

COVER PAGE

Title of Manuscript: Differences in areal bone mineral density between metabolically healthy and unhealthy overweight/obese children: the role of physical activity and cardiorespiratory fitness.

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Abstract

Objectives: 1) to examine whether areal bone mineral density (aBMD) differs between metabolically healthy (MHO) and unhealthy (MUO) overweight/obese children, and 2) to examine the role of moderate-to-vigorous physical activity (MVPA), and cardiorespiratory fitness (CRF) in this association. **Methods:** a cross-sectional study was developed in 188 overweight/obese children (10.4 ± 1.2 y) from the ActiveBrains and EFIGRO studies. Participants were classified as MHO or MUO based on Jolliffe and Janssen's metabolic syndrome cut-off points for triglycerides, glucose, high-density cholesterol, and blood pressure. MVPA and CRF were assessed by accelerometry and the 20-meters shuttle run test, respectively. Body composition was measured by Dual-energy X-ray absorptiometry. **Results:** in model 1 (adjusted for sex, years from peak high velocity, stature and lean mass), MHO children had significantly higher aBMD in total body less head (Cohen's *d* effect size, $ES=0.34$), trunk ($ES=0.43$) and pelvis ($ES=0.33$), than MUO children. These differences were attenuated once MVPA was added to the model 1 (model 2), and most of them disappeared once CRF was added to the model 1 (model 3). **Conclusions:** This novel research shows that MHO children have greater aBMD than their MUO peers. Furthermore, both MVPA and more importantly CRF, seem to partially explain these findings.

Introduction

About 200 million people suffer osteoporosis worldwide (1) and approximately 3.4 million people died because of overweight and obesity (2), and its prevalence is expected to increase. As in osteoporosis (3), obesity in childhood tracks into adulthood (4), and therefore, the importance of focusing on this population. Obesity and osteoporosis are different disorders seldom found in the same individual, but scientific evidence supports a controversial link between fat and bone. It has traditionally been considered that excess weight due to obesity has a positive effect on bone mineral density (BMD), which may decrease osteoporotic fracture risk (5); nevertheless, more recent findings suggest that BMD is compromised (6). From a mechanistic point of view, obese youngsters may have greater BMD than their normoweight peers due to their greater lean mass and the higher impact associated with it, consequence of the extra body weight they have to carry on their everyday life activities (7). However, evidence also suggests a negative association between abdominal adiposity and BMD in obese youngsters (8). This may be explained by the role of the abdominal adipose tissue in the release of adipokines into the bloodstream which negatively affects bone remodelling (8).

Also, childhood obesity is commonly associated with increased risk of cardiovascular and metabolic factors (9). Nevertheless, some obese individuals do not show any of these metabolic disorders and they are named as metabolically healthy but obese (MHO) (10). MHO individuals despite having a body mass index (BMI) ≥ 30 kg/m², present a normal/healthy metabolic and inflammation profile (11), and may represent for 30% of the obese population (10). Previous researches have shown that MHO adolescents and adults have better cardiorespiratory fitness (CRF) (12,13) and they are more active (14) than their metabolically unhealthy obese (MUO) peers, which was confirmed in a recent meta-analysis (15). Moreover,

adolescents with good cardiorespiratory fitness (CRF) have more bone mass than those with poor CRF (16) and those more active also present greater levels of bone mass (17). However, whether MHO children and adolescents have greater or lower bone mass than their MUO peers and whether moderate-to-vigorous physical activity (MVPA) or CRF contribute to these differences, has yet to be elucidated. In this regard, MVPA refers to a lifestyle behaviour while CRF refers to a physical state derived from a genetic component. Therefore, testing their independent contribution is necessary.

So far, the only study published examining the association between MHO and MUO with BMD (in adult population) showed that the MUO phenotype was associated with higher total BMD than the MHO, but no association was found with lumbar spine BMD (11). Thereby, there could be a novel link between BMD and MHO/MUO phenotype. To the best of our knowledge, there is no scientific evidence about this possible link in young population. Thus, the aim of the present study was two-fold: 1) to examine whether areal bone mineral density (aBMD) differs between MHO and MUO children; and 2) to examine the role of MVPA, and CRF in this association.

Methods

The present cross-sectional study was developed in a subsample of 188 overweight and obese children (10.4 ± 1.2 years old) with valid data in the variables of interest. Both the ActiveBrains and EFIGRO studies were developed from 2014 to 2017, under the same protocols and with data collection obtained in parallel. Details of design and methods have been published elsewhere (18,19).

Briefly, ActiveBrains (ClinicalTrials.gov ID's: NCT02295072) is a randomized controlled trial designed to examine the effects of an exercise program on brain, cognitive and academic performance, as well as on selected physical and mental health outcomes in overweight/obese children (18). A total of 110 overweight/obese children aged 8-11 years were recruited from Granada (south of Spain) according to the following inclusion criteria (18): 1) to be overweight or obese based on World Obesity Federation (formerly named International Obesity Task Force) cut-off points 2) to be 8 to 11 years-old, 3) not to have any physical disabilities or neurological disorder that affects their physical performance, and 4) in the case of girls, not to have started the menstruation at the moment of the assessments. EFIGRO (ClinicalTrials.gov ID's: NCT02258126) is a randomized controlled trial designed to evaluate the effect of a multidisciplinary intervention program on hepatic fat fraction, cardiometabolic risk factors, self-esteem and well-being in overweight or obese children (19). A total of 116 overweight/obese children aged 9-11 years were recruited from Vitoria-Gasteiz (north of Spain) according to the following inclusion criteria (19): 1) primary overweight or obesity status defined according to the International Obesity Task Force 2) aged between 9 and 11 years, 3) to have at least one parent or caregiver willing to participate in the program sessions, 4) not to have medical conditions or medications that would affect study results or limit physical activity, and 5) in the case of girls, not to have started the menstruation at the moment of the assessments.

The research was conducted according to the Declaration of Helsinki and the protocols were approved by the Review Committee for Research Involving Human Subjects at the University of Granada and the Ethic Committee of Clinical Investigation of Euskadi. Informed consent was obtained from all participants.

Outcome measures

Weight (kg) and stature (cm) were measured with an electronic scale (SECA 861 and 760, Hamburg, Germany) and a stadiometer (SECA 225 and 220, Hamburg, Germany), respectively. BMI was calculated as: weight (kg)/stature (m²), and classified into BMI categories (overweight and obesity), according to Cole and Lobstein (20) cut-offs. Years from peak height velocity (PHV) was used as a maturational landmark, and was predicted through age and stature using validated algorithms in boys and girls (21).

Physical activity was objectively measured by tri-axial accelerometers (GT3X+, ActiGraph, Pensacola, FL, USA) for seven consecutive days. The participants wore the accelerometers on the non-dominant wrist during 24 hours a day and removed it only while bathing or swimming. Accelerometer raw data was processed using the GGIR package for R (GGIR Package, v.1.5.-24, <https://cran.r-project.org/web/packages/GGIR/>). In brief, raw data was aggregated as the Euclidean Norm Minus One g (ENMO) over 5 second epochs with all negative values rounded to 0. The ENMO metric was used to determine the time spent in MVPA by applying previously proposed cut-points validated against metabolic equivalents (measured with indirect calorimetry) for MVPA (22).

CRF was assessed by the 20 m shuttle run test from the ALPHA (Assessing Levels of Physical fitness and Health in Adolescents) test battery, which has been shown to be valid, reliable, feasible, and safe for the assessment of health-related physical fitness in children (23). The last completed lap was recorded and used as an indicator of CRF levels.

Fat mass (%), lean mass (kg), and aBMD (g/cm^2) were measured by Dual-energy X-ray absorptiometry (DXA) using the Hologic QDR 4500W (EFIGRO study) and the Discovery Wi (ActiveBrains study) (both from the manufacturer Hologic Series Discovery QDR, Bedford, MA, USA). The total body scan was used to obtain aBMD of the total body less head (TBLH), trunk, lumbar spine, arms, legs, and pelvis. All DXA scans and analyses were performed using the GE encore software (version 4.0.2) and were completed following the same protocol by the same researcher within each study, following recommendations from the International Society of Clinical Densitometry (24). Data from DXA scans were normalized separately within each study and then data were combined.

Briefly, serum triglycerides (mmol/L), fasting glucose (GL, mmol/L), and HDL (mmol/L) cholesterol were measured from fasting blood samples using standard protocols. Venepuncture was performed to obtain blood samples and those were immediately centrifuged, aliquoted within one hour after collection and stored at $-70\text{ }^{\circ}\text{C}$ or below. Systolic and diastolic blood pressure was obtained with an automatic oscillometric device (Omron M6, The Netherlands).

We followed the metabolic syndrome definition proposed by Ortega et al. (9) and the cut-off points reported by Jolliffe and Janssen (25) linked to the International Diabetes Federation adult criteria and Adult Treatment Panel III (25). Participants who did not have any of the following cardiometabolic risk factors altered were classified as MHO: high triglycerides levels (>1.44 and >1.60 mmol/L in boys and girls, respectively), high fasting glucose levels (>5.60 mmol/L), low HDL cholesterol (<1.13 and <1.25 mmol/L in boys and girls, respectively) and elevated systolic (>121 mmHg) or diastolic (>76 and >80 mmHg in boys and girls, respectively) blood pressure. Accordingly, MUO were those who had one or more of these cardiometabolic risk factors altered. Waist circumference was excluded as a risk factor

following previous literature (12,14) since overweight and obese individuals usually present a waist circumference above age- and sex-specific cut-off points for the metabolic syndrome (9,13).

Statistical analysis

All statistical analyses were performed using SPSS version 20.0 for Windows (IBM Corp, New York, USA), and the significance level was set at $p < 0.05$. Data are presented for overweight and obese children. Descriptive analyses were performed by using independent samples *T*-test to detect differences between MHO and MUO within each study (ActiveBrains and EFIGRO).

Analysis of covariance (ANCOVA) was used to examine differences in normalised (Blom's transformation) aBMD between MHO and MUO phenotypes. Sex, years from PHV, stature and TBLH lean mass (normalised) were used as covariates (model 1). The contribution of MVPA, or CRF was tested in additional models (models 2, and 3, respectively). The effect size (ES, Cohen's *d*) on aBMD between MHO and MUO with 1 cardiometabolic risk factor or with 2 or more cardiometabolic risk factors, using the same models of covariates described above is shown. The interpretation of ES is: 0.2 small, 0.5 medium and 0.8 large (26).

The selection of the covariates was based on the association with the outcome and exposure variables. Sensitivity analyses including sedentary time, vitamin D and dietary calcium intake were done (exploratory analysis). These variables did not have influence in the outcome variables, so they were not included as part of the models. Finally, complementary information running the statistical tests only for obese participants is shown as supplementary material.

Results

The descriptive characteristics of the MHO and MUO children within each study are shown in Table 1. In the ActiveBrains study, 50.6% of the participants did not present any metabolic risk factors, 28.4% presented one and 21% presented at least two. In the EFIGRO study, 54.2% of the participants did not present any metabolic risk factor, 34.6% presented one and 11.2% presented two. Overall, MUO children weighed more and, had more BMI, fat mass, waist circumference and triglycerides; and lower HDL cholesterol, levels of MVPA and CRF than MHO children (all $p < 0.05$). In addition, MUO from the EFIGRO study were more mature and older and, had higher glucose levels and lean mass than MHO (all $p < 0.05$). Finally, aBMD did not differ between MHO and MUO children within each study.

Adjusted normalised values in aBMD between MHO and MUO overweight/obese children

Table 2 shows a comparison of the adjusted normalised values in aBMD between MHO and MUO. In model 1, MHO children had higher aBMD in TBLH (ES=0.34; $p=0.026$), trunk (ES=0.43; $p=0.005$) and pelvis (ES=0.33; $p=0.029$), compared to MUO children. Most differences remained significant after MVPA was added as a new covariate (model 2). Interestingly, most significant differences shown in model 1 disappeared once CRF was accounted for (model 3), except for trunk aBMD (ES=0.37; $p=0.016$).

In an attempt to find out whether differences in aBMD were related with the number of cardiometabolic risk factors altered, MUO children were split into two different groups (1 vs. ≥ 2 cardiometabolic risk factors) and compared against MHO children. Then, the ES in aBMD between MHO, MUO with 1 cardiometabolic risk factor and MUO with ≥ 2 cardiometabolic

risk factors were obtained (Figure 1). Taking MHO as the reference group, generally all ES negatively increased from MUO with 1 cardiometabolic risk factor to MUO with 2 or more cardiometabolic risk factors (model 1). This trend remained similar but with smaller ES when MVPA (model 2) or CRF (model 3) was added into the model. The ES change (model 2 - model 1) explained by the contribution of MVPA ranged from 0.00 to 0.01 in participants with 1 cardiometabolic risk factor and from 0.04 to 0.11 in participants with ≥ 2 cardiometabolic risk factors. Finally, the ES change (model 3 - model 1) explained by the contribution of CRF ranged from 0.06 to 0.14 in participants with 1 cardiometabolic risk factor and from 0.05 to 0.18 in participants with ≥ 2 cardiometabolic risk factors.

Sensitivity analysis only with obese children

Descriptive characteristics only for obese participants are shown in supplementary Table 1. Overall, MUO children had higher levels of triglycerides and lower HDL cholesterol (all $p < 0.05$) in both studies. In addition, MHO children from the ActiveBrains study spent more time in MVPA than their MUO peers ($p < 0.05$). Moreover, MHO children from the EFIGRO were less mature than MUO children ($p < 0.05$). Adjusted normalised values in aBMD (supplementary Table 2) showed that MHO children had higher trunk aBMD (ES=0.34, $p = 0.025$) in model 1, compared to MUO children. The addition of MVPA as a covariate (model 2) did not alter these differences. Finally, the significant differences in trunk BMD disappeared once CRF was accounted for (model 3).

Discussion

To the best of our knowledge, this is the first study reporting differences in aBMD in children with MHO and MUO metabolic profiles. Our findings indicate that 1) MHO children have greater aBMD in TBLH, trunk and pelvis than MUO children and, 2) MVPA and more importantly, CRF seems to partially explain these differences between groups. The ES of the differences observed between MHO and MUO ranged from 0.11 and 0.43, suggesting a small to medium effect size. Large effect sizes would have been unexpected due to the multifactorial aetiology of bone and fat status.

We found only one study examining the association between metabolic profiles and aBMD, and it was carried out in adults (11). The authors concluded that there could be a link between MHO/MUO phenotypes and aBMD, since MUO adults had better bone status at hip site than MHO (11). Albeit in adult population, their findings contradict those observed in our study with children, in which MHO children had better BMD. Both studies are difficult to compare as not only the target population is different, but also different confounding variables are taken into account, which may importantly influence the findings. The study by Mirzababaei et al. (11) used age, sex, and BMI as covariates, while a more complete set of confounding variables was used in the present study, such as sex, years from PHV, stature and TBLH lean mass (model 1). In this regard, the use of BMI as a confounder of bone mass is debatable. BMI is calculated using weight and stature where weight is consisting primarily of fat and lean mass. It is a well-known that lean mass is the strongest determinant of bone parameters in young population (27), as the development of the muscles produces a higher tension on the bones following the mechanostat theory (28). However, the association between fat and bone mass is debated (6). A study has shown that the association between fat and bone mass disappears once the effect of lean mass is controlled in adolescents (7).

Additionally, objectively measured MVPA was additionally taken into account in our study (model 2) whose role is clear in the development and maintenance of bone mass (29), and is positively associated with better cardiometabolic risk factor profiles in children and adolescents (30). In agreement with our findings, a previous study concluded that MVPA was higher in MHO adolescents compared to MUO adolescents, both in the group with overweight/obese and only obese (14). However, Camhi et al. (31) did not find significant differences in MVPA between MHO and MUO adolescents with obesity. These discrepancies may be due to the use of different methods to measure physical activity (objective vs subjective). Even so, due to the time spent in MVPA is considered a protective factor of cardiometabolic risk factors during youth (14), using MVPA as a covariate in the association between bone mass and metabolic profiles is recommended. This is supported by our data showing greater ES changes explained by the contribution of MVPA in participants with ≥ 2 cardiometabolic risk factors than in those with 1 cardiometabolic risk factor.

Importantly, we also took into account the effect of CRF (model 3) because of its association both with metabolic profiles (12,13,32), and bone parameters (16,33). In our study, the role of CRF is evident from the change in the ES already observed in participants with just 1 cardiometabolic risk factor. Recently published reviews and updates concluded that a better CRF was considered a characteristic of the MHO individuals and, it is considered a paramount in the prognosis of MHO adults and children (13,15). However, a study published in youth, showed no significant differences in CRF between metabolic profiles (14). Of note, differences in bone parameters have been reported according to fitness levels in adolescents, with those with better CRF having greater BMC in the whole body and extremities than those with poorer CRF (16). Therefore, CRF could be considered a predictor of aBMD in youth (34) and its use as a confounder is justified.

With this in mind, the association between the MHO and MUO phenotypes with CRF and as a consequence with bone parameters, may be due to the known association between CRF with some of the cardiometabolic risk factors, i.e., HDL and triglycerides (35), peculiar of the aforementioned metabolic profiles. In our study, CRF was positively associated with HDL and negatively associated with triglycerides ($r=0.27$ and -0.20 respectively, data not shown), which agrees with the only study published with MHO/MUO in European adolescents (32). In addition, changes in CRF were negatively correlated with changes in triglycerides and HDL/total cholesterol ratio in adolescents, even after controlling for age, gender, fat-free mass and pubertal status (36). Also, young adults with high CRF (≥ 60 th percentile) showed lower triglycerides levels and higher HDL levels compared to young adults with low CRF (< 20 th percentile) (37). In this regard, CRF seems to explain the link between metabolic profiles (MHO and MUO) and aBMD in our study with children.

Furthermore, our findings may be explained through the bone-regulating role of hormones and nutrients. A novel study showed that MHO adults had higher serum concentrations of ionized magnesium, dietary magnesium intakes, and serum osteocalcin while lower serum PTH compared to MUO adults (38). Magnesium, together with the thyroid and parathyroid glands support bone health by stimulating the production of calcitonin, known to act as a bone-preserving hormone and regulating PTH (39). It is well known that PTH regulates serum calcium through its effects on bone, kidney, and the intestine (39). In bone, PTH enhances the release of calcium from the large reservoir and leads to bone resorption (39).

Some limitations of this study deserve to be commented. Firstly, the results cannot establish a cause-effect relationship due to its cross-sectional design. Secondly, supplementary material

data have been obtained using a relatively small sample of obese children (MHO =54 and MUO=69) and, therefore, some of the differences between MHO and MUO are non-significant but borderline. Thirdly, despite the fact that physical activity was objectively measured by tri-axial accelerometers, the obtained data may be affected by various factors and generally produce greater errors than those observed for physical fitness (40). Fourthly, data from observational studies generally show a stronger association between fitness and health parameters than for physical activity and health parameters (40). This might also explain why MVPA did not affect the association between bone parameters and metabolic profile to the same extent than CRF.

In conclusion, this novel research shows that MHO children have greater aBMD than their unhealthy peers (MUO). Furthermore, both MVPA and more importantly CRF, seem to partially explain these differences. Taking into account that children are in a very sensitive period to changes, further longitudinal studies such as clinical trials and cohort studies are needed. These types of studies would allow a better understanding of the development of bone health in overweight and obese metabolic profiles over time.

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Author Contributions

MM, CDS, LA, JHM, JMG and MMM obtained the data. EUG, MM and JMG analysed the data. EUG drafted the manuscript under the supervision of IL and FBO (principal investigators). LGM, IT, VEM and MO contributed and edited the manuscript. EUG had full access to the data in the study and final responsibility for the decision to submit for publication. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Disclosure Statement

The authors have no conflicts of interest relevant to this article to disclose.

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Figure 1. Adjusted differences on aBMD (g/cm^2) between MHO (as reference, $n=99$) and MUO with 1 ($n=60$) or with 2 or more cardiometabolic risk factors ($n=29$). Model 1 adjusted for sex, years from PHV, stature and TBLH lean mass (normalised); Model 2 for model 1+MVPA; and Model 3 for model 1+CRF.