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Relationship Between Total Body Mass, Fat-Free Mass, Fat Mass, and Bone Mineral Density of the Hip In Middle-Age Women: The Roles of Diet, Physical Activity, and Menopause

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ABSTRACT

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Objective: This study was conducted to investigate the relationship between hip bone mineral density (BMD), fat-free mass (FFM), fat mass (FM), and total body mass (TBM) and the extent to which these relationships were modified by various confounding factors. The cross-sectional analysis included 262 healthy females (mean age 41.6±3.0 years). ***Methods:*** BMD of the hip and body composition were assessed by the Hologic 4500W dual energy x-ray absorptiometry (DXA) system. Total and intensity of physical activity (PA) were objectively measured using an Actigraph accelerometer. Dietary calcium and vitamin D from food and beverages, as well as from supplements, were measured separately using the Block food frequency questionnaire. Menopause status and prescription bone drug use were measured by a questionnaire. ***Results:*** The relationship between FFM and hip BMD was strong and robust ($F=24.5$, $P<0.0001$). Using the pooled standard deviation revealed a large effect size of 1.2 when comparing hip BMD of women with low FFM and high FFM. Potentially confounding variables, considered individually and collectively, did not change this relationship. The association between FM and hip BMD was also substantial ($F=9.9$, $P<0.0001$) and remained significant when controlling for all potentially confounding variables, except differences in FFM. The relationship between TBM and hip BMD was also strong and dose-response ($F=21.5$, $P<0.0001$) and remained significant, except when differences in FFM were controlled. ***Conclusion:*** The relationships between body mass (total, fat, and fat-free) and BMD of the hip in middle-age women are strong and significant. The associations are not influenced by differences in age, height, menopause status, calcium or vitamin D intake, volume or intensity of PA, or the use of bone enhancing prescription drugs. The findings suggest that women with low body mass, particularly low FFM, tend to have low hip BMD and there is little that can be done to change this association.

Keywords: osteoporosis, body composition, cross-sectional, DXA, calcium, vitamin D, premenopausal

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INTRODUCTION

Osteoporosis is a skeletal disease characterized by extremely low bone mineral density accompanied by increased fracture risk (1). In osteoporosis, bone tissue is lost which makes bones porous, fragile, and brittle and much more likely to fracture (2). Osteopenia is a less severe type of bone loss where individuals have lower bone mineral density than normal, but do not have osteoporosis (3). Osteoporosis influences millions of people each year and rates continue to increase, causing an economic burden of nearly $20 billion (4). The incidence of fractures related to osteoporosis is at an all-time high throughout the world with current osteoporosis estimates at roughly 200 million, including approximately 44 million estimated cases in the United States alone (5).

Some studies show that bone mineral density is the single greatest predictor of future fracture risk, suggesting that maximizing bone density is a key for osteoporosis prevention (6). Bone mineral density (BMD) increases in response to stresses placed on the bones (7). Body mass is one such stressor that seems to have a positive influence on BMD, according to the literature (8, 9). In short, excess body mass puts increased stress on bones which tends to increase their strength. Those who have a low body mass tend to have lower BMD, particularly in women (10).

Individuals with higher risk of developing osteoporosis and osteopenia include those who have low body mass, less fat-free mass (FFM), the elderly, and postmenopausal women (11). Among these characteristics, body composition plays a key role in BMD status, with FFM being the most predictive of BMD levels (12-14). Recent research shows a strong and robust relationship between FFM and BMD and a less clear relationship between fat mass (FM) and

BMD. Apparently, an elevated body mass may not be as protective against osteoporosis as previously proposed, if that body mass is comprised of a large percentage of body fat (15-17).

Other factors that are predictive of BMD include age (18-21), physical activity (22-26), calcium and vitamin D intake (27-32), menopause status (33, 34), and the use of bone enhancing prescription drugs (35-38). The extent to which these factors influence the strong link between FFM and BMD has not been studied. Yet, these are critical questions. If individuals have low body mass, or particularly low FFM, are they destined to have low BMD? Are the strong associations between total body mass and FFM, as they relate to BMD, unchangeable? Can calcium and vitamin D intake, physical activity levels, menopause status, or other factors influence the association between FFM and BMD? To date, research has neglected to address these important questions.

The objective of the present study was to investigate the relationships between total body mass, FFM, fat-free mass minus bone mineral content (FFM-BMC), FM, and BMD of the hip and the extent to which these associations were influenced by the following potentially confounding variables: age, height, objectively measured total, vigorous, and moderate physical activity, calcium and vitamin D intake from food, beverages, and supplements, menopause status, and bone enhancing prescription drug use, studied individually and collectively. In short, if any of these variables was found to significantly influence the relationship between body mass and BMD, then osteoporosis prevention efforts could focus on that factor.

METHODS

Subjects

A cross-sectional design was employed and recruitment of participants was accomplished through newspaper advertisements in approximately 20 different cities throughout the Mountain

West region of the US. The final sample for this study included 262 women who varied in their menopause status, and had a mean age of 41.6±3.0 years. Subjects were free from chronic diseases as assessed by the Physical Activity Readiness Questionnaire (PAR-Q). Sample characteristics included the following: non-smokers, 90% non-Hispanic White, 80% married, and 37% had some college education. The study was approved by the Institutional Review Board at Brigham Young University. All subjects signed an informed consent document before participating in the study.

Procedures

Measurements were taken at the university Human Performance Research Center by trained research assistants during two separate appointments spaced by one week. Participants were asked to fast three hours prior to their arrival for the first appointment, however water intake was encouraged. Each participant changed into a form-fitting, one-piece lab-issued swimsuit to wear for the weighing and the DXA scan, and was instructed to eliminate any body waste before measurements were taken. Using a calibrated electronic scale (Tanita, Tokyo, Japan), subjects were weighed to the nearest 0.05 kg. After this, each subject had a total body scan using a Hologic 4500W DXA system (Hologic, Bedford, MA) to measure bone mineral density and body composition.

Upon completion of the scan, subjects were given two questionnaires: the Block food frequency questionnaire (Nutrition Quest, Berkeley, CA) and a questionnaire designed to assess menopause status and bone enhancing prescription drug use. Before leaving the laboratory, subjects were issued an Actigraph model 7164 accelerometer (Health One Technology, Fort Walton Beach, FL) which recorded the volume and intensity of physical activity over the

following seven days. Subjects received detailed verbal and written instructions regarding how to properly wear and use the accelerometer.

One week later, subjects returned to the lab with their completed questionnaires and the accelerometer. Data from the accelerometer were downloaded and checked for errors. If there were any errors on the questionnaire or accelerometer, subjects were contacted and the appropriate corrections were made.

Instrumentation and Measurements

In the present study, bone mineral density of the hip was the criterion variable. Various measurements of body mass were employed as predictor variables. Age, height, total and intensity of physical activity, dietary and supplemental intake of calcium and vitamin D, menopause status, and the use of bone enhancing prescription drugs were studied as potential mediating variables.

Bone Mineral Density

Bone mineral density (BMD) was measured using dual energy x-ray absorptiometry (DXA), with a Hologic QDR 4500W bone densitometer (Hologic, Bedford, MA). This instrument is considered a reliable and valid measurement of bone mineral density, as well as a safe measurement tool that exposes individuals to minimal amounts of radiation (39-41).

In a cross-sectional study involving 210 postmenopausal women, the Hologic QDR 4500 was used to assess bone mineral density (42). Researchers found that precision error was only 2% for the lumbar spine, 1.8% for the femoral neck, and 1.5% for whole body bone mineral density (42). Others have found precision error of the QDR 4500 to be less than 1% (43).

The 4500W was calibrated at the beginning of each testing day. To confirm accuracy, laser light cross-hairs emitted by the 4500W enabled the licensed technician to consistently

position subjects beneath the same spot of the scanning arm. Scans of the left hip were made after ensuring that the laser cross-hairs bisected the thigh at a position directly even with the pubic bone. To minimize the view of the lesser trochanter and femoral shaft, the subject's left leg was internally rotated and slightly abducted and then attached to a positioning aide.

Body Composition

The DXA screening included body composition results as an additional assessment. Specifically, fat-free mass (FFM), fat mass (FM), and fat-free mass minus bone mineral content (FFM-BMC) were analyzed. Total body mass included both FFM and FM. FM was the portion of total body mass that only included fat tissue mass. BMD was a measure of BMC per unit of volume of bone. FFM was defined as all components that give mass to the body, excluding fat tissue, including skeletal and muscle tissue, bone, and water. Because FFM includes bone mass, and because BMD is partly a function of bone mass, it follows that FFM and BMD tend to be correlated. To remove the direct effect of bone mass on FFM in the present study, a new variable was created, FFM-BMC (FFM minus bone mineral content). In short, the variable FFM-BMC was comprised of all components of body mass minus fat mass and bone mass.

Body composition analysis performed by DXA is considered reliable and valid (44-46). Test-retest reliability of the instrument when measured on 100 subjects from the present study resulted in a high intraclass correlation ($r=0.999$) (47). When the DXA results were compared to Bod Pod results for the same 100 subjects, a Pearson correlation of 0.94 ($P<0.001$) and an intraclass correlation of 0.97 ($P<0.001$) were found (48).

Total and Intensity of Physical Activity

For seven consecutive days, each subject wore an Actigraph model 7164 accelerometer (Health One Technology, Fort Walton Beach, FL) to assess total and intensity of physical

activity. Actigraph accelerometers are considered a reliable and valid instrument to measure physical activity (49-55).

Correctly wearing the accelerometer consisted of placing it in a small, nylon pouch attached to a waist belt that was worn at the level of the umbilicus directly over the left hip. Participants were instructed to wear the accelerometer continuously during each day and night of the measurement period except when they were bathing or engaging in other water-related activities. Failure to wear the accelerometer as instructed resulted in the subject having to wear it again on the day(s) of the week they did not wear it properly. A failed day was defined as not wearing the accelerometer for two or more hours. For the analysis, a day of wear was defined as activity counts derived between the hours of 7 am to 11 pm. Mean wear time during that time (a 15 hr period) was 13.9 hrs (93% wear time compliance) over the seven days.

The 2007 American College of Sports Medicine (ACSM) and American Heart Association (AHA) physical activity recommendations indicate that accumulating 10-minute bouts of moderate intensity aerobic activity totaling at least 30 minutes daily is sufficient activity to improve health (56). Therefore, analysis time was divided into 10-minute bouts, or epochs, which resulted in a total of 144 epochs for each day, and 1008 epochs for the seven day period. Total physical activity (tPA) was calculated as the sum of all physical activity counts over the seven consecutive days. Intensity of physical activity (iPA) was calculated by analyzing the physical activity counts obtained during each 10-minute bout, and categories were created based on these totals used in other investigations (57-59). Sedentary activity included 10-minute bouts with <10,000 activity counts. Low intensity activity (slow walking) included 10-minute bouts with 10,000-30,000 counts. Moderate intensity activity (slow to fast walking) included epochs with 30,000-50,000 counts, and Vigorous intensity movement (fast walking to running) included

10-minute bouts with >50,000 counts. The number of minutes subjects engaged in each intensity level of activity, vigorous, moderate, low, and sedentary, was used to differentiate among subjects.

Dietary and Supplemental Intake of Calcium and Vitamin D

In the present study, subjects completed the Block food frequency questionnaire (Nutrition Quest, Berkeley, CA), originally developed by the National Cancer Institute. The instrument was employed in the present study to assess dietary calcium and supplemental calcium intake. Similarly, vitamin D derived from foods and beverages, along with supplemental vitamin D, were measured using the Block questionnaire.

The Block food frequency questionnaire is eight pages in length and includes questions about serving size and serving frequency for more than 100 different foods. Additionally, the instrument includes questions about dietary supplement use. A full-page illustration, showing various common portion sizes, was given to each subject so that portion sizes could be more easily interpreted. The questionnaire relies on national dietary data, including data from the National Health and Nutrition Examination Survey (NHANES), and it is considered to be a valid and reliable instrument for assessing dietary intake (60, 61).

Menopause and Bone Enhancing Prescription Drugs

To assess other possible confounding variables in the study, each subject completed a questionnaire about menopause status and bone enhancing prescription drug use. Six questions were asked regarding menopause status specifically ascertaining symptoms of menopause, characteristics and duration of the menstrual cycle, time since the last menstrual cycle, and other related factors. According to their answers, subjects were labeled as premenopausal, perimenopausal, postmenopausal, or hysterectomy. Blood test results from a sample of 198

women in the study were used to validate the questionnaire. The follicle-stimulating hormone blood test results, which are indicative of ovarian function, were highly correlated with menopause status ($F=52.3$, $r^2=0.45$, $P<0.0001$)(62). The questionnaire also included questions regarding any physician-prescribed bone enhancing drugs used to enhance bone mineral density during the previous 10 years from that point in time.

Data Analysis

A power analysis indicated that 246 subjects were needed to detect a small effect size with 0.80 power with subjects divided into three groups and alpha set at 0.05. Using 262 participants, statistical power was 0.90 to detect a small linear association between body mass and BMD with alpha set at 0.05.

To differentiate among levels of hip bone mineral density (BMD), subjects were divided into quartiles and the two middle categories were collapsed to form three BMD categories (Low, Moderate, High). Mean levels of total body mass (TBM), fat-free mass (FFM), fat-free mass minus bone mineral content (FFM-BMC), and fat mass (FM) were compared across the three BMD categories, with and without control of the potentially confounding variables, which included: age, height, total and intensity of physical activity, dietary and supplemental intake of calcium and vitamin D, menopause status, and the use of bone enhancing prescription drugs. Means were adjusted for differences in the potential confounding variables and compared across the BMD groups using partial correlation and least squared means. Additionally, multiple regression analysis was employed to estimate BMD using TBM, FFM, FFM-BMC, and FM, considered separately as predictors. Potential confounders were controlled statistically and regression coefficients (*b*) were adjusted according to the influence of the covariates, considered individually and collectively. Statistical significance was determined with alpha set at the 0.05

level. Data analysis for this study was conducted using SAS, version 9.3 (SAS Institute, Inc., Cary, NC, 2010).

RESULTS

Descriptive characteristics of the 262 females included in the analyses are given in Table 1. Additional findings indicate that 191 of the women (73%) were premenopausal, 34 (13%) were perimenopausal, 15 (6%) were postmenopausal, and 22 (8%) had a hysterectomy. A total of 22 (8%) of the subjects reported taking physician-prescribed bone enhancing drugs in the previous 10 years. Mean total body mass (TBM), fat-free mass (FFM), and fat mass (FM) were 65.9±10.8 kg, 44.1±5.2 kg, and 21.8±7.7 kg, respectively. Mean bone mineral density (BMD) was 0.934 ± 0.112 g/cm^2.

As shown in Table 2, mean differences in hip BMD across low, moderate, and high FFM categories indicated a strong and significant relationship ($F=24.5$, $P<0.0001$). When using the pooled standard deviation, there was a large effect size of 1.2 when comparing hip BMD of women with low FFM and high FFM. After controlling for each potentially confounding variable individually, the relationship remained significant in all instances. Potentially confounding variables considered collectively in two separate models were also controlled and did not change the relationship.

In Table 3, the relationship between hip BMD across low, moderate, and high FM categories is displayed. The results indicated that this relationship was both strong and significant as well ($F=9.9$, $P<0.0001$). Controlling for the potentially confounding variables, including age, height, calcium and vitamin D intake, volume and intensity of physical activity, menopause status, and prescription drug use for bones, did not change the significance of the relationship. However, the relationship between hip BMD across the three FM categories was

not significant after controlling for FFM (F=2.1, P=0.1213) or Full Model 1, which included FFM (F=1.0, P=0.3692). After adjusting for differences in TBM, the relationship remained statistically significant, albeit not as strong as the other associations (F=3.6, P=0.0287).

According to Table 4, mean differences in hip BMD across low, moderate, and high TBM categories revealed a significant and dose-response association, with no potential confounders controlled statistically (F=21.5, P<0.0001), similar to the other relationships shown in Tables 2 and 3. After controlling for each of the potentially confounding variables separately, only one of the associations became non-significant. Specifically, adjusting for differences in FFM weakened the association by 94% (F=1.3, P=0.2693). Similarly, adjusting for differences in the Full Model 1 weakened the hip BMD and TBM association to the point of non-significance, because the model included FFM (F=0.3, P=0.7188). However, adjusting for all the potential confounders except FFM left the association strong and dose-response (F=15.3, P<0.0001).

Additional findings in Table 5 show the relationships between hip BMD and FFM, FFM-BMC, FM, or TBM, with and without control of potentially confounding variables. Analyses performed using regression coefficients (*b*) reflect differences in hip BMD for each 1 kg difference in the body mass variables treated separately (FFM, FFM-BMC, FM, and TBM). Without control of the potentially confounding variables, a highly significant relationship was unveiled between BMD and each body mass variable (P<0.0001). For every 1 kg difference in FFM, FFM-BMC, FM, or TBM, BMD differed by 0.0100, 0.0101, 0.0036, and 0.0042 g/cm^2, respectively. After controlling for each of the potentially confounding variables, the relationship between BMD and body mass (FFM, FFM-BMC, FM, and TBM) remained highly significant in almost every instance (P<0.0001). However, after controlling for FFM, the relationship between BMD and FM, and BMD and TBM, were not statistically significant (P=0.1316). Also, after

controlling for every variable in the Full Model, which included either FFM or FM depending on the association evaluated, the relationships between BMD and FM, and BMD and TBM, were not significant (P=0.4109).

DISCUSSION

The key finding of the present study was that fat-free mass (FFM) is a strong and robust predictor of hip BMD in middle-aged women, and this association is not modified or influenced by any of a large number of potentially confounding factors, considered individually or in combination. Evidently, women with low FFM tend to have low hip BMD, and it does not matter whether or not they are older or younger, taller or shorter, premenopausal or postmenopausal, physically active or inactive, or whether they consume significant levels of calcium and vitamin D or not. In short, it appears that FFM is the driving factor that predicts hip BMD, independent of demographics, lifestyle, and other factors.

Results indicate that total body mass (TBM) and fat mass (FM) were also strong predictors of hip BMD in the present sample. Similar to the FFM relationship, the TBM and FM connections with hip BMD do not appear to be strengthened or weakened by age, height, menopause status, physical activity, diet, prescription drug use, or other factors. However, the associations between hip BMD, TBM and FM seem to be eliminated when differences in FFM are controlled. Hence, when it comes to hip BMD, FFM seems to be the key.

Because the present study employed a cross-sectional design, cause-and-effect cannot be inferred. However, if a causal relationship is assumed, then women with low FFM are at risk of developing low hip BMD, and from the results of this study, it appears that there is little that women can do to alter this unhealthy connection, other than increase their body mass, particularly their FFM. Increasing FM may also help to increase hip BMD, although probably

not as much as FFM, but this would be ill-advised because of the many health problems associated with obesity.

As presented in the literature, there are a number of studies that have examined the relationship between FFM and BMD. This study supports the hypothesis that BMD is largely a function of individual FFM (12-14), as well as TBM (8, 9) and FM (15-17). However, to date, very few if any investigations have examined the extent to which the body mass and BMD relationship is modified by potential confounding factors.

The present study had many strengths. First, several high quality measurement methods were employed. Calcium and vitamin D intake were assessed using a validated food frequency questionnaire. Additionally, physical activity was measured objectively using accelerometry, whereas hip BMD and body fat percentage were evaluated using dual energy x-ray absorptiometry (DXA). Furthermore, many potential confounding variables were studied individually and collectively to determine the extent to which the body mass and hip BMD relationship is influenced by these factors, which has never been evaluated in the past. Lastly, statistical power was excellent in the present investigation, increasing the probability of detecting significant relationships when present.

The current investigation was not without weaknesses, however. The cross-sectional design prevented cause-and-effect conclusions. Also, participants were somewhat homogeneous, potentially limiting the generalizability of the results. Lastly, although physical activity was measured objectively, participation in strength training was not assessed, which could have influenced participant FFM and may have modified the relationship between FFM and BMD.

More research is needed in this area. Although difficult, lengthy randomized controlled trials or prospective cohort investigations could help to decipher the extent to which the strong

relationship between FFM and hip BMD can be modified by diet, exercise, or other factors. Additional research would be valuable focusing on the amount of FFM women need to prevent risk of low BMD at the hip. The present study indicates that the association is linear and there is no threshold effect. Lastly, examination of the relationship between FFM and BMD and the effect of strength training to build FFM in women without increasing FM could also be enlightening and lead to additional insights regarding improvement of hip BMD.

In conclusion, body mass, particularly FFM, seems to contribute significantly to hip BMD in middle-aged women. Those with high levels of FFM tend to have high levels of BMD, potentially reducing risk of osteopenia and osteoporosis. Conversely, women with low levels of body mass, particularly FFM, tend to have low levels of hip BMD, possibly increasing risk of osteopenia and osteoporosis. It appears that the body mass and BMD relationship is not influenced in any way by a number of factors, making it challenging for women with low FFM to avoid risk of low BMD.

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Table 1 Descriptive information for all participants (n=262)

Variables	MEAN	SD	MIN	25th percentile	MED	75th percentile	MAX
Age (yrs)	41.6	3.0	36.0	39.0	42.0	44.0	47.0
Height (cm)	166.4	6.9	143.5	162.3	166.1	170.4	189.2
BMD (g/cm^2)	0.934	0.112	0.659	0.855	0.932	1.010	1.354
Total Body Mass (kg)	65.9	10.8	42.7	58.2	64.6	73.0	96.4
Fat-Free Mass (kg)	44.1	5.2	32.4	40.3	44.2	47.4	63.5
Fat Mass (kg)	21.8	7.7	7.0	15.5	21.1	27.5	45.9
FFM-BMC	42.4	5.0	301.0	38.8	42.4	45.5	61.1
Dietary calcium (mg)	839.2	372.4	100.1	554.1	782.1	1072.8	2051.5
Suppl. calcium (mg)	326.7	419.0	0	0	92.9	714.5	1130.0
Total calcium (mg)	1165.9	553.0	230.0	709.1	1075.9	1526.4	3074.8
Dietary vitamin D (IU)	147.8	122.0	0.430	549.8	102.6	203.9	587.1
Suppl. vitamin D (IU)	141.3	165.7	0	0	0	285.8	400.0
Total vitamin D (IU)	289.1	218.8	0.430	93.7	255.4	448.6	987.1
Total PA (counts)	26.6	9.5	8.3	19.6	25.0	31.4	66.4
Vigorous PA (min)	32.7	64.2	0	0	0	40.0	420.0
Moderate PA (min)	40.3	48.8	0	0	20.0	60.0	250.0
MVPA (min)	73.1	89.1	0	0	40.0	110.0	470.0

Note: Total PA counts were divided by 100,000 to yield a manageable data set. Vigorous PA, Moderate PA, and MVPA indicate the number of minutes per week engaged in the specific intensity of activity.

SD: standard deviation, **MIN**: minimum, **MED**: median, **MAX**: maximum, **BMD:** bone mineral density, **FFM-BMC:** fat-free mass minus bone mineral content, **PA:** physical activity, **MVPA**: moderate and vigorous physical activity

Table 2 Mean differences in hip BMD across three categories of fat-free mass, without and with the effect of potential confounders

	Fat-Free Mass Category							
	Low FFM n=66		Moderate FFM n=130		High FFM n=66			
Outcome: Hip BMD	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**P**
Variable controlled								
None	0.871[a]	0.089	0.933[b]	0.096	0.997[c]	0.128	24.5	<0.0001
Age (yrs)	0.871[a]		0.933[b]		0.997[c]		24.3	<0.0001
Height (cm)	0.863[a]		0.934[b]		1.004[c]		19.9	<0.0001
Fat Mass (kg)	0.877[a]		0.934[b]		0.990[c]		17.5	<0.0001
Total Body Mass (kg)	0.896[a]		0.934[b]		0.970[c]		4.8	0.0091
Dietary calcium (mg)	0.873[a]		0.933[b]		0.996[c]		23.0	<0.0001
Suppl. calcium (mg)	0.871[a]		0.933[b]		0.997[c]		24.5	<0.0001
Total calcium (mg)	0.871[a]		0.933[b]		0.997[c]		24.2	<0.0001
Dietary vitamin D (IU)	0.872[a]		0.932[b]		0.997[c]		24.4	<0.0001
Suppl. vitamin D (IU)	0.872[a]		0.933[b]		0.997[c]		24.3	<0.0001
Total vitamin D (IU)	0.872[a]		0.932[b]		0.997[c]		24.3	<0.0001
Total PA (counts)	0.869[a]		0.934[b]		0.998[c]		25.1	<0.0001
Vigorous PA (min)	0.870[a]		0.933[b]		0.997[c]		24.8	<0.0001
Moderate PA (min)	0.871[a]		0.933[b]		0.997[c]		24.2	<0.0001
MVPA (min)	0.870[a]		0.933[b]		0.998[c]		24.7	<0.0001
Bone drugs (yes/no)	0.839[a]		0.891[b]		0.959[c]		23.8	<0.0001
Menopause status	0.876[a]		0.939[b]		1.003[c]		24.3	<0.0001
Full Model 1	0.879[a]		0.935[b]		1.004[c]		14.3	<0.0001
Full Model 2	0.875[a]		0.937[b]		1.013[c]		19.5	<0.0001

Note: Means on the same row with the same superscript letter are not significantly different.

BMD: bone mineral density, **FFM**: fat-free mass, **PA**: physical activity, **MVPA**: moderate and vigorous physical activity

Low FFM: ≤40.27 kg, Moderate FFM: >40.27 kg and ≤47.44 kg, High FFM: >47.44 kg

Full Model 1 includes statistical control of the following variables: age, height, menopause status, bone drug use, total vitamin D intake, total calcium intake, time spent in moderate and vigorous physical activity, and fat mass.

Full Model 2 includes all of the covariates of Full Model 1, except fat mass.

Table 3 Mean differences in hip BMD across three categories of fat mass, without and with the effect of potential confounders

	Fat Mass Category							
	Low FM n=66		Moderate FM n=130		High FM n=66			
Outcome: Hip BMD	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**P**
Variable controlled								
None	0.910[a]	0.115	0.920[a]	0.102	0.985[b]	0.113	9.9	<0.0001
Age (yrs)	0.908[a]		0.920[a]		0.985[b]		10.1	<0.0001
Height (cm)	0.913[a]		0.920[a]		0.980[b]		8.3	0.0003
Fat-Free Mass (kg)	0.925		0.926		0.957		2.1	0.1213
Total Body Mass (kg)	0.974[a]		0.928[b]		0.904[b]		3.6	0.0287
Dietary calcium (mg)	0.909[a]		0.921[a]		0.983[b]		9.4	<0.0001
Suppl. calcium (mg)	0.910[a]		0.920[a]		0.984[b]		9.6	<0.0001
Total calcium (mg)	0.909[a]		0.920[a]		0.985[b]		10.0	<0.0001
Dietary vitamin D (IU)	0.908[a]		0.921[a]		0.984[b]		10.1	<0.0001
Suppl. vitamin D (IU)	0.910[a]		0.920[a]		0.985[b]		9.9	<0.0001
Total vitamin D (IU)	0.908[a]		0.920[a]		0.985[b]		10.1	<0.0001
Total PA (counts)	0.908[a]		0.920[a]		0.986[b]		10.1	<0.0001
Vigorous PA (min)	0.907[a]		0.920[a]		0.986[b]		10.2	<0.0001
Moderate PA (min)	0.909[a]		0.920[a]		0.985[b]		10.1	<0.0001
MVPA (min)	0.907[a]		0.920[a]		0.986[b]		10.4	<0.0001
Bone drugs (yes/no)	0.874[a]		0.881[a]		0.985[b]		8.9	0.0002
Menopause status	0.910[a]		0.918[a]		0.984[b]		10.0	<0.0001
Full Model 1	0.934		0.934		0.955		1.0	0.3692
Full Model 2	0.912[a]		0.924[a]		0.980[b]		7.7	0.0006

Note: Means on the same row with the same superscript letter are not significantly different.

BMD: bone mineral density, **FM**: fat mass, **PA**: physical activity, **MVPA**: moderate and vigorous physical activity

Low FM: ≤15.52 kg, Moderate FM: >15.52 kg and ≤27.45 kg, High FM: >27.45 kg

Full Model 1 includes statistical control of the following variables: age, height, menopause status, bone drug use, total vitamin D intake, total calcium intake, time spent in moderate and vigorous physical activity, and fat-free mass.

Full Model 2 includes all of the covariates of Full Model 1, except fat-free mass.

Table 4 Mean differences in hip BMD across three categories of total body mass, without and with the effect of potential confounders

	Total Body Mass Category							
	Low TBM n=66		Moderate TBM n=130		High TBM n=66			
Outcome: Hip BMD	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**P**
Variable controlled								
None	0.876[a]	0.085	0.932[b]	0.109	0.995[c]	0.112	21.5	<0.0001
Age (yrs)	0.875[a]		0.933[b]		0.994[c]		21.8	<0.0001
Height (cm)	0.879[a]		0.932[b]		0.992[c]		16.6	<0.0001
Fat Mass (kg)	0.857[a]		0.929[b]		1.018[c]		13.7	<0.0001
Fat-Free Mass (kg)	0.916		0.932		0.953		1.3	0.2693
Dietary calcium (mg)	0.873[a]		0.933[b]		0.996[c]		23.0	<0.0001
Suppl. calcium (mg)	0.876[a]		0.932[b]		0.994[c]		21.0	<0.0001
Total calcium (mg)	0.875[a]		0.932[b]		0.994[c]		21.5	<0.0001
Dietary vitamin D (IU)	0.877[a]		0.931[b]		0.995[c]		21.2	<0.0001
Suppl. vitamin D (IU)	0.874[a]		0.933[b]		0.995[c]		22.0	<0.0001
Total vitamin D (IU)	0.874[a]		0.933[b]		0.995[c]		22.1	<0.0001
Total PA (counts)	0.875[a]		0.932[b]		0.995[c]		21.5	<0.0001
Vigorous PA (min)	0.874[a]		0.933[b]		0.996[c]		22.1	<0.0001
Moderate PA (min)	0.876[a]		0.932[b]		0.995[c]		21.3	<0.0001
MVPA (min)	0.875[a]		0.932[b]		0.995[c]		22.0	<0.0001
Bone drugs (yes/no)	0.847[a]		0.896[b]		0.956[c]		19.0	<0.0001
Menopause status	0.871[a]		0.928[b]		0.991[c]		21.4	<0.0001
Full Model 1	0.930		0.938		0.950		0.3	0.7188
Full Model 2	0.879[a]		0.932[b]		0.989[c]		15.3	<0.0001

Note: Means on the same row with the same superscript letter are not significantly different.

BMD: bone mineral density, **TBM**: total body mass, **PA**: physical activity, **MVPA**: moderate and vigorous physical activity

Low TBM: ≤58.23 kg, Moderate TBM: >58.23 kg and ≤73.00 kg, High TBM: >73.00 kg

Full Model 1 includes statistical control of the following variables: age, height, menopause status, bone drug use, total vitamin D intake, total calcium intake, time spent in moderate and vigorous physical activity, and fat-free mass.

Full Model 2 includes all of the covariates of Full Model 1, except fat-free mass.

Table 5 Relationship between hip BMD, FFM, FFM-BMC, Fat Mass, and Total Body Mass, without and with control of potential confounders

	Body Mass Category n=262							
	FFM		**FFM-BMC**		**FM**		**TBM**	
Criterion: Hip BMD	***b***	**p**	***b***	**p**	***b***	**p**	***b***	**p**
Variable controlled								
None	0.0100	<0.0001	0.0101	<0.0001	0.0036	<0.0001	0.0042	<0.0001
Age (yrs)	0.0100	<0.0001	0.0100	<0.0001	0.0037	<0.0001	0.0042	<0.0001
Height (cm)	0.0127	<0.0001	0.0125	<0.0001	0.0032	0.0004	0.0042	<0.0001
Fat Mass (kg)	0.0103	<0.0001	0.0093	<0.0001	N/A	N/A	0.0093	<0.0001
Fat-Free Mass (kg)	N/A	N/A	N/A	N/A	0.0013	0.1316	0.0013	0.1316
Dietary calcium (mg)	0.0100	<0.0001	0.0110	<0.0001	0.0035	<0.0001	0.0041	<0.0001
Suppl. calcium (mg)	0.0100	<0.0001	0.0101	<0.0001	0.0036	<0.0001	0.0042	<0.0001
Dietary vitamin D (IU)	0.0100	<0.0001	0.0110	<0.0001	0.0036	<0.0001	0.0042	<0.0001
Suppl. vitamin D (IU)	0.0100	<0.0001	0.0101	<0.0001	0.0036	<0.0001	0.0042	<0.0001
Total PA (counts)	0.0103	<0.0001	0.0103	<0.0001	0.0038	<0.0001	0.0042	<0.0001
Vigorous PA (min)	0.0101	<0.0001	0.0102	<0.0001	0.0039	<0.0001	0.0043	<0.0001
Moderate PA (min)	0.0101	<0.0001	0.0101	<0.0001	0.0036	<0.0001	0.0042	<0.0001
MVPA (min)	0.0102	<0.0001	0.0102	<0.0001	0.0038	<0.0001	0.0042	<0.0001
Bone drugs (yes/no)	0.0096	<0.0001	0.0107	<0.0001	0.0032	0.0003	0.0039	<0.0001
Full Model	0.0120	<0.0001	0.0116	<0.0001	0.0007	0.4109	0.0007	0.4109

Note: *b*=regression coefficient. Values in the columns showing regression coefficients (*b*) reflect differences in BMD for each 1 kg difference in the body mass category.

BMD: bone mineral density, **FFM**: fat-free mass, **FFM-BMC**: fat-free mass minus bone mineral content, **FM**: fat mass, **TBM**: total body mass, **PA**: physical activity, **MVPA**: moderate and vigorous physical activity

Full Model included the following covariates: age, height, total vitamin D intake, total calcium intake, time in moderate and vigorous physical activity, use of bone drugs, and either fat-free mass or fat mass, depending on the criterion variable.