

Effect of Caffeine and Vitamin D₃ on Body Weight of Balb/C Mice

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Abstract

Background: To evaluate effect of caffeine and vitamin D₃ on body weight of BALB/c mice.

Methods: In this experimental study thirty BALB/c mice, three weeks old with average weight 12-14 g were taken and divided into three groups with 10 mice (5 male, 5 female) in each group. The control group G₁ was given normal diet prepared at National Institute of Health Islamabad with liberal water intake. Each animal in 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. All animals of G₃ were given caffeine 10mg/100g body weight on alternate day, three days a week and vitamin D₃ 0.1µg per day by oral gavage for 60 days. The effect of caffeine and vitamin D₃ was assessed by measuring the body weight of the mice at the end of the experiment.

Results: Mean final body weight of mice of control group G₁ was 31.2 ± 1.932 g while the mean final body weight of experimental groups G₂ and G₃ was found to be 27.1 ± 2.025 g and 29.2 ± 2.098 g. Mean final body weight of mice of experimental group G₂ was less as compared to control group G₁ and experimental group G₃.

Conclusion: The body weight of the mice was reduced due to caffeine consumption while vitamin D₃ administration ameliorated the deleterious effect of caffeine on the body weight.

Key Words: Body weight, Caffeine, Vitamin D₃.

Introduction

Caffeine is one of the most researched food components, with the vast majority of dietary contributions coming from beverage consumption. Caffeine intake varies across different types of beverages and in different population groups. Caffeine is one of many constituents in foods that can exert physiological effects. Global consumption of caffeine has been estimated at 120,000 tons per year, making it world's most popular psychoactive substance.¹

Caffeine consumption has been associated with weight loss and its ingestion provokes an increase in the metabolic rate.² Metabolic stimulation appears to be dose dependent and in rats it has been found to be

partly due to endogenous catecholamine release and partly to the intrinsic calorogenic effect of caffeine.³ There was an increase in both plasma free fatty acids (FFA) and urinary catecholamine excretion in man after caffeine ingestion. The FFA response was the result of catecholamine induced lipolysis.⁴ Caffeine was regarded as a thermogenic agent that in combination with slimming regimens could be of use in promoting the loss of body energy.⁵ Caffeine can be deemed as a noncaloric thermogenic agent that is usually consumed in many beverages. While a mild fever, anorexia, insomnia, and a loss of weight have been reported in women who frequently consumed the equivalent of 1.5 to 1.8 g caffeine per day as coffee, it is well tolerated by human subjects.⁶ There had not been any fatalities as a result of caffeine consumption, however, a toxic dose was believed to be about 10 g or more.²

Caffeine increases basal metabolic rate in adults.⁷ Lipid oxidation has also been shown to increase in subjects performing exercise after caffeine ingestion.⁸ The resting metabolic rate of lean and post-obese human volunteers was raised by 3-4% as a result of single-dose oral administration of 100 mg caffeine.⁹ The studies in animals evaluated that methylxanthines including caffeine were responsible for reduction of body weight as well as body fat by both anorectic and thermogenic effects.¹⁰

Vitamin D is a fat soluble vitamin and its chemical structure was determined in the 1930s. In the early 20th century, the therapeutic use of cod liver oil, containing vitamin D, led to a dramatic decrease in the incidence of rickets.¹¹ Vitamin D can be obtained from diet, supplements or from endogenous production in the skin. The cutaneous production is very effective and even a few minutes in the sun tends to increase circulating vitamin D concentration.¹² Severe deficiency of vitamin D can cause rickets in children and osteomalacia in adults.¹³ Vitamin D exists in two forms, namely ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Ergocalciferol (vitamin D₂) is obtained from plants, in particular mushrooms, whereas all other dietary sources as well as the cutaneous production of vitamin D are cholecalciferol (vitamin D₃). Vitamin D, and in particular 1,25(OH)₂D, is of critical importance to bone.¹⁴

Material and Methods

This laboratory based randomized control trial was conducted in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH) Islamabad from October 2014 to October 2015. Approval of ethical committee on animal experiments, of the Army Medical College, Rawalpindi was obtained for the study. A total of thirty, 15 male and 15 female BALB/c mice of three weeks weighing 12-14 g were studied. Mice were kept in a well ventilated room and under a temperature range of 20-26°C. They were randomly divided into three groups. Each group contained 5 male and 5 female mice (10 BALB/c mice in each group). Male and female mice were kept in separate cages to elude pregnancy.¹⁵ The mice of group G₁ served as controls and were given standard laboratory diet for 60 days. BALB/c mice in G₂ group were given caffeine at a dose of 10mg/100gm bw, on alternate day, 3 days a week for 60 days by oral gavage (Fig. 1). All animals of G₃ were given caffeine 10mg/100g body weight on alternate day, three days in a week and vitamin D₃ 0.1 µg per day by oral gavage for 60 days. The body weights of all the animals were recorded at the beginning of the study and at the end of the experiment. Data analysis was done using IBM-SPSS version 20 while ANOVA test was applied followed by Post Hoc Tukey's Test for intergroup comparison of quantitative variable based on means and standard deviations (mean ± SD). A p value < 0.05 was considered significant.



Fig 1. Photograph showing administration of drug through oral gavage

Results

The initial and final body weights (mean ± SD) of G₁ animals were found to be 13.1 ± 0.876 g and 31.2 ± 1.932 g, respectively. The initial and final body weights (mean ± SD) of G₂ animals were 13.1 ± 0.876 g and 27.1 ± 2.025 g, respectively. Similarly the initial and final body weights of G₃ were 13.1 ± 0.876 g and 29.2 ± 2.098 g, respectively (Table-1).

Table 1: Initial and final body weight of animals in control group G₁, experimental groups G₂ and G₃

Animal Weight (g)	Group G ₁ Mean±SD (n=10)	Group G ₂ Mean±SD (n=10)	p-value
Initial	13.1±0.876	13.1±0.876	1.0
Final	31.2±1.932	27.10±2.025	0.000472*

*p value ≤ 0.05 is statistically significant

The comparison of initial body weights of animals among control group G₁ and experimental groups G₂ and G₃ was statistically insignificant (Table -2); however, the change in final body weight of experimental groups G₂ and G₃ was found to be statistically significant (p<0.05) in comparison with control group G₁ (Table-2). The comparison of body weight of male and female animals within the groups G₁, G₂ and G₃ was statistically insignificant (Table - 3).

Table-2: Comparison of initial and final body weight of animals among groups G₁, G₂ and G₃

Animal Weight (g)	Group G ₁ vs. Group G ₂	Group G ₁ vs. Group G ₃	Group G ₂ vs. Group G ₃
	p-value	p-value	p-value
Initial	1.000	1.000	1.000
Final	0.000207*	0.039685*	0.035173*

*P value ≤ 0.05 is statistically significant

Table 3: Body weight of male and female animals within the groups G₁, G₂ and G₃

Animal Weight (g)	Male vs. Female G ₁	Male vs. Female G ₂	Male vs. Female G ₃
	p-value	p-value	p-value
Initial	0.7404	0.7404	0.6938
Final	0.1028	0.0836	0.0649

p- value ≤ 0.05 is statistically significant

Discussion

The consumption of caffeine is increasing day by day throughout the world. The public and the scientific community have raised voices against its detrimental effects on human health.¹⁶ A 2011 research determined that high caffeine intake was linked with an alteration in two genes that enhance the rate of caffeine breakdown. The individuals who had this transmutation on both chromosomes took 40 mg more caffeine per day as compared to others. In present study the initial body weight was found statistically insignificant among the groups G₁, G₂ and G₃, as well as during comparison between male and female

animals of groups G_1 , G_2 and G_3 . However, the final body weights were statistically significant when experimental group G_2 (caffeine fed) was compared with control group G_1 . Although, the final body weight was found insignificant in comparison between male and female groups of G_1 , G_2 and G_3 . The findings of this study are similar to one performed by Huang et al.(2002) on the effect of caffeine in male Wistar rats where the final body weight after exposure to caffeine was appreciably reduced than that of the control group. These results indicated that caffeine caused a significant decrease in accretion of body weight.¹⁷ Caffeine causes stimulation of the resting metabolic rate by enhancing the catabolism of fatty acid, thus inducing a greater loss of body weight.² It has been proposed that caffeine consumption which increased lipolysis was partially due to higher catecholamine discharge.⁴

Caffeine promotes lipolysis by restraining the cyclic nucleotide phosphodiesterase that is used for catalyzing the transformation of cyclic AMP to AMP. The high level of tissue cyclic AMP concentrations mobilize inactive hormone-sensitive lipase and stimulate lipolysis.¹⁸ Caffeine acts in the presence of lipolytic hormones, such as adrenaline, and causes an increase in cyclic AMP concentrations which is greater than that caused by the hormones alone. This shows that caffeine stimulates not only resting energy expenditure but lipid mobilization and fat oxidation also.⁴ In this study, improvement was noticed in the final animal weight of G_3 group co-treated with caffeine and vitamin D_3 . Sufficient dietary intake of vitamin D is necessary for building and maintaining healthy bones.¹⁹ Vitamin D supplementation results in decreased bone turnover and increased bone mineral density.²⁰ A 5 to 7% increase in the mean degree of mineralization of bone tissue was observed due to vitamin D.²¹ Vitamin D enhanced the absorption of calcium from gut and influenced the overall mineralization of the skeleton.²⁰ Vitamin D has been universally used for the prevention and treatment of osteoporosis and recognized as a bone resorbing hormone mostly in in-vitro studies.²²

Conclusion

Caffeine caused negative effect on the accretion of body weight of the mice. The deleterious effect of caffeine on body weight was ameliorated by the administration of vitamin D_3 . Hence, vitamin D_3 antagonized partially the detrimental effect of caffeine on the accretion of body weight.

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