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Urinary electrolyte analysis of non-vegetarians and vegetarians in a Nigerian university community

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This study compared the electrolyte concentrations of urine samples of vegetarians and non-vegetarians in a university community, by monitoring the urinary level of sodium, calcium, potassium and chloride ions. Sixty people participated in the study of which 17%, 23%, 40% and 20% were children, adolescents, adult non-vegetarians and adult vegetarians respectively. Participants were restricted to three square meals per day with no snack in-between. Urine samples were collected every twenty four hours for seven days, combined and refrigerated before use. The electrolyte concentrations were estimated using the simple Fautus test method for chloride, flame photometry for Na^+ and K^+ and a colorimetric method for Ca^{2+}. The results of the analysis showed that retention of Na^+, K^+ and Cl^- decreases with the age group of the children having the highest retention for the three ions. The average concentrations of the urinary electrolytes for the non-vegetarians were higher than those of the vegetarians except for Ca^{2+}. The potassium and chloride ion concentrations were within normal range for both groups of respondents. The calcium ion concentrations are lower than the normal for both groups.

Keywords: Potassium, chloride, sodium, calcium ion, urine, vegetarian, non-vegetarian.

INTRODUCTION

Urine has important chemical significance in the body of humans and other urinating living organisms. It helps to maintaining the electrolyte balance. Sodium (Na^+), potassium (K^+) and chlorine (Cl^-) ions are electrolytes of vital importance to the functioning of the body of these living organisms. This fact is occasionally overlooked resulting in potentially life-threatening consequences. Concentrations of electrolytes such as Na^+, K^+ and Cl^- ions in the body must remain within narrow ranges to preserve the delicate homeostatic balance required for good health. These electrolytes are distributed throughout the body to help maintain osmotic pressure, control fluid volume, regulate pH, blood pressure and to participate as cofactors in many enzymatic reactions. Normally 80-90% of ingested Na^+, K^+ and Cl^- ions are excreted by the kidney in the urine (Giuliano et al. 1988; Erich, 1990).

Chloride ion is the most abundant negatively charged ion in the extracellular fluid compartment of the human cell participating in the maintenance of acid-base balance through the chloride shift

(Jacobs et al. 1990; Meletis, 2014). Cl^- works with Na^+ to maintain serum osmolarity and fluid balance. It also functions in instituting an inhibitory post synaptic potential (IPSP) when the negativity of the neuron is beyond the normal resting membrane potential of -45mV threshold for excitation (Paradiso et al. 1995).

Potassium is an extremely important electrolyte that functions in the maintenance of water balance and distribution, electrical charge within the cell, acid-base balance, muscle and nerve cell functions, heart, kidney and adrenal functions. In addition to functioning as an electrolyte, potassium is also essential for conversion of blood sugar into glycogen as the storage form of sugar in the muscles and liver (Mc Donald, 1971; Creager, 1992).

Na^+ and K^+ ions function together in an electrogenic system in the body called the Na^+/ K^+ pump. This "pump" is used to transfer nutrients across cells, for muscle contraction and relaxation, and nerve action. The body needs to keep these two minerals in balance because they work opposite of each other. According to Kumar et al. (2011) Na^+ and K^+ have a relationship with blood pressure. If the content of Na^+ in the blood is high, this may affect water balance and may raise blood pressure.

Calcium present in milk products, fish, hard water and in bread is required for healthy bones and teeth. Calcium ions (Ca^{2+}) are especially important in maintaining the rhythmic contraction of the heart muscles. Calcium ions affect the nerve transmission by their stabilizing effect on the nerve membrane. A specific level of Ca^{2+} ions in the fluid of the brain is critical in maintaining body temperature. Too high a concentration of Ca^{2+} ions will result in the lowering of the body temperature. Calcium plays an important role in the clotting process of blood. It is also involved in the metabolism of vitamin D (Sourer, 1995; Kirsehmann and Murray, 1996; Pravina et al. 2013).

In this study, vegetarians are defined as people who predominantly consume foods of plant origin, in addition to eggs, and milk, while non-vegetarians are defined as people eating foods of both plant and animal origin (Wong et al. 2013). According to Ngozi et al. (2018), the nutritional status of lacto-ovo vegetarian young adults in the same institution, Babcock University, where this study was carried out showed that over 50% of the study population was normal, even though the nutrient intake of the respondents was generally below the RDI. The present study, therefore, intends to evaluate the effect of nutrition on electrolyte levels in humans by determining the levels of Na, K, Ca and chloride ions present in urine samples of non-vegetarians and vegetarians in a university community.

MATERIALS AND METHODS

For the purpose of this study, 60 urine samples from non-vegetarians and vegetarians in Babcock University, Nigeria were collected. The effect of gender and age groups were monitored within the non-vegetarian groups. Standard questionnaires were distributed to the subjects to indicate their gender, age and diet. An early morning urine sample was collected every twenty four hours from the respondents into sterile urine plastic containers for seven days combined and stored in a refrigerator at 4 ^oC until required for analysis. Table 1 indicates the population distribution used in this study; while Table 2 shows a typical daily diet of the participants. The data were collected from the community of Babcock University including staff and students.

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Table 1: Demography of Respondents

Classification	Age (year)	Number of Participants
Non-vegetarian	5-10	10
	13-18	14
	20-54	24
Vegetarian	20-54	12

N.B = There were no vegetarians for the lower age grade

Urine Analysis

According to Adrogué and Madias (2012), measuring urine electrolytes and computing the urine/serum (U/S) electrolyte ratio, which is the sum of the urinary concentrations of sodium and potassium divided by the serum sodium, can point to the effect of the urine output on the level of serum sodium at the time of evaluation. In alignment to this viewpoint, Furst et al. (2000), also presuppose that the urinary electrolyte levels usually reflect the serum levels.

Chloride ion

The urine samples were analyzed using the Fantus (1936) clinical test as described by Davidson and Henry (1974) for chloride ion concentration. The Fantus test is a simple test for measuring urinary output of chloride ion. It is widely believed that the urinary chloride electrolyte levels usually reflect the serum levels,

and the Fantus test is commonly employed as a guide to electrolyte therapy. Briefly, one drop of freshly prepared 20 % K_2CrO_4 solution was added to 20 drops of urine and titrated drop wise with 2.9 % $AgNO_3$ solution. The number of drops of $AgNO_3$ solution that turned the yellow color of the K_2CrO_4 - urine mixture to chocolate brown color was recorded.

Calculation of Cl^- ion concentration

$Ag^+_{(aq)} + Cl^-_{(aq)} \rightarrow AgCl(s)$

1 drop of $AgNO_3$ is equivalent to 0.5 g of NaCl per liter

g of NaCl per liter = Drops of $AgNO_3 \times 0.5$

g of Cl^{-1} per liter of urine $= \frac{\text{g of NaCl per liter}}{1.65}$

Where 1.65 is a constant that relates the amount of Cl^- in NaCl.

Sodium and potassium Test

Sodium and potassium were determined using the Flame photometer from B-Bran Scientific and Instrument Company England.

Calcium ion (Ca^{2+}) Test

Calcium ion concentration was determined by the O-cresolphthaleincomplexone colorimetric method using the Randox Laboratories (Crumlin, UK) Colorimetric Kit CA 590 as described by Barnett et al. (1973).

Preparation of urine samples for calcium ion test

200 µL of urine sample, 1.0 mL each of the buffer, 2-amino-2-methylpropan-1-ol (3.5 mol/L, pH 10.7) and o-Cresolphthaleincomplexone (0.16 mmol/L) and 200 µL of 0.9% saline NaCl were pipetted into a test tube. The mixture was swirled gently and left to stand for 10 minutes. Thereafter, the absorbance of the mixture (A) and the Standard (B) were measured at 580 nm wavelength against a reagent blank, using spectrophotometer Spectronic 20 (B-Bran Scientific and Instrument Company, England).

Calculation of calcium ion concentration in the urine samples

Calcium ion concentration (mg/100ml) $= \frac{\text{A(sample)}}{\text{B(standard)}} \times 10.0\ \text{mg/dL} \times 2$

Where,

A (sample) = Absorbance of sample.

B (standard, calcium solution, 10.0 mg/dL or 2.5 mmol/L) = Absorbance of standard.

10.0 mg/dL= Concentration of standard

2 = Dilution factor

The results for all quantitative variables are expressed as the mean ±standard deviation (SD).

Table 2: Representative Diet Menu for the Study

Classification	Break fast	Launch	Dinner
Non-vegetarian	Bread (4 slices) **or** a bowl of corn meal (500 ml) **or** 4 pieces of yam/potato (200g each) + one egg **or** 2 pieces of bean cake (40g each), a cup of milk/sugar (240ml) **or** a sachet of water (500ml)	Two wraps of Eba/fufu/ semolina (500g/wrap) + tomato sauce mixed with vegetables (ewedu, okra, greens, melon, ogbono) + two pieces of meat/fish (35g each) + one sachet of water (500ml) **or** one bottle of soft drink (75cl)	Two pieces of meat/fish (35g each) + one plate of rice (800g) **or** beans + plantain (800g) + one sachet of water (500ml) **or** one 75 bottle of soft drink (75cl)
Vegetarian	Bread (4 slices) **or** a bowl of corn meal (500 ml) **or** 4 pieces of yam/potato (200g each) + one egg **or** 2 pieces of bean cake (40g each) + a cup of milk/sugar (240ml) **or** a sachet of water (500ml)	Two wraps of Eba/fufu/ semolina (500g/wrap) + tomato sauce mixed with vegetable (ewedu, okra, greens, melon, ogbono) + 3 pieces of tofu + one sachet of water (500ml) **or** one bottle of soft drink (75cl)	One plate of rice (800g)/ beans/plantain + 3 pieces of tofu + one sachet of water (500ml) **or** one bottle of soft drink (5cl)

N/B A slice of bread = 35g A loaf of bread = 360g

RESULTS AND DISCUSSION

The results obtained from this study are shown in Tables 3 and 4. The demographic background of the respondents shown in Figure 1, indicates that there were no children and adolescent vegetarians within the Babcock University community. Urinary sodium and potassium and other electrolyte outputs estimated by analysing the first voided morning urine were found to be useful indices of the dietary intakes of these cations (Woo et al. 1992). Table 3 shows the electrolyte retention among the age groups of non-vegetarians. Retention of Na^+, K^+ and Cl^- ions among the non-vegetarian respondents did not follow a definite pattern. The K^+ and Cl^- ion concentrations were found to be very high among the 5-10 year old children compared to other groups; while Na^+ and Ca^{2+} ions concentrations were high in the urine of the adolescents and adults respectively. This may be because adults use more chloride metabolically than the young people (Jacobs et al.1990; Paradiso et al.1995). Furthermore, the pattern of Na excretion, which seems to decrease with increasing age, could explain the high rate of cardiovascular diseases prevalent in the elderly (Madukosiri, 2011). Retention of the Ca^{2+} ions is almost the same with children and adolescents but almost double with adults. All electrolytes are within their normal limits except for Na^+ which is higher than the normal blood concentration limit.

Table 4 shows the gender and average electrolyte retention in the population. Except Ca^{2+},theurinaryNa^+, K^+ and Cl^- ions concentrations are higher in male non vegetarians than their female counterparts. Their concentrations are twice the normal limit of level concentrations. High concentration of Na^+ is a condition known as hypernatremia; which might be due to subjects' reduced intake of fluid and consumption of diets that are rich in Na. Generally, fresh and frozen vegetables and fruits and eggs are relatively poor in Na while animal foods are rich in Na (Shelton, 1998; Morais et al. 2018).

The average electrolyte concentration for the vegetarian and non-vegetarian is also shown in Table 4. Non-vegetarians in this study had slightly higher urinary electrolytes concentrations than the vegetarians except for calcium ions. This may be attributed to abundant calcium in the vegetarian diet (Woo et al. 1998). However, the calcium ion concentrations are lower than the normal limit for both non-vegetarian and vegetarians. The potassium and chloride ion concentrations are within normal limit for both classes of respondents.

The chloride ion concentration is slightly higher in males compared to their female counterparts and is within the normal blood level concentrations necessary for proper metabolic functions. It has been reported that females in menstruating age group have low chloride ion concentration in their urine. This could be attributed to premenstrual syndrome and menstrual disorders that can stimulate hypochloremia (Chapman et al. 1980).

Table 3: Electrolytes concentration in urine for age group non-vegetarians

Age (year)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/24hr)	Cl^- (g/L)
5-10	207.11±77.03	54.60±40.45	1.22±0.68	6.07±2.28
13-18	211.21±56.88	49.31±29.61	1.20±0.53	5.55±2.59
20-54	170.60±75.08	48.03±28.53	2.05±0.89	4.81±1.80
Blood level concentrations (Normal Limit)	130-145	20-120	2.3-6.2	2.0-13

N/B A slice of bread = 35g A loaf of bread = 360g

Table 4: Electrolytes Concentration in Urine for vegetarians and non-vegetarians (Data are averages of two experiments NVG = Non Vegetarian)

Group/ Gender	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/24hr)	Cl^- (g/L)
Male (NVG)	208.05±58.53	51.48±26.42	1.58±0.80	5.44±2.15
Female (NVG)	183.67±77.22	48.52±34.97	1.88±0.94	4.89±2.16
Average values for non-vegetarians	198.05±69.47	49.91±30.94	1.73±2.15	5.17±0.88
Average values for vegetarians	123.08±90.3	23.18±15.47	2.13±1.46	2.43±1.35
Blood level concentrations (Normal Limit)	130-145	20-120	2.3-6.2	2.0-13

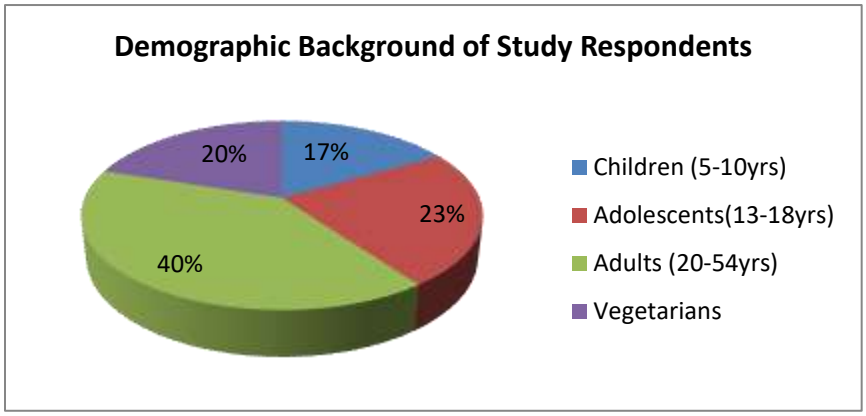

Figure 1: The demographic background of the respondents (subjects)

It was also observed that vegetarians had low chloride and sodium ions concentration in their urine compared to the non-vegetarians. Edible plants generally have less than 0.5mg/g of chloride ion while the value can be as high as 1.0 to 1.5mg/g in meat and fish; apart from the added table salt during cooking (Meletis, 2014; Capuano et al. 2013). To this effect it might be postulated that strict vegetarians feeding mainly on vegetables have reduced chloride ion consumption compared to their non-vegetarian counterparts who feed on fish, meat as well as vegetables. Consequently, due to the low urinary profile of Na^+ and Cl^- in vegetarian subjects, it could be argued that the vegetarian diet may have preventive role in hypertension – (Tripathi, et al. 2010). The average concentration of sodium ion is higher than normal for non-vegetarians but normal for vegetarians.

Urinary calcium ion concentrations in all the urine samples are lower than normal blood level concentration. Urinary calcium excretion depends on the amount of calcium reaching the glomeruli, the glomerular filtration rate and on the tubular function. If the renal glomerular function is impaired, calcium excretion is low (Philip, 1999). Normally the renal tubules reabsorb 99% of the filtered calcium and about 10% is excreted in the urine. Reabsorption of calcium is also selective, depending on the calcium ion concentration in the blood. When concentration is low, reabsorption is so great that almost no calcium is lost through the urine (Guyton and Hall, 2000). However, high urinary Na^+ and low Ca^{2+} concentrations in the non-vegetarians might predispose them to hypertension (Kwok et al. 2003; Yoshida et al. 2012).

CONCLUSION

This study reveals that the non-vegetarian subjects have higher dietary electrolyte intake and increased urinary electrolyte than the vegetarian respondents. The low urinary Na^+ profile in vegetarians showed that vegetarians are less likely to have hypertension compared to non-vegetarians. Both non-vegetarians and vegetarians will need food supplements to rectify

low calcium level status in their urine. In general, vegetarians are less likely to develop electrolyte related diseases than non-vegetarians.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Amos-Tautua BMW designed and conceptualized the idea; supervised the project and prepared the first draft of the paper. Adeyemi OT; Elegbede OK; Falade A and Femi-Ojo O carried out the experimental aspect. Onigbinde AO wrote part of the paper. Data analysis was done by Madukosiri while Songca SP carried out the editorial work.All authors have read and approved the final version.

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