**Original Article**

# Impact Of Caffeine And Vitamin D3 On The Body-Weight Of Pregnant Balb/C Mice

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## Abstract

**Objective:** To investigate the effect of caffeine and Vitamin $D_3$ on the body weight of pregnant BALB/c mice.
**Design of Study:** An RCT (Randomized control trial) in the Lab.
**Place and Duration of Study:** The study was carried out at Anatomy Department, Army Medical College (AMC), Rawalpindi, in collaboration with the National Institute of Health (NIH), Islamabad, from Oct 2018 to Oct 2019.
**Material and Methods:** Six-week-old pregnant BALB/c mice, thirty (30) in number, weighing 26-28g, were taken and divided into three groups with 10 pregnant mice in each group. The control group $G_1$ was given a standard diet with water ad libitum for 21 days. The animals in experimental group $G_2$, in addition to the standard diet, were given 10mg of caffeine per 100g body weight once a day on alternate days by oral gavage for 21 days. Similarly, mice in group $G_3$, in addition to the diet of group $G_2$, were given 0.1µg of vitamin $D_3$ per day by oral gavage for 21 days. The body weights on 1st day, 7th day, 14th day, and 21st day of gestation in all the groups were measured to determine the influence of caffeine and vitamin $D_3$.
**Results:** Mean bodyweights of mice in control group $G_1$ were noted as 26.8 ± 0.789g, 30.7 ± 0.949g, 36 ± 0.667g, and 42 ± 1.054g on the 1st, 7th, 14th, and 21st day of gestation, respectively. The mean bodyweights of experimental group $G_2$ were recorded to be 26.7 ± 0.675g, 29.9 ± 0.738g, 34.3 ± 0.823g, and 39.5 ± 0.972g on 1st, 7th, 14th, and 21st day of gestation, respectively. The mean bodyweights of experimental group $G_3$ were determined as 26.8 ± 0.632g, 30.4 ± 0.699g, 34.6 ± 0.516g, and 40.5 ± 0.850g on 1st, 7th, 14th, and 21st day of gestation, respectively. In comparison to the control group $G_1$, the bodyweights of animals in experimental group $G_2$ showed more decrease in the accrual of body weight than noted in experimental group $G_3$.
**Conclusion:** Caffeine intake has a decreasing influence on the growth of body weight in pregnant mice while intake of vitamin $D_3$ somewhat nullifies the harmful effect of caffeine on body weight.
**Keywords:** Body weight, Caffeine, Pregnant mice.

## Introduction

Caffeine is one of the most commonly consumed (pharmacologically active) matter on this planet. Carbonated soft drinks are the major intake cause of caffeine in kids while coffee is the main source of caffeine in adults[1]. Similarly, energy drinks have additional quantities of caffeine through preservatives. Worldwide caffeine intake in foods, drinks, and medicines used for pain or headache remedies has also increased with the passage of time. The principal use of caffeine is to amplify attentiveness and enhance short-term memory[2].

Epidemiological research has concluded that weight loss and an increase in the metabolic rate are directly linked to caffeine ingestion. The results of metabolic stimulation in rats show that it is associated with the ingested dose, partially because of endogenous catecholamine discharge and partly due to the inherent heat-generation influence of caffeine[3]. A rise in plasma free fatty acids (FFA), as well as urinary catecholamine emission, was observed in human post-caffeine intake. The FFA reaction was the outcome of catecholamine-instigated lipolysis. Caffeine was regarded as a heat-generative agent that along with slimming prescriptions could be used to promote the reduction in body energy4. Caffeine can be deemed as a non-caloric thermo-genic agent that is usually taken in many drinks. Although slight fever, loss of appetite, sleeplessness, and a decrease in weight have been reported in women who frequently took around 1.5 to 1.8g of caffeine per day in coffee, however, it is well endured by humans. No deaths have been noted due to caffeine consumption, however, a lethal dose was known to be well above 10g [5].

Caffeine had thermogenic and appetite suppressant effects both of which resulted in weight reduction in habitual consumers[6]. Caffeine causes an increment in basal-metabolic rate in grown-ups. It is known that lipid oxidation tends to rise in people during exercise post-caffeine intake. The metabolic rate of thin and post-obese individuals during resting state was increased by 4% as a consequence of one oral dose of 100 mg caffeine[7]. Studies on animals have shown that methylxanthines plus caffeine both were causes for the decrease in body weight and body fat through anorectic and thermogenic effects. Previous epidemiologic research has supported the relationship between caffeine ingestion in pregnancy and high risks of being underweight and/or pre-mature births[8].

The chemical arrangement of fat-soluble vitamin D was found at the beginning of the 20th century. Utilization of vitamin D present in cod liver oil to treat diseases was determined in the 1940s which led to a rapid decline in the cases of rickets[9]. Dietary, supplementary, and endogenic production in the skin is a common source of vitamin D. Production in the skin is very efficient, and even slight exposure to sunlight helps to raise vitamin D absorption. The absence of vitamin D is a reason causing rickets in kids and osteo-malacia in adults[10]. Vitamin D exists in two forms, namely ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$). Ergocalciferol is extracted from shrubs usually mushrooms, while cholecalciferol is found in various other dietary sources including skin production of vitamin D. Vitamin D, particularly $1,25(OH)_2$ D, has universal importance in human health[11].

## Materials and Methods

This laboratory-based randomized control trial was performed in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with the National Institute of Health (NIH) Islamabad. The study was carried out from October 2018 to October 2019 with the approval of the ethical committee on animal experiments, of the Army Medical College, Rawalpindi. Six-week-old thirty (30) pregnant BALB/c mice were taken with initial weights ranging between 26 - 28g. The convenience non-probability sampling technique was used, and these mice were divided into three groups of 10 pregnant mice each. The temperature was maintained between 20 - $26^0$C through environmental control system installed in the laboratory. A standard laboratory diet along with water ad libitum was administered for 21 days to the mice of the control group (G1). The experimental group (named G2) in addition to the standard laboratory diet, was given a single dose of caffeine 10mg/100gm body weight, on alternate days for 21 days. Animals in group $G_3$, along with the diet of group $G_2$, were given 0.1µg of vitamin $D_3$ per day by oral gavage for 21 days. The body weights of all the animals were noted on the 1st day, 7th day, 14th day, and 21st day of gestation.

IBM -SPSS version 20 was used for data analysis. A one-way ANOVA test was applied for intergroup comparison of a quantitative variable, which was taken as means and standard deviations (mean ± SD). A p-value ≤ 0.05 was taken as significant.

## Results

Mean body weights of pregnant mice in control group $G_1$, experimental group $G_2$, and experimental group $G_3$ were determined on 1st, 7th, 14th, and 21st days of the gestational period. The reduction in mean body weights of mice in experimental group $G_2$ was more than that noted in experimental group $G_3$, in comparison with the control group $G_1$ (Table / Figure). The p-value of the bodyweights in groups $G_2$ and $G_3$ on the 14th and 21st days in comparison with group $G_1$ was calculated to be ≤ 0.05 and therefore found statistically significant (Table)

**Table.** Comparison of Mean weights among the Groups $G_1$, $G_2$ and $G_3$

| *Animal Weight (g)* | *Group $G_1$ Mean ± SD (n=10)* | *Group $G_2$ Mean ±SD (n=10)* | *Group $G_3$ Mean ±SD (n=10)* | *p-value* |
|---|---|---|---|---|
| Day 1 | 26.8 ± 0.789 | 26.7 ± 0.675 | 26.8 ± 0.632 | 0.93 |
| Day 7 | 30.7 ± 0.949 | 29.9 ± 0.738 | 30.4 ± 0.699 | 0.10 |
| Day 14 | 36 ± 0.667 | 34.3 ± 0.823 | 34.6 ± 0.516 | < 0.001* |
| Day 21 | 42 ± 1.054 | 39.5 ± 0.972 | 40.5 ± 0.850 | < 0.001* |

*p- value ≤0.05 is considered as significant

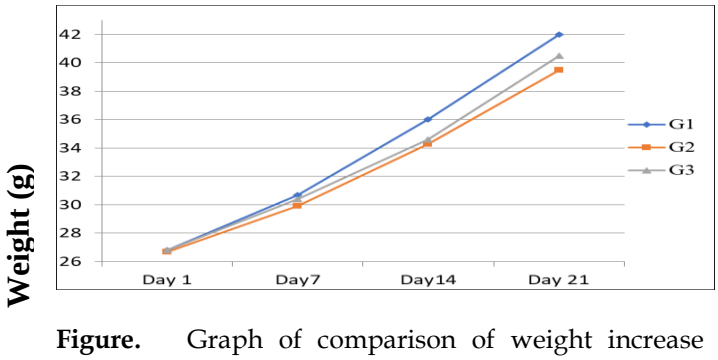


**Figure.** Graph of comparison of weight increase among the Groups $G_1$, $G_2$ and $G_3$

## Discussion

The consumption of caffeine is quite common in our daily life. Being a psychoactive substance, researchers have expressed great concerns regarding its injurious effects on human health[12]. Its consumers also consist of expectant females, 82% of whom have been found to take caffeine every day in the United States and 91% in France[13]. Moreover, in the United Kingdom and France, the effect of caffeine intake during pregnancy has also been reported showing the presence of caffeine in the blood of most of newborns as pharmacologically active concentrations[14]. In 1980, the USA Food and Drug Administration (FDA) in response to the findings of caffeine-induced teratogenic effects in rodents, issued an advisory to pregnant ladies to limit or refrain from this drug[15]. Awareness regarding caffeine pharmacology also recommends high vulnerability for possible fetal harm through motherly consumption[16]. Taken during the expectancy period, caffeine passes through the placenta, thus endangering the fetus to concentrations of the drug similar to maternal systemic levels[17].

The rationale of this experiment was to find out the impact of caffeine and its combined influence with vitamin $D_3$ on the body weight of pregnant BALB/c mice. The control group was marked as $G_1$ while the experimental group $G_2$ was administered caffeine and the experimental group $G_3$ was provided with caffeine as well as vitamin $D_3$. Results have shown that caffeine caused a noteworthy drop in the build-up of body weight. Caffeine raises the resting metabolic rate by improving the catabolism of fatty acid, thus causing a greater drop in body-weight[18].

Caffeine consumption increases lipolysis partially due to higher catecholamine discharge and partially by limiting the cyclic nucleotide phosphodiesterase, used for catalyzing the transformation of cyclic AMP to AMP[19]. The high level of tissue cyclic AMP concentrations subsequently activate dormant hormone-sensitive lipase and excite lipolysis. Caffeine performs in the presence of lipolytic hormones, such as adrenaline, to cause a rise in cyclic AMP concentrations which is much higher than what was due to only the hormones[20]. This manifests that caffeine excites the static energy expenditure as well as lipid mobilization and fat oxidation[21]. In the current study, progress was noted in the animal weight of experiment group $G_3$ given both caffeine and vitamin $D_3$. Thus, adequate dietary supplementation of vitamin D is considered essential for building and maintaining healthy bones[22]. Moreover, vitamin D plays a vital role in adipogenesis within the body and alleviates the negative effects of caffeine on body weight[23]. The genetically modified models highlighted the action of vitamin D and its receptor in adipose tissue energy homeostasis[24]. Vitamin D presence would improve calcium assimilation and

could even cause an indirect influence on body-weight[25].

## Conclusion

Caffeine caused a deleterious influence on the accumulation of body weight in pregnant mice. The harmful effect was somewhat reduced by the administration of vitamin $D_3$. Hence, vitamin $D_3$ partially antagonized the detrimental effect of caffeine on the build-up of body weight.

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