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Biochemical Effects of Dietary Caffeine on Urinary Excretion of Minerals in Adolescent Females

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ABSTRACT

Caffeine is widely available and the U.S. Food and Drug Administration (FDA), says about 80 percent of the population in the world take some form of caffeine every day to stay active. But caffeine does so much more than just keeping you awake. It's a central nervous system stimulant that affects your body in numerous ways. Female college students of age group 17-19 were involved and self-descriptive caffeine consumption questionnaire were distributed to them. Twelve of them were selected and their fasting urine samples and two hours of post consumption of coffee were collected for creatinine and mineral analysis. The test was performed for a period of 14 days. No significant alterations were observed in the creatinine levels signifying the perfect functioning of kidney. The level of sodium in urine has been found to be significantly increased in the test urine sample when compared with the fasting levels. A very high significant increase in the level of potassium by a margin of 15meq/L was recorded and levels of calcium and magnesium have also shown significant alteration when compared with that of the fasting samples. The study also showed a high significant increase in level of excretion of phosphorus in the urine.

KEYWORDS: Caffeine, Urinary minerals, natriuretic effect, Mineral excretion

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INTRODUCTION

Caffeine is consumed by many people all over the world in the form of coffee, beverages, foods and medicines. The most coveted drink in the world is coffee which contains more amount of caffeine than any other food substances. It is consumed by many people for many purposes and most of them prefer coffee because it gives instant alertness, free from drowsiness, makes mind fresh, free of sleep and to overcome stress. Caffeinated beverages are among the most frequently consumed beverages by teenagers due to their mentally stimulating effect. People consume caffeine without clearly understanding its possible side effects. Caffeine produces no nutritional value on its own[1].

Caffeine is present in a number of dietary sources consumed worldwide, i.e., tea, coffee, cocoa beverages, chocolate bars, and soft drinks. The content of caffeine of these various food items ranges from 40 to 180 mg/150 ml for coffee to 24 to 50 mg/150 ml for tea, 15 to 29 mg/180 ml for cola, 2 to 7 mg/150 ml for cocoa, and 1 to 36 mg/28 g for chocolate [2].

Caffeine easily passes through the membrane so from the first sip caffeine enters the bloodstream through the lining of the mouth, throat and stomach. It only takes 45 minutes for 99% of caffeine to be absorbed through the membrane. In humans the average life of caffeine is 4 to 6 hours on average which explains why the average energy drink or coffee's effect lasts up to 4-6 hours[3]. The caffeine is excreted from the body when the caffeine metabolites are filtered by the kidneys and then they are excreted through the urine. Because of its widespread use as an ergogenic aid and its ubiquitous availability, caffeine is no longer on the banned substance list of the International Olympic Committee. The general consensus of research findings indicates that caffeine improves continuous exercise time [4].

Caffeine acts as central nervous system stimulant [5]. When it reaches the brain the most noticeable effect is its alertness. But people are oblivious to the fact that caffeine in coffee contributes to mineral excretion such as calcium, magnesium, iron, sodium though the urine.Harpaz et al., (2014) reported that the relative dietary contribution of caffeine from sodas has decreased (62% to 38%) whereas coffee has increased (from 10% to 24%) and "energy" drinks have emerged as a conspicuous source.Substance abuse and proper nutrition continue to be of concern among adolescents[6]. Therefore, the purpose of this present study was to record the adolescent nutritional knowledge the history of caffeine and its effect on human physiology and caffeine intoxication. The aim and objective of the present study is to document the dietary caffeine intake pattern of adolescent females (healthy volunteers of our college students) and to ascertain the post consumption pattern of minerals excretion in urine samples of test subjects.

MATERIALSAND METHOD

Selection of Subjects

The caffeine intake pattern and identification of the subjects were done based on standard questionnaire which was distributed among the undergraduate students of the department of Biochemistry, Justice Basheer Ahmed Sayeed College for Women, Chennai. In the questionnaire in addition to demographic details, information regarding different types of caffeinated products and frequency of consumption, multiple-choice questions concerning the benefits and side effects of caffeine were covered. Participation was voluntary and were well informed about the protocol.

Collection of samples

Baseline studies were conducted, by collecting 24 hours non fasting urine samples. For a test period of 2weeks the samples were collected from the subjects two hours after the consumption of coffee (Birukov*et al.*,2016)[7]. Samples were collected in sterile containers which were sealed and refrigerated and the tests for various minerals were done using standard protocols.

Estimation of Creatinine

The serum creatinine was determined using Jaffe's reaction by following the method of Owen *et al.,* (1954)[8].0.2 ml of the serum and 2.0 ml of the reagent mixture were pipetted out into a test tube. It was mixed well and the change in absorbance (A) was recorded after 30 seconds at 490nm against a reagent blank, which was taken as A1 and exactly after 2 minutes the absorbance was read and it was taken as A2. A1 - A2 gave the measure of the creatinine present in the sample. The results were expressed as mg/dl.

Estimation of Sodium and potassium

In accordance with the standard procedure, urinary sodium and potassium were measured using ion selecting electrode method by Olympus AU 680 autoanalyser (coefficient of variation was 1.5% for sodium and 2.5% for potassium). A difference in sodium or potassium ion concentration between the Na^+/K^+ solution inside the electrode and the sample causes an electrochemical potential to form across the membrane of the active electrode. The urine sample is then processed and measured as per the instruction manual. The sodium ion or potassium ion concentration measured is expressed in terms of mEq/L.

Estimation of Magnesium and Calcium in Urine

Magnesium and Calcium was estimated with kit procedure using Biosystem RA 50 analyzer.The procedure consists of precipitation of urinary calcium from an acid aliquot of urine containing an

excess of ammonium oxalate, by adjusting the pH to 5.0 using methyl red as the indicator. The urine is then centrifuged and the supernatant is used for the determination of magnesium. The precipitate, which is dissolved with hydrochloric acid, is used for the determination of calcium. The calcium and magnesium ion concentration measured is expressed in terms of mg/dl.

Estimation of phosphorus

Urinary phosphates was estimated using Fiske and Subbarow (1925) method[9]. The standards are pipettedout in the range of 0.2-1.0 ml into different tubes. 0.4ml of Urine sample was also pipetted into a test tube and volume in all the tubes were made upto 4.3 ml using double distilled water. A blank tube with 4.3ml of double distilled water alone was taken then to all the tubes 0.5ml of ammonium molybdate and 0.2ml of ANSA were added. The tubes were allowed to stand for 15minutes. The intensity of the blue color was read at 640nm. The results are expressed as mg/dl.

Statistical Analysis

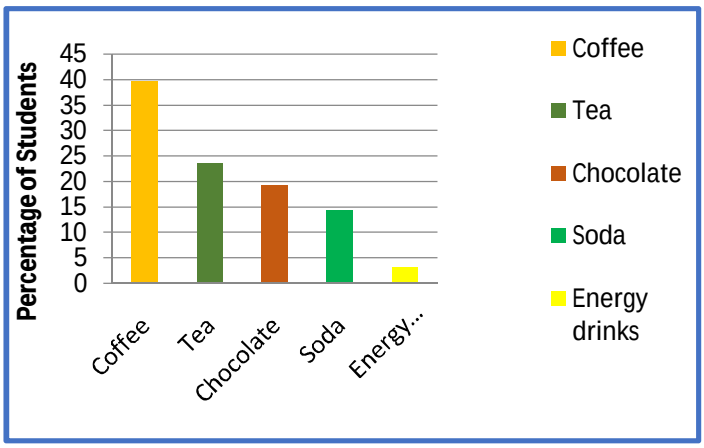
For statistical analysis data was entered in Microsoft excel spread sheet and analyzed statistically using Graph Pad Software Quick calcs: test calculator. Results were considered significant if the ‘p’ value was below 0.05.

RESULTS

The data for our study was gathered from the UG students of the Department of Biochemistry restricted to adolescent female between the age group of more than 17-19 years. Figure 1 shows the distribution of caffeinated beverage consumption among selected group. The majority of the participants (77%) stated that they ingested caffeinated beverages of these, 39.7% consumed coffee, 23.6% consumed tea, 19.2% consumed chocolate, 14.3% consumed Soda, and 3.2% consumed energy drinks. They differed slightly with regard to the sources of caffeinated beverages. Our students 32.7% reported that the main reason for their caffeinated beverage consumption was to sense increased alertness and 19.6% expressed a preference for the taste. 18.3% of the caffeine consumers indicated that they consumed caffeinated products because it enhanced their mood, 5.1% as a social pastime, 18.1% to treat a headache or a hangover and 6.2% stated other reasons such as habit (Figure 2). Among the participants, the majority (49.3%) had the most consumption in the morning, 3.8% in the afternoon, 38.1% in the early evening and 8.8% at night (Figure 3). The frequency of consumption was also recorded and it was observed that 40.6% consumed 4-5 times a week, 28.2% consumed 2 times a day, 15.5% 1 time a day and 15.7% did not answer (Figure 4).

The measured urinary sodium, potassium and creatinine excretion values in the baseline and post caffeine consumption are shown in figure 5. The levels of urinary sodium were very high

significantly elevated (P<0.001) than that in the baseline collection this finding probably reflects the sodium diuresis of volume expansion. The results of potassium excretion also showed significant increase (P < 0.01) in the excretion levels after consumption of coffee whereas the creatinine illustrates no significant increase only a mild variation in the excretion rates when compared with the control.



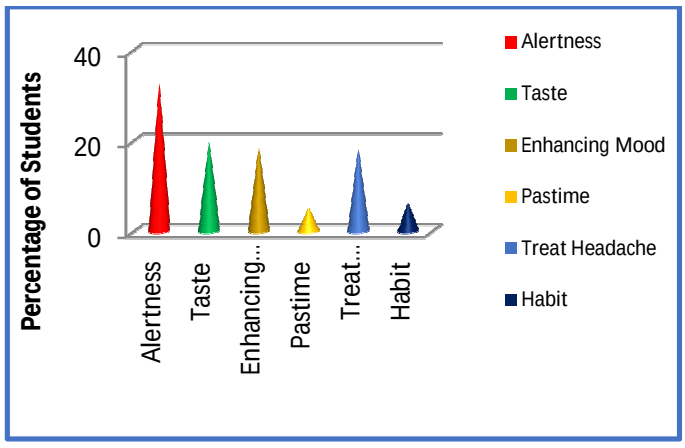
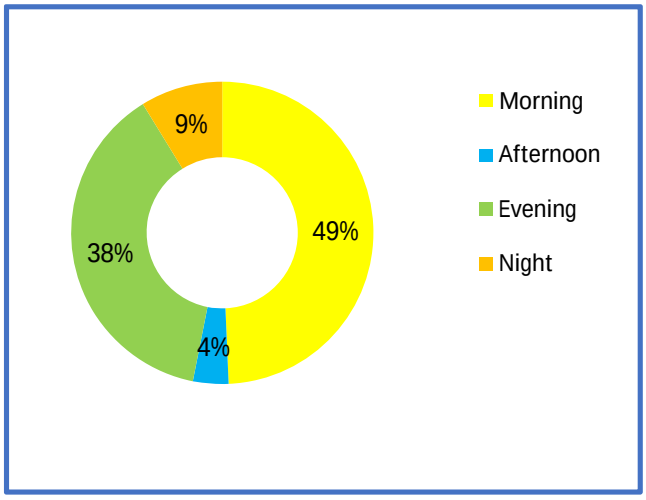


Figure 1: Distribution of Caffeinated Beverage Consumption **Figure 2: Reasons for Consumption of Caffeinated Beverages**



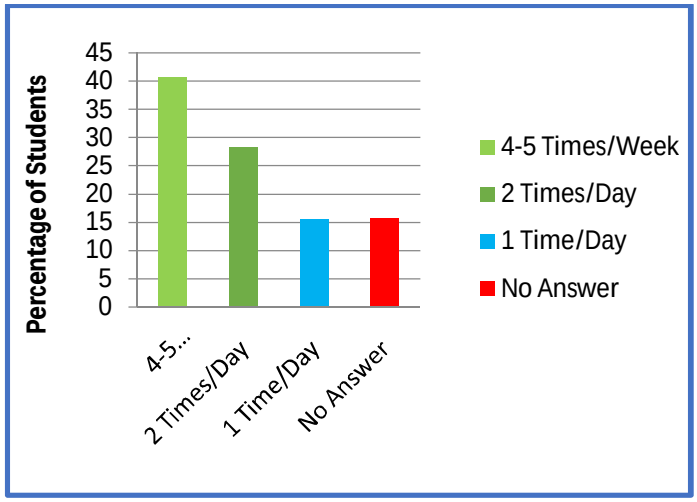


Figure 3: Time of Consumption of Caffeinated Beverages Consumption

Figure 4: Frequency of Caffeinated Beverage

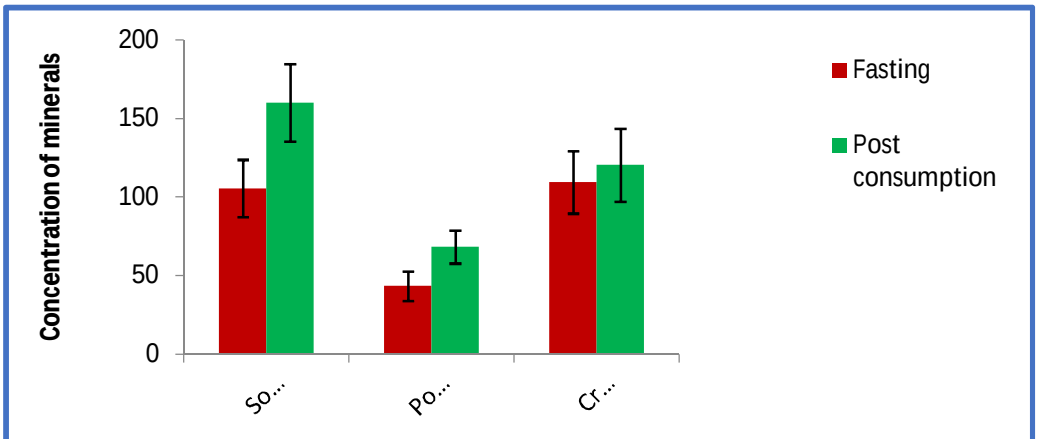


Figure 5: Effects of Caffeine Consumption on Urinary Excretion of Sodium, Potassium & Creatinine

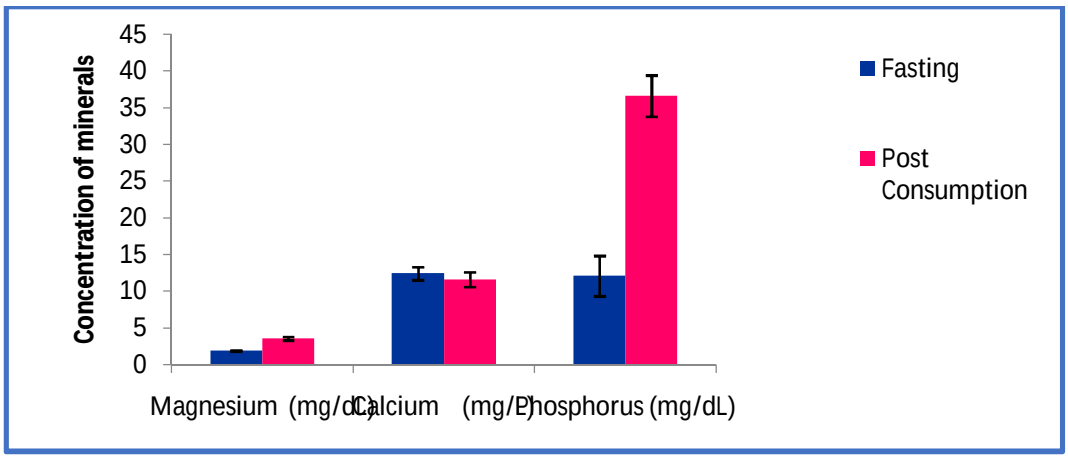


Figure 6 : Effects of Caffeine Consumption on Urinary Excretion of Calcium, Magnesium and Phosphorus

The observed urinary calcium, magnesium and phosphorus excretion values in the baseline and post caffeine consumption are documented in figure 6. The levels of urinary magnesium was very high significantly elevated ($P<0.001$) than that in the baseline collection. The results of calcium excretion also demonstrated a significant increase ($P < 0.05$) in the excretion levels after consumption of coffee. and the phosphorus illustrates very high significant increase ($p< 0.001$) in the excretion rates when compared with the control.

DISCUSSION

The students are vulnerable to multiple stressors whether academically or socially. This study has revealed a significant percentage (66.3%) of caffeine addicted students. Creatinine is produced as a result of muscle metabolism. Creatinine measurements ensure the physiological condition of subject being tested [10]. Urine samples with a creatinine of less than 20 mg/dL should be considered dilute and a dilute sample does not accurately reflect the recent drug use history of the person being

tested. Normal human creatinine levels will vary during the day based upon fluid intake - healthy individuals will rarely produce urine samples with creatinine of less than 20 mg/dL. In the present study creatinine clearance did not change significantly and the results were in close agreement with Elhady, 2018[11].

According to the present study the level of sodium in urine has been found to be increased. This can be attributed to the natriuretic effect of caffeine. Adenosine is an important regulator of kidney function and is involved in the regulation of glomerular filtration rate (GFR)[12], medullary blood flow[13] and renal water and electrolyte transport[12,13]. Derivatives of caffeine (methyl xanthines)may antagonize the adenosine receptors and are also known to inhibit phosphodiesterases in the proximal tubule that may contribute to the diuretic and natriuretic effects[14,15]. Though the sodium excretion level is in the normal range, compared to the baseline study the excretion level has considerably increased. This shows that frequent caffeine consumption may lead to sodium deficiency. Excessive excretion of sodium causes hyponatremia. The symptoms include nausea and vomiting, headache, confusion, loss of energy and fatigue, restlessness and irritability, muscle weakness, lethargy, malaise, spasms or cramps, seizures and coma.

Most apparent in these data is the rise in urinary potassium after the ingestion of caffeinated beverages. The level of potassium is found to have increased by a margin of 15meq/L. This most likely means that an increased loss of potassium via the urine stream is caused by the diuretic action of caffeine [16]. The homeostasis of salt and water involves different segments of the nephron, in which adenosine plays complex roles depending on the differential expression of AR (Adenosine receptor). Hence, caffeine increases glomerular filtration rate by opposing the vasoconstriction of renal afferent arteriole mediated by adenosine via type1 AR during the tubuloglomerular feedback[17] herby slowly leading to hypokalemia. The symptoms of hypokalemia are extreme fatigue, muscle spasms, weakness or cramping, lessened reflexes, irregular heartbeat, constipation, nausea, vomiting.

Also, in the present study it was observed that the levels of calcium and magnesium were not much disturbed however, a slight increase in the level of excretion of magnesium of approximately 0.5 mg/dl was recorded and no significant increase in calcium excretion was noted. Similar results have been reported by James *et al* 1986. On contrary Bergman *et al.*, 1988and Wikoff*et al.*, 2017 reported that caffeine reduces the reabsorption of calcium and magnesium in the kidney, causing minerals to be excreted in the urine[18,19,20]. The direct action of caffeine to increase urinary calcium excretion has also been demonstrated by Massey and Wise 1984. Caffeine is also found to negatively affect intestinal magnesium absorption [11,16]. Although coffee does not directly deplete magnesium from the body, the decreased absorption of magnesium can cause the body to gradually lose

magnesium, potentially resulting in a magnesium deficiency. Deficiency of calcium and magnesium increases the risk of developing osteoporosis.

The data from the study shows significant increase in level of excretion of phosphorus in the urine. The decrease in phosphorus balance with caffeine administration appears to have been caused primarily by an elevation in the urinary excretion of phosphate. Unlike calcium, which had only 1.4% of the ingested calcium excreted in the urine, a relatively large percentage (21%) of the ingested phosphorus was excreted in the urine. Caffeine had a greater effect on the amount of urinary phosphorus excreted than on the amount of calcium excreted.In normal human adults, most dietary phosphorus is absorbed from the intestine and 60-80% of the ingested phosphorus is excreted in urine[14,21]. Therefore, the possibility that caffeine to produce clinical problems as a result of its phosphaturic effect cannot be overlooked.This can lead to phosphorus deficiency or hypophosphatemia**.**

CONCLUSION

Caffeine drinking is a harmful trend that affects the entire society, particularly its young members. The prevalence of caffeinated beverage consumption proved to be substantially high in our study, with coffee, chocolate and carbonated drinks being the most frequently consumed. The amount of ingestion of these caffeinated beverages readily and particularly increases during the stressful period of exams, thus allowing students to maintain a significant mental state of alertness. This results in students becoming unconsciously addicted to these seemingly harmless beverages. The present study has demonstrated that caffeine exerted only a minor diuretic effect therefore according to Indian scenario the concerns regarding mineral or fluid loss associated with caffeine consumption are unwarranted. Though the mineral metabolism was found to be not much affected in young women there is a need to develop a beneficial awareness program for students about the manner in which to consume these caffeinated products moderately in order to prevent future health problems.

RECOMMENDATION

The role of caffeine as a risk factor for mineral loss especially calcium is controversial. Moderate consumption of caffeinated beverages has no effect on bone health however further studies in adult women are needed to confirm these findings as assessment of the health risks and health related endpoints.

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