ORIGINAL ARTICLE

The Effect of Caffeine on the Weight and Length of Femur of Balb/C Mice

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ABSTRACT

Objective: To determine the effect of caffeine on the weight and length of femur of BALB/c mice.

Study Design: A Laboratory based randomized control trial.

Place and duration of study: The study was conducted at Anatomy Department, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad for a duration of one year from 6th October 2014 to 5th October 2015.

Materials and Methods: Twenty BALB/c mice (10 male, 10 female) , three weeks old, weighing 12-14 g, were taken and divided into two groups with 10 mice (5 male, 5 female) in each group. The control group G1 was given normal diet and water ad libitum. Each animal in the experimental group G2 was given 10mg of caffeine per 100g body weight on alternate day, three days in a week by oral gavage for 60 days. The effect of caffeine was evaluated by measuring the weight and length of femur of the BALB/c mice at the end of study. IBM-SPSS version 20 was used for data analysis. The student's T-test was applied for intergroup comparison of quantitative variable, which was taken as means and standard deviations (mean ± SD). A p value < 0.05 was taken as significant.

Results: The mean femur weight of BALB/c mice of control group G1 was observed as 0.387±0.019 g while the mean femur weight of experimental group G2 was found to be 0.316±0.020 g. However, the mean femur length of control group G1 was 20.70±0.609 mm and experimental group G2 was 24.382±1.087mm. The weight of femur was decreased in experimental group G2 while the length of femur was increased in experimental group G2 as compared to control group G1.

Conclusion: Caffeine consumption causes reduction in femur weight and increase in femur length.

Key Words: *Caffeine, Femur, Length, Weight.*

Introduction

Caffeine is the most commonly consumed stimulant in the world. It is found in coffee, tea, cocoa products, soft drinks and energy drinks. Caffeine (1, 3, 7-trimethylxanthine), in addition to being food constituent, is also a common analgesic adjuvant.[1] The pandemic consumption of caffeine in food, beverages, and pharmaceutical preparations, such as decongestants, muscle relaxants, and allergy drugs, has developed special attention in demonstrating the multitude of effects and mechanisms of action of this drug of daily life.[2]

The laboratories based researches have illustrated that caffeine impairs bone development by decreasing the mineral content and calcium absorption. The low bone mass and microarchitectural deterioration of osseous tissue that leads to bone fragility as well as an increased susceptibility to fractures.[3] Caffeine is anti-proliferative towards osteoblasts and it debilitates some important events in osteogenesis.[4] The excessive coffee consumption was associated with a small but significant reduction in number of teeth with periodontal bone loss.[5] There is a significant association between caffeinated beverages and dental erosion.[6] The intake of caffeine in amounts >300 mg/day (514 g, or 18 oz, brewed coffee) accelerated bone loss at the spine in elderly postmenopausal women.[7] It is also investigated that if young, rapidly growing rats are exposed to caffeine, disruption of osteoblasts and retarded bone development occur. An in-vitro study showed that caffeine may enhance the rate of osteoblast apoptosis and has potential deleterious effect on the osteoblast viability .[8]

Caffeine could easily permeate through placenta and

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it could interrupt the development as well as mineralization of the osseous tissue. Consequently the process of skeletal ossification was delayed in fetal animals.[9] During lactation, maternal exposure to caffeine resulted into specific effects on the enamel of the molar teeth of young animals and enhanced the sensitivity to dental caries. There would be fewer osteocytes per area of femur cross section, retarded structural remodeling of the lateral tibial metaphysis, abnormal osteoblasts and osteocytes with swollen mitrochondria.[10] Caffeine invariably lowered the Zn content and altered the bone tissue mass that caused fragility as well as predisposition to fractures.[11] The excessive dietary caffeine is responsible to increase urinary calcium output, most probably as a result of the acidic load which is favored by it. The bones counterbalance against acidosis by the buffering capacity of a large reservoir of calcium salts.[12]

The effects of coffee on bone metabolism are contentious, although caffeine intake is associated with an eloquent increase in risk of periodental disease, osteoporosis and fracture.[13] The animal studies have evaluated that rats exposed to caffeine during gestational period exhibited structural disturbances of bone with a decreased number of osteocytes and smaller cross-sectional area of bone. The histological manifestations showed immature bone trabeculae and inhibition of osteoblasts proliferation.[14] Caffeine reduces calcium balance which is either by increased urinary excretion or decreased intestinal calcium absorption. [15]

Caffeine is consumed in Pakistan in different forms through foods as well as beverages. Over the past decades, intake of caffeine is increasing day by day and it has become a part of our daily diet. However, the society is generally unaware regarding its deleterious effects on human health especially the bony tissues. This is because of non-availability of data on its various adverse effects. Until now, there have been limited local studies on the subject of amount of caffeine consumption and its effects on health. The present study is an effort towards generating this understanding by gathering information and demonstrating detrimental effect of high caffeine consumption on the developing thigh bone of animals (BALB/c mice).

Materials and Methods

The study was a laboratory based randomized control trial. It was carried out at Anatomy Department, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad. It was spanned from 6th October 2014 to 5th October 2015 with the approval of ethical committee on animal experiments. The healthy three week old male and female BALB/c mice were taken for the experiment. The total number was twenty (20), 10 male and 10 female weighing 12-14 g. The simple random sampling technique was applied. They were kept in a well ventilated room and under a temperature range of 20-260C. Mice were randomly divided by lottery method into two groups. Each group contained 5 male and 5 female mice (10 animals in each group). Male and female mice were kept in separate cages to avoid pregnancy.[16] The mice of group G1 served as controls, they were given standard laboratory diet for 60 days. Mice in G2 group were given caffeine at a dose of 10mg/100gm body weight, on alternate day, 3 days a week for 60 days by oral gavage. At the end of experiment, the animals were euthanized with ether anesthesia. They were dissected and right femur was removed after separating from hip and knee joints. Femur was weighed by electrical balance while its length was measured by digital vernier calliper from greater trochanter to lateral condyle[17] (Fig 1). IBM-SPSS version 20 was used for data analysis. Student's T test was applied for intergroup comparison of quantitative variable which was taken as means and standard deviations (mean ±SD). A p value < 0.05 was considered significant.

Results

The mean femur weight of mice in experimental group G2 was considerably decreased as compared to control group G1. However, the mean femur length of experimental group G2 was appreciably increased as compared to control group G1 (Table I). The p-values of both femur weight and length of group G2 in comparison with group G1 were calculated to be < 0.05 and therefore found statistically significant (Table I).

Table I: Mean values of weight and length of femur in control group G1 and experimental group G2

Femur	Group G_1 Mean±SD (n=10)	Group G_2 Mean±SD (n=10)	p-value
Weight (gm)	0.387±0.019	0.316±0.020	< 0.05*
Length (mm)	20.70±0.609	24.382±1.087	< 0.05*

*P value < 0.05 is statistically significant

Fig 1: Photograph showing measurement of length of the right femur with vernier calliper

Discussion

Caffeine is one of the most commonly consumed substances in the entire world. The scientists have investigated its effects and they have expressed apprehension regarding its deleterious effects on human health.[18] The U.S. Food and Drug Administration (FDA) reported that more than 90 percent of the world's population ingested some form of caffeine. Caffeine intake has been linked to a variety of health issues, both short and long term. Increased caffeine ingestion is syndicated with an alteration in two genes that increase the rate of caffeine metabolism. Subjects who has this variation on chromosomes imbibe 40mg more caffeine per day as compared to others.[19] The current study is designed to determine the effect of caffeine on femur length and weight of the BALB/c mice.

The gross findings concerning femur length and weight have illustrated significant differences between G1 (control) and G2 (experimental) groups. The mean length of femur was greater in experimental group G2 (caffeine fed) as compared to control group G1 (Table I). The results of present experiment are comparable with an earlier international study done on male Wristar rats where the length of tibia after exposure to caffeine is significantly greater than that of the control group.[20] There is another study which has demonstrated that caffeine significantly modified quantitative composition and biomechanical parameters of the bone.[21] In a long bone, the cells of growth plate are amenable for the longitudinal growth of bone.[22] The growth hormone secretion increases due to acute effect of caffeine.[23] Caffeine stimulates the secretion of growth hormone (GH) through different mechanisms. Methylxanthines inhibit phosphodiesterase (PDE), which leads to an increase in pituitary cyclic adenosine monophosphate (cAMP) responsible for growth hormone release.[22] Caffeine also effects neurotransmitters. Caffeine increases the turnover of norepinephrine [23] and serotonin [24] in the brain. Norepinephrine as well as serotonin cause GH secretion in adult rats and humans.[25]

The weight of femur was found less in experimental group G2 than control group G1. An earlier international study shows that caffeine reduces the weight of the leg bones of rat.[26] Yet another study illustrates that intake of caffeine diminishes the volume and weight of femur.[27] The caffeine consumption lowers the BMD and hence weight of skeletal bones.[8] There is impairment of weight and longitudinal growth of bones by caffeine.[28] The excessive caffeine ingestion is usually a marker for a low calcium intake.[15] Caffeine decreases mineral and hydroxyproline content in bones. The amount of hydroxyproline in-turn indicates the collagen content of bones.[2] Caffeine consumption effects the normal metabolism of bones, including lower bone mineral density (BMD), lighter bone weight and decrease in calcium content of the bone.[27] The lower calcium content is also connected with caffeine induced defective development of bone.8 Moreover, caffeine has negative effects on normal growth and development of the osseous tissue.[28] Caffeine also decreases zinc levels in several tissues including bones.[29] The deficiency in zinc concentration in caffeine fed animals changes bone metabolism and permanently alters bone cytoarchitecture.[13]

The future work can be conducted by using different doses/duration of caffeine and studying the teratogenic effect of caffeine on genetic / chromosomal changes.

Conclusion

It was observed in the present study that caffeine altered the development of femur of BALB/c mice. High intake of caffeine caused increase in femur length and decrease in femur weight.

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