

Variation of Major Cations and Na^+K^+ -ATPase in Erythrocytes and Its Relation Between the Serum Level of These Cations Along with Glucose, Urea and Creatinine in Vegetarians and Non-Vegetarians - A Cross Sectional Study

¹Mohit Adhikary, ²Smita S. Sonoli

^{1,2}Department of Biochemistry, J N Medical College, K L E University, Belgaum, Karnataka, India

Abstract: Lacto-vegetarians, lacto-ovo-vegetarians and pesco-vegetarians are put together as vegetarians and people who consume animal products such as meat, poultry, fish, seafood at least once a week are considered as non-vegetarians. The diet is the key factor for tolerance to glucose, urea, salt retention and many enzymes. Na^+K^+ -ATPase, an enzyme present on the surface of RBC membrane, Na^+ and K^+ are seen to alter with levels of urea and creatinine, with an altered glucose level and blood pressure. Hence, this study was undertaken to analyse the above hypothesis. The objective of this cross-sectional study was to estimate the levels of serum Na^+ , K^+ in association with erythrocyte membrane Na^+K^+ -ATPase and the status of serum glucose, urea and creatinine in healthy vegetarians and non-vegetarians and a co-relation was done with the diet that individual has. 50 vegetarians and 50 non-vegetarians within the age group 30-40 years were selected with the help of a questionnaire and the analysis of Na^+K^+ -ATPase in erythrocytes and Na^+ , K^+ , urea, creatinine and glucose in serum was done in these individuals. ATPase activity was measured by the method of *Racchah et al.* while serum glucose was estimated by Glucose Oxidase-PerOxidase's method, urea by DiAcetyl Monoxime's (DAM) method and creatinine by alkaline creatinine picrate. The Na^+ and K^+ were measured flame photometrically. Mean and standard deviation of all the serum and erythrocytic parameters were computed and compared using a paired 't' test. The *p* value was calculated for the comparison among the two groups, i.e., non-vegetarians and vegetarians. The results obtained showed an decrease in the activity of Na^+K^+ -ATPase with an increased Na^+ , glucose and urea, which was observed to be higher in non-vegetarians when compared to vegetarians. The K^+ and creatinine levels did not show any change when compared with the fooding habit of these individuals. Vegetarianism is advantageous as depicted by the lower levels of glucose, urea, Na^+ , K^+ and higher activity of Na^+K^+ -ATPase was observed in this study when compared to the non-vegetarians who had a comparatively higher level of these values and lower activity of Na^+K^+ -ATPase. It strengthens the notion of vegetarianism being preferred to non-vegetarianism with the other studies done on similar fooding habit.

Keywords: Vegetarians, Non-vegetarians, glucose, urea, Na^+K^+ -ATPase.

1. INTRODUCTION

“Non-vegetarians: Good at life or Vegetarians: Good at heart”. The debate for the non-vegetarians enjoying food and living life than that of the vegetarians and the vegetarians being at decreased risk of heart disease than that of the non-vegetarians has always been done. Though there are several advantages of the former over the latter and the vice versa, the association of vegetarian's diet with lower risk of several chronic diseases has been well documented.

The mortality rate has been seen to be lower in vegetarians who consume more fruits, vegetables, cereals, nuts, seeds and pulses than the non-vegetarians.¹ In earlier studies, it has been found that the non-vegetarians have high concentration of cholesterol and glucose levels as compared to that of the vegetarians and are at a higher risk of cardiovascular disease when compared to that of the vegetarians. The vegetarian diet contains substantially less saturated fats than the non-vegetarian diet, and saturated fatty acids have been shown to reduce insulin sensitivity.²

The vegetarian diet has typical inclusion of foods that have a low glycemic index such as beans, legumes, and nuts. In low-glycemic-response diets associated with less prevalence of type II diabetes, cohort studies have found a relation between dietary glucose and the risk of hypertension.^{3, 4} Observational studies have suggested that cardiovascular morbidity is ~2 times higher in diabetic patients than in the general population, and that cardiovascular disease accounted for ~70% of all such deaths.⁷ The constellation of abnormalities that include obesity, hypertension, diabetes and dyslipidemia is called metabolic syndrome, and this condition has been associated with high cardiovascular risk, morbidity and mortality.⁸ It has been generally accepted that hypertension and other vascular pathologies increase in diabetics, generally non-vegetarians, as a result of renin-angiotensin-aldosterone system,⁹ which plays a key role in the regulation of fluid and electrolyte balance. Protection against hypertension associated with vegetarian diet has been partly due to the lower BMI of vegetarians, where the effects of diet when not adjusted for BMI was greater.

Disentangling the effects of diet on insulin sensitivity independent of lower adiposity among vegetarians is difficult. Only sparse data have investigated whether vegetarians matched to non-vegetarians with regard to adiposity differ in insulin resistance or sensitivity. In a study that matched vegetarians and non-vegetarians, non-vegetarians had higher insulin, glucose, and homeostasis model assessment values than that of the vegetarians.⁵

Vegetarians weighed less and had low blood pressure than their non-vegetarians counterpart. It has been proved that plant sources contribute to adequate proteins, less total fat, saturated fat, cholesterol and more carbohydrates and fibers.¹ Concentration of trace elements like folate and magnesium has been seen generally to be high among vegetarians with low levels of iron and variable levels of selenium and zinc.⁶ The reduced risk of disease that has been found amongst vegetarians is influenced by diet.^{1, 6} Therefore much attention has been currently focused on the beneficial effect of vegetarian versus non-vegetarian diets.¹

The angiotensin converting enzyme inhibitors have been shown to be effective in many cardiovascular diseases particularly hypertension.¹⁰ The levels of glucose being high in the non-vegetarians as compared to that of the vegetarians has lead us to hypothesize that increased glucose level may lead to diabetes in non-vegetarian and to cardiovascular risk than that of a vegetarian. The levels of urea and creatinine are found to be high in non-vegetarians than that of it in vegetarians.¹¹

The Na^+ , K^+ , and Na^+K^+ -ATPase activity have been seen to be in positive co-relation to the dietary intake of an individual in relation to increased blood pressure. Na^+ , K^+ , and Na^+K^+ -ATPase activity levels in the serum and erythrocytes have been seen to alter in people with an altered glucose level. The altered levels of urea and creatinine also contribute to the level of Na^+ , K^+ and Na^+K^+ -ATPase activity.¹² The present study was planned to compare the Na^+ , K^+ , urea, creatinine in serum and glucose in plasma and Na^+K^+ -ATPase in erythrocyte of healthy vegetarians and non-vegetarians. The levels of Na^+ , K^+ were assessed by estimating it in the serum and the activity of Na^+K^+ -ATPase was assessed in erythrocytes. The urea, creatinine was assessed by estimating it in serum and glucose in plasma. The aim and objective of the study was to estimate the levels of serum Na^+ , K^+ in association with membrane Na^+K^+ -ATPase activity as well as the status of serum glucose, urea and creatinine in healthy vegetarians and non-vegetarians. The serum levels Na^+ , K^+ in association with red cell membrane Na^+K^+ -ATPase activity and status of serum glucose, urea, BUN and creatinine in vegetarians and non-vegetarians were then later co-related.^{13, 15, 16-19}

The diet is the key environmental factor implicated in health and disease and the people who consume these diets are classified into two as vegetarians and the non-vegetarians. The former's division of classification is little difficult to find, so a number of people with the same practice of feeding habit have been broadly put under the head of vegetarianism which majorly includes some of the types that has been mentioned below:

Vegetarians:

1. Lacto-vegetarians: People who consume vegan diet along with milk (and its products). A vegan diet constitutes of the various cereals, fruits and vegetables that we have belonging to that of the plant origin. It also includes the types of the food that are broadly put under *Fungi*, for example the mushroom.

2. Lacto-ovo-vegetarians: People who consume eggs along with milk (and its products) and the vegan diet included.
3. Pesco-vegetarians: People who consume vegan diet along with fish and milk (and its products).^{14, 17}

Non-vegetarian: People who consume animal products such as meat, poultry, fish, other seafood and (eggs and milk along with a vegan diet) at least once in a week.

The factors affecting dietary variation (food choice)^{12, 21, 23:}

1. Biological and Physiological e.g. age, gender
2. Personal and Psychological e.g. attitude
3. Cultural and Religious e.g. belief
4. Food e.g. taste, appearance
5. Socio economic e.g. affordability
6. Educational factors
7. Extrinsic e.g. media, time, season

IMPLICATIONS OF VEGETARIAN/NON-VEGETARIAN DIET IN DISEASES:

Relationships between a vegetarian diet and reduced risk for several chronic degenerative diseases and conditions including obesity, coronary artery disease, hypertension, diabetes mellitus and some types of cancer is positive according to the scientific data.³⁰ Studies indicate that vegetarians often have lower morbidity and mortality rates from several chronic degenerative diseases than do non-vegetarians.^{22, 26 and 15}

Death rate ratios are with 95% confidence intervals for vegetarians versus non-vegetarians.^{24, 25}

	Death rate	ratios
Ischaemic heart disease	0.76	0.62-0.94
Cerebrovascular diseases	0.93	0.74-1.17
Stomach cancer	1.02	0.64-1.62
Colorectal cancer	0.99	0.77-1.27
Lung cancer	0.84	0.59-1.18
Breast cancer	0.95	0.55-1.63
Prostate cancer	0.91	0.60-1.39
All causes	0.95	0.82-1.11

Intake of fruits and vegetables which provide antioxidants that might act as beneficial supplements in humans are positive health effects of vegetarian diets.²⁷⁻²⁹

2. MATERIALS AND METHODS

Source of the data:

Blood samples were collected from age and sex matched healthy individuals (vegetarian and non-vegetarian) in KLE's Prabhakar Kore Charitable Hospital and the staff of the J N Medical College and the KLE University. The entire participants were within the age group 30-40 years. 100 participants of the age group 30-40 were taken, of which 50 were vegetarians and 50 were non-vegetarians . The determination of vegetarianism and non-vegetarianism was done on the basis of the response given by the interested participants to the questions poised in a questionnaire. The interested participants consisted of age and sex matched healthy individuals in KLE's Prabhakar Kore Charitable Hospital and the staff of the J N Medical College and the KLE University, Belgaum between the ages of 30-40 years.

CRITERIA FOR SELECTION OF THE STUDY GROUP:

A participant who was willing to participate in the study was selected. The selection was done under the inclusion and exclusion criteria of the study. S/he was asked to fill in the consent for participation in the study. The participant was asked then to fill in a questionnaire. Healthy individuals between the age group 30-40 years, who were either vegetarians: lacto-vegetarians, lacto-ovo-vegetarians and pesco-vegetarians, i.e., people who consume vegan diet along with milk (and its products), people who consume eggs along with milk (and its products) and people who consume fish along with milk (and its products) respectively or non-vegetarians: people who consume animal products such as meat, poultry, fish, other seafood and (eggs and milk along with a vegan diet) at least once in a week were the ones included in the study. Participants who were alcoholics, hypertensive, had Diabetes Mellitus, Coronary artery disease and/or dyslipidemia, Metabolic syndrome, suffering from any other systemic disease (liver disease, renal disease etc.) were excluded from the study.

Permission to conduct the study was obtained from all the concerned authorities viz. Institutional ethics committee on human subjects' research of Jawaharlal Nehru medical college, Belgaum. Informed consent was taken from all the participants in the study.

PHYSICAL PARAMETERS: Height and Weight was measured using a measuring scale and weighing machine with an accuracy of ± 100 grams, respectively. The BMI was then calculated using the formula weight in kgs/ height in meter squares. The blood pressure (BP) was measured with the help of a sphygmomanometer.

COLLECTION OF SAMPLE:

Patient preparation: Fasting 5 ml of blood was withdrawn under aseptic precautionary measures using sterile disposable syringe by vein-puncture from the participating vegetarians and non-vegetarians and the blood collected was distributed in three vials.

1. Plain vial (2ml) for estimation of urea, creatinine, Na^+ , K^+ .
2. Fluoride vial (1ml) for the estimation of glucose.
3. EDTA vial (2ml) for the estimation of erythrocytic Na^+ , K^+ and membrane $\text{Na}^+-\text{K}^+-\text{ATPase}$.

Serum: 2 ml of blood was collected in a plain vial and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of vene-puncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes.

Plasma: 1 ml of blood was collected in a fluoride vial for the estimation of glucose. Plasma was separated by centrifuging the tubes at 3,000 r.p.m for 10 minutes.

RBCs: 2 ml of blood was collected in an EDTA vial. Plasma was separated by centrifuging the tubes at 3,000 r.p.m for 10 minutes and the RBCs obtained was stored in temperature of -20°C .

STATISTICAL TESTS USED:

Mean and standard deviation of all the serum and the erythrocytic parameters were computed and compared by using a paired 't' test. The p value was calculated for the comparison among the two groups, i.e., non-vegetarians and vegetarians.

The estimation of glucose was done from plasma with the help of glucose oxidase and peroxidase method. The estimation of urea was done by diacetyl monoxime method from serum and creatinine was done by the alkaline creatinine picrate method using a spectrophotometer to obtain the readings at their respective wavelengths. Na^+ and K^+ were estimated with the help of a flame photometer while the estimation of $\text{Na}^+-\text{K}^+-\text{ATPase}$ was done with the method explained by Raccah et al., 1996.³⁴

Preparation of erythrocyte membrane: The red cell pack extracted by centrifugation at 3,000 r.p.m for 10 minutes were thawed at 4°C and re-suspended by diluting it in 10 volumes of Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged at 3,000 rpm and the membrane pellet was suspended in 5 ml of 0.11 mol L-1 Tris-HCl buffer and the centrifugation step was repeated thrice. It was stored at -20°C until the assay was performed. **Erythrocyte $\text{Na}^+-\text{K}^+-\text{ATPase}$ activity measurement (Raccah et al., 1996)³⁵:** ATPase activity was measured in a final volume of 1 ml as

follows: The suspension were preincubated for 10 minutes at 37°C in a mixture. (Mixture composition: 92 mmol L-1 Tris-HCl (pH=7.4), 100 mmol L-1 NaCl, 20 mmol L-1 KCl, 5mmol L-1 MgSO₄.H₂O and 1 mmol L-1 EDTA)

Assays were performed with and without Ouabain, a specific inhibitor of Na-K-ATPase. The reaction was stopped by adding ice-cold trichloroacetic acid (5%) after incubation in 4 mmol L-1 ATP at 37°C for 10 minutes. Na⁺-K⁺-ATPase activity was calculated as the difference between inorganic phosphate released during the 10-minute incubation with and without Ouabain. After centrifugation 3000 g for 10 minutes, the amount of inorganic phosphate in the supernatant was determined (Dryer and Tammes, 1957).³⁶

3. RESULTS

The study comprised of 100 subjects out of which 50 were vegetarians and 50 were non vegetarians. All the values are expressed as Mean \pm S.D. Comparison of the studied parameters among groups was done by paired 't' test. P value <0.05 was considered significant. Results are given in Table 1.

Mean age for vegetarians was 33.96 \pm 2.78 years and for non-vegetarians were 33.28 \pm 2.85 years. There was no difference in the mean age among the two groups. (*P-value 0.231*) Out of the 100 subjects 50 were males and 50 were females, of which 25 males and 25 females were vegetarians and non-vegetarians. The sex distribution among the groups was equal.

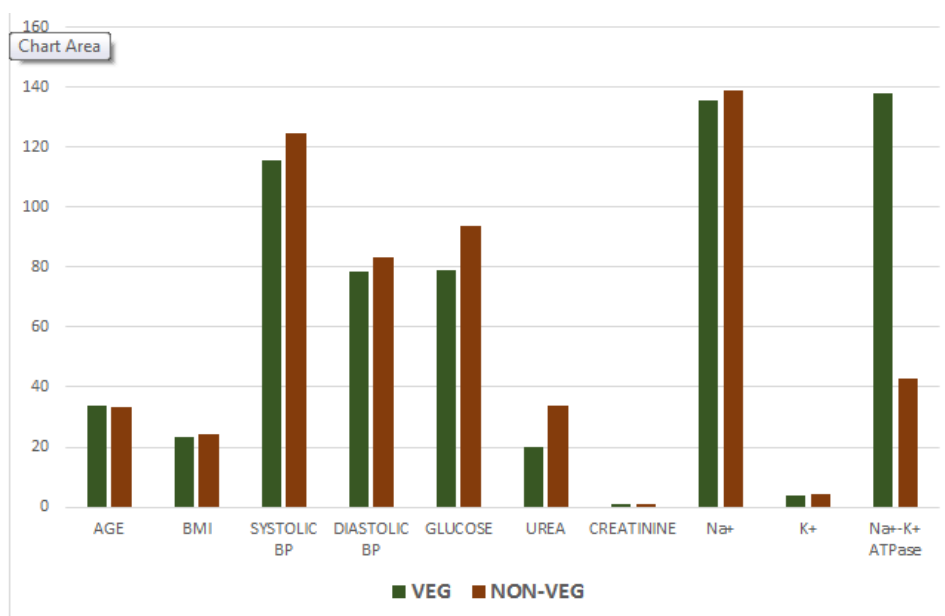
The BMI in vegetarians and non-vegetarians were equal. The BMI in vegetarians was 23.58 \pm 2.29 and that in the non-vegetarians was 24.39 \pm 2.27. There was no difference seen in the BMI level. (*P-value 0.077*) The systolic BP in vegetarians was 115.76 \pm 5.04 and the diastolic BP was 78.52 \pm 4.58. The systolic BP in non-vegetarians was 124.68 \pm 4.36 and the diastolic BP was 83.24 \pm 3.93. The systolic and the diastolic blood pressure in vegetarians were normal than that in the non-vegetarians which was seen to be increased significantly (*P-value <0.001*).

The glucose levels in vegetarians were 79.12 \pm 9.65 and that in non-vegetarians were 93.52 \pm 8.46. The glucose levels in non-vegetarians was seen to be increased significantly than that in the vegetarians (*P-value <0.001*). The urea levels in vegetarians were 19.84 \pm 5.18 and that in non-vegetarians were 33.82 \pm 10.29. The urea levels in non-vegetarians was seen to be increased significantly than that in the vegetarians (*P-value <0.001*). The creatinine levels in vegetarians and non-vegetarians were equal. The creatinine levels in vegetarians were 0.91 \pm 0.24 and that in the non-vegetarians was 0.97 \pm 0.27. There was no difference seen in the creatinine level. (*P-value 0.220*)

The Na⁺ levels in vegetarians were 135.78 \pm 2.80 and that in non-vegetarians were 139.02 \pm 3.61. The Na⁺ levels in non-vegetarians was seen to be increased significantly than that in the vegetarians (*P-value <0.001*). The K⁺ levels in vegetarians and non-vegetarians were equal. The K⁺ levels in vegetarians were 4.12 \pm 0.31 and that in the non-vegetarians was 4.15 \pm 0.22. There was no difference seen in the K⁺ level. (*P-value 0.059*) The Na⁺K⁺-ATPase levels in vegetarians were 138.07 \pm 43.65 and that in non-vegetarians were 42.61 \pm 26.33. The Na⁺-K⁺-ATPase levels in vegetarians was seen to be increased significantly than that in the non-vegetarians (*P-value <0.001*).

Table 1 with age distribution, physical (BMI, SBP and DBP) and biochemical (glucose, urea, creatinine, Na⁺, K⁺ and Na⁺,K⁺-ATPase) parameters

	VEG		NON-VEG		P value
	50 participants		50 participants		
	25 M and 25 F		25 M and 25 F		
Physical Parameters					
Age	33.96 \pm 2.78		33.28 \pm 2.85		0.231
Bmi	23.58 \pm 2.29		24.39 \pm 2.27		0.077
Systolic BP	115.76 \pm 5.04		124.681 \pm 4.36		<0.05
Diastolic BP	78.52 \pm 4.58		83.24 \pm 3.93		<0.05
Biochemical Parameters					
Glucose	79.12 \pm 9.65		93.52 \pm 8.46		<0.05
Urea	19.84 \pm 5.18		33.82 \pm 10.29		<0.05
Creatinine	0.91 \pm 0.24		0.97 \pm 0.27		0.22
Na ⁺	135.78 \pm 2.80		139.02 \pm 3.61		<0.05
K ⁺	3.77 \pm 0.31		4.15 \pm 0.22		0.059
Na ⁺ ,K ⁺ -ATPase	138.07 \pm 43.65		42.61 \pm 26.33		<0.05



4. DISCUSSION

The results obtained from the study shows that the levels of glucose, urea, Na^+ , K^+ is comparatively higher in non-vegetarians and the activity of the Na^+-K^+ ATPase is lower when compared to that of the vegetarians. A decreased activity of Na^+-K^+ ATPase further fortifies previous and similar studies done by Shahid and Tabassum, 2008 and Shahid, Jawed and Tabassum 2008.^{31, 32} Vegetarianism is advantageous as lower levels of glucose, urea, Na^+ , K^+ and lower activity of Na^+-K^+ ATPase was observed from this study when compared to the non-vegetarians who had a comparatively higher level of these values. It strengthens the notion of vegetarianism being preferred to non-vegetarianism with the other studies done on similar feeding habit of humans. It also enlightens and provides information that there are several other biochemical parameters that are seen to be increased with the increase in glucose and hypertension as discussed in the review by Buemi et al.³³

ACKNOWLEDGEMENT

The study was carried out in the laboratory of the Department of Biochemistry, J N Medical College, Belgaum, India. We would like to thank M. D. Mallapur, Department of Community Medicine, J N Medical College for the statistics and results and all the staff and colleagues of the Department of Biochemistry, J N Medical College.

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