assessed for all participants since 2015, therefore was used as a main variable for MS. Using a hand dynamometer (Jamar+ digital hand dynamometer, Patterson Medical, Chicago, IL, U.S.), participants performed three maximal voluntary contractions of each hand with 90° at the elbow and 90° at the middle two fingers with 1 minute of rest between contractions (Figure 4). The highest force of the three contractions of each hand was averaged, and the average value was used for analysis.

c. 400-Meter Walk Test

CRF was assessed with the 400-meter walk test that showed high correlations with measured VO₂max, thus walking speed from the 400-meter walk test was used as CRF variable (Gabriel et al., 2010; Simonsick et al., 2006). Two cones were placed 20 meters apart at opposite ends of a walking lane (Figure 5). Participants were instructed to walk as quickly as possible (without running) for 10 laps around the cones, totaling 400 meters. Before testing, participants were encouraged to warm-up. A stopwatch was used to record the total time taken for the participant to cover the full 400 meters,
including any periods of rest. The test could also be terminated by the participant at any point during the procedure.

d. 1-Repetition Maximum Chest Press

1-RM chest press (kg) was measured in 2015 only using a seated isotonic chest press machine (Figure 6). The seat was adjusted so that participants’ upper arms were parallel to the ground. Participants completed a sub-maximal warm-up of 8-10 repetitions. After a 1-minute resting period, 10-20 lbs or 5-10% of the participant’s body weight was added, after which the participants performed a second warm-up set of 3-5 repetitions. Following this, 2-minutes of rest ensued prior to the 1-RM procedure itself. This phase sought to determine the maximum load (kg) a participant could move only one time. A starting load was determined by the tester and the participant was asked to complete just a single repetition. If the load could be moved easily and with good form, the participant would rest for 2-4 minutes and an increase in load equivalent to 10-20 lbs or 5-10% body weight was added for the next attempt. If an attempt was unsuccessful, a rest period of 2-4 minutes ensued, the load was decreased by 5-10lbs or 2.5-5% body weight, and the participant was instructed to complete a single repetition. The last successfully lifted load was recorded as the 1 RM.
e. 1-Repetition Maximum Leg Press

1-RM leg press (kg) was measured in 2015 only using a seated isotonic leg press machine (Figure 6). Seat and foot placements were adjusted so that participants’ knees were at 90 degrees, and their toes were in line with their knees. The same warm-up and series of 1-RM attempts as the chest press were completed for the leg press. We plan to use 1-RM bench and leg presses in addition to grip strength as an additional MS variable in subsample from 2015.

2) Visit 2: *Visit 2 occurred one week later in the morning after a 12-hour fast and included anthropometric measurements.*

a. Dual-energy X-ray Absorptiometry Scan (Hologic Horizon W, Hologic, Malborough, MA, U.S.)

A whole-body DXA scan was conducted to assess BMD. During the orientation, study participants were fully informed of the minimal radiation exposure experienced during scanning. Participants changed into scrubs and were asked to take off metals such as rings. Low BMD was defined as a t-score < -1.0 in analyses (WHO, 1991).

b. Body Mass Index (BMI)

Body weight was measured using a digital weighing scale, and height was measured unshod using a stadiometer. BMI was calculated as body weight (kg) divided by height-squared (m^2).

c. Step Counts

Physical activity was assessed using an accelerometer-based pedometer (Omron,
Model HJ-321, IL, U.S.). The pedometer was worn at the waist for 7 days to determine if participants met the physical activity recommendation (≥5,000 steps/day) (Physical Activity Guidelines for Americans 2nd edition, 2018; Tudor-Locke at al., 2011).

**Statistical Analyses**

1) *Descriptive Statistics*

Descriptive statistics for MS, CRF, BMD, and covariates were reported as means and standard deviations for continuous variables and n and percentage for categorical variables. Characteristic differences in groups (i.e., between MS or CRF tertiles) were calculated using χ² test for categorical variables or ANOVA test for continuous variables by BMI-specific MS and CRF tertiles in men and women separately. Because BMI was found to be significantly associated with CRF in both women and men (p<0.001) and similar trends were found with MS, although not significant (Table 1), BMI-specific tertiles of each MS and CRF were used in all logistic regressions to control for the effect of BMI on MS and CRF. We used BMI-specific tertiles of MS although BMI was not significantly associated with MS because this study was to compare the effect of MS vs. CRF on bone health. To assign participants to BMI-specific MS/CRF tertiles, participants were assigned to normal weight (<25 kg/m²), overweight (25-29.9 kg/m²), or obese (≥30 kg/m²) categories, and in each BMI category, participants were divided into tertiles of MS/CRF. MS/CRF tertiles from each BMI category were combined to create the final BMI-specific tertiles of MS/CRF. Also, considering menopause that differently affects
bone change in women than men, all analyses were conducted in men and women separately.

2) **Aim 1**

To test the hypothesis that MS will be positively associated with BMD independent of CRF in Aim 1, we used two statistical methods: 1) linear regression was conducted to find unstandardized and standardized betas (βs) with MS (i.e., grip strength) as an independent variable, and then BMD (g/cm^2) as well as t-scores as dependent variables, adjusting for potential covariates including CRF and 2) logistic regression was conducted to calculate ORs and 95% CIs of having low BMD (i.e., t-score < -1.0) across MS tertiles after adjusting for the covariates including CRF.

3) **Aim 2**

To test the hypothesis that CRF will be positively associated with BMD independent of MS in Aim 2, we used the same two statistical methods described in Aim 1: 1) linear regression was conducted to find unstandardized and standardized βs with CRF (time to complete the 400-meter walk test) as an independent variable, and t-scores and BMD as dependent variables, adjusting for potential covariates including MS and 2) logistic regression was conducted to calculate ORs and 95% CIs of having low BMD across CRF tertiles after adjusting for covariates including MS.

4) **Aim 3**

To test the hypothesis that the combined association of MS and CRF with BMD will be stronger that either MS or CRF alone in Aim 3, logistic regression was conducted to calculate ORs and 95% CIs of having low BMD across combined categories by MS and CRF following earlier studies (Artero et al., 2011; Ruiz et al., 2008). MS and CRF were
dichotomized into upper two thirds and lower one third 1) to keep the sufficient numbers of participants for the joint stratification analyses and 2) based on its association with BMD (Tables 5 and 7), and then 4 combined categories such as “strong and fit”, “strong and unfit”, “weak and fit”, or “weak and unfit” were created based on the associations between MS or CRF with BMD from Aims 1 and 2.

5) Covariates

Based on literature reviews on similar studies, we considered the following covariates: age, current smoking status (yes/no), heavy alcohol consumption (yes/no, >14 and >7 alcohol drink per week for men and women, respectively), hormone therapy (yes/no, women only), meeting physical activity recommendation (yes/no, ≥5,000 steps/day), and BMI.

6) Exploratory Analyses

Statistical analyses were repeated using 1-RM chest press and leg press in addition to grip strength as MS variables with data collected at NWRC from fall 2015 through spring 2017 to test if regional strength (grip strength, 1-RM chest and leg press) is more strongly related to whole-body and regional (arm, pelvis, leg, and lumbar) BMD (e.g., grip strength is more strongly related to arm BMD than leg BMD). Correlations between each covariate and exposures and outcome were examined in men and women separately after adjusting for age to check a possible multicollinearity (also collinearity) issue. Whole-body DXA scans typically underestimate the prevalence of osteoporosis by generating higher t-scores, compared with partial DXA scans on specific body regions such as femoral neck or lumbar spine that are usually used for diagnosis of osteoporosis (Graat-Verboom et al., 2010; Rajaei et al., 2016). Therefore, additional logistic regression
analyses were conducted using a different t-score cut-point (t-score < -0.5 instead of < -1.0). It has been reported that a t-score of -0.5 was an acceptable alternative cut-point for whole-body DXA scans, yielding a sensitivity of 90% and a specificity of 91% for detecting low BMD by regional DXA scans (Rajaei et al., 2016). Also, interaction tests were conducted to see if the associations of MS and CRF with BMD are modified by artificial joints, since artificial joints typically erroneously exaggerate whole body t-scores.

All the analyses were done separately in men and women. Statistical tests were two-sided, and p < 0.05 were accepted to indicate statistical significance using SAS software (version 9.4).
CHAPTER 4
RESULTS

From October 2017 to February 2019, 502 older adults participated in the Physical Activity and Aging Study (PAAS). Among the 502 participants, those who had a history of cancer (other than skin cancer) within the past five years (n=31), did not complete main exposure measurements (i.e., grip strength or 400-meter walk test) or outcome measurement (i.e., DXA scan) (n=34), or had an artificial joint (n=84) were excluded from the data analyses. Therefore, 353 participants (189 women and 164 men) were included in the primary analyses.

Mean values and cut-points for MS and CRF tertiles are provided in Table 1. Based on the significant associations of BMI with CRF (p<0.001) in both women and men and similar trends with MS as well, although not significant, BMI-specific tertiles of each MS and CRF were used in all logistic regressions to control for the effect of BMI on MS and CRF. Participant characteristics by BMI-specific MS and CRF tertiles in women and men are reported in Tables 2 and 3, respectively. Participants in the upper MS and CRF tertiles tended to be younger, stronger, and fitter in both women and men (all p<0.01).
Table 1. Means and cut-points for sex-specific muscular strength and cardiorespiratory fitness tertiles by body mass index

<table>
<thead>
<tr>
<th>BMI</th>
<th>Lower (Muscular Strength: kg)</th>
<th>Middle (Muscular Strength: kg)</th>
<th>Upper (Muscular Strength: kg)</th>
<th>Overall (Muscular Strength: kg)</th>
<th>P value</th>
<th>Lower (Cardiorespiratory Fitness: minutes)</th>
<th>Middle (Cardiorespiratory Fitness: minutes)</th>
<th>Upper (Cardiorespiratory Fitness: minutes)</th>
<th>Overall (Cardiorespiratory Fitness: minutes)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>Mean (cut-point)</td>
<td>19.4 (&lt;23.3)</td>
<td>25.8 (23.3-28.1)</td>
<td>35.5 (&gt;28.1)</td>
<td></td>
<td>5.1 (&gt;4.7)</td>
<td>4.5 (4.3-4.7)</td>
<td>3.9 (&lt;4.3)</td>
<td>4.4 (NA)</td>
<td>0.417</td>
</tr>
<tr>
<td>25 – 29.9</td>
<td>Mean (cut-point)</td>
<td>19.7 (&lt;23.0)</td>
<td>25.4 (23.0-27.6)</td>
<td>33.2 (&gt;27.6)</td>
<td>26.6 (NA)</td>
<td>5.3 (&gt;4.7)</td>
<td>4.4 (4.2-4.7)</td>
<td>3.9 (&lt;4.2)</td>
<td>4.6 (NA)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ 30</td>
<td>Mean (cut-point)</td>
<td>19.2 (&lt;23.0)</td>
<td>25.1 (23.0-27.0)</td>
<td>34.1 (&gt;27.0)</td>
<td>27.6 (NA)</td>
<td>5.7 (&gt;4.7)</td>
<td>4.5 (4.2-4.7)</td>
<td>4.1 (&lt;4.2)</td>
<td>5.0 (NA)</td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>Mean (cut-point)</td>
<td>33.9 (&lt;38.7)</td>
<td>41.9 (38.7-45.8)</td>
<td>51.7 (&gt;45.8)</td>
<td>42.4 (NA)</td>
<td>4.9 (&gt;4.5)</td>
<td>4.3 (4.0-4.5)</td>
<td>3.7 (&lt;4.0)</td>
<td>4.1 (NA)</td>
<td></td>
</tr>
<tr>
<td>25 – 29.9</td>
<td>Mean (cut-point)</td>
<td>32.6 (&lt;39.3)</td>
<td>41.6 (39.3-45.7)</td>
<td>53.0 (&gt;45.7)</td>
<td>42.5 (NA)</td>
<td>5.1 (&gt;4.5)</td>
<td>4.2 (4.0-4.5)</td>
<td>3.7 (&lt;4.0)</td>
<td>4.2 (NA)</td>
<td>0.877</td>
</tr>
<tr>
<td>≥ 30</td>
<td>Mean (cut-point)</td>
<td>34.3 (&lt;38.6)</td>
<td>41.6 (38.6-45.7)</td>
<td>54.0 (&gt;45.7)</td>
<td>43.3 (NA)</td>
<td>5.2 (&gt;4.5)</td>
<td>4.3 (4.0-4.5)</td>
<td>3.7 (&lt;4.0)</td>
<td>4.6 (NA)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index (kg/m²).
Muscular strength: grip strength (kg).
Cardiorespiratory fitness: minutes to complete 400-meter walk test.
Table 2. Participant characteristics by muscular strength and cardiorespiratory fitness in women

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=189)</th>
<th>Tertile of Muscular Strength</th>
<th>Tertile of Cardiorespiratory Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.8 (5.5)</td>
<td>73.5 (6.3) 70.6 (4.3) 71.2 (5.5)</td>
<td>0.006 74.9 (6.1) 71.0 (4.9) 69.5 (4.0)</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>26.4 (7.1)</td>
<td>19.4 (2.6) 25.7 (1.7) 34.0 (5.9)</td>
<td>&lt;0.001 23.3 (6.3) 26.7 (7.3) 29.1 (6.4)</td>
</tr>
<tr>
<td>400-meter walk completion (minutes)</td>
<td>4.6 (0.7)</td>
<td>4.9 (0.8) 4.5 (0.7) 4.3 (0.5)</td>
<td>&lt;0.001 5.3 (0.7) 4.5 (0.2) 4.0 (0.3)</td>
</tr>
<tr>
<td>Whole-body bone mineral density (g/cm²)</td>
<td>1.047 (0.091)</td>
<td>1.033 (0.090) 1.053 (0.081) 1.053 (0.102)</td>
<td>0.395 1.034 (0.098) 1.056 (0.094) 1.050 (0.083)</td>
</tr>
<tr>
<td>T-scorea</td>
<td>-0.8 (1.2)</td>
<td>-1.0 (1.1) -0.7 (1.0) -0.7 (1.3)</td>
<td>0.395 -0.9 (1.2) -0.7 (1.2) -0.8 (1.0)</td>
</tr>
<tr>
<td>Low BMDb, n (%)</td>
<td>90 (48)</td>
<td>35 (56) 27 (42) 28 (44)</td>
<td>0.229 33 (53) 29 (45) 28 (44)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>2 (1)</td>
<td>0 (0) 2 (3) 0 (0)</td>
<td>0.330 0 (0) 1 (2) 1 (2)</td>
</tr>
<tr>
<td>Heavy alcohol consumptionc, n (%)</td>
<td>19 (10)</td>
<td>4 (6) 7 (11) 8 (13)</td>
<td>0.489 4 (6) 10 (16) 5 (8)</td>
</tr>
<tr>
<td>Physical inactivityd, n (%)</td>
<td>97 (51)</td>
<td>32 (52) 31 (48) 34 (54)</td>
<td>0.822 47 (76) 26 (41) 24 (38)</td>
</tr>
<tr>
<td>Bone supplement/medicatione, n (%)</td>
<td>146 (77)</td>
<td>51 (82) 45 (70) 50 (79)</td>
<td>0.247 48 (77) 49 (77) 49 (78)</td>
</tr>
<tr>
<td>Hormone therapy, n (%)</td>
<td>19 (10)</td>
<td>5 (8) 8 (13) 6 (10)</td>
<td>0.714 4 (7) 9 (14) 6 (10)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 (4.7)</td>
<td>26.4 (4.4) 26.6 (5.2) 25.9 (4.5)</td>
<td>0.699 27.0 (5.3) 26.4 (4.4) 25.6 (4.3)</td>
</tr>
</tbody>
</table>

Data are means (SD) unless indicated otherwise.

a T-score = standard deviation difference in bone mineral density (g/cm²) compared with young (age=20-29), white female from National Health and Nutrition Examination Survey, 2012.

b Low BMD, low bone mineral density (t-score < -1.0).

c Determined by alcohol consumption per week (> 7 drinks/week).

d Determined by steps per day (< 5,000 steps/day).

e Includes calcium, vitamin D, and/or medications to stimulate bone growth.
Primary Analyses

Table 4 shows the association of MS with t-scores after adjusting for potential confounders including CRF. Multivariable linear regression indicated that MS was significantly associated with t-score when adjusting for age in women and men (p<0.05). However, when covariates including smoking status, heavy alcohol consumption, physical inactivity, hormone therapy, and BMI were added to the model, the relationship was no longer significant in women.
(p>0.05). With further adjustment for CRF, the association remained non-significant (p>0.05). In contrast, in men, MS was significantly associated with t-score when controlling for all covariates, including CRF (all p<0.05).

Table 4. Association of muscular strength with t-scores: multivariable linear regression

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
</tr>
<tr>
<td>Muscular strengtha (kg)</td>
<td>0.03 (0.01)</td>
<td>0.18</td>
<td><strong>0.017</strong></td>
<td>0.02 (0.01)</td>
<td>0.11</td>
<td>0.145</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.02 (0.02)</td>
<td>-0.07</td>
<td>0.315</td>
<td>-0.02 (0.02)</td>
<td>-0.07</td>
<td>0.346</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.95 (0.83)</td>
<td>-0.08</td>
<td>0.253</td>
</tr>
<tr>
<td>Heavy alcohol consumptionb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.55 (0.29)</td>
<td>0.14</td>
<td>0.057</td>
</tr>
<tr>
<td>Physical inactivityc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.18 (0.18)</td>
<td>-0.08</td>
<td>0.326</td>
</tr>
<tr>
<td>Bone supplement/medicationd</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.30 (0.20)</td>
<td>-0.11</td>
<td>0.146</td>
</tr>
<tr>
<td>Hormone therapy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.15 (0.28)</td>
<td>0.04</td>
<td>0.591</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.03 (0.02)</td>
<td>0.13</td>
<td>0.092</td>
</tr>
<tr>
<td>Cardiorespiratory fitness (minutes)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscular strength (kg)</td>
<td>0.04 (0.01)</td>
<td>0.27</td>
<td><strong>0.001</strong></td>
<td>0.04 (0.01)</td>
<td>0.25</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.0017 (0.03)</td>
<td>0.0051</td>
<td>0.948</td>
<td>0.01 (0.03)</td>
<td>0.03</td>
<td>0.763</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.12 (1.50)</td>
<td>0.06</td>
<td>0.456</td>
</tr>
<tr>
<td>Heavy alcohol consumption</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.12 (0.57)</td>
<td>0.02</td>
<td>0.836</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.12 (0.24)</td>
<td>-0.04</td>
<td>0.633</td>
</tr>
<tr>
<td>Bone supplement/medication</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.21 (0.26)</td>
<td>-0.07</td>
<td>0.416</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.04 (0.03)</td>
<td>0.12</td>
<td>0.141</td>
</tr>
<tr>
<td>Cardiorespiratory fitness (minutes)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SE, standard error; STD, standardized.

T-score = standard deviation difference in bone mineral density (g/cm²) compared with young (age=20-29), white female from National Health and Nutrition Examination Survey, 2012.

aGrip strength (kg).
bDetermined by alcohol consumption per week (> 7 and >14 drinks/week for women and men respectively).
cDetermined by steps per day (< 5,000 steps/day).
dIncludes calcium, vitamin D, and/or medications to stimulate bone growth.

Model 1: adjusted for age.
Model 2: model 1 further adjusted for current smoking status, heavy alcohol consumption, physical inactivity, supplement/medication for bone health, hormone therapy (women only), and body mass index.
Model 3: model 2 further adjusted for cardiorespiratory fitness (minutes to complete 400-meter walk test).
Logistic regression was conducted to investigate the odds of having low BMD across the MS tertiles (Table 5) after adjusting for potential confounders including CRF (Model 3). In both women and men, higher MS appeared to be related to reduced odds of having low BMD; however, none of the associations were significant (all ORs and p-trends>0.05).

Table 5. Odds ratios of low bone mineral density by muscular strength

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Casea (%)</th>
<th>Model 1 OR (95% CI)</th>
<th>Model 2 OR (95% CI)</th>
<th>Model 3 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Lower MS</td>
<td>62</td>
<td>35 (56)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td></td>
<td>Middle MS</td>
<td>64</td>
<td>27 (42)</td>
<td>0.59 (0.28-1.21)</td>
<td>0.60 (0.28-1.29)</td>
</tr>
<tr>
<td></td>
<td>Upper MS</td>
<td>63</td>
<td>28 (44)</td>
<td>0.64 (0.31-1.30)</td>
<td>0.75 (0.35-1.61)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value for linear trend</td>
<td>0.227</td>
<td>0.471</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Lower MS</td>
<td>50</td>
<td>4 (7)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td></td>
<td>Middle MS</td>
<td>54</td>
<td>2 (4)</td>
<td>0.50 (0.08-2.96)</td>
<td>0.51 (0.08-3.09)</td>
</tr>
<tr>
<td></td>
<td>Upper MS</td>
<td>53</td>
<td>1 (2)</td>
<td>0.26 (0.03-2.47)</td>
<td>0.23 (0.02-2.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value for linear trend</td>
<td>0.210</td>
<td>0.188</td>
</tr>
</tbody>
</table>

CI, confidence interval; MS, muscular strength (grip strength, kg); OR, odds ratio.
*aCase: low bone mineral density (t-score < -1.0).
Model 1: adjusted for age (years).
Model 2: model 1 further adjusted for current smoking status, heavy alcohol consumption (> 7 and > 14 drinks/week for women and men respectively), physical inactivity (< 5,000 steps/day), supplement/medication for bone health, hormone therapy (women only), and body mass index (kg/m²).
Model 3: model 2 further adjusted for cardiorespiratory fitness (minutes to complete 400-meter walk test).

Table 6 shows the association of CRF with t-score after adjusting for potential confounders including MS. In both women and men, CRF was not significantly associated with t-score, in all models.
Table 6. Association of cardiorespiratory fitness with T-scores: multivariable linear regression

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
<th>Model 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
</tr>
<tr>
<td>Cardiorespiratory fitness&lt;sup&gt;a&lt;/sup&gt; (minutes)</td>
<td>0.02 (0.13)</td>
<td>0.01</td>
<td>0.885</td>
<td>-0.17 (0.15)</td>
<td>-0.10</td>
<td>0.254</td>
<td>-0.10 (0.16)</td>
<td>-0.06</td>
<td>0.524</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.02 (0.02)</td>
<td>-0.11</td>
<td>0.168</td>
<td>-0.01 (0.02)</td>
<td>-0.07</td>
<td>0.417</td>
<td>-0.01 (0.02)</td>
<td>-0.06</td>
<td>0.482</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.98 (0.83)</td>
<td>-0.09</td>
<td>0.239</td>
<td>-0.97 (0.83)</td>
<td>-0.09</td>
<td>0.243</td>
</tr>
<tr>
<td>Heavy alcohol consumption&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.58 (0.29)</td>
<td>0.15</td>
<td>0.042</td>
<td>0.55 (0.29)</td>
<td>0.14</td>
<td>0.057</td>
</tr>
<tr>
<td>Physical inactivity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.25 (0.18)</td>
<td>-0.11</td>
<td>0.182</td>
<td>-0.21 (0.19)</td>
<td>-0.09</td>
<td>0.274</td>
</tr>
<tr>
<td>Bone supplement/medication&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.30 (0.20)</td>
<td>-0.11</td>
<td>0.143</td>
<td>-0.29 (0.20)</td>
<td>-0.11</td>
<td>0.149</td>
</tr>
<tr>
<td>Hormone therapy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.13 (0.28)</td>
<td>0.03</td>
<td>0.639</td>
<td>0.15 (0.28)</td>
<td>0.04</td>
<td>0.595</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.04 (0.02)</td>
<td>0.17</td>
<td>0.044</td>
<td>0.04 (0.02)</td>
<td>0.15</td>
<td>0.075</td>
</tr>
<tr>
<td>Muscular strength&lt;sup&gt;e&lt;/sup&gt; (kg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02 (0.01)</td>
<td>0.09</td>
<td>0.267</td>
</tr>
</tbody>
</table>

Women

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
<th>Model 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
<td>β (SE)</td>
<td>STD β</td>
<td>P Value</td>
</tr>
<tr>
<td>Cardiorespiratory fitness&lt;sup&gt;a&lt;/sup&gt; (minutes)</td>
<td>-0.02 (0.17)</td>
<td>-0.01</td>
<td>0.920</td>
<td>-0.18 (0.19)</td>
<td>-0.08</td>
<td>0.355</td>
<td>-0.01 (0.20)</td>
<td>0.0035</td>
<td>0.971</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.02 (0.03)</td>
<td>-0.06</td>
<td>0.457</td>
<td>0.00013 (0.03)</td>
<td>0.0038</td>
<td>0.997</td>
<td>0.01 (0.03)</td>
<td>0.03</td>
<td>0.765</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.12 (0.15)</td>
<td>0.06</td>
<td>0.472</td>
<td>1.12 (1.51)</td>
<td>0.06</td>
<td>0.461</td>
</tr>
<tr>
<td>Heavy alcohol consumption</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.10 (0.59)</td>
<td>-0.01</td>
<td>0.859</td>
<td>0.12 (0.58)</td>
<td>0.01</td>
<td>0.838</td>
</tr>
<tr>
<td>Physical inactivity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.26 (0.25)</td>
<td>-0.09</td>
<td>0.303</td>
<td>-0.12 (0.25)</td>
<td>-0.04</td>
<td>0.636</td>
</tr>
<tr>
<td>Bone supplement/medication&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.30 (0.27)</td>
<td>-0.09</td>
<td>0.260</td>
<td>-0.21 (0.26)</td>
<td>-0.07</td>
<td>0.421</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05 (0.03)</td>
<td>0.15</td>
<td>0.096</td>
<td>0.04 (0.03)</td>
<td>0.12</td>
<td>0.180</td>
</tr>
<tr>
<td>Muscular strength&lt;sup&gt;e&lt;/sup&gt; (kg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.04 (0.01)</td>
<td>0.25</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SE, standard error; STD, standardized.

T-score = standard deviation difference in bone mineral density (g/cm<sup>2</sup>) compared with young (age=20-29), white female from National Health and Nutrition Examination Survey, 2012.

<sup>a</sup>Minutes to complete 400-meter walk test.

<sup>b</sup>Determined by alcohol consumption per week (> 7 and >14 drinks/week for women and men respectively).

<sup>c</sup>Determined by steps per day (< 5,000 steps/day).

<sup>d</sup>Includes calcium, vitamin D, and/or medications to stimulate bone growth.

<sup>e</sup>Grip strength (kg).

Model 1: adjusted for age.

Model 2: model 1 further adjusted for current smoking status, heavy alcohol consumption, physical inactivity, supplement/medication for bone health, hormone therapy (women only), and body mass index.

Model 3: model 2 further adjusted for muscular strength (grip strength).
The odds of having low BMD by CRF tertiles are reported in Table 7. In women, middle and upper CRF tertiles appeared to have lower odds of having low BMD, however, none of the associations were significant (all ORs and p-trends>0.05). In men, higher CRF was related to higher odds of having low BMD; however, none of associations were significant as well (all ORs and p-trends>0.05). This unexpected result may possibly be related to the very limited numbers of low BMD in men in all tertiles (n=2, n=2, and n=3 cases in each Lower, Middle, and Upper CRF, respectively).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Case (%)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower CRF</td>
<td>62 33 (53)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Middle CRF</td>
<td>64 29 (45)</td>
<td>0.77 (0.37-1.60)</td>
<td>0.65 (0.28-1.48)</td>
</tr>
<tr>
<td>Upper CRF</td>
<td>63 28 (44)</td>
<td>0.76 (0.35-1.63)</td>
<td>0.56 (0.24-1.32)</td>
</tr>
<tr>
<td>P value for linear trend</td>
<td>0.489</td>
<td>0.196</td>
<td>0.463</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower CRF</td>
<td>55 2 (4)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Middle CRF</td>
<td>54 2 (4)</td>
<td>1.13 (0.15-8.58)</td>
<td>1.48 (0.18-11.99)</td>
</tr>
<tr>
<td>Upper CRF</td>
<td>55 3 (5)</td>
<td>2.01 (0.28-14.22)</td>
<td>2.53 (0.34-18.78)</td>
</tr>
<tr>
<td>P value for linear trend</td>
<td>0.480</td>
<td>0.360</td>
<td>0.117</td>
</tr>
</tbody>
</table>

CI, confidence interval; CRF, cardiorespiratory fitness: minutes to complete 400-meter walk test; OR, odds ratio.

*Case: low bone mineral density (t-score < -1.0).

Model 1: adjusted for age (years).
Model 2: model 1 further adjusted for current smoking status, heavy alcohol consumption (> 7 and > 14 drinks/week for women and men respectively), physical inactivity (< 5,000 steps/day), supplement/medication for bone health, hormone therapy (women only), and body mass index (kg/m²).
Model 3: model 2 further adjusted for muscular strength (grip strength, kg).

In addition to linear regression with t-score as the dependable variable, linear regression with whole-body BMD (g/cm²) was also conducted to investigate independent associations of MS and CRF with BMD, which is the original source data of the t-score computation (i.e., the raw BMD data not standardized to the “young, white female” reference value (Table 8)). There
was not a significant association of MS with whole-body BMD in women (p>0.05) after adjusting for potential confounders including CRF for MS and MS for CRF; however, there was a significant association in men (p<0.05) similar to the result of t-score in men (Table 4). This result indicates that every 1 kg increase in MS was associated with 0.003 g/cm² increase in whole-body BMD in men. CRF was not significantly associated with whole-body BMD in both women and men (all p>0.05) similar to the results of t-score in both women and men (Table 6).

Table 8. Association of muscular strength and cardiorespiratory fitness with whole-body bone mineral density: multivariable linear regression

<table>
<thead>
<tr>
<th></th>
<th>β (SE)</th>
<th>STD β</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscular strength</td>
<td>0.001 (0.001)</td>
<td>0.09</td>
<td>0.267</td>
</tr>
<tr>
<td>Cardiorespiratory fitness</td>
<td>-0.010 (0.010)</td>
<td>-0.06</td>
<td>0.524</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscular strength</td>
<td>0.003 (0.001)</td>
<td>0.25</td>
<td>0.003</td>
</tr>
<tr>
<td>Cardiorespiratory fitness</td>
<td>-0.001 (0.020)</td>
<td>-0.004</td>
<td>0.971</td>
</tr>
</tbody>
</table>

SE, standard error; STD, standardized.
Bone mineral density (g/cm²).
^Grip strength (kg).
^Minutes to complete 400-meter walk test.
Model: adjusted for age, current smoking status, heavy alcohol consumption (> 7 and 14 drinks/week for women and men respectively), physical inactivity (< 5,000 steps/day), supplement/medication for bone health, hormone therapy (women only), and body mass index (kg/m²).

Joint analyses were used to investigate the combined association of MS and CRF with low BMD (Figure 7). In women, compared to the weak & unfit (reference) group, the weak & fit, strong & unfit, and strong & fit groups showed ORs (95% CIs) of 0.48 (0.16-1.49), 0.53 (0.18-1.59), and 0.44 (0.17-1.13), respectively, after adjusting for potential confounders.

Although not significant, this result suggests a possible additive association of MS and CRF with low BMD with the lowest OR of 0.44 in the strong and fit group. In men, compared to the weak & unfit (reference) group, the ORs (95% CIs) for the weak & fit, strong & unfit, and strong & fit groups were 7.90 (0.60-104.81), 1.50 (0.08-29.45), and 1.17 (0.08-16.34), respectively, after adjusting for potential confounders. However, this unexpected result may possibly be related to
the very limited number of low BMD in men in most groups (i.e., n=3, n=1, and n=2 cases in weak & fit, strong & unfit, and strong & fit group, respectively).

Figure 7. Odds Ratios of Low Bone Mineral Density by Combined Muscular Strength and Cardiorespiratory Fitness in Women (above) and Men (below).

Odds ratios (95% confidence intervals) of low bone mineral density by muscular strength and cardiorespiratory fitness joint analysis. Low bone mineral density is defined as t-score below -1.0. Muscular strength is grip strength (kg). Cardiorespiratory fitness (CRF) is the time to complete 400-meter walk test (minutes). Participants were assigned to one of “weak and unfit”, “weak and fit”, “strong and unfit”, and “strong and fit” group, where weak and unfit were the lower 1/3 of MS and CRF, respectively, based on its independent associations with BMD (Tables 5 and 7).

The model adjusted for age, current smoking status, heavy alcohol consumption (> 7 and > 14 drinks/week for women and men respectively), physical inactivity (< 5,000 steps/day), supplement/medication for bone health, hormone therapy (women only), and body mass index (kg/m²).
Exploratory Analyses

The logistic regression was repeated using a different t-score cut-point that defines low BMD (t-score < -0.5, instead of t-score < -1.0). This prevented an underestimation of low BMD by the whole-body DXA scan, also increased the case numbers for low BMD from 90 (48%) to 121 (64%) in women and from 7 (4%) to 11 (7%) in men. When the alternative cut-point was used, compared to the lower MS tertile, ORs (95% CIs) of having low BMD for the middle and upper MS tertiles were 1.13 (0.51-2.53) and 0.77 (0.34-1.78) in women. In men, the ORs for the middle and upper MS tertiles were 0.54 (0.12-2.41) and 0.37 (0.06-2.19). For the association of CRF with low BMD, ORs for middle and upper CRF in women were 0.83 (0.34-1.99) and 0.97 (0.37-2.51). In men, having higher CRF seemed to start turning inversely related to the prevalence of low BMD showing ORs of 0.69 (0.11-4.24) for middle CRF, although ORs was still positively associated with upper CRF (1.93 [0.35-10.59]). However, none of the results in women or men were significant (all p>0.05).

With a sample of 235 older adults (134 women and 101 men) who came to the NWRC for the study between fall 2015 and spring 2017, the associations of different MS measurements (grip strength, 1-RM chest press, and 1-RM leg press) with whole-body and regional (arm, pelvis, leg, and lumbar) BMD were examined to see if there is a stronger or weaker association by different MS measurements (Table 9). Overall, MS showed mostly positive associations with whole-body and regional BMD in both women and men. Only lower-body strength (1-RM leg press) was associated with whole-body BMD in both women and men (p<0.05). With arm BMD, lower-body strength was most strongly associated in women and men (p<0.05). In the pelvic region, lower-body strength was associated with pelvis BMD in women (p<0.05), but in men, grip strength was positively associated with pelvis BMD (p<0.05). In the leg and lumbar regions,
all the MS measurements showed positive, but non-significant, associations with BMD in women, (all \( p>0.05 \)). In men, lower-body strength showed the strongest associations with leg and lumbar BMD (all \( p<0.05 \)).

**Table 9.** Associations of whole-body and different regional (arm, pelvis, leg, and lumbar) bone mineral density with different muscular strength measurements (grip strength, 1-repetition maximum chest press, and 1-repetition maximum leg press), independent of cardiorespiratory fitness

<table>
<thead>
<tr>
<th></th>
<th>Whole-body BMD</th>
<th>Arm BMD</th>
<th>Pelvis BMD</th>
<th>Leg BMD</th>
<th>Lumbar BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ) (SE)</td>
<td>STD ( \beta ) (P value)</td>
<td>( \beta ) (SE)</td>
<td>STD ( \beta ) (P value)</td>
<td>( \beta ) (SE)</td>
</tr>
<tr>
<td>Grip strength (n=133)</td>
<td>0.001 (0.002)</td>
<td>0.08 (0.41)</td>
<td>0.04 (0.002)</td>
<td>0.24 (0.01)</td>
<td>0.004 (0.002)</td>
</tr>
<tr>
<td>1-RM CP (n=121)</td>
<td>0.001 (0.002)</td>
<td>0.05 (0.69)</td>
<td>0.04 (0.001)</td>
<td>0.35 (0.01)</td>
<td>0.003 (0.002)</td>
</tr>
<tr>
<td>1-RM LP (n=123)</td>
<td>0.001 (0.001)</td>
<td>0.25 (0.04)</td>
<td>0.001 (&lt;0.001)</td>
<td>0.36 (0.01)</td>
<td>0.002 (0.001)</td>
</tr>
<tr>
<td>Grip strength (n=99)</td>
<td>0.003 (0.001)</td>
<td>0.20 (0.07)</td>
<td>0.003 (0.001)</td>
<td>0.26 (0.02)</td>
<td>0.004 (0.002)</td>
</tr>
<tr>
<td>1-RM CP (n=93)</td>
<td>0.001 (0.001)</td>
<td>0.10 (0.39)</td>
<td>0.001 (0.001)</td>
<td>0.17 (0.14)</td>
<td>&lt;0.001 (0.001)</td>
</tr>
<tr>
<td>1-RM LP (n=95)</td>
<td>0.001 (&lt;0.001)</td>
<td>0.31 (&lt;0.001)</td>
<td>0.001 (0.001)</td>
<td>0.34 (0.01)</td>
<td>0.001 (0.001)</td>
</tr>
</tbody>
</table>

BMD, bone mineral density (g/cm\(^2\)); CP, chest press (kg); LP, leg press (kg); RM, repetition maximum; SE, standard error; STD, standardized.

Grip strength (kg).

Bone mineral density was calculated by dual-energy X-ray absorptiometry.

Model: adjusted for age, current smoking status, heavy alcohol consumption (> 7 and 14 drinks/week for women and men respectively), physical inactivity (< 5,000 steps/day), supplement/medication for bone health, hormone therapy (women only), and body mass index (kg/m\(^2\)), and cardiorespiratory fitness (minutes to complete 400-meter walk test).

To examine multicollinearity, correlations between the main exposure variables and covariates were explored. The strongest correlation was found between CRF (minutes to complete 400-meter walk test) and BMI (\( r=0.48 \) [p<0.001] and \( r=0.44 \) [p<0.001] in women and men), respectively, which is consistent with the results from Table 1. In women, correlations between MS and CRF, between CRF and physical activity, and between BMI and physical
activity were significant ($r=-0.29$-$0.27$ [all $p<0.001$]). In men, correlations between MS and CRF, between CRF and physical activity, between BMI and physical activity, and between BMI and smoking status were significant ($r=-0.23$-$0.18$ [all $p<0.05$]). All other variables showed only weak and not significant correlations ($r=-0.13$-$0.14$ in women [all $p>0.05$] and $-0.13$-$0.12$ in men [all $p>0.05$]). Variance inflation factors were below 5 for all covariates in both women and men, indicating minimal multicollinearity.

To examine the effect of artificial joints on the associations of MS and CRF with BMD, the interaction test was conducted, in which participants who were excluded because of artificial joints (n=84) were added back to the sample. In linear regression including all the covariates, artificial joint status was a significant predictor of higher t-scores ($\beta=2.42$, $p<0.01$) in women, but the interaction between MS and artificial joints on t-scores was not significant ($p=0.67$). In men, there was a significant interaction between MS and artificial joint status on t-scores ($p=0.01$). There was no interaction between CRF and artificial joints on t-scores in women and men (all $p>0.05$). In logistic regression with all the covariates, the interactions of artificial joints with MS or CRF on low BMD were not found in both women and men (all $p>0.05$).
CHAPTER 5
DISCUSSION

There were three major findings in this study. First, higher MS measured by grip strength was significantly associated with higher t-score after adjusting for potential confounders including CRF in men (p=0.003), but not in women (p=0.267), from multivariable linear regression (Table 4). Although not significant, logistic regression also showed similar trends suggesting lower odds of having low BMD in the middle and upper MS tertiles compared to the lower MS tertile in both women and men (Table 5). Second, CRF measured by the 400-meter walk test was not associated with t-score in both women and men from multivariable linear regression (Table 6). However, although not significant, we found lower odds of having low BMD in the middle and upper CRF tertiles compared to the lower CRF tertile in women, but not men, from logistic regression (Table 7). Lastly, the combined category of having higher MS and higher CRF appeared to be associated with the lowest odds of having low BMD, compared to other categories such as weak and fit, strong and unfit, and weak and unfit in women, but not in men possibly due to the limited number of low BMD in men from the joint analyses (Figure 7).

In the linear regression analyses, the positive association between MS and t-score was found more consistently in men in all three models (Models 1, 2, and 3) after adjusting for all possible confounders including CRF (Table 4). The association was found in women when adjusting for age only (Model 1), but no longer significant after adjusting for other potential confounders including CRF in Models 2 and 3. We found similar trends in BMD instead of t-score with a significant association between MS and BMD in men (p=0.003), but not in women (p=0.267) from multivariable linear regression (Table 8). The results align with previous findings.
demonstrating that improving or having higher MS is associated with higher BMD in both women (Blain et al., 2001; Huovinen et al., 2016; Marques et al., 2013; Zhou et al., 2013) and men (Kukuljan et al., 2011; Marques et al., 2013).

There are some potential reasons why a significant association between MS and low BMD was not found in women. One is not adjusting for years since menopause in our study. Ravn et al. (1994) pointed out that years since menopause is the strongest factor that influences bone loss within 10-15 years since menopause. It is often considered in bone-related studies (Bayramoğlu et al., 2005; Zhou et al., 2013). However, the information of years since menopause was not collected in our study. Another potential explanation is the whole-body DXA scan, which is not commonly used for diagnosing osteopenia or osteoporosis. Whole-body DXA scans are affected by BMD from the head region. After menopause, women’s head BMD also decreases like BMD in other regions (Looker et al., 2013). However, the degree of BMD decrease in the head is less than other regional BMD. Head BMD remains high even after menopause due to the greater proportion of cortical (hard) bone in the skull while other regional BMD decreases to the half level of head BMD (Yao et al., 2001). Because of head BMD that remains high, the association of MS with low whole-body BMD could be obscured, specifically in post-menopausal older women.

In the linear regression analyses, no significant association of CRF with BMD was found in both women and men (all p>0.05). In the logistic regression analyses, an inverse association of CRF with BMD was found in men. However, the association was not significant and the relationships should be interpreted with caution due to the small number of cases. In women, having higher CRF appeared to reduce the odds of having low BMD although not significant. These results were not consistent with previous findings that having higher CRF is associated
with the lower odds of having low BMD (DeFina et al., 2016; Wainstein et al., 2016).

There are some potential explanations for the lack of significant findings between CRF and BMD. As previously mentioned, missing an influential covariate (i.e., years since menopause) and using the whole-body DXA scan could have influenced our results. Another possible explanation is the method for measuring CRF, the 400-meter walk test. For the sake of safety, time, cost efficiency, and familiarity with walking on the ground rather than on a treadmill, the 400-meter walk test (or 6-minute walk test) is routinely used when measuring CRF in older adults. Correlations between the results of the 400-meter walk test (minutes to complete 400-meter walking) and maximal treadmill test (VO\textsubscript{2max}) were found to be moderate to strong in previous studies (r= -0.56 - -0.79) (Gabriel et al., 2010; Simonsick et al., 2006). Despite the correlations, the walking test may not accurately reflect CRF as the maximal treadmill test does, which could be a possible explanation of no significant finding in CRF with t-score and BMD. Previous studies that found 68% (OR=0.32, 95% CI: 0.21-0.51) and 57% (OR=0.43, 95% CI: 0.29-0.65) reduced odds of having low BMD in upper CRF tertile employed the maximal treadmill test (DeFina et al., 2016; Wainstein et al., 2016). These results suggest that walking tests might need to be carefully considered when investigating the relationship between CRF and bone health in older adults. Considering that the 400-meter walk test is less likely to have the participants exert their maximal aerobic capacity within 4.6 minutes (the average minutes to complete the test in women as shown in Table 2), it could be more appropriate to think that the 400-meter walk test is possibly more related to functionality of lower extremity. Additionally, considering that participants can control their walking speed during the test unlike the maximal treadmill test, the 400-meter walking test records could reflect this variability in preferred walking speed, even though participants are told to “walk as fast as possible.” Another possible
explanation is the distribution of minutes to complete 400-meter walking in our cohort. About ninety percent of our participants’ 400-meter walk times fell within the lower half (faster walking times) of the 400-meter walk test times of a larger cohort of 3,075 older adults in the Health Aging and Body Composition Study (Newman et al., 2006) indicating that our participants may be fitter than other older adults. Also, because of the narrow distribution of 400-meter walking records, a clear association of CRF with low BMD might not have been found. Conversely, grip strength values in our sample were relatively widely distributed compared to a large cohort of 1,947 older adults (Anstey et al., 2001).

The most unique component of our study is the investigation of the combined association of MS and CRF with BMD using the joint analysis. Men showed increased OR of having low BMD in all the groups (ORs= 7.90, 1.50, and 1.17 in weak & fit, strong & unfit, and strong & fit, respectively) compared to weak and unfit group (reference), which was unexpected. However, as mentioned earlier, the results are difficult to interpret due to the small numbers of case (low BMD) in men since BMD is higher in men than women. In women, even though the result was not significant, being strong and fit appeared to be associated with a lower prevalence of low BMD, showing the lowest OR of having low BMD found in the strong & fit group (ORs=0.44, 0.53, and 0.48 in strong & fit, strong & unfit, and weak & fit groups, respectively), consistent with the results from logistic regression by MS and CRF.

In an exploratory analysis, we compared the associations between different MS measurements (grip strength, 1-RM chest press, and 1-RM leg press) and whole-body and different regional (arm, pelvis, leg, and lumbar) BMD, trying to find which MS measurement is more strongly related to whole-body or regional BMD. It was found that lower-body strength (measured by 1-RM leg press) was more strongly related to whole-body BMD and regional
BMD in both women and men in general. Grip strength was related to arm BMD in women and all the regional (arm, pelvis, leg, and lumbar) BMD in men, which is consistent with previous studies that have shown that grip strength is moderately associated with regional BMD (Di Monaco et al., 2000; Kim et al., 2012). The correlations between grip strength and 1-RM chest press and between grip strength and 1-RM leg press were 0.44 and 0.28 in women and 0.34 and 0.33 in men after adjusting for age (all p<0.01).

Overall, in women and men, regional MS seemed to be associated with regional BMD. For example, arm BMD was significantly related to grip strength in women and men, pelvis BMD was related to 1-RM leg press in women, and 1-RM leg press was related to leg BMD and lumbar BMD in men.

In our study, there were some limitations. First, our cohort is comprised of mostly white, highly-educated, independently living, and generally healthier and fitter older adults. This confirms that people who actively volunteer to participate in health-related research studies tend to be healthier than those who do not actively participate. Data derived solely from these healthier individuals would not necessarily reflect the general population. To increase the external validity of PAAS, we recently conducted on-site data collection in the community at locations such as retiree clubs. However, the sample size from those sites was still quite small (n=17) in these analyses. Second, we conducted the whole-body DXA scan that is not usually done for diagnoses of osteopenia and osteoporosis. The whole-body DXA scan is known to underestimate the prevalence of low BMD (Graat-Verboom et al., 2010; Rajaei et al., 2016). To overcome this limitation in an exploratory analysis, we used an alternative t-score of -0.5, instead of -1.0, that was reported to show a sensitivity of 90% and a specificity of 91% for detecting low BMD by regional DXA (e.g., femoral neck) scans (Rajaei et al., 2016). However, in men, the
scarcity of low BMD cases was still present. Also, in both women and men, the statistical results with the alternative cut-point did not change substantially (all p-trends>0.5). Third, the 400-meter walk test that measured CRF may not have been the optimal method for investigating bone health in older adults. The 400-meter walking time in our sample is relatively fast and that previous studies that found the expected results used maximal treadmill test (DeFina et al., 2016; Wainstein et al., 2016).

In conclusion, having higher MS was associated with higher t-score after adjusting for potential confounders including CRF in men. The positive association of MS with t-score appeared to exist in women although not significant after further adjustment for potential confounders including CRF. In both women and men, although not significant, having higher MS or higher CRF appeared to reduce odds of having low BMD, independent of each other from logistic regression, which is consistent with the findings for t-score from multivariable linear regression. The combined association of having higher MS and higher CRF appeared to be more strongly associated with the lower odds of having low BMD in women than men. Our study suggests that higher levels of MS and CRF may be associated with better bone health in older adults although most results did not reach statistical significance in this relatively healthy and fit population. While we observed several expected findings, such as positive directional associations between MS and BMD in women and men, these results will have to be replicated and confirmed in larger samples representing general older adult population using gold-standard measurements of MS (e.g., isokinetic dynamometry), CRF (e.g., direct VO₂max measurement), and BMD (e.g., femoral neck scan).
REFERENCES


APPENDIX: IRB APPROVAL

Date: 08/03/2018
To: Duck-Chul Lee, PhD
From: Office for Responsible Research
Title: Physical Activity and Aging Study (PAAS)
IRB ID: 15-430
Submission Type: Continuing Review & Modification Review Type: Expedited
Approval Date: 08/03/2018 Date for Continuing Review: 08/09/2020

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University according to the dates shown above. Please refer to the IRB ID number shown above in all correspondence regarding this study.

To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- Use only the approved study materials in your research, including the recruitment materials and informed consent documents that have the IRB approval stamp.

- Retain signed informed consent documents for 3 years after the close of the study, when documented consent is required.

- Obtain IRB approval prior to implementing any changes to the study.

- Inform the IRB if the Principal Investigator and/or Supervising Investigator end their role or involvement with the project with sufficient time to allow an alternate PI/Supervising Investigator to assume oversight responsibility. Projects must have an eligible PI to remain open.

- Immediately inform the IRB of (1) all serious and/or unexpected adverse experiences involving risks to subjects or others; and (2) any other unanticipated problems involving risks to subjects or others.

- Stop all human subjects research activity if IRB approval lapses, unless continuation is necessary to prevent harm to research participants. Human subjects research activity can resume once IRB approval is re-established.

- Submit an application for Continuing Review at least three to four weeks prior to the date for continuing review as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.

IRB 09/2018
• Please be aware that IRB approval means that you have met the requirements of federal regulations and ISU policies governing human subjects research. Approval from other entities may also be needed. For example, access to data from private records (e.g., student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. IRB approval in no way implies or guarantees that permission from these other entities will be granted.

• Please be advised that your research study may be subject to post-approval monitoring by Iowa State University’s Office for Responsible Research. In some cases, it may also be subject to formal audit or inspection by federal agencies and study sponsors.

• Upon completion of the project, transfer of IRB oversight to another IRB, or departure of the PI and/or Supervising Investigator, please initiate a Project Closure to officially close the project. For information on instances when a study may be closed, please refer to the IRB Study Closure Policy.

Please don’t hesitate to contact us if you have questions or concerns at 515-294-4566 or IRB@iastate.edu.