Biochemical effects of caffeine on bone of growing rats

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Abstract: This study investigated the biochemical effects of caffeine on bone of growing rats. The safety of caffeine use among children is understudied and poorly understood. Given that some caffeine-containing beverages are marketed directly to children and that caffeine use is growing among children, it is important to understand the potential effects of caffeine use within this population. Caffeine; 1, 3, 7 trimethylxanthine, a purine alkaloid, is a key component of many popular drinks, mainly tea and coffee, but most phytochemists know little about its biochemistry and molecular biology. A total of 25 rats (8 weeks old) were divided randomly into three groups: Group 1 (n =10), caffeine-high dose; group 2 (n = 10), caffeine-low dose; group 3 (n = 5), serve as the control group. The caffeine was fed via the animals' dietary water and the high dose of caffeine=10 mg/100 g body weight/day, and the low dose of caffeine=2.5 mg/100g body weight/day. Body weight was measured weekly. After sacrifice, blood samples were collected in tubes, and separated the serum for the determination of Ca, ALP, Zn and Mn. The results showed that serum Ca level for high dose group is significantly lower than the low dose than the control group, serum Zn level for high dose is significantly lower than the low dose than control group, and serum Mn level of high dose group is significantly lower than the low dose group than the control group. The Alkaline phosphatase in low dose group is not significant smaller than the control group, but the high dose group has significantly elevation value than the control group. It is reported that the oral administration of caffeine lead to significant reduction in serum Ca, Zn and Mn and a significant elevation in serum ALP according to the increase of caffeine dose.

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1. Introduction:

Caffeine is the most commonly used psychoactive substance throughout the world (Nehlig, 1999). The safety of caffeine use among children is understudied and poorly understood. Given that some caffeine-containing beverages are marketed directly to children (Bramstedt, 2007) and that caffeine use is growing among children (Frary *et al*., 2005), it is important to understand the potential effects of caffeine use within this population. There is also a strong belief that the people consuming high amounts of caffeine tend to carry a higher risk of developing bone problems, including osteoporosis, as well as problems in metal absorption, excretion and reabsorption processes in intestines and in kidney (Chen and Whitford, 1999; Massey, 2001; Borse *et al*., 2002; Pan *et al*., 2003), and iron deficiency anemia (Hallberg and Rossander, 1982). Thus caffeine is probably the most commonly consumed pharmacologically active compound in the world, certainly in Europe and North America. Probably it is partly for that reason that caffeine has often been a target of opportunity for investigators seeking to identify environmental factors that may contribute to the burden of chronic disease. Caffeine and the other methyl xanthines act in a variety of tissues, generally by interfering with the action of phosphodiesterase and there by potentiating the activity of agonists acting through the adenylate cyclase – cAMP pathway. At sufficient doses, therefore, they could theoretically exert effects directly on the cellular apparatus controlling bone remodeling. In high doses caffeine interferes with fetal rat skeletal development (Nakamoto *et al*., 1989; Schneider *et al*., 1990). The first publication showing a negative effect of caffeine on the calcium economy came from this author's laboratory (Heaney and Recker, 1982). Shortly thereafter, Massey and colleagues (Massey and Wise, 1984; Massey and Hollingbery, 1988; Bergman *et al*., 1990) showed that a caffeine-induced diuresis increased urinary calcium loss acutely. In the clinical setting, alkaline phosphatase (ALP) is frequently used as a biochemical marker for osteopenia due to its ease of measurement. ALP is a routine marker in the diagnosis of hepatic disorders and metabolic bone diseases.

There is ample evidence that zinc plays an important role in bone metabolism and is required for normal bone development (Follis and Mccollum, 1941; Calhoun, and Smith, 1968; Calhoun *et al.,* 1974;

Calhoun *et al*., 1975). Moreover, zinc deficiency has been shown to impair collagen biosynthesis (Fernandez *et al*., 1973; Yamaguchi *et al*., 1982; Yamaguchi and Yamaguchi, 1986; Yamaguchi and Ehara, 1995; Yamaguchi and Ehara, 1996) and the lower activity of zinc is dependent on enzymes in the bone (Prasad *et al*., 1967; Roth and Kirchgessner, 1974). Zinc deficiency has also been implicated to play a role as a risk factor in the development of human osteoporosis (Gaby and Wright, 1990; Fushimi *et al*., 1993; Saltman and Strause, 1993). Manganese is an essential trace nutrient in all forms of life. The human body contains about 12 mg of manganese, which is stored mainly in the bones; it is mostly concentrated in the liver and kidney (Emsley and John, 2001). In recent years there has been a rebirth of interest in studies concerning the role of trace elements in the development and maintenance of the skeleton (Asling and Hurley, 1963). A relationship among manganese, copper and skeletal growth has been observed in the abnormal fetal development of rats in deficient dams (Hurley, 1981).The dose of caffeine given to rats in the present study was equivalent to slightly more than four cups of coffee a day in the human based on metabolic body weight (Kleiber, 1961). Caffeine increases urinary calcium excretion by reducing renal reabsorption and, possibly, reducing calcium absorption, leading to a negative calcium balance (Bergman, 1990; Massey and Whiting, 1993; Ilich *et al*., 2002). Other study find that a high caffeine dose may influence vitamin D receptor protein (VDR) expression stimulated by vitamin 1,25(OH)2D3 and controlled by vitamin 1,25(OH)2D3 activity of human osteoblast cells by reducing alkaline phosphatase activity (Rapuri *et al.,* 2007).

Caffeine-containing beverage consumption has been reported to be associated with reduced bone mass and increased fracture risk in some, but not most, observational studies. Human physiological studies and controlled balance studies show a clear but only a very small depressant effect of caffeine itself on intestinal calcium absorption, and no effect on total 24-h urinary calcium excretion (Heaney, 2002). Liu *et al.,* (2011) reported that the calcium contents in tibia and femur of caffeine-treated rats were also lower than that in the control group. The osteoclastogenesis of bone marrow cells isolated from caffeine-treated rats was markedly enhanced as compared with the control group.

The aim of the present experiment was to investigate the biochemical effect of oral administration of caffeine on the serum level of Ca, Zn, Mn and ALP. The bad effect of caffeine on bone of growing rats was also studied.

2. Material and Methods

Animals

Twenty-five 7-week old Wistar rats with a mean body weight of 100 g at the beginning of the experiment, and were all kept under controlled conditions. After_1 week of adaptation; animals were fed with Purina Laboratory Rodent Diet (PMI; St. Louis, MO) (0.95% calcium) and distilled water *ad libitum*. This approach was used because many younger adults and more than 50% of older adults living in the United States, including those who use calcium-containing antacids or supplements, consume diets that are considered to be inadequate in calcium (Ervin and Kennedy, 2002), and thus may be at higher risk of loss of bone mass during weight loss (Riedt *et al*., 2005). The experiment began when the rats were 8- weeks old.

Experimental Design

The animals were randomly divided into three groups: Group1 (n =10), caffeine-high dose; group 2 (n = 10), caffeine-low dose; group 3 (n = 5), serve as the control group.

Caffeine Feeding

The dose of caffeine was delivered to each rat in its drinking water daily throughout the duration of the 8 week study.

We prepare 0.5% gm caffeine (Sigma Aldrich, St. Louis, Mo, USA) solution, the high dose of caffeine=10 mg/100g body weight/day, and the low dose of caffeine=2.5 mg/100g body weight/day.

Measurements

At the end of the experiment, 8-weaks after the start of the experiment, all animals were killed by decapitation under light anesthesia with diethyl ether. Blood samples were collected in tubes, and serum was separated for the determination of Ca, ALP, Zn and Mn. Zn and Mn were measured by atomic absorption spectrophotometry (Perkin-Elmer 560, Norwalk, CT). Ca was measured by Colorimetric method based on formation of color complex between calcium and o-cresolphtalein in alkaline medium (Young, 2001). ALP were measured by "Optimised standard method"according to the recommenations of the German Clinical Association (Deutsche Gesellschaft f ur Klinische Chemie) (Klin, 1972). Ca and ALP are measured by Biosystem BTS 330 spectrophotometer analyzer.

Statistical analysis

All values were expressed as means±SD. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Differences with a value of $P < 0.05$ were considered as statistically significant.

3. Results

At 8 weak after caffeine feeding, Calcium (Ca), Zinc (Zn), Manganese (Mn), and Alkaline phosphatase (ALP) serum levels are measured. Serum Ca level for low dose group (8.94 ± 0.17) mg/dl were significantly decreased compared with the control group (10.5 ± 0.34), and the high dose group (7.7 ± 0.23) were significantly decreased lower than the low dose group (table 1).

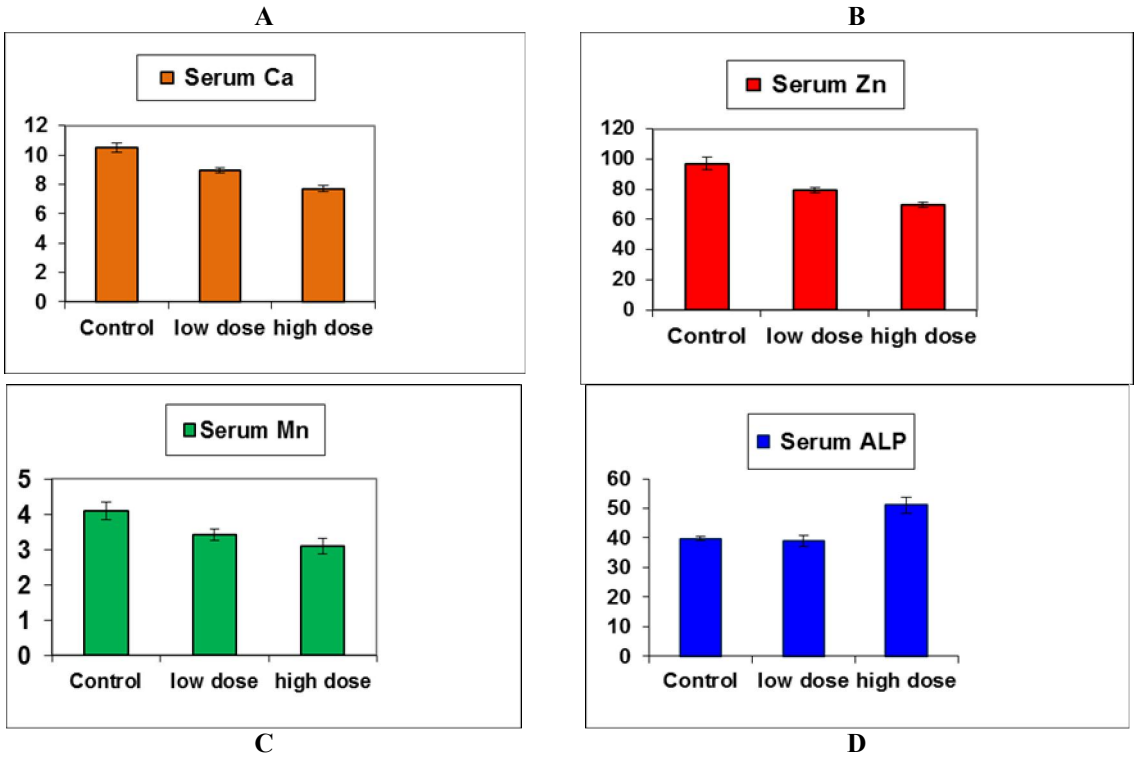

Figure 1. A) Serum Ca level of growing rats. **B)** Serum Mn level of growing rats. **C)** Serum Zn level of growing rats.
D) Serum ALP level of growing rats.
Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Table 1. Significant decrease of serum Ca level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Ca	10.5±0.34	8.94±0.17*	7.7±0.23*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Serum Zn level for low dose group (79.14± 1.71) µg/dl were significantly decreased in compared with the control group (97± 4.2), and the high dose group (69.66 ± 1.87) were significantly decreased lower than the low dose group (table 2).

Table 2. Significant decrease of serum Zn level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Zn	97 ± 4.2	79.14±1.71*	69.66±1.87*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

Serum Mn level for low dose group (3.42 ± 0.17) µg/dl were significantly decreased compared with the control group (4.1±0.24), and the high dose (3.1± 0.22) were significantly decreased lower than the low dose group (table 3) .

Table 3. Significant decrease of serum Mn level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum Mn	4.1±0.24	3.42±0.17*	3.1±0.22*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

The Alkaline phosphatase in low dose group (39 ± 1.93) UI/L is lower than the control group (39.75 ± 0.62) without significant difference, but the high dose group (51.16±2.72) is significantly increased compared with the control group (table 4).

Table 4. Change of serum ALP level of growing rats feeding low dose and high dose of caffeine.

	Control	Low dose	High dose
Serum ALP	39.75±0.62	39±1.93	51.16±2.72*

Data are expressed as the mean ±SD; *P < 0.05 versus control group.

4. Discussion

The dose of caffeine used in this study (10 mg/100 g body weight) was similar to those used in previous investigations (Yeh and Aloia, 1986; Glajchen *et al*., 1988). Yeh and Aloia, (1986) reported also that this dose was equivalent to 1360 mg/ 70 kg in humans. There is a belief among the public that those who drink too much tea or coffee are disposed to have mineral deficiency problems (Conlisk *et al*., 2000; Horie *et al*., 2002). There is some experimental evidence supporting a negative effect of caffeine on bone. Following caffeine administration, teratogenic effects on ossification of the fetus have been observed in some rodent studies (Nakamoto and Shaye, 1984). Whiting and Whitney (1987) reported that the administration of coffee or caffeine to rats was followed by a negative calcium balance, an effect that could possibly be explained by an increase in the excretion of urinary and faecal calcium.

Possible explanations for the injurious role of the caffeine on bone, observed in these studies, may be due to the action of this compound on calcium metabolism and on the proliferation of osteoblast-like cells. Caffeine increases urinary calcium excretion by reducing renal reabsorption and, possibly, reducing calcium absorption, leading to a negative calcium balance (Bergman, 1990; Massey *et al*., 1993; Ilich *et al*., 2002).

Currently, Rapuri *et al*. (2007) observed that the caffeine dose dependently decreases vitamin D receptor expression and alkaline phosphatase enzyme activity in human osteoblasts; constituting a possible mechanism by which caffeine may affect bone metabolism. The effect of caffeine on bone tissue is related to calcium metabolism. Caffeine slightly impairs calcium absorption from intestines; however it has no effect on calcium excretion with urine (Heaney, 2002). In addition, Kamagata *et al.* (1999) demonstrated that caffeine has an inhibitory effect on the proliferation of osteoblast-like cells *in vitro*. Tsuang *et al.* (2006) concluded that caffeine may induce apoptosis and decrease the viability of osteoblasts. In this work; alkaline phosphatase level does not differ at low dose of caffeine; this finding is in agreement with (Liu *et al*., 2011) who showed that the low concentration of caffeine (0.005-0.1 mM) did not affect the bone marrow cell viability and alkaline phosphatase activity during osteoblast differentiation from bone marrow stromal cells, but it effectively enhanced the osteoclastogenesis from bone marrow hematopoietic cells and the bone resorption activity by pit formation assay.

Rapuri *et al*. (2007) concluded that 1,25-Dihydroxyvitamin D3 (1,25(OH)2D3) performs a fundamental role in the regulation of bone metabolism. A receptor for this vitamin (VDR, Vitamin D Receptor) occurs in osteoblast cells. This means that a high caffeine dose may influence VDR expression stimulated by vitamin 1,25(OH)2D3 and controlled by vitamin 1,25(OH)2D3 activity of human osteoblast cells by reducing alkaline phosphatase activity.

Zinc plays an important role in the maintenance of cell membrane structure and function (Bettger and O'Dell, 1993). Zinc deficiency causes a reduction in osteoblastic activity, collagen and chondroitin sulfate synthesis, and alkaline phosphatase activity (Calhoun *et al*., 1974). The elements, such as zinc, copper, magnesium and calcium have relatively low intestinal absorption efficiency and are excreted primarily in feces (Avioli, 1980; Li and Vallee, 1980; Shils, 1980).

Manganese absorption is influenced by many factors such as chemical form, presence of chelating or complexing agents and interactions between different micronutrients. It has been suggested that manganese and iron share a common mechanism of absorption and transport in the digestive tract (Keen and Zidenberg, 1996). This finding is in consistent with Leach and Harris (1997) who found that Mn and Fe share mechanisms of transport and cell uptake.

Nowadays, it is impossible to avoid caffeine intake, and its effect on bone tissue is not fully understood. Researchers' opinions on the influence of caffeine on bone tissue differ. Further studies are in progress to elucidate cellular and biochemical mechanisms by which trace elements participate in bone metabolism.

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