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Original article

Body composition phenotypes and bone health in young adults: A cluster analysis

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SUMMARY

Background and aims: Lean mass is considered the best predictor of bone mass, as it is an excellent marker of bone mechanical stimulation, and changes in lean mass are highly correlated with bone outcomes in young adults. The aim of this study was to use cluster analysis to examine phenotype categories of body composition assessed by lean and fat mass in young adults and to assess how these body composition categories are associated with bone health outcomes.

Methods: Cluster cross-sectional analyses of data from 719 young adults (526 women) aged 18–30 years from Cuenca and Toledo, Spain, were conducted. Lean mass index (lean mass (kg)/height $(m)^2$), fat mass index (fat mass (kg)/height $(m)^2$), bone mineral content (BMC) and areal bone mineral density (aBMD) were assessed by dual-energy X-ray absorptiometry.

Results: A cluster analysis of lean mass and fat mass index z scores resulted in a classification of a five-category cluster solution that could be interpreted according to the body composition phenotypes of individuals as follows: high adiposity-high lean mass (n = 98), average adiposity-high lean mass (n = 113), high adiposity-average lean mass (n = 213), low adiposity-average lean mass (n = 142), and average adiposity-low lean mass (n = 153). ANCOVA models showed that individuals in clusters with a higher lean mass had significantly better bone health (z score: 0.764, se: 0.090) than their peers in other cluster categories (z score: −0.529, se: 0.074) after controlling for sex, age, and cardiorespiratory fitness ($p < 0.05$). Additionally, subjects belonging to the categories with a similar average lean mass index but with high or low-adiposity levels (z score: 0.289, se: 0.111; z score: 0.086, se: 0.076) showed better bone outcomes when the fat mass index was higher ($p < 0.05$).

Conclusions: This study confirms the validity of a body composition model using a cluster analysis to classify young adults according to their lean mass and fat mass indices. In addition, this model reinforces the main role of lean mass on bone health in this population and that in phenotypes with high-average lean mass, factors associated with fat mass may also have a positive effect on bone status.

Introduction

Bone mass gain during growth is a very important predictor of osteoporosis and frailty fractures in the later years [1–3]. A large amount of evidence supports that the optimization of nutritional status is an effective strategy to improve bone health among young people [4]. Body composition reflects nutritional status, and

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variables such as lean mass and fat mass are key to understanding bone development during this period [5].

Lean mass is considered the best predictor of bone mass, as it is an excellent marker of bone mechanical stimulation, and changes in lean mass are highly correlated with bone outcomes in young adults [6,7]. However, the interaction between fat mass and bone health is complex. It is well known that weight-bearing exercises positively affect bone accrual [8], but excessive fat mass could negatively influence bone remodelling through several mechanisms, including alterations in the hormonal bone-regulating system, increased oxidative stress, inflammation, and altered bone cell metabolism [9,10]. Additionally, it has been postulated that the adverse effects of fat mass on the skeleton might be more pronounced during growth, possibly due to the disruption of skeletal adaptation to mechanical loading [11].

Excess fat mass has been traditionally determined through body mass index (BMI) as a standard measurement of nutritional status [12,13]. However, the limits of sensitivity and inability of this index to differentiate lean versus fat mass could confound health outcomes and misclassify individuals. Thus, compared with BMI, the implementation of a body composition analysis that allows the clustering of individuals according to their lean/fat body composition phenotype could help to better understand the health risks associated with excess fat mass [14].

In view of the body composition variability of individuals, several paradigms of body composition phenotypes have been proposed for a more comprehensive categorization of people according to their lean and fat mass [15–18]. Most models have been studied in children [15], in older populations [16] or for cardiovascular diseases [17,18] using the lean mass index and fat mass index (lean mass or fat mass (kg)/height squared (m^2)) as approaches to define body composition indices. However, the bone health profiles of the different body composition phenotypes using statistical clustering techniques have been poorly studied in young adults, where it is well established that a high lean mass is favourable for bone health [6,7], but the role of fat mass is unclear [11].

Therefore, the aims of this work were: i) to identify, using clustering analysis procedures, the body composition phenotypes of young adults according to their body lean and fat mass, and ii) to compare the differences in bone mineral content (BMC) and areal bone mineral density (aBMD) among these phenotypes as empirical proof of the validity of the model.

Methods

Study design and participants

This was a multicentre cross-sectional study involving first-year undergraduate students from the University of Castilla-La Mancha, Spain (Cuenca and Toledo campuses). Initially, a total of 1330 first-year undergraduate students (aged 18–30 years) were invited to participate in the study, and 1043 (78.42%) accepted the invitation. Finally, for the present study, we used data from a subsample of 719 students (aged 18–30 years) in which the body composition measures were determined.

Ethics approval and informed consent

The study protocol was approved by The Clinical Research Ethics Committee of the "Virgen de la Luz" of Cuenca and complied with the principles of the Declaration of Helsinki (REG: 2016jPI1116). This study was a part of the research "*Lifestyle, adiposity and vascular function in college students from Castilla-La Mancha, Spain*" from the 2017/2018 academic year. All students read and signed the informed consent form as a condition to participate in the study.

Variables

All determinations were conducted by trained nurses and researchers under standardized conditions as a structured written assessment plan was developed for the study. The assessments are described in detail as follows:

Anthropometry was measured twice, with a 5-min interval, and their average was considered for the final analyses. Height was measured with the subject barefoot and standing upright with the sagittal midline at the midline of the 100 Seca-222 stadiometer. Body mass was measured with the subject barefoot and wearing light clothing using a Seca-770 scale. BMI was calculated as the body mass in kilograms divided by the square of the height in metres (kg/m^2). The categorization of BMI was performed according to the cut-offs established by the World Health Organization (WHO) [19]. Waist circumference was assessed by the average of three determinations taken with flexible tape located at the midpoint between the last rib and the iliac crest at the end of a normal expiration.

Body composition. The lean mass (kg), fat mass (kg), BMC (kg) and aBMD (g/cm2) were determined using dual-energy X-ray absorptiometry (DXA). From a bone health point of view, DXA is the most widespread clinical and research technique for the measurement of peak bone mass [20], which is reached from children to young adults [2]. DXA Lunar iDXA, GE Medical Systems Lunar, Madison, WI 53718, USA at the Cuenca campus and DXA Hologic 100 Discovery Series QDR, Bedford, USA at the Toledo campus were used. On the campus of the University of Cuenca, the analyses were performed using enCoreTM 2008 software version 12.30.008. On the campus in the city of Toledo, the DXA equipment was regulated using a lumbar spine phantom following the Hologic guidelines. All DXA scans were examined using Physician's Viewer, APEX System Software Version 3.1.2 (Bedford, USA). DXA equipment precision was examined daily before each checking session using the GE Lunar calibration phantom, as suggested by the manufacturer. As two different DXA devices were used to evaluate body composition, the z-scores of these variables were calculated according to the measuring device to adjust for possible variability. In addition, all body composition variables were normalized for sex.

Additionally, the lean mass index and fat mass index were calculated using lean mass (kg) and fat mass (kg) divided by height (m) squared.

Physical fitness was evaluated after a 4-min warm-up and included assessments of cardiorespiratory fitness (CRF) and musculoskeletal fitness. For the CRF evaluation, we used the Course–Navette test (20-m shuttle run test). Participants had to run between the two lines for 20 m, starting with 8.5 km/h and increasing their speed progressively (0.5 km/h each minute) based on a sound signal of a pre-recorded tape. Maximal oxygen intake (VO2 max; ml/kg/min) was determined using Leger's formula ($[31.025 + (3.238 \times \text{velocity}) - (3.248 \times \text{age}) + (0.1536 \times \text{age} \times \text{velocity})]$) [21]. For the musculoskeletal evaluation, handgrip strength was measured with a TKK 5401 Grip-DW handgrip dynamometer (Takeya, Tokyo, Japan). The evaluation was completed twice with the left hand and twice with the right hand and the best score for each hand was registered in kilograms; the mean average of the two measurements was estimated.

Covariates information was also collected on potentially confounding covariates including age, vitamin D (in μg/day), total

energy intake (in kcal/day) (both obtained with the 137-item Food Frequency Questionnaire), and physical activity (using GENEactive accelerometers (ActivInsights) for 7 consecutive full days, with a fixed frequency of 30.0 Hz to record the data raw of acceleration measured in "g" for each movement axis (x, y, and, z).

Statistical analysis

All statistical analyses were completed using SPSS software v.28 for Windows (IBM Corp., Armonk, NY). Statistical significance was set at 0.05.

The chi-squared test (categorical variables) or Student's t test (continuous variables) was used to describe the characteristics of the study sample by sex. Prior to the analyses, we tested the distribution of the continuous variables for normality using both statistical (the Kolmogorov–Smirnov test) and graphical (normal probability plot) approaches. Subsequently, we estimated partial correlation coefficients to examine the associations among body composition, physical fitness, and bone-related variables, controlling for age and sex.

To detect similar groups according to the body composition variables, based on the normalized z scores by sex of the lean mass index and fat mass index, hierarchical and non-hierarchical clustering approaches were used. First, a hierarchical cluster analysis was conducted using Ward's method based on the squared Euclidean distance; because of the high sensitivity of Ward's method, the values of two individuals were removed because they were outliers (±3 SD). Thus, a final sample of 719 was included in the analysis. The number of clusters was determined by visual inspection of the dendrogram and according to the conceptual model. Additionally, a non-hierarchical k-means procedure was used to achieve a final solution of five body composition phenotypes: (i) high adiposity-high lean mass; ii) average adiposity-high lean mass; iii) high adiposity-average lean mass; iv) low adiposity-average lean mass, and v) average adiposity-low lean mass (Fig. 1).

Additionally, mean differences in body composition, physical fitness, and bone mineral expressed through BMC and aBMD (dependent variables) between cluster categories (fixed factor) were analyzed using ANOVA (model 0) and ANCOVA with adjustment for age, body height, vitamin D, total energy intake, and total physical activity (model 1). Pairwise post hoc multiple comparisons were examined using the Bonferroni post hoc test. Lastly, to test mean differences by gender a subgroup analysis was performed.

Results

Table 1 shows the descriptive characteristics of the analysed sample by gender. Of the 719 young adults included in the study, 193 (26,84%) were men. This gender distribution was similar to that of the whole university campus population. The participants who agreed to participate, i.e. those who agreed to have their body composition assessed by DXA, did not differ in the variables analysed from those who did not (Table S1 in the Supplement).

Correlations among study variables

Table 2 shows the partial correlation coefficients among body composition, physical fitness and bone-related variables controlling for sex and age. Total body weight was negatively associated with CRF and positively related with handgrip strength and bone-related variables. Specifically, the lean mass index was positively correlated with the fat mass index, CRF, handgrip strength and bone-related variables (aBMD and BMC). Otherwise, the fat mass

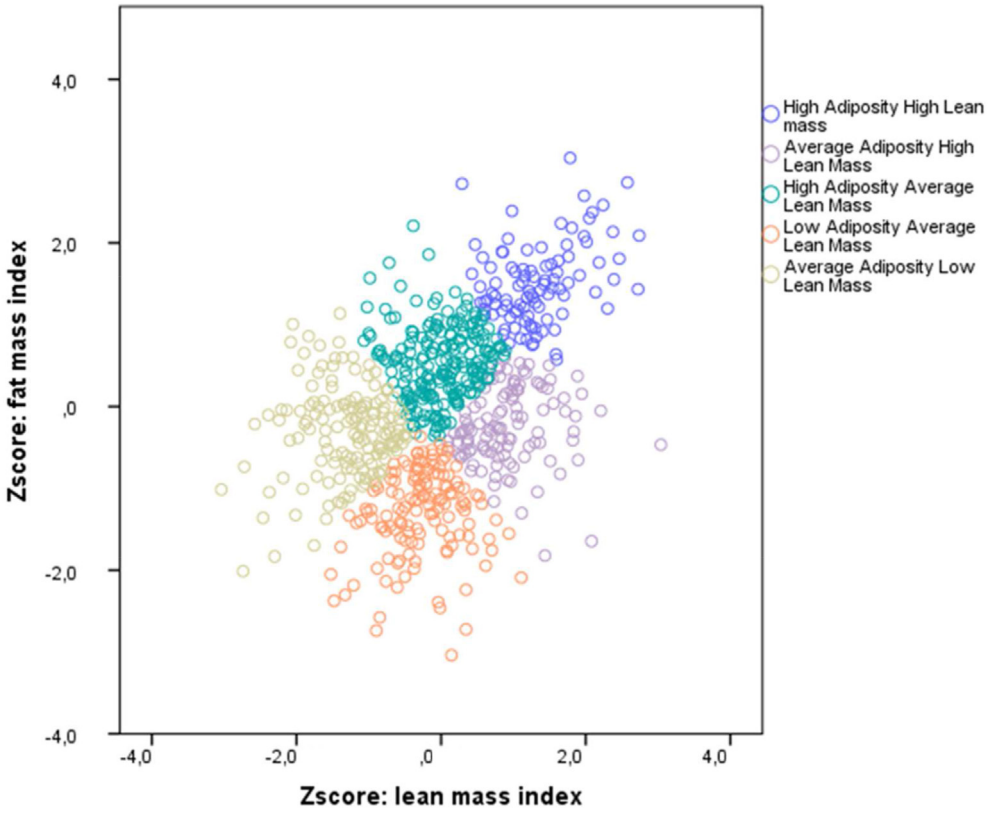

Fig. 1. Clustering individuals according to body composition phenotypes using fat mass and lean mass index.

Table 1
Characteristics of the study sample.

	All n = 719	Men n = 193	Women[a] n = 526
Age (years)	20.63 ± 4.19	20.61 ± 3.05	20.65 ± 4.54
Total body weight (kg)	62.81 ± 11.75	72.12 ± 10.93	59.47 ± 10.34
Body height (cm)	166.09 ± 8.39	175.21 ± 7.25	162.63 ± 5.88
BMI (kg/m^2)	22.70 ± 3.44	23.42 ± 2.88	22.45 ± 3.68
Underweight (%)	4.8	0.5	6.4
Normal weight (%)	75.1	73.6	75.7
Overweight (%)	16.2	23.3	13.5
Obesity (%)	3.9	2.6	4.4
Waist circumference (cm)	79.71 ± 8.64	83.05 ± 7.89	78.54 ± 8.76
Lean mass (kg)	42.01 ± 8.93	54.09 ± 6.65	37.55 ± 4.39
Lean mass index (kg/m^2)	15.09 ± 2.06	17.58 ± 1.55	14.10 ± 1.36
Fat mass %	29.92 ± 7.87	21.42 ± 6.15	33.05 ± 5.92
Fat mass (kg)	18.94 ± 6.80	15.78 ± 6.27	20.10 ± 6.62
Fat mass index (kg/m^2)	6.94 ± 2.62	5.13 ± 2.01	7.60 ± 2.51
VO_2max (mL/kg/min)	33.68 ± 8.04	41.10 ± 8.57	30.47 ± 5.15
Handgrip strength (kg)	28.90 ± 8.37	39.68 ± 7.17	24.73 ± 3.85
Total body BMC (kg)	2.42 ± 4.49	2.90 ± 4.73	2.25 ± 2.81
Total body aBMD (g·cm^2)	1.153 ± 0.110	1.228 ± 0.125	1.124 ± 0.090

Values are means ± SD (quantitative variables) or n (%) (categorical variables).
Abbreviations: BMI, body mass index; BMC: bone mineral content; aBMD: areal bone mineral density; VO2 max: maximal oxygen consumption.
[a] All variables differed by sex except for age and % of underweight $p < 0.05$.

index was negatively associated with CRF and handgrip strength and positively correlated with the bone-related variables.

Validity of the cluster analysis

Figure 1 reveals the visual structure of the five solutions based on the body composition phenotypes: (i) high adiposity-high lean mass (n = 98); ii) average adiposity-high lean mass (n = 113); iii) high adiposity-average lean mass (n = 213); iv) low adiposity-average lean mass (n = 142): and v) average adiposity-low lean mass (n = 153).

Table 3 shows the mean differences in body composition and physical fitness variables by the five previously identified body composition phenotypes tested using ANCOVA models. Overall, those who have a higher body weight and adiposity showed a lower CRF (clusters 1 and 3) and those who have a low muscularity showed a lower musculoskeletal fitness (cluster 5). Post hoc statistical significance for mean differences between categories was achieved in most cases.

Body composition phenotypes and bone-related variables

Concerning the ANCOVA models testing mean differences in bone mineral according to body composition phenotypes (Fig. 2), was observed that young adults belonging to categories with a higher lean mass index (clusters 1 and 2) had higher values for the bone-related variables than their peers of other categories with less lean mass ($p < 0.05$). Additionally, it was observed that subjects belonging to the categories with an average fat mass index (clusters 2 and 5) showed better bone mineral outcomes when the lean mass index was higher ($p < 0.05$). Conversely, individuals belonging to the categories with an average lean mass index (clusters 3 and 4) presented better bone mineral outcomes when the fat mass index was higher ($p < 0.05$). Overall, significant differences were maintained after adjusting for age, body height, vitamin D, total energy intake, and total physical activity in Model 1. The results by gender were like the whole sample (Figs. S1 and S2 in the Supplement).

In general, correlation coefficients between fitness and bone-related variables across body composition phenotypes, were higher with handgrip strength than with CRF and participants with high and average lean mass had higher coefficients than those with low lean mass regardless of their adiposity. Additional ANCOVA models testing mean differences in bone-related variables according to total body weight categories showed that participants with a higher total body weight had better bone values than their peers with lower values (data not shown).

Discussion

This study provides two novel findings in relation to nutritional status and its relationship with bone health in young adults. First, it proposes a five-category cluster solution of body composition using statistical clustering techniques to classify individuals according their lean and fat mass index profiles. Second, our analyses support that lean mass is the main contributing factor for bone health, although some fat mass-related factors could have a positive effect on it.

Our clustering analysis in young adults comparing different body composition phenotypes allowed us to identify the role of fat and lean mass on bone health in this population, showing that individuals with a higher lean mass have significantly better bone mineral outcomes than their peers with lower lean mass, an observation consistent with the literature [22]. Moreover, young adults in categories with similar fat mass index (average adiposity-high lean mass and average adiposity-low lean mass) showed very different bone outcomes associated with the change in the lean mass index, confirming that lean mass is a strong determinant for bone health in young adults [20,23].

With respect to the role of fat mass, the impact of obesity during growth on cardiovascular risk in adults has been proven [24], but its association with bone health is complex. The effect of fat on bone outcomes is mediated by both mechanical and metabolic factors. A mechanism that can explain why people with obesity show higher BMD is the increased mechanical loading and strain associated with this condition. Usually, people with obesity have increased fat mass but also have higher lean mass; therefore, passive loading and muscle-induced strain are enhanced [13,25]. In this line, our analysis showed the best bone outcomes for the high adiposity-high lean mass phenotype that included overweight/young adults with obesity with not only the highest fat mass index but also the highest lean mass index and a high muscular strength level.

Table 2
Partial correlation coefficients among body composition, physical fitness and bone-related variables controlling for sex and age.

	Lean mass index	Fat mass index	Total body weight	VO_2max	Handgrip strength	Total body aBMD	Total body BMC
Lean mass index	1.00	0.496[a]	0.661[a]	0.493[a]	0.740[a]	0.582[a]	0.713[a]
Fat mass index		1.00	0.774[a]	−0.533[a]	−0.253[a]	0.330[a]	0.305[a]
Total body weight			1.00	−0.203[a]	0.376[a]	0.490[a]	0.652[a]
VO_2max				1.00	0.457[a]	0.189[a]	0.352[a]
Handgrip strength					1.00	0.527[a]	0.754[a]
Total body aBMD						1.00	0.842[a]

Values indicate the correlation coefficient (r). Abbreviations: aBMD: areal bone mineral density; BMC: bone mineral content.
[a] $p < 0.001$.

Table 3
ANCOVA models comparing means of body composition and physical fitness variables by 'body composition phenotype.

	Cluster (fat mass index, lean mass index)						Pairwise post hoc comparisons									
	High adiposity, high lean mass [1]	Average adiposity, high lean mass [2]	High adiposity, average lean mass [3]	Low adiposity, average lean mass [4]	Average adiposity, low lean mass [5]	P value	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
N	98	113	213	142	153											
Body composition																
Total body weight (kg)	78.9 ± 0.8	64.9 ± 0.8	65.4 ± 0.6	54.9 ± 0.7	54.6 ± 0.7	<0.001	>	>	>	>	-	>	>	>	>	-
Body height (cm)	164.7 ± 0.8	166.8 ± 0.8	166.7 ± 0.6	166.1 ± 0.7	165.1 ± 0.7	0.164	-	-	-	-	-	-	-	-	-	-
Lean mass (kg)	46.4 ± 8.1	46.6 ± 9.2	42.4 ± 8.8	40.6 ± 8.0	36.5 ± 6.6	<0.001	-	>	>	>	>	>	>	-	>	>
Lean mass index (kg/m^2)	17.0 ± 1.7	16.6 ± 2.0	15.1 ± 1.6	14.6 ± 1.6	13.3 ± 1.4	<0.001	-	>	>	>	>	>	>	-	>	>
Fat mass %	38.5 ± 6.3	25.7 ± 6.4	32.7 ± 6.2	23.0 ± 5.7	29.2 ± 6.0	<0.001	>	>	>	>	<	>	>	>	>	<
Fat mass (kg)	30.7 ± 6.1	16.3 ± 3.6	21.2 ± 4.2	12.4 ± 2.7	16.4 ± 3.35	<0.001	>	>	>	>	<	>	–	>	>	<
Fat mass index (kg/m^2)	11.2 ± 2.6	5.9 ± 1.3	7.7 ± 1.6	4.5 ± 1.1	6.1 ± 1.3	<0.001	>	>	>	>	<	>	-	>	>	<
BMI (kg/m^2)	29.0 ± 2.8	23.2 ± 1.5	23.4 ± 1.6	19.8 ± 1.2	20.0 ± 1.3	<0.001	>	>	>	>	-	>	>	>	>	-
Waist circumference (cm)	92.5 ± 8.3	79.6 ± 5.0	82.4 ± 6.0	72.6 ± 4.8	74.6 ± 4.9	<0.001	>	>	>	>	<	>	>	>	>	<
Physical fitness																
VO_2max (mL/kg/min)	30.8 ± 8.0	36.6 ± 8.2	31.6 ± 6.6	36.8 ± 9.2	32.1 ± 7.1	<0.001	<	-	<	-	>	-	>	<	-	>
Handgrip strength (kg)	30.8 ± 8.7	31.2 ± 8.9	29.3 ± 9.0	28.2 ± 7.1	25.8 ± 6.8	<0.001	-	-	-	>	-	-	>	-	>	-

Values are means ± SD. Comparisons between phenotypes adjusted for age. Symbols: >, < indicate statistical significance (P < 0.05) and symbol - indicates no statistical significance (P > 0.05) in pairwise mean comparisons using Bonferroni post-hoc test. Abbreviations: BMI, body mass index.

Muscular strength, which is closely related to lean mass [26], has been recognized as a useful skeletal marker. Additionally, young adults with a high level of fat mass and high levels of muscular strength (individuals with excess of fat but fit) showed significantly better bone health than their peers with lower values of muscular fitness [27].

Unfavourable metabolic changes associated with excessive fat accumulation may make the bones more fragile. Mechanisms underpinning these changes may be related to high levels of proinflammatory cytokines leading to osteoclast formation and activation, the replacement of osteoblasts by fat cells in bone marrow or reduced calcium absorption associated with high fat intake, among others [28]. However, our data suggest that, in this age group, the detrimental role of high fat mass on bone health is neither strong nor consistent in profiles with high lean mass (high adiposity-high lean mass and average adiposity-high lean mass) or average lean mass (high adiposity-average lean mass and low adiposity-average lean mass), where those with phenotypes with higher adiposity achieved better bone outcomes than their peers with lower levels.

Apart from the mechanical relationship between high adiposity and bone outcomes, the apparently protective effect of adiposity on bone outcomes in phenotypes with high-average lean mass could be due, at least partly, to the presence of insulin sensitivity which has been described in individuals with obesity and linked to exercise. The insulin sensitivity could be related with bone health through an increase in serum osteocalcin [29]. In addition, in these phenotypes, the mechanical stimuli of high-average lean mass and high adiposity may influence mesenchymal stem cells promoting the osteoblastogenesis. These pluripotent cells have the potential to differentiate into osteoblasts, adipocytes, chondrocytes or myocytes, and mechanical stimulation has been proven to regulate these cells toward osteogenic lineage [30].

Lean mass usually constitutes ~ 50% of the body mass in young adults but progressively declines with age. This age-related loss in skeletal muscle mass in combination with high fat mass and low muscle function (low muscular strength and performance) is known as sarcopenic obesity [31] Although previous studies have related low BMD or osteoporosis with sarcopenic obesity [32], this phenotype combining high adiposity and low lean mass did not appear in our study, so it was not possible to assess its effect on bone status. However, our study clearly showed that individuals with a low lean mass index and low muscular fitness had the worst bone outcomes. This association of low lean mass index and low muscular fitness has been related to sarcopenia in adolescents [33].

Limitations

This study presents some limitations. First, the analysis was cross-sectional; therefore, we cannot make cause-effect inferences. Second, although the total body less head is the recommended whole-body region for the assessment of bone health [34], in our sample, this variable was not available for the analyses. Third, we explored the interplay between fat and lean mass on total bone as recommended by the International Society for Clinical Densitometry [35]; however, we did not examine its influence in other specific bone sites. Fourth, we tested the effect of the fat mass index to elucidate its influences on bone outcomes, but we did not explore other adipose tissue distributions, such as visceral vs. subcutaneous or android vs. gynoid. Fifth, two different DXA devices were used to measure body composition variables, which could bias our results; however, to control this source of variability, we used z scores by device in our analyses. Finally, the relationships between body composition and bone health could be potentially confounded by other hormonal or health conditions variables not included in the models because that information was not collected.

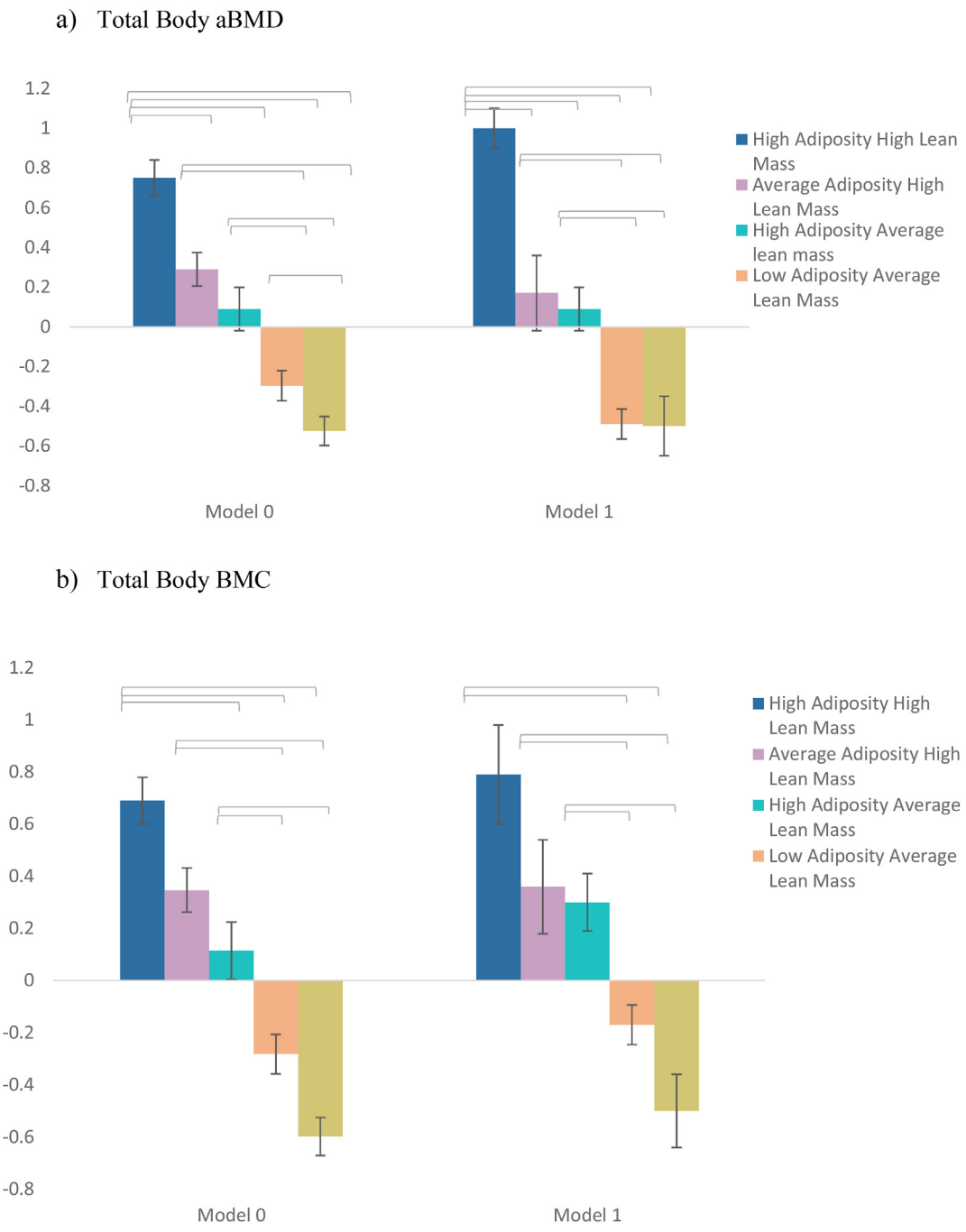

Fig. 2. Mean differences in sex-normalized z-score: a) total body aBMD (areal bone mineral density) and b) total body BMC (bone mineral content) by 'body composition phenotypes' categories. Brackets indicate significant differences in mean ($p < 0.05$) between categories in the Bonferroni multiple comparison post hoc test. Error bars represent standard error. Model 0: crude data; Model 1: adjusted for age, body height, vitamin D, total energy intake, and total physical activity.

Conclusions

Our study confirms the validity of a body composition model using a cluster analysis to classify young adults according to their lean mass and fat mass index. This model reinforces that, lean mass is the most relevant body composition compartment for bone mineral health, in this group of young adults. In addition, in presence of higher body weight and high-average lean mass, the fat mass may have a positive effect on bone status. Therefore, from a bone health point of view, interventions should be designed to motivate young adults towards improving lean mass levels, especially for those phenotypes with high adiposity.

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Authors' contributions

All authors have made substantial contributions to all of the following: i) the conception and design of the study, acquisition of

data, analysis and interpretation of data, ii) drafting the article or revising it critically for important intellectual content, iii) final approval of the version to be submitted.

Conflicts of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.05.006.

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