Obesity Effects on Bone Femur Tissue of Animals Subjected to Obesity through High-fat Diet

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors CMN, FAF, MLB and SLB did the experimental studies, data and statistical analysis, manuscript preparation, editing and review. Authors ALCAR and DSL did the experimental studies, data analysis and manuscript preparation. Authors CAN and PON did the definition of intellectual content, design, experimental studies, data and statistical analysis, manuscript preparation, editing and review. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Adipose corporal tissue accumulation may cause alterations in the bone metabolism and might influence its properties. However, there are still many controversies, in addition to how the adipose tissue can cause these changes.

Aims: To evaluate the femur bone tissue behavior of animals submitted to obesity through cafeteria diet.

Place of Study: The Ethics Committee on Animal Experiments and Practical Classes (CEEAAP) of UNIOESTE has approved the project, which was developed at the same university.

Methodology: It was used twenty-four 8-week-old male Wistar rats, which were obtained at UNIOESTE’s central vivarium and kept in the Endocrine Physiology and Metabolism Lab of this institution. The animals were divided in: CONTROL group (CG) that received a standard diet and OBESE group (OG) that underwent a high-fat diet, during 28 weeks. We analyzed the obesity parameters, such as body weight and Lee index and femur bone properties like the quantity of osteocytes, the thickness of the cortical bone mass and the epiphyseal line’s thickness. All data were analyzed and evaluated using the Student-T Test ($p<0.01$).

Results: The results pointed that the animals that underwent a high fat diet became obese (OG = 624.00±4.00 g vs CG = 466.00±1.50 g). The obese animals presented a statistically significant ($p<0.01$) decrease of all analyzed bone parameters, when compared to the non-obese animals, including the amount of osteocytes (OG = 27.20±0.90 vs CG = 30.30±0.70), the thickness of the epiphyseal line (OG = 171.70±0.92 µm vs CG = 175.50±1.07 µm) and the thickness of femur cortical bone mass (OG = 434.10±2.03 µm vs CG = 495.50±2.55 µm).

Conclusions: The obesity had negative influences on the femur bone tissue of the animals that received a high-fat diet, due to a finding of a decrease on all bone parameters analyzed. We could highlight, too, that the applied cafeteria diet was effective to induce obesities in the animals.

Keywords: Obesity; bone; femur; histology.

1. INTRODUCTION

Obesity is considered an epidemic condition around the world and it has already been highlighted as one of today’s most blatantly disease [1,2,3]. Recently, a possible relationship between bone mass and adipose tissue [4,5] has been recognized.

The bone tissue is a dynamic tissue that is in a constant process of formation and resorption [6,7], and which requires the balance among several factors to ensure the maintenance of its structure and functionality [4,8]. Obesity seems to affect the bone metabolism through multiple mechanisms, contributing to the increase [9,10,11,12] or decrease of the bone mass [6,7].

Traditionally, clinical epidemiological studies show a positive association between body weight increasing and fat mass, resulting in bone mineral density [9,10,11,12] and lower risk of fractures [13,14,15].

Thus, obesity is considered as a protective factor for the bone. The probable mechanisms responsible for this relationship are: high mechanical load on bone due to increased body weight, stimulating bone formation [4,6], stimulation of estrogen synthesis by adipose tissue [7], and changes in the insulin metabolism, often associated with obesity [4].

On the other hand, other studies link the increase of body fat mass to the decrease of total bone mineral density and total bone mineral content, this way, obesity would lose its role of bone protector against osteoporosis [6,8,16].

The physiological mechanisms that may explain this negative relationship between bone tissue and obesity are: first, the common origin from stem cells marrow to osteoblasts and osteoclasts, so that obesity can reduce the formation of bone cells while increases the formation of adipocytes [6,7]. Furthermore, the adipose tissue may stimulate the production of inflammatory cytokines and adipocytes [6,7,17,18], which are able to stimulate the osteoclastogenesis through receptor activator of NF-κB (RANK)/RANK ligand pathway [6,17].

Added to these factors, there is also an increase of leptin secretion and/or a decrease of adiponectin common in obesity, which may
contribute to the accumulation of macrophages, and, thus, intensify the cytokines inflammatory role on bone resorption [6,7]. The high-fat diet, which generally is the cause of obesity, has been associated to the decrease of calcium absorption by the intestine [19].

This way, the increased tissue resulting profile is a vast production of bioactive molecules that may act in a paracrine way increasing the inflammatory state of adipose tissue and, at the same time, releasing cytokines in the circulation, promoting an important inflammatory effect system important in promoting circulation systemic inflammatory effect [18].

Despite several evidences, there are still a lot of issues about the relationship between obesity and bone metabolism. While most clinical studies demonstrate protective role of obesity on bone tissue [10,11,12], some research with animal models establish negative effects [6,7,20,21]. It is evident that there are several controversies between the results of various studies [5], there are important differences when comparing data of epidemiological studies with physiological mechanisms.

Understanding how this intricate relationship studies can help identify the mechanisms involved in the process of bone formation and resorption. This way, there can be a contribution to the development of therapeutic interventions for the prevention and treatment of obesity and processes of osteopenia and osteoporosis, collaborating to the life quality of people affected by the diseases in discussion, and beyond that, the early intervention, the reduction of these diseases incidence and their complications can greatly contribute to the decrease of public expenses related to it. All in all, enabling to target these resources to promote health and quality of life to these individuals.

1.1 Objectives

To evaluate the femur bone tissue behavior of animals that was subjected to obesity through cafeteria diet.

2. MATERIALS AND METHODS

This was a quantitative and experimental research. The research was approved by Ethics Committee on Animal Experimentation (CEUA/UNIOESTE) UNIOESTE. To conduct this experiment, 24 8-week-old male Wistar rats were used. They were obtained at the central vivarium of UNIOESTE and kept at the Laboratory of Endocrine Physiology and Metabolism of the institution under controlled light (12 h of light / darkness) and temperature (21°C±2°C). The animals were randomly divided into two groups:

1) Control Group (CON) received standard diet and ad libitum access to water during 28 weeks.
2) Cafeteria Group (OBESE) received high-fat diet and ad libitum access to soda during 28 weeks.

To induce obesity in the animals, the exogenous obesity model, known as the westernized diet, fast-food or cafeteria diet, was used. This model consists of the administration of a hypercaloric diet with an increased amount of fats and carbohydrates [22,23]. The exact composition of the diet is shown on Table 1.

The amount of each type of food offered to each animal was weighed daily to prevent any difference in the food supply among the animals.

To control the animals’ weight, they were individually weighed once a week throughout the experimental period.

At the end of the 28th week of the experiment, the animals were weighed and the nasoanal length was obtained for the Lee index calculation (cube root of body weight (g)/nasoanal length (cm) [24]. Subsequently, the animals underwent desensitization by CO2 and euthanasia by decapitation.

Afterwards, the right femur of the animals was removed by dissection and then fixed in a 10% formaldehyde solution for 24 hours. The standard histological process was performed, and the histological sections measuring 5 µm was stained with hematoxylin and eosin.

Quantification of osteocytes from five consecutive fields/areas of the alveolar bone crest starting from the highest point were performed. The samples were viewed at × 100 magnification. Two observations per field were performed per field, then calculated foe each animal of each group [25].

We used the Shapiro-Wilk test to evaluate the normality of distribution. The data obtained were analyzed and evaluated using Student-T Test, considering $P<0.01$. 

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Table 1. Cafeteria diet (High-fat diet) composition

|                        | Energy value kj/100 g | Carbohydrates g/100 g | Proteins g/100 g | Fat g/100 g | Sodium mg/100 g |
|------------------------|-----------------------|------------------------|------------------|-------------|----------------|
| Cheetos Snack (Pepsico, Brazil) | 1948                  | 72                     | 6,4              | 17,2        | 676             |
| Bacon Snack / Troféu (Santa Helena, Brazil) | 2200                  | 56                     | 8,8              | 30          | 1040            |
| Cornstarch cookie (Zadimel, Brazil) | 1793                  | 73                     | 8                | 10,7        | 300             |
| Chocolate cake (Renata, Brazil) | 1798                  | 55                     | 5                | 21,7        | 141,7           |
| Soda/Coke (Coca-cola, Brazil) | 178                   | 11                     | 0                | 0           | 5               |
| Soda/Guaraná (Antarctica, Brazil) | 168                   | 10                     | 0                | 0           | 5,5             |
| Italian salami (Sadia, Brazil) | 1822                  | 2                      | 22               | 38          | 1140            |
| Sausage (Sadia, Brazil) | 1554                  | 1,4                    | 16               | 34          | 1342            |
| Bisnaguinha (Nutrella, Brazil) | 1328                  | 54                     | 11,2             | 6,2         | 300             |
| Chocolate Waffer (Bauducco, Brazil) | 2176                  | 63                     | 5                | 27          | 113             |
| Bologna (Frimesa, Brazil) | 845                   | 2                      | 12               | 16          | 1545            |
| Marshmallow (Fini, Brazil) | 1423                  | 80                     | 5                | 0           | 46              |

Table 2. Body weight values, nasoanal length and lee index

| Groups      | Final weight (g)     | Nasoanal length (cm) | Lee index (g/cm) |
|-------------|----------------------|-----------------------|------------------|
| Control     | 466.00±1.50          | 24.00±1.00            | 318.80±1.25      |
| Obese       | 624.00±4.00          | 25.90±1.00            | 329.00±1.20      |

Different letters mean that the results are statistically different, in the same parameter of evaluation. The values represent average ± standard deviation (P<0,01)

3. RESULTS

Above Table 2 shows the mean values of final body weight, nasoanal length, and Lee index with respect to the groups of rats. The results significantly demonstrated that the rats subjected to the cafeteria diet had a mean increase of 33.90% in total body weight in the control group, an average increase around 158 g. Regarding the Lee index, it corresponded to an increase of 10.2 g per centimeter to the obese animals. These results show that the diet model used was effective to induce obesity in animals.

Table 3 shows the average result of the number of osteocytes in the animals according to the groups. As the results show, induced-periodontal disease resulted in a 10% decrease in the number of osteocytes in the animals with induced obesity, when compared to the Control Group, indicating that group obese had a bone mass decrease.

Table 3. Average of counting – Femur osteocytes of animals

| Groups      | Averages (unities) |
|-------------|--------------------|
| Control     | 30.30 ± 0.70       |
| Obese       | 27.20 ± 0.90       |

Different letters mean that the results are statistically different. The values represent average ± standard deviation (P<0,01)

Table 4 presents the measurement of the epiphyseal femur line’s thickness and the cortical femur layer’s thickness. According to these results, it is possible to observe that the obesity contributed to a decrease, concerning the thickness of the epiphyseal line and resulted in a mean decrease of 12.4% on the cortical femur thickness of the obese group.

Table 4. Average values of the epiphyseal femur line’s thickness and the cortical femur layer’s thickness

| Groups      | Epiphyseal femur line’s thickness (µm) | Cortical femur layer’s thickness (µm) |
|-------------|----------------------------------------|--------------------------------------|
| Control     | 175.50 ± 1.07                        | 495.50 ± 2.55                        |
| Obese       | 171.70 ± 0.92                        | 434.10 ± 2.03                        |

Different letters mean that the results are statistically different. The values represent average ± standard deviation (P<0,01)
4. DISCUSSION

From the 1990s, obesity has become associated with an inflammatory state [6,7,17,18]. At the beginning of this same decade, several studies arose, particularly in the field of clinical epidemiology, linking obesity and changes in bone mass [4,5].

According to the results of this study, it was possible to verify the effectiveness of the cafeteria diet to induce obesity. In addition, the animals subjected to the cafeteria diet showed a significant increase in the weight of 33%, when compared to the group control. Same results are observed when it comes to the Lee index, in which the values of the group obese were significantly higher than the group control's. Other studies which used similar models of diet also similar results if compared to the present study (Table 2) [26,27,28].

Regarding the bone tissue evaluation, all the evaluated bone parameters had a significantly decrease of values related to the group obese. These results indicate that obesity contributed to changes in the bone tissue, tending to resorption process thereof (Tables 3 and 4).

Studies show that the excessive increase in body fat is related to a decrease of bone mineral density [16,29]. Several studies indicate that the increase of proinflammatory cytokines production contributes to the development of osteopenia and osteoporosis [8,30].

It is also recognized that obesity is associated to a degree of inflammation and this inflammatory chronic response is characterized by abnormal production of cytokines, increase in acute phase reactants and activation of inflammatory signaling pathways [6,7,18,31].

The data obtained pointed that obese animals subjected to high-fat diet had impairment in morphological properties, diminution of cortical thickness and reduction of the tibia’s maximum load support [21]. These results were attributed to increased activity of osteoclasts stimulated by the inflammatory state of obesity (Table 4).

High-saturated fat diets can impair calcium absorption and bone mineralization in growing animals, causing consequences for bone health [32]. We could observed that the administration of high-fat diet for a long term caused to the animals a reduction in femur cortical bone mass [33] and changes in bone morphology of the tibia and metatarsus, suggesting that bone tissue would not adapt itself positively to this diet [34].

The total body weight and body composition can be important determinants of bone quality [20]. Overweight and obese female adolescents had higher bone mineral content and bone mineral density compared with normal-weight adolescents. Although, when adjustments were performed for lean body mass, there were no differences between groups. The authors suggest that the bone adapts itself to lean mass increase [35].

Cao et al. [20] evaluated the effect of administration of a high fat diet on bone on the tibia and osteoprogenitor cells of rodents. The authors found higher levels of osteoprogenitor marrow cells in the group subjected to obesity; however, there were no structural differences in the animals’ cortical bone, while the trabecular bone had its volume reduced.

Obesity seems to affect bone metabolism through multiple mechanisms, therefore it can induce adipogenesis while decreasing osteoblastogenesis. The direct or indirect production of leptin and adiponectin related to obesity may influence bone metabolism. The action of leptin on bone seems to be variable, however, it seems to negatively affect the bone metabolism of animals subjected to high-fat-diet-induced obesity [6,7] (Tables 3 and 4).

The subcutaneous and visceral fats seem to have distinct effects on bone density and strength. Gilsanz et al. [36] pinpointed that, in young women, the subcutaneous fat was positively associated with the bone mass peak and bone strength, while the visceral fat was negatively associated with the bone health. Pollock et al. [37], showed that the metabolic changes associated with overweight and visceral adiposity may negatively influence bone mass in adolescents. These data indicate that the influence of the body fat mass on bone depends on where the fat is deposited.

Dytfeld et al. [38] researched the influence of lean mass and body fat mass on bone mineral density in postmenopausal women, diagnosed with osteoporosis. The authors drew the conclusion that as much fat mass as lean mass are crucial for increasing bone mineral density. However, the presence of obesity does not discard an association with osteoporosis.
On the other hand, the evaluation of the correlation between body mass index (BMI) and the risk of fractures in 60,000 men and women have concluded that low BMI is a risk factor for all types of fractures, independent of age and sex of the individuals, but, dependent upon the bone mineral density. The effect of adipose tissue causing increased load on the femur would provoke an extra protection factor against hip fractures [15].

In fact, there are a large number of studies about the relationship between the amount of fat mass and bone mineral density, however, the various evaluation methods and various forms of statistical analysis adds to the large variability of results found in the literature and make it difficult to compare studies [5]. It is necessary to conduct more studies that confront the clinical epidemiological data with biochemical and structural characteristics.

5. CONCLUSION

It could be concluded that, for this study, obesity negatively influenced the femur bone tissue concerning the animals subjected to a high-fat diet, due to its contribution to the thickness decrease of the cortical bone mass, epiphyseal line and amount of osteocytes reduction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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