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Urinary Amino Acids Profile of Vegetarians and Non-vegetarians

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ABSTRACT

The objective of the study was to quantify and to profile the amino acids content in urine samples. The amino acids content in urine was determined in 162 individuals (62 young non-vegetarians aged 15-45 years, 24 elderly non-vegetarians aged 46-70 years, 40 young vegetarians and 36 elderly vegetarians) by high performance liquid chromatography (HPLC). The most common amino acids detected in the young and elderly individuals on vegetarian and non-vegetarian diets were phenylalanine, threonine, arginine and asparagine, while leucine, aspartic acid and alanine were not found in any urine samples in both groups. Isoleucine was not detected in the urine of vegetarians. The concentrations of the majority of essential amino acids were between 0.10 - 2.00 mg/24hrs except for histidine which had a range of 4.1 - 5.0 mg/24hrs. The concentrations of non-essential amino acids varied. Proline, glycine and tyrosine concentrations were between 0.10 - 1.00 mg/24hrs, while cysteine, glutamine, glutamic acid and cystine concentrations were between 11.0 - 21.0 mg/24hrs. Asparagine and hydroxy-proline had a range of 0.10 - 5.00 mg/24hrs, while serine and arginine ranged between 31.0 - 50.0 mg/24hrs. Isoleucine and serine were not detected in elderly vegetarians while histidine, glycine, glutamic acid and hydroxy-proline were not detected in elderly non-vegetarians. Isoleucine, glycine and hydroxy-proline were detected in young non-vegetarians but not in young vegetarians. The levels of amino acids showed no significant statistical differences between young vegetarians and non-vegetarians as well as between elderly vegetarians and non-vegetarians. Phenylalanine, threonine and trypthophan were commonly detected in the lacto-ovo and lacto vegetarians, while valine, cysteine, arginine and asparagine were commonly detected in vegans. In conclusion, except for isoleucine, general differences were seen in urinary amino acid excretions between vegetarians and non-vegetarians even though the differences were statistically not significant. Therefore lacto-ovo diets could be nutritionally adequate as the nutrients were substituted by dairy or plant products.

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INTRODUCTION

Quantification of total and individual amino acids in biological fluids such as plasma, urine and cerebrospinal fluid is an important diagnostic tool in laboratory medicine (Babu *et al.*, 2002). The total amino acid contents and amino acid profiles in plasma and urine also reflect the nutritional / metabolic status of an individual (Babu *et al.*, 2002).

Amino acids are the primary components of proteins and they are essential to life (Elliot and Elliot, 2001). Humans ingest far more protein (amino acids) than they need for replacement of endogenous proteins. These excess amino acids cannot be stored and thus are catabolised. The ultimate fate of the carbon skeletons derived from these amino acids is converted to carbon dioxide (CO_2), water (H_2O) and energy (ATP) via the citric acid cycle and the respiratory chain (Nelson and Cox, 2000).

The kidney plays a major role in amino acid metabolism and nutrition. Amino acid reabsorption at the renal proximal tubules is mediated by the specialised amino acid transport systems. Amino acids are filtered and reabsorbed in the proximal tubule. Active transport is the key to the reabsorptive processes in the proximal tubule. Glucose and amino acids are reabsorbed across the luminal face of the proximal tubule by sodium-coupled secondary active transport (Pesce *et al.*, 1980).

The eating patterns of vegetarians may vary considerably. The lacto-ovo vegetarian diet is based on grains, vegetables, fruits, legumes, seeds, nuts, dairy products and eggs but excludes meat, fish, and fowl. The lacto-vegetarian diet excludes eggs as well as meat, fish, and fowl. The vegan, or total vegetarian, eating pattern is similar to the lacto-vegetarian pattern, as it is the complete exclusion of animal products (Griffith and Omar, 2003). Even within these patterns, considerable variations may exist to the extent in which animal products are avoided (Griffith and Omar, 2003).

As not much information could be found in the scientific literature on the urinary amino acids excretion in vegetarians and non-vegetarians, this study was undertaken to determine the urinary amino acid profiles in individuals on a vegetarian or normal diet. Vegetarians in this study included lacto-ovo, lacto-vegetarians and vegans.

MATERIALS AND METHODS

Sampling

The total number of subjects studied was 162 with 102 young volunteers and 60 elderly volunteers. In the present study, young individuals were between 15 - 45 years old while elderly individuals were between 46 - 70 years old. There were 62 young individuals and 24 elderly individuals who were on a normal diet. In the vegetarian group, there were 40 young individuals and 36 elderly individuals (Figure 1). In the present study, there were 33 lacto-ovo volunteers, 19 lacto volunteers and 24 vegan volunteers. There were 46 female volunteers and 40 male volunteers on a normal diet, while there were 34 female volunteers and 42 male volunteers on a vegetarian diet.

24-hour urine samples were collected in clean plastic bottles with toluene as a preservative (Varley, Gowenlock and Bell, 1998). All participants were in a good nutritional state, consuming their usual food; no special precautions were taken regarding drug therapy during the time of sample collections. Collected urine samples were frozen at -20 °C until assayed, usually within 2 weeks.

The volunteers were requested to complete a self-administered food frequency questionnaire. Frequencies of consumption of food items (number of

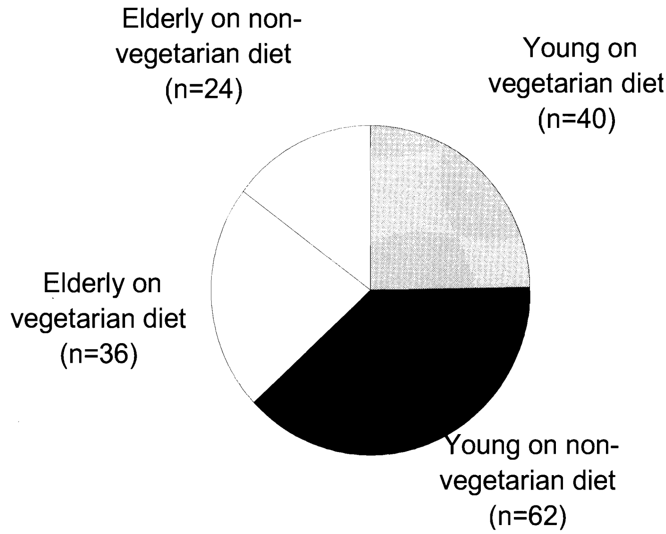

Figure 1. Distribution of different group of volunteers in present study

servings) were reported on an increasing, four-level scale, including never, once a day, 1-3 times a day and 4 or more times a day. The respondents indicated their average portions of food sizes (i.e., small, medium and large) (Lindmark *et al.*, 2005) based on the described standard food portion in Table 1. However no quantification of food intake was recorded.

High performance liquid chromatography procedure

In this study, a reversed-phase HPLC (RP-HPLC; Perkin Elmer) was used as it offered high sensitivity, versatility and speed of analysis (Griffith and Omar, 2003). Standard amino acids (2.5 μmoles/ml) were dissolved in 0.1N HCl. 0.4mM methionine sulfone was prepared as an internal standard. 1.4 ml of urine sample was filtered using an Acrodisc CR PTFE syringe filter (0.45 μm; 13mm) and a 1ml latex free syringe into a test tube. No filtration was done for the standard. 400 μl of Milli-Q water and 700 μl of internal standard were added to the sample preparation. The solution was mixed by inversion. 25 μl of the solution was vacuum-dried using the ISS110 SpeedVac® System.

10 μl of the re-drying solution (methanol: 1M sodium acetate: triethylamine; 2:2:1) was added, vortexed and vacuum-dried. 20 μl of derivatising solution (methanol: milli-Q water: triethylamine: phenylisothiocyanate; 7:1:1:1) was added and it was allowed to stand at room temperature for 10 minutes before being vacuum-dried. 50μl of Pico-Tag diluent (acetonitrile: sodium acetate trihydrate: milli-Q water; 2:1:97) was added before the standard/sample was analysed. The solution was mixed to ensure that all substances were dissolved completely.

The HPLC system was set up according to the manual (Pico-Tag method; Waters). The method was chosen according to the conditions tested. A standard

Table 1. Standard food portion list used in this study

Food Group/ Number of Servings in a Day	*Food Item*	*Food portion size (S, M and L) / Standard portion description*
Eggs (2 servings)	-	(M) / 2 eggs (USDA, 2002)
Meats / Poultry (2 servings)	Chicken Pork Beef	(M) / ~3 ounces (USDA, 2002) [3 ounces is estimated to be about the size and thickness of a deck of playing cards (Allina Hospitals & Clinics, 2005)]
Seafood (2 servings)	Prawn Crab Fish Squid Shell seafood (clam, etc)	(M) / ~3 ounces (USDA, 2002) [3 ounces is estimated to be about the size and thickness of a deck of playing cards (Allina Hospitals & Clinics, 2005)]
Vegetables (4 servings)	Leaf vegetable (spinach, etc)	(M) / 4 cups (USDA, 2002)
	Fungi/ Seaweed Root vegetable (carrot, etc) Onion-family vegetables Tubers (potato, etc)	(M) / ~ 2 cups (USDA, 2002)[1 cup is estimated to be about the size of a fist (Allina Hospitals & Clinics, 2005)]
	Juices	(M) / ~ 3 cups (USDA, 2002)
Beans (2 servings)	Soy beans (tofu, etc) Sprouts Lentils	(M) / ~ 1 cup (USDA, 2002)
	Nuts	(M) / ~ 2/3 cup (USDA, 2002)
	Others (peanut butter)	(M) / 2 tablespoons (USDA, 2002) [1 tablespoon is estimated to be about the size of the tip of a thumb (Allina Hospitals & Clinics, 2005)]
Fruits(3 servings)	-	(M) / 1 medium size (USDA, 2002) [1 medium size is estimated to be about the size of a tennis ball (Allina Hospitals & Clinics, 2005)]
	Juices	(M) / ~ 3 cups (USDA, 2002)
Rice/ Noodles (6 - 9 servings)	-	(M) / ~ 3 cups (USDA, 2002)
Grains (6 - 9 servings)	-	(M) / ~ 6 cups (USDA, 2002)
Dairy Products (2 - 3 servings)	Milk/ Cheese/ Yogurt	(M) / ~ 2 cups of milk/yogurt or 1 ½ ounces of cheese (USDA, 2002) [1 ounce is estimated to be about 4 stacked of dice (Allina Hospitals & Clinics, 2005)]
Pastry/ Bread (6 - 9 servings)	-	(M) / ~ 6 slices (USDA, 2002)
Snacks	Chocolate/ Chips	-
Soft Drinks / Alcoholic Drinks	-	-
Supplements		-

(S) = small - half the standard portion, (M) = medium - standard portion, (L) = large - double the standard portion (Shimizu *et al.*, 1999) gradient was created (Table 2) and was programmed into the software system (Total Chromatogram Navigator Software-Perkin Elmer). An absorbance detector was set at 254 nm. After the method was set up, the pump was turned on. The detector had to be auto-zeroed before every run was carried out. 35 μl of standard/sample were injected and the run was started. The phenylthiocarbamyl amino acid derivatives were separated on the C18 Pico-Tag physiological free amino acid column [RP-18 endcapped- 5μm] using a stated binary gradient at a flow rate of 1.0 mL/min.

Amino acid derivatives were identified by their relative retention times based on the reference peaks for retention time of

Table 2. Standard gradient for amino acids in urine

Time	*Flow*	*%A*	*%B*	*Curve Number*
0.5	1.0	100	0	0
2.5	1.0	100	0	0
63	1.0	0	100	1

Table 3. Essential and non-essential amino acids in individuals on vegetarian and non-vegetarian diets

	Mean ± S.D. (mg/24hrs)	
	Vegetarian (n = 76)	*Non-vegetarian (n = 86)*
histidine	4.44 ± 2.4	5.91 ± 2.3
isoleucine	-	0.94 ± 0.1
leucine	-	-
lysine	2.51 ± 2.7	0.81 ± 1.4
methionine	0.93 ± 1.9	0.94 ± 1.2
phenylalanine	1.92 ± 2.4	1.68 ± 1.8
threonine	1.94 ± 2.4	0.42 ± 0.5
trypthophan	1.46 ± 2.1	0.90 ± 1.2
valine	1.88 ± 1.3	0.62 ± 1.0
cysteine	35.32 ± 24.0	22.3 ± 15.0
proline	0.99 ± 1.2	1.19 ± 1.1
glycine	2.57 ± 2.4	0.12 ± 0.0
arginine	28.87 ± 19.9	38.41 ± 20.8
asparagine	5.13 ± 6.8	2.11 ± 4.4
serine	67.65 ± 0.0	37.20 ± 14.6
glutamine	39.85 ± 31.6	18.788 ± 9.2
cystine	34.79 ± 27.2	17.02 ± 8.9
glutamic acid	2.20 ± 2.6	1.10 ± 1.5
tyrosine	1.39 ± 0.9	0.25 ± 0.1
hydroxy-proline	1.79 ± 3.4	6.46 ± 5.8
alanine	-	-
aspartic acid	-	-

(-) = not detectable

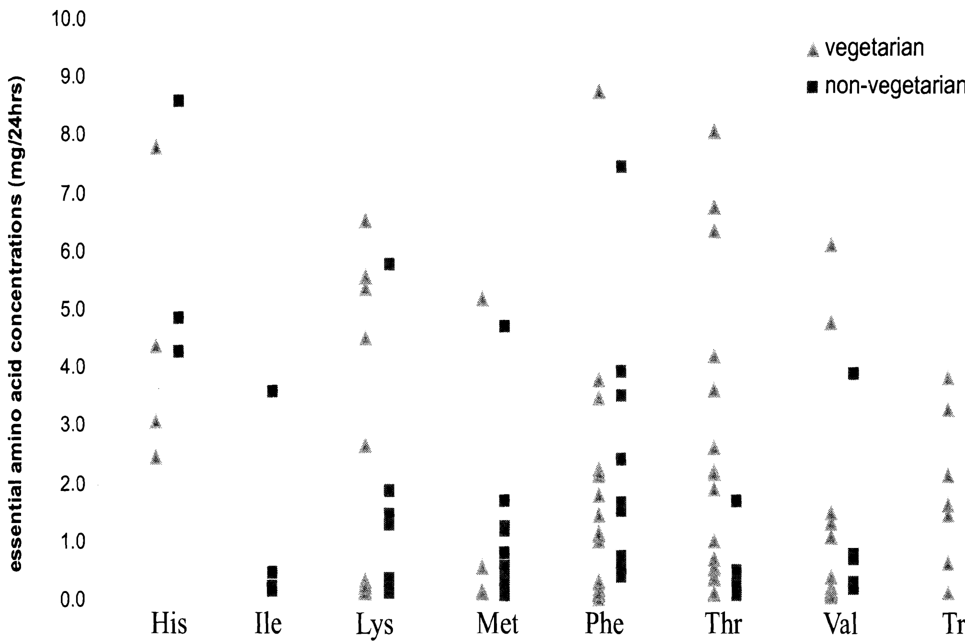

Figure 2. Distribution of essential amino acids in urine of vegetarian and non-vegetarian individuals.
His = Histidine; Ile = Isoleucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Trp = Trypthophan; Val = Valine

the internal standard (methionine sulfone, which was added as an internal standard solution). The ratio of the peak area of each amino acid to the internal standard (methionine sulfone) was used to calculate the amino acid concentrations and the final results were expressed as mg/24hours of amino acids in urine.

Statistical Analysis

The statistical analysis between the groups was determined by student's t-test and chi-square. A p-value ≤ 0.05 was considered significant.

RESULTS

In this study, urinary amino acids of two groups on different diets, namely vegetarian and non-vegetarian, were determined. Distribution patterns of essential and non-essential amino acids in the urine of vegetarian and non-vegetarian individuals are shown in Figures 2 and 3. As can be seen, leucine, aspartic acid and alanine were not detected in both groups while isoleucine was not detected in the vegetarian group.

As shown in Table 3, individuals on vegetarian diets had a higher percentage of frequency in the excretion of phenylalanine (68% vs. 35%), threonine (61% vs. 44%), cysteine (69% vs. 50%), glutamic acid (21% vs. 16%), tyrosine (13% vs. 9%) and hydroxy-proline (16% vs. 5%) while those on non-vegetarian diets had a higher percentage of frequency in the excretion of lysine (31% vs. 26%), methionine (34% vs. 19%), proline (31% vs. 21%), arginine (68%

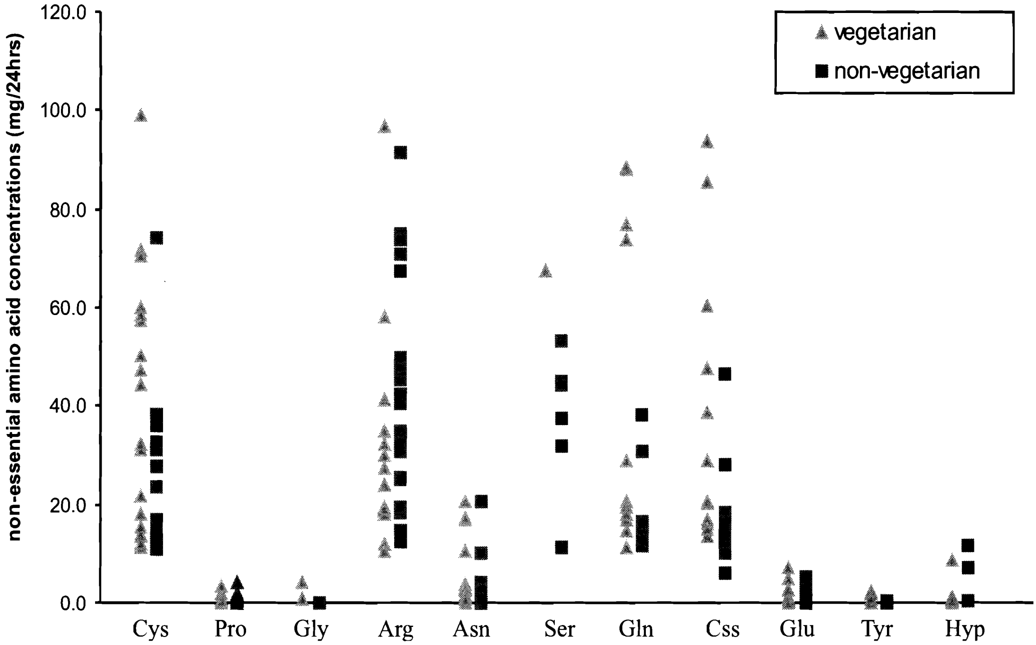

Figure 3. Distribution of non-essential amino acids in urine of vegetarian and non-vegetarian individuals.
Cys = Csyteine; Pro = Proline; Gly = Glycine; Arg = Arginine; Asn = Asparagine; Ser = Serine; Gln = Glutamine; Css = Cystine; Glu = Glutamic acid; Tyr = Tyrosine; Hyp = Hydroxy-proline

vs. 51%), asparagines (54% vs. 43%), serine (12% vs. 3%), and cystine (40% vs. 37%). However, no differences were observed in all amino acids between vegetarians and non-vegetarians ($p>0.05$).

Among the vegetarians, histidine, glycine and tyrosine were only found in lacto-ovo and vegans while serine was only detected in lacto individuals and not in the lacto-ovo and the vegans. The other amino acids were commonly detected in lacto-ovo, lacto and vegan individuals.

The concentrations of the majority of essential amino acids in both groups were between 0.10 and 2.00 mg/24hrs, except histidine which had a range of 4.1 - 5.0 mg/24hrs. The concentrations of non-essential amino acids in both groups varied. Proline, glycine and tyrosine ranged between 0.10 and 1.00 mg/24hrs, while cysteine, glutamine, glutamic acid and cystine ranged between 11.0 and 21.0 mg/24hrs. Asparagine and hydroxy-proline had a range of 0.10 - 5.00 mg/24hrs, while serine and arginine ranged between 31.0 and 50.0 mg/24hrs. Cysteine, glutamine and hydroxy-proline were found to have differences between the two groups, where cysteine and glutamine had a higher concentration range in vegetarians while hydroxy-proline had a higher concentration range in non-vegetarians (p>0.05).

Young individuals on non-vegetarian diets excreted isoleucine, glycine and hydroxy-proline in their urine, which were not found in the young vegetarians (Table 4). Isoleucine and serine were found in the urine of elderly non-vegetarian individuals but not in elderly vegetarian individuals. However histidine, glycine, glutamic acid and hydroxy-proline were not found in the urine of elderly non-vegetarians but were found in elderly vegetarians.

A comparison of mean and standard deviation computed from individuals on vegetarian and non-vegetarian diets, and percentage frequency (presence of amino acid in a group/ total of samples in a group) of both essential and non-essential amino acids in individuals on vegetarian and non-vegetarian diets are seen in Tables 3, 4 and 5 respectively. Concentrations of lysine, phenylalanine, threonine, trypthophan, valine, cysteine, glycine, asparagine, serine, glutamine, cystine, glutamic acid and tyrosine were seen to be higher among vegetarian individuals while histidine, methionine,

Table 4. Essential and non-essential amino acids in young and elderly individuals on vegetarian and non-vegetarian diets.

	Mean ± S.D.			
	Young non-vegetarian (n = 62)	*Young vegetarian (n = 40)*	*Elderly non-vegetarian (n = 24)*	*Elderly vegetarian (n = 36)*
histidine	5.91 ± 2.3	4.89 ± 2.7	-	3.1 ± 0.0
isoleucine	0.28 ± 0.2	-	1.92 ± 2.4	-
leucine	-	-	-	-
lysine	0.43 ± 0.5	2.37 ± 3.0	3.84 ± 2.7	3.93 ± 2.2
methionine	0.62 ± 0.4	0.17 ± 0.0	1.5 ± 1.9	1.52 ± 2.1
phenylalanine	1.74 ± 1.2	2.02 ± 2.4	3.94 ± 5.0	1.92 ± 2.6
threonine	0.37 ± 0.5	2.18 ± 2.9	0.95 ± 0.5	2.08 ± 2.0
trypthophan	0.55 ± 0.3	1.35 ± 2.4	1.48 ± 2.1	1.58 ± 1.7
valine	0.75 ± 1.1	3.27 ± 0.0	0.11 ± 0.0	1.65 ± 1.2
cysteine	21.37 ± 10.1	39.2 ± 21.0	32.53 ± 24.8	37.49 ± 28.8
proline	1.52 ± 1.5	0.31 ± 0.0	1.34 ± 0.7	1.88 ± 1.7
glycine	0.12 ± 0.0	-	-	2.57 ± 2.4
arginine	42.85 ± 24.3	36.4 ± 34.8	40.17 ± 15.7	29.26 ± 13.2
asparagine	2.08 ± 3.35	4.49 ± 8.9	7.38 ± 9.0	4.61 ± 5.6
serine	34.0 ± 13.8	67.65 ± 0.0	53.2 ± 0.0	-
glutamine	12.85 ± 1.7	34.4 ± 28.3	22.46 ± 11.3	50.9 ± 36.6
cystine	17.45 ± 10.6	53.69 ± 35.8	17.61 ± 11.1	37.56 ± 27.3
glutamic acid	1.10 ± 1.5	2.87 ± 2.7	-	0.18 ± 0.1
tyrosine	0.21 ± 0.1	1.0 ± 1.2	0.39 ± 0.0	1.65 ± 0.9
hydroxy-proline	4.02 ± 5.3	-	-	2.51 ± 4.1
alanine	-	-	-	-
aspartic acid	-	-	-	-

(-) = not detectable

Table 5. Percentage detection frequency of both essential and non-essential amino acids in individuals on vegetarian and non-vegetarian diets.

	Detection frequency (%)		
	Vegetarian (n = 76)	*Non-vegetarian (n = 86)*	*p-value*
histidine	10	5	0.878
isoleucine	-	13	0.298
leucine	-	-	-
lysine	26	31	0.995
methionine	19	34	0.903
phenylalanine	68	35	0.631
threonine	61	44	0.988
trypthophan	29	21	0.983
valine	19	11	0.922
cysteine	69	50	0.987
proline	21	31	0.981
glycine	8	2	0.668
arginine	51	68	0.987
asparagine	43	54	0.994
serine	3	12	0.613
glutamine	34	33	0.998
cystine	37	40	0.998
glutamic acid	21	16	0.987
tyrosine	13	9	0.966
hydroxy-proline	16	5	0.653
alanine	-	-	-
aspartic acid	-	-	-

(-) = not detectable

$$\text{Detection frequency} = \frac{\text{Presences of amino acid in a group}}{\text{Total samples in a group}}$$

proline, arginine and hydroxy-proline were higher among non-vegetarian individuals. No statistically significant differences were observed in any of the levels of amino acids between vegetarians and non-vegetarians (p>0.05).

DISCUSSION

Different foods contain different proteins, each with their own unique amino acid composition (Nelson and Cox, 2000). The proportions of essential amino acids in foods differ from the proportions needed by the body to synthesise proteins. Most foods contain at least some protein (Nelson and Cox, 2000). Therefore, the amount of amino acids excreted in the urine will be dependent on the food consumed and its requirement by the individual.

The advantages of urine analysis are that they reveal more distinctive patterns related to problems in enzymatic activity, nutrient cofactor adequacy and transport. In fact, urine is also not subjected to the circadian rhythm variation in amino acids that are present in blood, and excesses or deficiencies over a period of time can be more easily assessed (Carroll and Temte, 2000).

Amino acid analysis is an extremely difficult task as there are some 40 to 50 amino acids or amino acid conjugates in biological fluids, with more than a 1000-fold range of concentrations (Walker and Mills, 1995). However, there are only 20 amino acids which are necessary for proper human growth and functions (Elliot and Elliot, 2001; Nelson and Cox, 2000). In this study, 22 amino acids were analysed, as cystine and hydroxy-proline were added in the detection.

A fresh random urine sample is adequate for amino acid analysis. The excretion rates of amino acids may vary independently over 24 hours. Therefore, a 24 hour collection is necessary if accurate quantitative data are required (Walker and Mills, 1995).

Very little work has been done on the amino acid profiles in the urine of vegetarian and non-vegetarian individuals. The range of distribution in essential and non-essential amino acids concentrations in this present study varied among vegetarians and non-vegetarians.

The majority of the individuals on vegetarian and non-vegetarian diets who excreted phenylalanine were found to have a dietary intake of eggs and dairy products. The detection frequency of methionine was higher in individuals on non-vegetarian diets. Methionine is normally activated and converted to homocysteine (Gomber, Dewan and Dua, 2004). Homocysteine is then further converted to either cysteine or methionine. Normally homocysteine is an intracellular intermediate and is not detectable in plasma or urine (Gomber, Dewan and Dua, 2004).

Tryptophan was significantly higher in vegetarians compared to non-vegetarians ($p<0.05$). This could be due to the consumption of different diets where vegetarians in this study consumed more soybeans than non-vegetarians (Dwyer, 1999).

Isoleucine was detected in the urine of non-vegetarians, but not in the urine of any vegetarians. This could be due to the consumption of seafoods by the non-vegetarians as seen from their diet, since it contains a high concentration of valine and isoleucine (Dwyer, 1999). No leucine, alanine and aspartic acid were detected in both groups. These amino acids are found moderately in poultry and in low levels in soy products (USDA, 2005). Therefore, it could be due to low consumption (small serving size) of poultry among the non-vegetarians while among vegetarians it could be due to the low content of these amino acids in the food itself.

Neither cysteine nor cystine is essential in the diet of humans; cystine and cysteine are interconvertible and cysteine is synthesised in the body from serine and methionine (Nelson and Cox, 2000). The vegetarians had higher mean values probably due to the consumption of more cysteine-rich foods such as soy beans, legumes, wheat germ, broccoli, brussel sprouts and oats than the non-vegetarians (Dwyer, 1999).

Proline and hydroxy-proline are amino acids found in collagen in tendons and ligaments (Nelson and Cox, 2000). Therefore, non-vegetarian individuals were found to have a higher mean value compared to the vegetarians. This could be due to the consumption of animal products like poultry and meats by the non-vegetarians (Dwyer, 1999).

Individuals who consumed dairy products in the vegetarian group (lacto-ovo and lacto) excreted more phenylalanine, cystine, cysteine and glycine than vegans. This is because phenylalanine can be found in milk, whereas cystine, cysteine and glycine are found in cheese, yogurt and also in milk (Dwyer, 1999; Davis *et al.*, 1994).

As for lacto-ovo vegetarians who consumed eggs, they excreted higher amounts of glutamine, cystine, histidine and phenylalanine. This could be due to the consumption of eggs as it is the best sources of cystine and histidine (Dwyer, 1999; Davis *et al.*, 1994). On the other hand, vegans were found to excrete a higher amount of arginine and asparagine probably due to the fruits and vegetables they consumed, as these contain high concentrations of arginine and asparagine eg. broccoli, oranges and apples (Harris, 2001).

In conclusion, concentrations and patterns of amino acids excreted in the urine by non-vegetarians and vegetarians were similar except for isoleucine, which was not found in vegetarians but in non-vegetarians. No significant correlations were observed between urinary amino acids in both groups. However, trends of differences in the patterns of amino acid excretion between vegetarians and non-vegetarians were seen. Concentrations of lysine, phenylalanine, threonine, trypthophan, valine, cysteine, glycine, asparagine, serine, glutamine, cystine, glutamic acid and tyrosine were seen to be higher among vegetarian individuals while histidine, methionine, proline, arginine and hydroxy-proline were higher among non-vegetarian individuals.

Thus even when nutrient bioavailability is compromised (meatless diets), lacto-ovo diets can be nutritionally adequate as they are substituting meat products with meat alternatives, plant or dairy products in their diets (Venti and Johnston, 2002).

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