Interaction of bone mineral density, adipokines and hormones in obese adolescents girls submitted in an interdisciplinary therapy

Abstract: Obesity is a chronic inflammatory condition with numerous metabolic consequences to the organism, highlighting its influence on bone mass. Therefore, the aim of this study was to verify the role of visceral fat, leptin, adiponectin and ghrelin on bone mineral density in obese post-puberty adolescents girls, submitted to an interdisciplinary therapy. The study involved 20 post-puberty obese adolescent girls: 16±1.5 years of age, 98.9±15.8 kg (weight), 1.60±0.72 m (height) and 37.2±4.8 kg/m² [body mass index (BMI)]. Anthropometric measurements, body composition, visceral fat, subcutaneous fat, bone mineral density and content were determined. Ghrelin, leptin and adiponectin were analyzed and the leptin/adiponectin ratio was calculated. Our findings showed a significant increase in adiponectin concentration and a reduction in body weight, BMI, total fat mass, visceral and subcutaneous fat. In addition, ghrelin (r²=−0.53; p=0.02) visceral fat (r²=−0.46; p=0.04) (r²=−0.66, p=0.001) and leptin/adiponectin ratio (r²=−0.56, p=0.01) were negative predictors for bone mineral density and content in obese adolescent girls, respectively. It provides a novel physiologically concept that may shed light on the etiology of osteoporosis and help to identify new therapeutic targets. However this should be confirmed in a large cohort study.

Keywords: adipokines; adolescents; bone mineral density; hormone; obesity.

Introduction

The epidemic of obesity has increased significantly over recent decades, including among children and adolescents. This increase represents a public health problem, since being overweight is directly linked to metabolic complications (1). European countries demonstrated a high prevalence of this worldwide epidemic disease (2), as shown in the USA, including the pediatric population. It is predicted that the percentage of obese children will double in numbers by 2030 (3). In Brazil, recent government research demonstrated that 20% of adolescents were overweight according to the references values of World Health Organization (WHO) (4).

Although obesity is itself a disease with serious metabolic consequences, it appears that obesity may be a protective factor against bone fractures and osteoporosis, as obese individuals may have higher bone mineral density when compared to normal individuals (5). However, is well-known that chronic inflammatory diseases are commonly associated with bone mass loss in adults, caused by an excessive increased resorption activity and decreased bone formation (6).

Bone mineralization starts in the fetus, extends throughout childhood and peaks in adolescence. The period of childhood and adolescence is marked by a higher rate of bone formation, with a predominance of formation over resorption. In adults both processes are stabilized, and from age 45 to 50 years, especially in females, there is a predominance of bone resorption (7).

Body mass has been considered one of the strongest predictors of bone mineral content (BMC) and bone mineral density (BMD) (8). The protective effect of fat over the bone may be mediated by hormonal signals that involve several biochemical adipokines related to obesity (9). Some data suggest that body fat distributions – especially visceral adipocytes – are linked to the secretion of pro-inflammatory adipokines that can act negatively on bone metabolism (10–12). In a previous study by our group, we demonstrated...
that visceral fat, as well as the visceral/subcutaneous ratio, were negatively independent predictors of BMD in boys and girls, respectively. However, subcutaneous fat had a protective influence in BMD only in boys (13).

Adiponectin, the most important anti-inflammatory protein, is secreted by adipose tissue. It increases insulin sensitivity and the oxidation of free fatty acids. Adiponectin concentrations inversely correlate with body fat and are reduced in obese subjects. It is believed that adiponectin play a key role in bone formation, acting specifically on the osteoblasts metabolism (14).

Leptin is a protein that is produced and secreted by adipose tissue. Beyond its effect on inhibiting food intake and increasing energy expenditure at the central level, leptin appears to play a pro-inflammatory function. Leptin, presents reversed results to adiponectin – its performance seems to be related to the inhibition of bone resorption, predominantly osteoblastic activity (15).

Ghrelin is a polypeptide secreted mainly by neuroendocrine cells of the fundus of the stomach. It is known that ghrelin plays a key role in energy balance and caloric intake and is inversely related to BMI. Moreover, some investigations showed a controversial role of ghrelin in bone metabolism (16). However, the mechanisms of these adipokines are still not sufficiently clarified in the literature.

Therefore, the aim in the present investigation is to analyze possible interactions between obesity and bone metabolism in adolescents undergoing interdisciplinary therapy; these interactions are unclear and controversial regarding the role of visceral fat, ghrelin, leptin and adiponectin.

## Material and methods

### Population

The study involved 20 post-puberty obese adolescent girls: 16 ±1.5 years of age, 98.9±15.8 kg (weight), 1.60±0.72 m (height) and 37.2±4.8 kg/m² [body mass index (BMI)]. Inclusion criteria were Tanner stage 5, primary obesity, BMI >30 kg/m² (BMI >95th percentile of the CDC reference growth charts). Medicaments with cortisone can promote metabolic changes in the human organism, such as an increase in the body mass. Exclusion criteria were: the use of birth control pills, cortisone, use of anti-epileptic drugs, history of renal disease, alcohol intake, smoking, secondary endocrine disorders caused by obesity, history of fractures and fixed assets and long-term supplementation of calcium and/or other drugs that can affect bone metabolism. Thus, the use of cortisone was considered an exclusion criteria of this study. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee on research at the Universidade Federal de São Paulo (number #0135/04) and registered as a clinical trial at www.clinicaltrials.gov NCT 0135/7883.

### Anthropometric measurements

The patients were weighed wearing light clothes and no shoes on a scale (Filizola – M150/4 – Brazil), and their weight was recorded to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm using a stadiometer – Holtain Limited – Crymych, Dyfed, made in Britain.

### Serum analysis

Blood samples were collected at the clinic after an overnight fast. The concentrations of leptin, ghrelin and adiponectin were evaluated by commercial kits for immunoassays (eBioscience, San Diego, CA and R&D Systems, Minneapolis, MN) according to the manufacturer’s manuals.

### Measurement of visceral and subcutaneous fat

The examination was performed by a specialist in imaging diagnostics using a multifrequency transducer (broadband) 3.5 MHz, which reduces the risk of error analysis. The ultrasound measurement of subcutaneous fat tissue was defined as the distance between the skin and external face of the rectus abdominal. The visceral fat tissue was defined as the distance between the inner face of the rectus abdominal and the anterior wall of the aorta (17).

### Bone mineral density and bone mineral composition

The examination for the determination of BMC (g) and BMD (g/cm²) was performed by a unit of bone densitometry by attenuation of X-ray double energy (DXA), using a Hologic device QDR4200 (Hologic, Bedford, MA) and appropriate software for bone assessment (17).

### Statistical analysis

Statistical analysis was performed using the program STATISTICA version 7.0 for Windows Vista. The adopted significant value was α<5%. Data normality was verified with the Shapiro-Wilk test. Parametric data were expressed as mean±SD, and non-parametric data were expressed as median, minimum and maximum values. To verify the sample, a homogeneity between groups t-test was applied. A student t-test was applied to analyze the effects of therapy over 1 year. For the non-parametric data the Friedman test was used and if necessary we applied the Wilcoxon test. Correlation analyses were established through a simple linear regression.

### Clinical intervention

### Medical monitoring

The adolescent subjects underwent a diagnostic clinical evaluation of their general health (family history and obesity) and were assessed for sexual maturation. Subsequently, they were followed monthly.
Physical intervention

During the intervention (1 year), the adolescents followed a program of combined training (aerobic and resistance exercise), which included sessions of 60 min (30 min aerobic and 30 min resistance) three times a week (180 min/week), under the supervision of an exercise physiologist.

Nutritional intervention

Food consumption was set at the recommended levels of dietary intake for individuals with low levels of physical activity, based on age, gender and a balanced diet. No medication or supplement for weight loss were prescribed. Once a week, the adolescents attended classes on topics related to improving food consumption and all received individual consultations during the intervention program. At the start and end of the long-term multidisciplinary therapy each adolescent filled in a 3-day dietary record with the help of his/her parents. Portions were measured in familiar volumes and sizes. The nutritionist taught the parents and the adolescents how to record food consumption. These dietary data were transferred to a computer by the same nutritionist, and the nutrient composition was analyzed using a PC program developed at the Universidade Federal of São Paulo (Nutwin Software, for Windows, version 1.5, 2002) based on western and local food tables. In addition, the parents were encouraged to call if they needed further information (17).

Results

Effects of interdisciplinary therapy

The analysis of the intervention effect showed a significant reduction of: body weight (from 98.9±15.8 to 90.3±13.5 kg; p=0.003); BMI (from 37.4±4.89 to 34.0±4.24 kg/m²; p=0.000); total fat mass (from 49.9±10.6 to 42.6±8.4 kg; p=0.000); visceral fat (from 3.4 to 2.3 cm; p=0.030); subcutaneous fat (from 3.6 to 3.1 cm; p=0.001) and total BMD (from 1.3±0.1 to 1.2±0.1 g/cm²; p=0.001). Moreover, there was a significant increase in the adiponectin concentration (from 8.7±2.6 to 10.6±4.3 ng/mL; p=0.000). However, the values of ghrelin, leptin, lean body mass, BMC and the leptin/adiponectin ratio were not significantly changed (Table 1).

Regression analysis

The simple linear regression showed that visceral fat was seen as a predictor for reduction of total BMD (r²=-0.40, p=0.04) and BMC (r²=-0.66, p=0.001). Another important result observed in this study was that ghrelin can be considered as a predictor for reduced total BMD, as its concentration was negatively associated with total BMD in girls (r²=-0.53, p=0.02). The leptin/adiponectin ratio was negatively correlated with BMD (r²=-0.56, p=0.01) (Figure 1A–D). The regression analysis of adiponectin and leptin concentrations with BMD and BMC did not showed significantly correlations.

Discussion

The main purpose of this investigation was to analyze the interactions between hormonal regulations and bone metabolism in obese adolescents. Therefore, the most important finding is that ghrelin concentration is negatively correlated with total BMD, which corroborates a previous study (18). However, another study previously demonstrated a positive association between ghrelin concentration and BMD in children and adolescents (19). These contradictory results in the literature are supported by the possible roles of ghrelin, either in acylated or des-acylghrelin form, in mediating its biological actions (20). Data showed that 80%–90% of circulating ghrelin is in the des-acylghrelin form, which seems to be a potent stimulator of a proliferation of osteoblasts in humans (21). It is also important to highlight the role of ghrelin as an orexigenic hormone on increased release of neuropeptide Y (NPY); which culminates in the inhibition of hypothalamic osteoblast factor, finally promoting a reduction in the expression of osteoblastic cells, responsible for bone formation (16).

Interestingly, we observed a negative correlation between visceral fat and total BMD and BMC. Indeed, it is noteworthy that visceral fat presents itself as a close relation to plasma levels of leptin and that both act on the mechanisms of bone metabolism. These findings corroborate with others authors, who demonstrated that visceral fat was a negative predictor for BMD and this correlation was stronger in females. Moreover, this result reinforces the necessity of an early intervention to combat obesity; because of the importance of early prevention of osteoporosis in women (22, 23).

It is known that visceral fat is involved in the secretion mechanism of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and they work in the activation of osteoclast differentiation, resulting in a decrease of BMD because of increased bone resorption (22). Therefore, we emphasize with our findings the intimate relationship between high visceral
fat and increased leptin concentration, agreeing that both factors act unfavorably on bone metabolism in girls. Moreover, the regulatory loop of bone remodeling, which involves the hormone leptin, may help to explain the effect of obesity on bone metabolism (24).

Leptin plays a very important role in bone metabolism, although the literature regarding this is contradictory (25). Some authors argue that their action on bone metabolism is directly related to the depletion of osteoblastic activity without any influence on the osteoclasts. In fact, our data demonstrated that BMD in girls decreased significantly, which can be particularly explaining by a state of hyperleptinemia that remained, even at the end of intervention. These data corroborate with previous findings from our group, where leptin concentration in adolescents correlated negatively with BMC (17). This should be confirmed in a large cohort study, as we were not able to show a correlation between leptin and bone metabolism.

The highlight result from this study was the increase of adiponectin concentration after therapy, favoring its anti-inflammatory effects on this analyzed population. In agreement, it has been demonstrated that adiponectin can reduce osteoclasts numbers and can favor bone formation via activation of osteoblastogenesis (26). Moreover, multiple clinical, meta-analysis and genetic studies have correlated hypoadiponectinemia with insulin resistance and higher adiponectin concentrations associated with lower risk of type II diabetes (26–29). These findings support a primary role for adiponectin in preventing metabolic disease in humans (30), especially in obese adolescents (17). However, in the present study we did not show a correlation between adiponectin and bone metabolism, probably because states of hyperleptinemia may contribute to the increase in the pro-inflammatory conditions even in obese adolescents.

In agreement with these hypotheses, recent studies show that the leptin/adiponectin ratio presents a potent...
biomarker of the pro-inflammatory state related to obesity, including in adolescents (31, 32). Therefore, another interesting result observed in our study is that the leptin/adiponectin ratio was a negative predictor of BMC. This is an important finding, as the role of this pro-inflammatory marker in bone metabolism can be useful in clinical practice. However, after the interdisciplinary therapy the values of the leptin/adiponectin ratio did not change significantly, probably because of the state of hyperleptinemia in obese adolescents (Table 1 and Figure 1), as mentioned above.

Finally, the small sample size represents a limitation of this study, although we can conclude that ghrelin, visceral fat and the leptin/adiponectin ratio were considered to be negative predictors of BMD and BMC, respectively. These are important findings that show that increase values of these variables promote decreased in BMD and BMC values in obese adolescent girls. It provides a novel physiological concept that may shed light on the etiology of osteoporosis and help to identify new therapeutic targets. However our findings should be confirmed in a large cohort study.

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### References


### Table 1  Variables examined in obese adolescents girls during the interdisciplinary therapy.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Year</th>
<th>p-Value</th>
<th>Δ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>98.9±15.8</td>
<td>90.3±13.5*</td>
<td>0.003</td>
<td>–8.6±8.1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.60±0.72</td>
<td>1.62±0.74</td>
<td>0.329</td>
<td>0.005±0.002</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>37.4±8.49</td>
<td>34.0±4.24</td>
<td>0.000</td>
<td>–3.2±3.03</td>
</tr>
<tr>
<td>Fat mass total,%</td>
<td>51.3±4.3</td>
<td>47.6±4.2*</td>
<td>0.000</td>
<td>–3.7±2</td>
</tr>
<tr>
<td>Fat mass total, kg</td>
<td>49.9±10.6</td>
<td>42.6±8.4*</td>
<td>0.000</td>
<td>–7.3±5.6</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>3.4 (1.7–6.5)</td>
<td>2.3 (1.2–8.4)*</td>
<td>0.030</td>
<td>–1.1 (–3.0 to 4.1)</td>
</tr>
<tr>
<td>Subcutaneous fat, cm</td>
<td>3.6±0.7</td>
<td>3.1±0.6*</td>
<td>0.001</td>
<td>–0.5±0.6</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>29.7±11.5</td>
<td>30.6±14.4</td>
<td>–</td>
<td>0.9±3.8</td>
</tr>
<tr>
<td>BMD total, g/cm²</td>
<td>1.3±0.1</td>
<td>1.2±0.1*</td>
<td>0.001</td>
<td>0±0.1</td>
</tr>
<tr>
<td>BMC total, kg</td>
<td>26.7±3.2</td>
<td>27±5.1</td>
<td>–</td>
<td>2.4±3.8</td>
</tr>
<tr>
<td>Adiponectin, ng/mL</td>
<td>8.7±2.6</td>
<td>10.6±4.3*</td>
<td>0.000</td>
<td>1.8±2.8</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>34.9±14</td>
<td>31.4±11.4</td>
<td>–</td>
<td>0.1±12.1</td>
</tr>
<tr>
<td>Leptin/adiponectin ratio</td>
<td>4.41±2.5</td>
<td>3.73±2.5</td>
<td>–</td>
<td>–0.68±1.9</td>
</tr>
<tr>
<td>Ghrelin, ng/mL</td>
<td>1.4 (0.7–7.8)</td>
<td>1.4 (0.9–17.9)</td>
<td>–</td>
<td>0.02 (–3.6 to 15.6)</td>
</tr>
</tbody>
</table>

BMI, body mass index; BMD, bone mineral density; BMC, bone mineral content. *p<0.05 comparison of basal vs. year. ‘non-parametric data described as median and minimum and maximum values.


