

# Influence of creatine supplementation on bone mass of spontaneously hypertensive rats

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## ABSTRACT

**Introduction:** Recent evidence has suggested that creatine supplementation (Cr) can increase the bone mineral density (BMD) of the femur in healthy growing rats. Nevertheless, studies assessing the efficacy of the Cr supplementation in conditions characterized by bone mass loss are scarce. **Objective:** To investigate the effect of Cr supplementation on BMD and bone mineral content (BMC) in spontaneously hypertensive rats (SHRs), an experimental model of osteoporosis. **Materials and methods:** Sixteen 8-month-old male SHRs were randomly allocated into two groups matched by body weight: 1) Pl group: SHRs treated with placebo (distilled water; n = 8); and 2) Cr group: SHRs treated with Cr (n = 8). After nine weeks of supplementation, the animals were euthanized and their femur and spine (L1-L4) were analyzed by use of densitometry (Dual Energy X-Ray Absorptiometry). **Results:** No significant difference was observed between the groups regarding either the spine or the total femur measures as follows: spine – BMD (Pl =  $0.249 \pm 0.003$  g/cm<sup>2</sup> vs. Cr =  $0.249 \pm 0.004$  g/cm<sup>2</sup>; P = 0.95) and BMC (Pl =  $0.509 \pm 0.150$  g vs. Cr =  $0.509 \pm 0.017$  g; P > 0.99); and total femur – BMD (Pl =  $0.210 \pm 0.004$  g/cm<sup>2</sup> vs. Cr =  $0.206 \pm 0.004$  g/cm<sup>2</sup>; P > 0.49) and BMC (Pl =  $0.407 \pm 0.021$  g vs. Cr =  $0.385 \pm 0.021$  g; P > 0.46). **Conclusion:** In this study, using the experimental model of osteoporosis, Cr supplementation had no effect on bone mass.

**Keywords:** osteoporosis, creatine, bone mineral density.

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## INTRODUCTION

The spontaneously hypertensive rat (SHR) has been considered the arterial hypertension genetic model most similar to the primary hypertension observed in humans.<sup>1,2</sup> There is evidence of a reduction in bone mineral density (BMD) in that model, resulting from changes in calcium metabolism, which cause an increase in bone resorption.<sup>3–5</sup> While the bone mass of healthy Sprague Dawley rats peaks around the age of 30–36 weeks,

SHRs stabilize their bone growth at the age of only 18 weeks. In addition, the peak bone mass in SHRs is approximately 40 mg/cm<sup>2</sup> lower than that in healthy rats.<sup>6</sup> Thus, SHRs are considered an experimental model for the study of osteoporosis.<sup>7</sup>

The energy need of bone cells to survive, proliferate, differentiate, and synthesize extracellular matrix is known to be high.<sup>8</sup> Evidence has shown that part of the energy required for those processes originates from creatine (Cr;  $\alpha$ -methyl guanidine-acetic acid), which plays a central role in maintaining

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ATP and ADP levels in several tissues, such as skeletal muscle, brain, testicles, cartilage, and bone (for a recent and comprehensive review, see Wallimann et al.<sup>9</sup>).

The hypothesis that Cr could play an important role in bone metabolism was first suggested based on the identification of creatine kinase isoforms (CK), enzyme responsible for the reversible reaction as follow: phosphocreatine + ADP + H<sup>+</sup> ↔ creatine + ATP in the bone.<sup>9,10</sup> In addition, *in vitro* assays have shown that stimuli that can induce the development of bone mass (i.e., insulin growth factor-1 and parathyroid hormone) concomitantly increase CK activity, suggesting that the Cr/CK system is associated with the process of bone remodeling.<sup>11,12</sup> In fact, a study has shown that incubating Cr in a culture medium with primary osteoblasts has stimulating effects on the differentiation, metabolic activity, and bone mineralization, elevating the phosphorylcreatine/Cr ratio and preserving the ultrastructure and mitochondrial function of osteoblasts.<sup>8</sup>

*In vivo* evidence<sup>13,14</sup> has corroborated those findings. Supplementation with Cr can increase the BMD and cause beneficial biomechanical adaptations in the femur of healthy rats.<sup>15</sup> In humans, there is preliminary evidence that the Cr supplementation can prevent bone mass loss in patients with Duchenne dystrophy<sup>14</sup> and in the elderly undergoing physical training.<sup>16</sup>

However, the role of the dietary Cr supplementation remains little explored. Aiming at increasing the understanding about the effects of Cr on possible bone mass loss conditions, the present study assessed the effect of that nutrient on the BMD and bone mineral content (BMC) of SHR.

## MATERIALS AND METHODS

### Sample

The sample comprised 16 8-month-old male SHRs, which were maintained at the animal facility of the Laboratory of Nutrition and Metabolism Applied to Motor Activity (Escola de Educação Física e Esporte of the Universidade de São Paulo – EEF/USP) in plastic cages (three to four animals per cage), at room temperature of 22.0 °C–24.0 °C and 12-hour cycle (inverted light and dark). The rats were fed a normal protein diet (12% of protein) and water *ad libitum*.

### Experimental design

The animals were randomized into two experimental groups matched by body weight, as follows: 1) PI group: SHRs treated with placebo (n = 8); and 2) Cr group: SHRs treated with Cr (n = 8). After nine weeks of intervention the animals were

sacrificed by decapitation. The bone specimens [right femur and lumbar spine L1–L4] were removed by use of surgical tools, immersed in saline solution, and frozen at –80 °C for later BMD analysis.

The procedures were approved by the Ethics Committee in Research of the EEF/USP (protocol 2011/10).

### Creatine supplementation

The Cr group received, through gavage, Cr supplement daily (Ethika, Ribeirão Preto, SP, Brazil) for nine weeks. Creatine powder was diluted in water (room temperature) at the proportion of 200 g for each liter of water, and the dosage used was 5 g/kg weight/day.<sup>13</sup> The animals were weighted daily for the required corrections. The PI group received distilled water through gavage to simulate the stress imposed to the Cr group.

### Bone densitometry

Bone densitometry (Dual Energy X-Ray Absorptiometry; DXA) was used to assess the BMD and BMC of the spine (L1–L4) and total femur (total femur length, including diaphysis and epiphyses). The device Discovery-A SN: 80999 Hologic (Bedford, MA, USA) in the high resolution mode was used, with the aid of the *small animal software*, provided by the same manufacturer. The accuracy of the DXA for assessing BMD was previously analyzed by measuring the coefficient of variation, expressed as a percentage of the mean.<sup>17,18</sup> The coefficient of variation was 1.9% for the spine and 0.6% for the total femur. Together, those data indicate high accuracy of measures.

### Statistical analysis

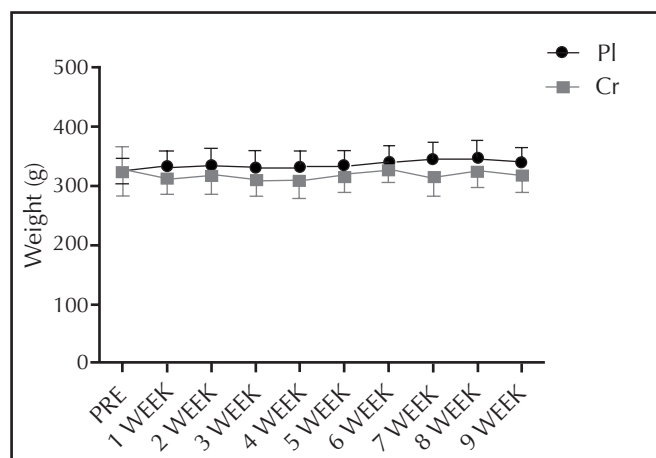
Data were expressed as mean ± standard deviation. The non-paired *t* test was used to compare BMD and BMC of the groups and two-way ANOVA to assess body weight every week. The significance level adopted to reject the null hypothesis was P < 0.05.

## RESULTS

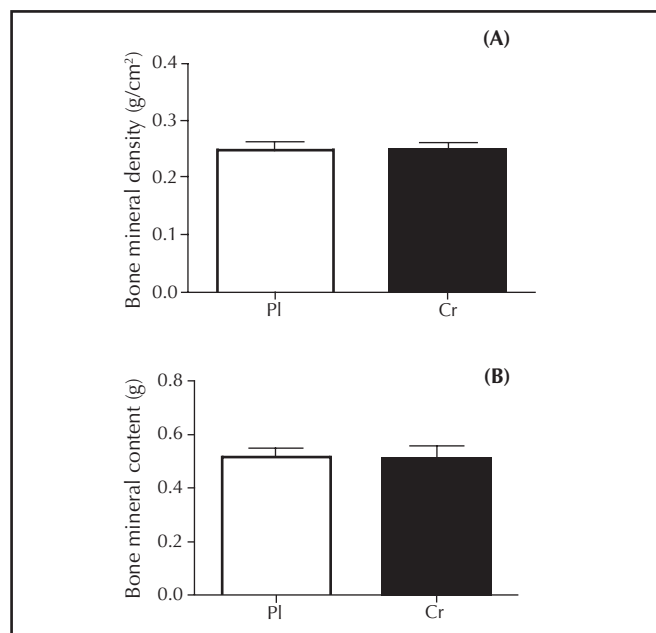
Only one rat (PI) died during follow-up. The body weight did not significantly differ between both groups during the study (P = 0.48; Figure 1).

No significant difference was observed between the groups regarding either the spine or the total femur measures as follows: spine – BMD (PI = 0.249 ± 0.003 g/cm<sup>2</sup> vs. Cr = 0.249 ± 0.004 g/cm<sup>2</sup>; P = 0.95; Figure 2A), and BMC (PI = 0.509 ± 0.150 g vs. Cr = 0.509 ± 0.017 g; P > 0.99;

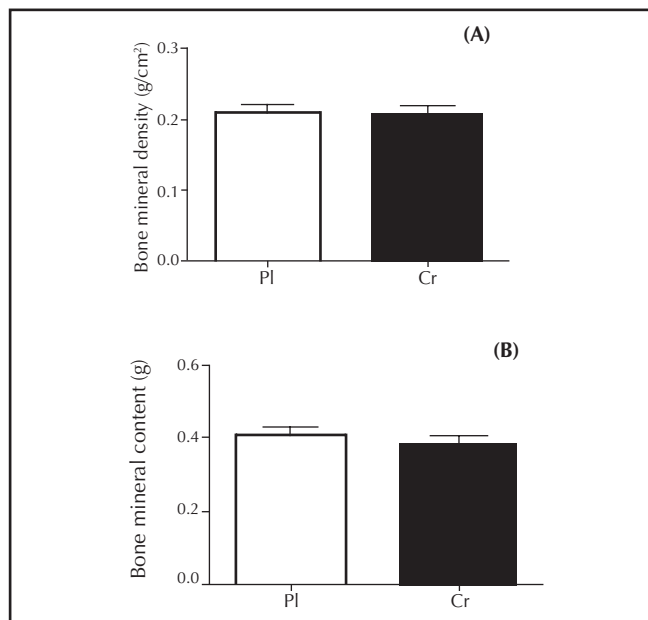
Figure 2B); and total femur – BMD (PI =  $0.210 \pm 0.004$  g/cm<sup>2</sup> vs. Cr =  $0.206 \pm 0.004$  g/cm<sup>2</sup>; P > 0.49; Figure 3A), and BMC (PI =  $0.407 \pm 0.021$  g vs. Cr =  $0.385 \pm 0.021$  g; P > 0.46; Figure 3B).



**Figure 1**  
Body weight of the two experimental groups (g). PI: SHR treated with placebo; Cr: SHR treated with creatine. No statistically significant difference was found between the groups during all the follow-up.



**Figure 2**  
Bone mineral density (A) and bone mineral content (B) in lumbar (L1–L4) spine of the experimental groups. PI: SHR treated with placebo; Cr: SHR treated with creatine. No statistically significant difference was found between the groups.



**Figure 3**  
Bone mineral density (A) and bone mineral content (B) in the total length of the femur of the experimental groups. PI: SHR treated with placebo; Cr: SHR treated with creatine. No statistically significant difference was found between the groups.

## DISCUSSION

This study aimed at assessing the influence of Cr supplementation in the bone mass of SHRs, a well-described experimental model to study low bone mass.<sup>3-5</sup> Our results are not in accordance with those of Antolic et al.,<sup>15</sup> who have reported beneficial effects of Cr supplementation on the bone mass of Sprague Dawley rats. Some methodological differences can explain the contradictory results. Antolic et al.<sup>15</sup> have reported benefits with Cr supplementation to growing rats, while the present study used adult rats. The process of bone growth and development is characterized by high bone turnover, a period more susceptible to environmental influences on bone mass.<sup>19</sup> The gains with Cr supplementation might have been intensified in that phase. In addition, it is worth emphasizing that the SHR model is known to have high bone resorption, and, thus, low bone mass. On the other hand, the Sprague Dawley model studied by Antolic et al.<sup>15</sup> has no change in bone metabolism. Based on the differences of the experimental models, one can speculate that Cr supplementation might be more effective in potentiating bone mass gain in healthy growing rats than in attenuating bone mass loss in rats undergoing bone mass loss.

This study has some limitations. The tissue capture of Cr, to guarantee the success of supplementation, was not assessed. However, the dosage used in this study (5 g/kg weight/day) has been considered high in the literature<sup>20,21</sup> and effective to increase the musculoskeletal content of Cr in Wistar rats.<sup>22</sup> Future studies have to assess whether the Cr supplementation can also increase Cr concentrations in bone tissue. Finally, it is worth emphasizing that our study assessed only male rats. Knowing that gender is a factor that influences directly the bone mass response,<sup>22</sup> and, considering the impossibility of generalizing those data for both genders, further studies should also assess the therapeutic potential of Cr in bone mass remodeling in females.

Despite the evidence that Cr supplementation can promote important therapeutic effects, such as bone mass increase,<sup>23</sup> our findings indicate that SHRs supplemented with Cr do not experience such gains. Considering the evident difficulty in carrying out large longitudinal clinical assays, other models characterized by bone mass loss (for example, polycystic rats or rats treated with corticoids) should be investigated to more deeply assess the therapeutic potential of Cr on the preservation of BMD in low bone mass conditions. However, it is worth emphasizing that the Cr metabolism seems to differ substantially between species, and, thus, studies in humans should be conducted to confirm all preclinical findings.

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