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## Original article

# Is swimming able to maintain bone health and to minimize postmenopausal bone resorption?

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## ABSTRACT

**Objective:** We studied the effect of swimming on the somatic and bone growth of female rats.

**Methods:** 40 neonate Wistar female rats were separated into: monosodium glutamate group (GluM, n = 20) and received MSG solution (4.0 mg/g) on alternate days during the first 14 days after birth, and Saline group (SAL, n = 20) which received saline solution for the same period of time and at the same dose. At 60 days of age, GluM group was ovariectomized (GluMO) and SAL group just suffered surgical stress. Subsequently, half the animals in each group started swimming, resulting in groups: sedentary saline (SALsed, n = 10), swimming saline (SALswi, n = 10), sedentary ovariectomized Glutamate (GluMOsed, n = 10) and swimming ovariectomized Glutamate (GluMOswi, n = 10). At the end of the experiment, we measured the animals' longitudinal length and weight; their radius was weighed and its length measured.

**Results:** The animals of the GluMOsed group had lower body weight and longitudinal length compared to SALsed. Swimming decreased body weight, but had no influence on the longitudinal length of the GluMOswi group compared to GluMOsed group. Longitudinal length and body weight were lower in SALswi animals compared to SALsed animals. Radius weight and length of GluMOsed animals were lower than in SALsed animals. There was no difference in these parameters between GluMOsed and GluMOswi groups; however, these parameters were lower in SALswi animals compared to SALsed animals.

**Conclusion:** Swimming does not influence previously affected bone tissue during the neonatal period, however it may cause damage to healthy bone tissue.

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## A natação é capaz de manter a saúde do tecido ósseo e minimizar a reabsorção óssea pós-menopausa?

### R E S U M O

#### Palavras-chave:

Tecido ósseo  
Glutamato monossódico  
Ovariectomia  
Natação

**Objetivo:** Estudou-se o efeito da natação sobre o crescimento somático e ósseo de ratas.

**Métodos:** usaram-se 40 ratas Wistar neonatas separadas em grupo glutamato monossódico (GluM, n = 20), que recebeu solução de MSG (4 mg/g), em dias alternados, nos primeiros 14 dias de vida; e Grupo Salina (SAL, n = 20), que recebeu solução salina na mesma dose e no mesmo período. Aos 60 dias de vida, o grupo GluM foi ovariectomizado (GluMO) e o SAL passou apenas pelo estresse cirúrgico. Posteriormente, metade dos animais de cada grupo iniciou o treinamento de natação, o que resultou nos grupos Salina sedentário (SALsed, n = 10), Salina natação (SALnat, n = 10), Glutamato ovariectomia sedentário (GluMOsed, n = 10) e Glutamato ovariectomia natação (GluMONat, n = 10). Ao término do experimento, os animais tiveram o comprimento longitudinal mensurado e foram pesados; o rádio foi pesado e o comprimento, avaliado.

**Resultados:** Os animais do grupo GluMOsed apresentaram peso corpóreo e comprimento longitudinal menores em relação ao SALsed. A natação diminuiu o peso corpóreo, porém não exerceu influência no comprimento longitudinal dos animais do grupo GluMONat em relação ao GluMOsed. Peso corpóreo e comprimento longitudinal foram menores nos animais do grupo SALnat quando comparados aos do SALsed. Peso e comprimento do rádio dos animais do grupo GluMOsed foram menores do que os do SALsed. Não houve diferença desses parâmetros entre os grupos GluMOsed e GluMONat. Contudo, foram menores nos animais do grupo SALnat em relação ao SALsed.

**Conclusão:** O treino de natação não exerce influência no tecido ósseo previamente afetado durante o período neonatal e ainda pode causar prejuízo ao tecido ósseo sadio

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## Introduction

The longitudinal bone growth is driven by genetic factors<sup>1</sup> and by a complex network of endocrine signals, including growth hormone, glucocorticoids, estrogens, vitamin D and leptin,<sup>2</sup> among others.

Studies have shown that the administration of monosodium glutamate (MSG) in neonatal period<sup>3,4</sup> affects the growth and development of rodents, especially during the period of sexual maturation.<sup>4</sup> This is possible because MSG causes permanent damage to the brain,<sup>3</sup> more specifically in the nucleus arcuate (ARC) and ventromedial (VMH) hypothalamic nuclei,<sup>5</sup> which leads to an anatomical and functional reorganization of the hypothalamus and adenyhypophysis.<sup>4</sup> As a result, the damage in ARC is associated with a deficiency in the secretion of growth hormone-releasing hormone (GHRH) and gonadotropin-releasing hormone (GnRH), causing changes in hormone secretion from adenyhypophysis' growth hormone (GH) and also in the secretion of gonadotropins.<sup>5</sup> In other words, a reprogramming of the development of the neonatal animal occurs,<sup>4</sup> which explains the linear body growth delay and hypogonadism.<sup>5</sup>

Therefore, the bone metabolism in women, as well as in rats of both sexes suffering hormonal injury, is increased,<sup>6</sup> leading to a prevalence of resorption over bone formation, which is a condition found in osteoporosis.<sup>7</sup>

The ovariectomy in rats is a widely used model,<sup>8,9</sup> which lead to estrogen deficiency and therefore bone loss.<sup>9,10</sup> This model may provide information related to human bone loss in postmenopausal women.<sup>11-13</sup>

Among the preventive resources against osteoporosis, physical activity is one of the best non-pharmacological methods,<sup>14</sup> considering that bone tissue responds positively to mechanical stimulation such as exercise,<sup>15</sup> which stimulates osteogenesis,<sup>10</sup> increases and maintain bone mineral density,<sup>14</sup> reduces the fall of BMD,<sup>16</sup> in addition to providing greater resistance to the bone;<sup>17</sup> therefore, the physical activity is essential to decrease skeletal fragility.<sup>14</sup> On the other hand, some studies have shown that vigorous exercise can cause premature damage to bone tissue.<sup>18</sup>

Thus, we are not sure exactly the intensity, type and duration of exercise that can promote skeletal health. Studies on the influence of swimming on bone are still controversial and inconclusive;<sup>15,19</sup> the available literature shows that swimming is effective in preventing bone loss in the femur and vertebrae<sup>20</sup> but few studies investigate the effect of swimming in other bones, such as the radius, were published.<sup>21</sup>

Therefore, this study aimed to evaluate whether swimming could affect bone tissues previously exposed to conditions that would lead to a loss of bone mass.

## Materials e methods

### Animals

A total of 40 newborn Wistar female rats from the breeding colony of the Department of Nutrition, UFPE, were used. The animals were kept in a vivarium at the Department of Anatomy at room temperature of 22±1°C and at a 12/12 h

light/dark cycle in collective cages (maximum of four animals/cage) with free access to food and filtered water. This study was approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de Pernambuco (office No. 018024/2007-11), consistent with the guidelines suggested by Comitê Brasileiro de Experimentação Animal (COBEA).

### Experimental model

After birth, the animals were randomly divided into two groups: Monosodic Glutamate (GluM, n = 20) and Saline (SAL, n = 20). The GluM animals received a solution of monosodium glutamate subcutaneously at a dose of 4.0 mg/g body weight on alternate days during the first 14 days of their lives<sup>22</sup> and the SAL group received saline at the same dose and period of time.

### Surgical procedure

At the age of 60 days, all animals of GluM group were ovariectomized (GluMO) with bilateral removal of the ovaries. Initially, the animals were anesthetized with ketamine and xylazine chlorhydrate and then placed on a surface in ventral decubitus to perform a trichotomy. Then, in the middle region of the animal dorsum was incised and, with the aid of surgical scissors, the subcutaneous tissue was divided from the lateral abdominal muscle wall. Shortly afterwards, an incision 1 cm below the costal cage was performed. With the help of tweezers, the ovary was located; it was ligated at the end of the fallopian tube with suture, and the ovary was removed. Then, the muscles and skin of the animal were sutured. The animals of the SAL group were only subjected to surgical stress, without removal of the ovaries.

After surgery, the rats were treated with Pentabiotic, a topical veterinary antibiotic.

### Training of animals

One week before surgery, half the animals in each group started the adaptation phase of the freestyle swimming exercising (Table 1) program,<sup>10</sup> resulting in GluMOswi (n = 10) and SALswi (n = 10) groups, which were submitted to a swimming training held from Monday to Friday in a plastic tank with a capacity of 500 L, with a swimming surface area of 0.90 m<sup>2</sup> and with a resistance coupled to a thermostat, allowing the maintenance of the water temperature around 32°-34°C. The water was changed daily.

The female rats were monitored throughout the exercise, so that the animals did not touch the sides of the container.

After surgery, the animals were left to rest for a week, and after the third week, they resumed the swimming training with a progressive duration, starting with 15 minutes/day until reaching 60 minutes/day between the 5th and 12th weeks.<sup>10</sup>

The animals that were not submitted to the swimming protocol were kept into separate cages containing approximately 2 cm of water for the same period in which the other groups were submitted in the swimming training. Thus

**Table 1 – Training protocol.**

Weeks	Duration
1 <sup>a</sup>	1st day: 5 minutes 2nd day: 10 minutes 3rd day: 10 minutes 4th day: 15 minutes 5th day: 15 minutes
Week-end	Surgery
2 <sup>a</sup>	Surgical recovery
3 <sup>a</sup>	1st day: 15 minutes 2nd day: 20 minutes 3rd day: 25 minutes 4th day: 30 minutes 5th day: 40 minutes
4 <sup>a</sup>	1st day: 45 minutes 2nd day: 50 minutes 3rd day: 55 minutes 4 <sup>o</sup> day: 60 minutes 5 <sup>o</sup> day: 60 minutes
5 <sup>a</sup> – 12 <sup>a</sup>	60 minutes

they were subjected to a similar water stress, without performing the physical effort.

After swimming, the animals were dried with a towel and then encased in a timber heating lined chamber (surface area, 0.25 m<sup>2</sup>) with an average heating temperature of 32°-36°C for a period of 10 minutes.

### Material collection

After completing the swimming period, the animals were weighed using an electronic scale (Marte, model S-4000, with 0.1 g sensitivity). Then, the animals were anesthetized via intramuscular injection, using xylazine chlorhydrate (0.03 g 3 mL/100 g weight) and ketamine (0.25 mL/100 g weight). After that, the animals were placed on a flat surface in a prone position and had the longitudinal length measured from snout to the anus<sup>23</sup> using a calliper (Western, 0.02 mm). Then, an incision in the forepaw root region was performed. The muscles and tendons were removed and the radius was proximally and distally disjuncted with the aid of surgical scissors and, then, thoroughly dissected to remove soft tissues.

After a proper dissection of the bone, we measured its length with a calliper (Western, 0.02 mm). The radius was positioned with the anterior surface facing up and the measurement of the bone was done from the radial head to the styloid process. Soon after, the radius was weighed and its density was measured using a hydrostatic weighing digital balance (AND model HR-200, 0.1 mg sensitivity). After this procedure, the bone was fixed in buffered formalin (10 mL of 37% formalin and 27 mL of 0.1 M phosphate buffer pH 7.0) at 50 times the volume of the sample and then the bone was stored in glass containers.

### Data analysis

For data analysis, SigmaStat 32 program was used; Student t test was applied for parametric values, and Mann-Whitney test was used for non-parametric values. The significance level was set at 95%.

## Results

GluMOsed animals had lower body weight compared to SALsed group ( $p < 0.001$ ). The swimming exercise decreased the body weight of GluMOswi animals compared to GluMOsed group ( $p = 0.026$ ), and also caused a reduction in body weight of SALswi animals compared to SALsed ( $p < 0.001$ ) group (Table 2).

The longitudinal length of GluMOsed animals was lower compared to SALsed ( $p < 0.001$ ) group. There was no change in this parameter between GluMOsed and GluMOswi groups ( $p = 0.224$ ), nevertheless the exercise caused a reduction in longitudinal length of SALswi animals compared to SALsed group ( $p = 0.028$ ) (Table 2).

The weight of the radius in GluMOsed animals was lower compared to SALsed group ( $p < 0.001$ ). There was no difference in this parameter between GluMOsed and GluMOswi groups ( $p = 0.054$ ); however, the weight of the radius was lower in the SALswi animals versus SALsed group ( $p < 0.001$ ) (Table 3).

Likewise, the length of the radius of GluMOsed animals was lower when compared to SALsed ( $p < 0.001$ ) animals. There was no change in this parameter between GluMOsed and GluMOswi groups ( $p = 0.232$ ), but the length of the radius of SALswi animals was lower compared to SALsed group ( $p = 0.001$ ) (Table 3).

## Discussion

The pre and postnatal life, including weaning stage, is crucial for brain development.<sup>24</sup> Some studies have shown that animals subjected to the application of monosodic glutamate (MSG) in the postnatal period showed longitudinal growth

delay, as this substance is able to destroy specific cells in hypothalamus arcuate nucleus (ARC), which is the site of production of growth hormone-releasing hormone (GHRH); that in consequence leads to a decreased secretion of growth hormone (GH).<sup>3,5,24</sup> The injury occurred in the hypothalamus, generated by the application of high doses of MSG administered immediately after birth, is attributed to animals' brain immaturity.<sup>25</sup>

In the present study, the rats treated with MSG showed lower longitudinal length and body weight versus those in the control group. Maiteret al.,<sup>3</sup> Rol de Lama et al.<sup>26</sup> and Ćirić et al.<sup>4</sup> exposed animals to the same damage, using MSG in the same dose for the first ten days of life. These authors also observed a reduction in the linear growth of the animals. Furthermore, Schoelch et al.<sup>5</sup> found a reduction in body weight even in animals subjected to the application of MSG in lower dose, compared to that used in our experiment (3 mg/g body weight), and with a shorter duration (during the first nine days of life), indicating that MSG can play a negative role in the body size of the animal, even when applied in smaller quantities and for less time.

In addition to these effects, MSG also promotes impaired secretion of gonadotropin-releasing hormone (GnRH), interfering negatively in the secretion of sex hormones.<sup>5</sup> Because of that, the animals develop hypogonadism, which may lead to an increase of bone resorption and, therefore, to osteopenia.<sup>27,28</sup> In the present study, to further accentuate the hormonal loss, pubescent female rats underwent a surgical procedure to remove their ovaries. This experimental model has been applied in recent years,<sup>9,13,29</sup> to demonstrate the effect of postmenopausal estrogen deficiency in bone structure quality.<sup>11-13,30</sup>

As a result, the skeleton becomes unable to adapt to applied loads,<sup>31</sup> making it liable to fractures.<sup>32</sup> This study aimed

**Table 2 – Values of body weight and longitudinal length of the experimental groups.**

Groups	Body weight (g)	Longitudinal length (mm)
SALsed	257.6 ± 17.14	214.94 ± 4.50
SALswi	225.2 ± 16.71 <sup>a</sup>	209.53 ± 5.56 <sup>a</sup>
GluMOsed	220.2 ± 24.30 <sup>b</sup>	192.89 ± 4.87 <sup>b</sup>
GluMOswi	194.4 ± 23.30 <sup>c</sup>	189.81 ± 6.02

<sup>a</sup> Corresponds to the analysis between SALsed and SALswi groups.  
<sup>b</sup> Corresponds to the analysis between SALsed and GluMOsed groups.  
<sup>c</sup> Corresponds to the analysis between GluMOsed and GluMOswi groups.  
 Values expressed as mean ± standard deviation, using Student's t-test ( $p < 0.05$ ).

**Table 3 – Values of radius weight and length of the experimental groups.**

Groups	Radius weight (g)	Radius length (mm)
SALsed	0.098 ± 0.005	24.20 ± 0.31
SALswi	0.090 ± 0.002 <sup>a</sup>	23.61 ± 0.36 <sup>a</sup>
GluMOsed	0.060 ± 0.003 <sup>b</sup>	21.54 ± 0.50 <sup>b</sup>
GluMOswi	0.057 ± 0.001	21.26 ± 0.50

<sup>a</sup> Corresponds to the analysis between SALsed and SALswi groups.  
<sup>b</sup> Corresponds to the analysis between SALsed and GluMOsed groups.  
 Values expressed as mean ± standard deviation, using Student's t-test for parametric data and Mann-Whitney test for non-parametric data ( $p < 0.05$ ).

to evaluate the radius, because fractures in the distal region of this bone are among the most common in humans affected by osteoporosis, showing increasing incidence,<sup>33</sup> and with a higher prevalence in women.<sup>34</sup>

It was also noted that MSG associated with ovariectomy caused damage to the bone structure of the radius. The length and weight of this bone were lower in MSG-treated rats, these parameters were compared to the control animals. This result can be explained by the imbalance caused by MSG in the ovarian hormonal metabolism in female rats, considering that this substance alters the gonadal development, thus decreasing the secretion of estrogen.<sup>5,27,28</sup>

In mammals, longitudinal bone growth occurs rapidly in prenatal life and early postnatal period. However, after this phase, the growth rate declines and then ceases.<sup>35</sup> In rats, animals often used as experimental models, the critical period of growth occurs between 21-35 days after birth, and a decrease of growth occurs between 35-80 days.<sup>36</sup> In this study, it is assumed that early administration of MSG negatively affected the bone development process, considering that this substance was applied at a time when the bone had not yet undergone the more critical period of development; so, it is expected that the damaged bone tissue would behave in a negative way to the main period of bone development. According to Ćirić et al.,<sup>4</sup> the neonatal exposure to glutamate affects the growth and development of rats, especially during the period of sexual maturation. This means that the pubertal period is characterized by a greater vulnerability as well as more sensitivity to the manifestation of external influences, particularly in females.<sup>4</sup>

Both MSG-treated female rats that underwent surgery to remove their ovaries as well as those in the control group who underwent the same surgical stress, but had their ovaries preserved, were submitted to a swimming practice. There is evidence that high impact exercises are beneficial for providing an increase in bone mass,<sup>31</sup> and that aquatic exercises without impact, such as swimming, are considered to have relatively little effect in the prevention of osteopenia.<sup>37</sup>

The female rats that received MSG as well as those in the control group who practiced swimming, exhibited lower body weight at the end of the experiment. This may have occurred because of increased energy expenditure with the exercise. The increase in energy expenditure by moderate-intensity exercise can be effective in preventing or reducing weight gain.<sup>38</sup>

It was noted in this study that the swimming practice had no influence on the animal's longitudinal length, its weight and in the length of the radius of MSG-treated animals, as compared to animals that also received the substance, but did not swim. According to Frost,<sup>39</sup> Crossley et al.,<sup>40</sup> and Magkos et al.,<sup>15</sup> the bone tissue responds to mechanical loading: part of this force is absorbed by the body during the load exercise, being attenuated in the joint structures, whereas another part of the force is transmitted to the skeleton, causing deformation and possible increase in bone mass. Bone that undergoes little stress does not promote osteogenesis, and lacking bone cell response. Therefore, it is assumed that the bone tissue could be most benefited with high-impact exercises.<sup>41</sup>

Much research on the relationship between swimming and bone mass have been conducted in young people and in athletes, and few benefits were cited.<sup>10</sup> In this study, it was found that swimming training was capable of causing damage to healthy bone tissue of animals, by reducing the weight and length of the radius. These same animals also showed decreased longitudinal length at the end of the experiment, when compared to sedentary animals. Thus, although physical activity is a variable positively related to high values of bone mineral density,<sup>10</sup> strenuous exercise can have negative consequences on the skeleton, particularly in an immature skeleton, which can delay the maturation of collagen and decrease the bone development, as noted in the tibia of animals subjected to exhaustive treadmill exercise.<sup>18</sup>

In this study it is assumed that the frequency and duration of swimming had been gruelling for the animals, thus causing damage to the bony structure of the radius. These findings corroborate Bourring et al.<sup>42</sup> conclusions; these authors showed that physical activity can also cause deleterious effects on bone, such as lowering of its longitudinal length and reducing the height and number of bone trabeculae, with a consequent increase in the intertrabecular space and significant decrease of the average thickness of osteoid, suggesting a decreased osteoblastic activity at the cellular level.<sup>42</sup>

This result can also be explained by the study by Simkin et al.<sup>43</sup> These authors submitted 40 rats to swimming training, and observed that the movements made by the animals during the swimming practice differ from the usual movements made on land. During swimming, the mice not only flex and extend the upper limbs; swimming promotes their rotation and abduction as well. Whereas, in terrestrial locomotion there are only two phases (flexion and extension) and the animals are subject to the action of gravity only in flexion; on the other hand, during the swimming exercise a continued resistance of the water in all phases of the movement occurs, generating higher amount of weariness. Therefore, strenuous exercises can lead to muscle fatigue which, in turn, increases the risk of bone stress and of stress fractures. Thus, they should be avoided.<sup>44</sup>

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## Conclusion

In the conditions under which this experiment was conducted, the results suggest that swimming does not influence bone tissue previously challenged and may cause damage to the structure of healthy bone tissue.

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## Conflicts of interest

The authors declare no conflicts of interest.

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