

Mineral Contents and Somatometric Parameters in the Hemimandible, Tibia and Incisor of Rats Submitted to a Hypothalamic Obesity Condition

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Abstract

Background: Obesity is a degenerative disease, causing several metabolic disturbances in different systems of the body. However, the effects of obesity on the development of mineralized tissues have not been well explored.

Objectives: The aim of this study was to investigate the effects of hypothalamic obesity, induced by monosodium glutamate (MSG) on morphometric parameters in the tibia, hemimandible and incisor tooth in rats, as well as on the mineral content in the tibia, enamel and dentine.

Materials and Methods: Twelve male Wistar rats were separated in a Control and experimental groups (MSG). The Control group received a daily subcutaneous injection of saline solution, while MSG group received the dose of 4 g/kg of body weight, on the five first days after birth. On the 90th day, all rats were dead to be evaluated the Lee Index (LI), retroperitoneal fat deposit, the bone weight, density, volume, morphometric and mineral content.

Results: In the MSG group the LI, retroperitoneal fat deposits and bone density were significantly increased while the bone weight, volume, and morphometric parameters were significantly decreased. The percentage of phosphorus was reduced and calcium was increased in the MSG group.

Conclusion: In the obesity induced by MSG the mineral bone content is altered and produced significant changes in the somatometric parameters in the rat hemimandible, tibia and incisor.

Keywords

Bone, Incisor, Obesity, MSG, Morphometry

Introduction

Bone has three principal functions in the organism; to support the muscle for the locomotion, protect vital organs such as the heart and brain and to regulate calcium-phosphorus levels. Bone has also been considered a key factor for the regulation of energetic metabolism [1-6] and studies have been discussed the relationship between obesity and bone metabolism [7-9]. Currently, obesity is considered a degenerative disease which causes insulin resistance, type 2 diabetes (T2D), atherosclerosis, stroke, and certain types of cancer [10-12].

The hypothalamic obesity model which consists of daily subcutaneous injection of monosodium glutamate (MSG) during the first days after the birth [13-21] produces in rat, a reduction in the size of heart, lungs, spleen, pancreas, kidneys, testes, brain and submandibular glands [22] as well as in the number of intestinal

goblet cells and expression of alkaline phosphatase [23]. Associated to periodontitis, obesity increases the alveolar bone loss and the insulin resistance [24]. A decrease in the TNF- α gene expression and a consequent reduction in alveolar bone loss has been observed in MSG-obese rats [25]. The obesity does not exert a significant influence on the strength of the cortical bone [26] but, changes in cortical bone, in response to a high-fat diet, in young-to-adult mice [27] were reported. Therefore, poor knowledge and the existence of controversy about the effects of obesity on bone reinforce the need for further studies.

Thus, the aim of this study was to investigate the effects of hypothalamic obesity, induced by MSG, on morphometric parameters in the tibia, hemimandible and incisor tooth in rats, as well as on the mineral content in the tibia, enamel and dentine.

Materials and Methods

Animal's treatments

Twelve male Wistar rats with average of 25 g from the UEPG animal house were weaning on the 21st postnatal day and were maintained under conventional conditions of lights, room temperature, balanced nutrition food and water *ad libitum*. The animals were separated into two groups: an experimental group, denominated MSG and the control group (C). From day 0 to day 5 of life, the experimental group received a daily subcutaneous injection of MSG (4 g/kg of body weight) [28] and the C group received a subcutaneous injection of saline solution. All experiments were performed in the morning to avoid circadian variations and after approval by UEPG's Ethics Committee for Animal Experimentation (protocol number -02/2011). On the 90th postnatal day, all rats were anesthetized with halothane. The body mass (weight body) and the length from the cranial to caudal regions were recorded to estimate the Lee Index (LI) [29]. After that, the animals were died to obtain the hemimandible and tibia, and the retroperitoneal fat which was immediately weighed. Left hemimandibles and tibias were dissected, cleaned, fixed in 4% paraformaldehyde for one week and then stored in 70% alcohol. The body, retroperitoneal fat and bones weight were determined using a semi-analytical electronic balance; volume was determined by the Archimedes method [30]. Density was calculated from the formula $d = m/v$ where m = mass (g) and v = volume (cm³), obtained from the Archimedes method and by the bone tomographic images taken using the GE equipment (with eight channels) at the São Camilo Hospital (Ponta Grossa city) and expressed in Hounsfield Units - (HU). Hemimandible and tibia morphology were evaluated by radiographical analysis using a Sirona CSU imaging system (80kV, 11mA, Siemens, Germany) and a Kodak Lanex 20X25 frame (USA) and radiographic film (Kodak, Brazil). The morphometry was obtained using a calibrated ruler to take measurements of the previously defined axis (Figure 1) [31]. For the incisor, the measurements were made in three cross-sections (2 mm) per animal, obtained using a diamond cutting disc (7" diameter) and a precision cutting machine (Isomet 1000) [32]. Digital pictures were obtained and Image J software (public domain) was used for the morphometric measurements

were expressed in μm after the pixel calibration from a bar of 200 μm . The microanalysis of mineral content, expressed in percentage of atomic weight (Wt %), was estimated by Energy dispersive X-ray spectroscopy (EDS) using a Shimadzu SSX-550 Scanning Electron Microscope (SEM) [33] at one point of the cross-sections of tibia, hemimandible, enamel and dentin. For the EDS analysis, minerals present in large quantities are those with values higher than 10%, and in small quantities are those with values lower than 10%; elements with values lower than 1% indicate "traces" [34].

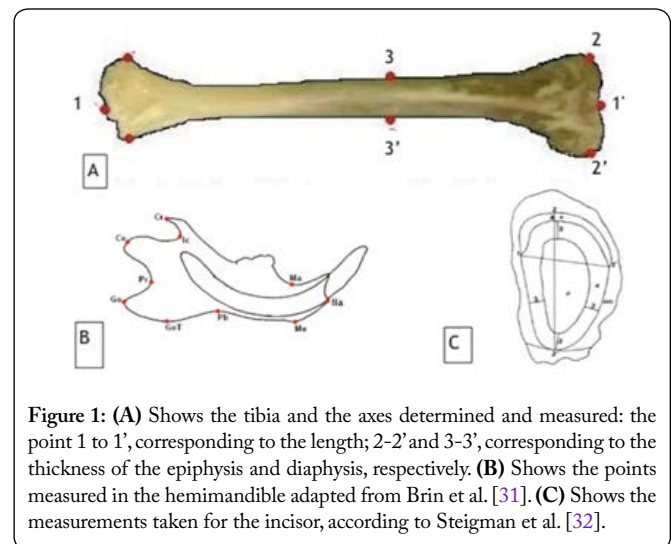


Figure 1: (A) Shows the tibia and the axes determined and measured: the point 1 to 1', corresponding to the length; 2-2' and 3-3', corresponding to the thickness of the epiphysis and diaphysis, respectively. (B) Shows the points measured in the hemimandible adapted from Brin et al. [31]. (C) Shows the measurements taken for the incisor, according to Steigman et al. [32].

Statistical analysis

The parameters were analyzed by Graphpad prism software 3.0 using the Tukey t test at a level of significance of $P < 0.05$. The mineral content was expressed as percentage of atomic weight (Wt %) relative to the control group (C), according to previous reported [34].

Results

Obesity increases the Lee index (LI) and the retroperitoneal fat deposit

The weight of body, LI and the retroperitoneal fat deposit results for both groups are shown in figures 2A-C respectively. The weight of body was decreased in MSG group, however the LI as well as the retroperitoneal fat deposit were increased in the MSG group ($P < 0.05$).

Obesity decreases the weight, volume and density of the hemimandible and tibia.

Figures 3A-D show a significantly reduction in the MSG group ($P < 0.05$) for weights and volumes of the hemimandible and tibia, respectively, when compared to the C group. Figures 3E-H demonstrate an increase for the density of the hemimandible and tibia in the MSG group respectively, when compared to the C group.

Obesity reduces the size of the hemimandible, tibia and incisor tooth

Figures 4A and 4D show the radiographs of the

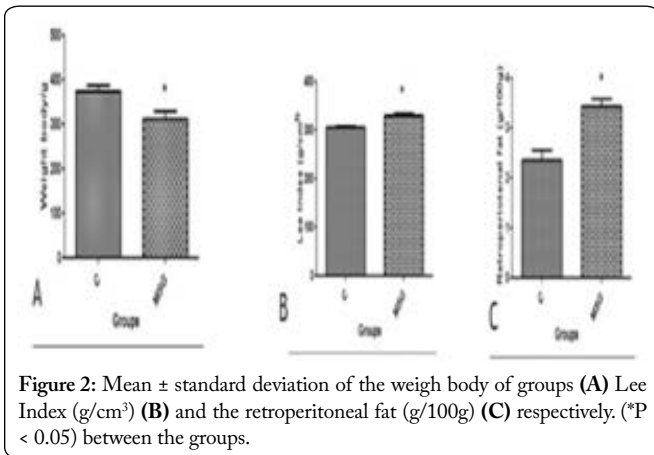


Figure 2: Mean ± standard deviation of the weigh body of groups (A) Lee Index (g/cm³) (B) and the retroperitoneal fat (g/100g) (C) respectively. (*P < 0.05) between the groups.

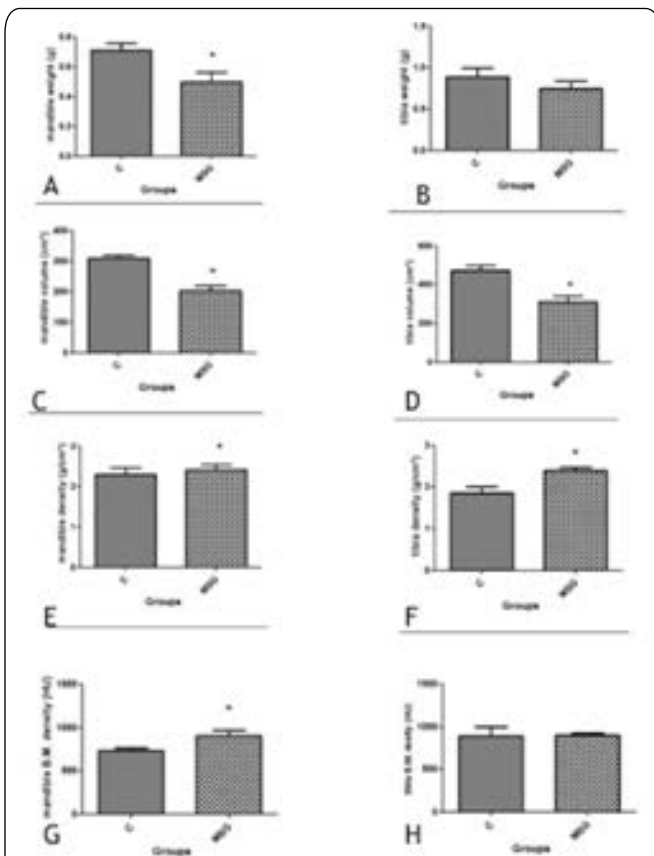


Figure 3: Mean ± standard deviation for the weight (g) in (A) and (B); volumes (g/cm³) in (B) and (D); for the bone density (g/cm³) in (E) and (F), measured by the Archimedes method, and in (G) and (H) for the bone mineral density (HU), measured by tomography in the hemimandible and tibia respectively. (*P < 0.05) between the groups.

hemimandible and tibia, and clearly demonstrate that their sizes were reduced in the MSG group. In figures 4B, 4C and 4E, the dashed lines show the distances that were significantly reduced in the MSG group (P < 0.05).

Obesity alters the percentual weight of mineral content of the enamel, dentine and tibia

Table 1 shows the percentages of weight of major minerals in the enamel, dentine and tibia for the C and MSG groups. EDS showed that, in the MSG group, the percentages of the

major of minerals, including phosphorus, were decreased; however, the percentage for calcium was increased in the MSG group, when compared to the C group.

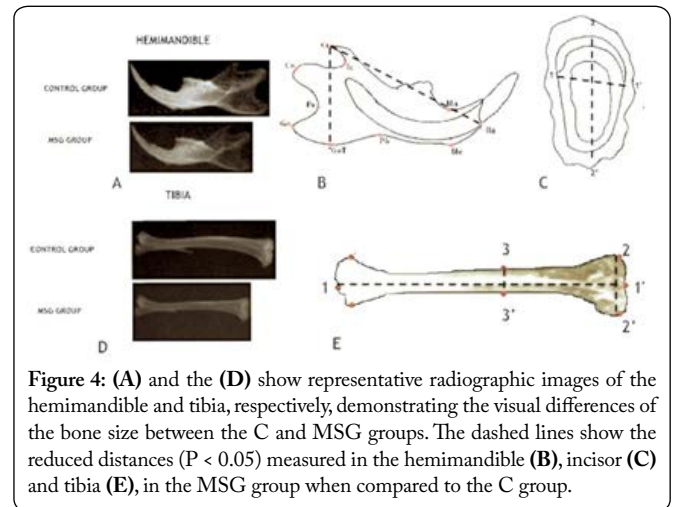


Figure 4: (A) and the (D) show representative radiographic images of the hemimandible and tibia, respectively, demonstrating the visual differences of the bone size between the C and MSG groups. The dashed lines show the reduced distances (P < 0.05) measured in the hemimandible (B), incisor (C) and tibia (E), in the MSG group when compared to the C group.

Table 1: Atomic weights, expressed in percentual (Wt %) for the principal minerals elements found in the bone matrix of the dentine, enamel and tibia for the Control and MSG groups. Bold values indicates the reduction of the mineral element weight.

	Dentin Control Group	Dentin MSG Group	Enamel Control Group	Enamel MSG Group	Tibia Control Group	Tibia MSG Group
Mineral Element	Wt%	Wt%	Wt%	Wt%	Wt%	Wt%
F	7, 8	6, 5	2, 8	2, 3	10, 7	7, 3
Na	3, 9	2, 8	1, 6	1, 2	8, 7	8, 0
Mg	2, 9	2, 1	0, 3	0, 3	4, 9	4, 3
P	44, 8	36, 7	37, 3	35, 6	33, 6	29, 5
Cl	2, 3	0, 5	0, 4	0, 6	2, 6	3, 6
K	0, 7	1, 2	0, 2	0, 2	3, 3	2, 3
Ca	31, 5	48, 3	56, 5	58, 4	27, 5	32, 9
Fe	6, 0	1, 9	0, 9	1, 4	8, 6	12, 2

Discussion

The evaluation of the bone mineral content may be an important approach used to diagnostic of metabolic diseases, especially in post-menopausal and advanced-age women, due to the risk of osteoporosis and consequently fractures [35, 36]. Recent findings also have demonstrated that in an obesity condition there is an increase of the alveolar bone loss, a reduction of the bone mineral density, an imbalance in bone remodeling with deterioration of trabecular bone structure, indicating a closed relationship between the overweight with osteoporosis and facture risk as well as with the energy metabolism [37-42].

In spite of this, there are few studies about the effect of obesity on the bone mineral content in MSG obesity-induced. Some studies demonstrated that when the obesity is associated

with periodontitis, there is a reduction in alveolar bone loss, suggesting a possible protective effect of obesity [24, 25] against bone loss but, this results seems to be contradictory in the literature and needs to be better evaluated yet.

In another study performed by Cirić et al. [28], the weights and lengths of the femur and of the tibia in rats were investigated using MSG-induced obesity; these authors did not report significant differences in the lengths of the femur and tibia in the MSG groups for both females and males, when compared to the control group. In spite of this, some measurements were significantly different between males and females, which could be explained in terms of the different effects of the sexual hormones on fat deposits in males and females. In contrast to the shallow findings of Cirić, in the present study we demonstrated a significant reduction in the weight and volume of the hemimandible and the tibia for the MSG-obese male rats, while the bone density was increased in this group. In addition, our results demonstrated that MSG-induced obesity was able to reduce somatometric parameters measured in the hemimandible, tibia and incisor tooth.

Our results also demonstrated a reduction in the content of phosphorus and an increase in calcium in the tibia, enamel and dentine, in the MSG-induced obesity model for the first time. These findings may corroborate those of a previous report that demonstrated the existence of a close relationship between bone structure and energy metabolism [6] and also suggest a close relationship between obesity and the metabolism of the bone mineral content. In general it is possible that reduced levels of different hormones, and/or their receptors in chondrocytes or osteogenic cells present in the proliferative region of the bone in development process result in a delay to the production of the bone extracellular matrix and consequently in the mineralization process. However, the mechanisms for this assumption must be explored in the future. Moreover, with regard to the phosphorus and calcium metabolism, our findings suggest that the cells involved in the mineralization process, i.e. osteoblasts, odontoblast and ameloblasts, may have a similar metabolic response in the MSG-group. Nevertheless, it is important to bear in mind that obesity, while presenting some “advantages” for bone loss (as described in different reports in humans), generally obesity result in a lower quality of life, due to the several degenerative diseases associated with this disorder.

Finally, our results demonstrated, in the MSG-obese model, a significant reduction in somatometric parameters, such as weight and volume, in both bones (hemimandible and tibia) and in the incisor tooth; and an increase in the bone density of the hemimandible and tibia, as well as changes in the mineral content.

Conclusion

We conclude that in the MSG obesity-induced model introduced disturbs in mineral content and, consequently, produced significant changes in the somatometric parameters of the hemimandible, tibia and incisor.

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Ethical Approval

Ethics Committee for Animal Experimentation (protocol number -02/2011).

Conflict of Interest

All authors state that they have no conflicts of interest.

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