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Normal Levels of Plasma Amino Acids among Sudanese Adults: The Effect of Gender

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For an accurate interpretation of amino acids results, well-documented amino acids reference values are required. To our knowledge, no reference values for plasma free amino acids have been published for Sudanese or neighbouring African countries. In this study, we intended to set reference values for fasting levels of plasma free amino acids among Sudanese males and females. Results obtained showed that out of the 22 studied amino acids, the males showed significantly higher mean values in 14 amino acids compared to the females including all Large Neutral Amino Acids (LNAA). However, only lysine and ornithine were significantly higher among the females compared to the males. Of notice, our results showed significantly higher level of tryptophan among the males, not only compared to the female group in this study but even to that reported among Western populations. In conclusion, our study confirmed that gender clearly affects plasma free amino acids levels among Sudanese. This should be taken into consideration when establishing base line values or interpreting results of amino acids profile.

Key words: Amino acids, Sudanese adults, amino acid analyser, reference values, gender

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INTRODUCTION

Fasting amino acids profile is required for the diagnosis and management of metabolic disorders which result from in-born errors of amino acids metabolism. It may also be useful in assessing nutritional status. It is well documented that lower ratio of essential to non-essential amino acids is associated with protein-calorie under nutrition. In addition, changes in the level of some amino acids may be considered as risk factors for developing certain diseases. Reduced arginine to citrulline and ornithine ratio, known as global arginine bioavailability ratio, is emerging as an important risk factor for cardiovascular diseases (Tang *et al*., 2009). Free plasma amino acids concentrations are also altered in liver and kidney diseases (Fernstrom *et al*., 1979; DeFronzo and Felig, 1980), diabetes (Halliday *et al*., 1979) and in obesity (Caballero *et al*., 1988).

For an accurate interpretation of amino acids results, well-documented amino acids reference values are required. Until now, no reference values for plasma free amino acids have been published for Sudanese. Thus, we do not know whether amino acids values from Western populations are applicable to our setting. Gender, age, food habits and ethnicity are among the factors that may influence the level of free plasma amino acids among normal individuals. Gender related differences in the level of plasma amino acids have been reported. Females, ranging from 21-31 years of age were reported to have lower Large Neutral Amino Acids (LNAA) levels compared to age matched males (Caballero *et al*., 1991). However, these differences were not observed among elderly subjects. Armstrong and Stave (1973) also found significant differences between males and females in their study on normal values of plasma free amino acids in children and adults. In their study, some amino acids such as isoleucine, leucine, methionine and tyrosine were clearly influenced by both gender and age. On the other hand, no significant difference between males and females was reported in a study of plasma free amino acids among Taiwan Chinese population (Chih-Kuang *et al*., 2002).

The present study sets reference values of amino acids and describes gender related differences in plasma amino acids concentrations observed in healthy fasting Sudanese subjects.

MATERIALS AND METHODS

A total of 169 healthy adults were included in this study. The study population was divided into males (n = 98) aged 20-48 year and females (n = 71) aged 21-47 year. The body mass index ranged from 18-30 among males and from 19-31 among females. None of the enrolled subjects was receiving any medication at the time of the study. Exclusion criteria included a history of kidney, liver or metabolic disease.

Venous blood samples were obtained in heparinised tubes after an overnight fast from each participant after signing a consent form. Plasma was separated within half an hour after collection by centrifugation at 3000 rpm for 5 min. Protein was precipitated by 20% sulfosalicylic acid, centrifuged at 4°C for 15 min at 12000 rpm and the clear supernatant was kept at -80°C until analysis. Plasma free amino acids were determined by automated ion-exchange chromatography with ninhydrin, using an amino acid analyser (Sykam S 334, Munich, Germany) following standard procedures. An amino acid standard solution was included in each run together with an internal control.

Statistical analysis: The independent sample t-test was used to compare the means of amino acids between male and female groups. Differences were considered significant with a 5% significance level ($p<0.05$). Normality, of distribution was determined by calculating the skewness and kurtosis for each amino acid. Distribution was considered normal with skewness and kurtosis range of -1 to +1. Statistical analysis was performed with the SPSS 15.0 for Windows software (SPSS Inc. Chicago, IL, USA).

RESULTS

A total of 22 plasma free amino acids were estimated among normal adult Sudanese males and females, values (range and Mean±SEM) are shown in Table 1. Normality of distribution as determined by Skewness (s) and Kurtosis (k) was calculated for each amino acid in both groups. All amino acids among females had normal distribution except for glutamic acid (s = 1.506, k = 2.124), methionine (s = 1.47, k = 1.036), phenylalanine (s = 1.441, k = 5.022), glycine (s = 1.01, k = 2.83) and homocystine (s = 1.638, k = 1.896). For the male group, all values were normally distributed except for cysteine (s = 1.032, k = 3.240) and homocystine (s = 2.508, k = 6.329). Males showed significantly higher levels in 12 of the 22 studied amino acids whereas only lysine and ornithine were significantly higher among females compared to the male group. The essential amino acids which showed higher levels among males included leucine, isoleucine, histidine, methionine, phenyalanine, tryptophan and valine. Arginine, glutamic acid, citrulline, proline and tyrosine were the non-essential amino acids that were significantly higher among the males in this study. Lysine was the only essential amino acid whereas ornithine was the only non-essential amino acid which showed significantly higher levels among the female group.

Table 1: Plasma free amino acids among normal Sudanese males and females

	Males (n = 98)			Females (n = 71)			
Amino acid	Range* (μmol L^{-1})	Mean (μmol L^{-1})	SEM	Range* (μmol L^{-1})	Mean (μmol L^{-1})	SEM	p-value
Taurine	34-88	56.5	1.4	30-82	52.3	1.7	0.10
Threonine	89-199	134.5	3.0	98-201	142.9	3.5	0.06
Serine	71-168	107.3	2.5	70-183	111.5	3.5	0.33
Glutamic acid	14-79	37.1	1.9	13-64	29.1	2.3	0.02
Glycine	126-361	221.3	6.3	126-369	226.1	7.6	0.58
Alanine	221-624	389.1	10.9	214-640	371.5	12.4	0.32
Citrulline	9-42	25.9	0.9	10-31	22.1	0.7	0.02
α-Aminobutyric	2-23	9.5	0.6	3-21	9.1	0.6	0.85
Valine	122-295	201.1	4.5	119-230	165.9	3.8	0.0001
Cystine	25-74	46.7	1.1	25-69	46.0	1.3	0.71
Methionine	15-55	30.4	1.0	17-50	25.7	1.3	0.004
Isoleucine	31-95	65.7	1.5	31-78	50.4	1.4	0.0001
Leucine	68-176	118.2	2.6	63-135	92.0	2.0	0.0001
Tyrosine	32-90	60.5	1.6	30-91	54.8	1.8	0.019
Phenylalanine	33-74	52.9	1.1	21-84	45.2	1.6	0.0001
Homocystine	0.6-48	7.8	1.1	1-39	10.8	1.5	0.17
Ornithine	4-90	34.0	2.7	5-78	44.0	2.6	0.05
Lysine	35-201	91.5	5.1	32-188	115.4	5.0	0.006
Histidine	56-207	128.2	4.2	57-178	101.6	4.0	0.0001
Tryptophan	34-228	86.6	4.4	27-137	62.3	4.2	0.001
Arginine	40-140	85.1	2.4	47-121	75.7	2.3	0.005
Proline	112-375	225.5	6.7	120-309	202.8	6.3	0.02

*Reference ranges were defined as the 2.5 to 97.5th centile of the distribution

DISCUSSION

Setting reference values for free amino acids is a prerequisite for proper interpretation of results obtained from amino acids analysis. Gender, age, food habits and ethnicity are among the factors that may influence the levels of amino acids. Hence, reference values may differ between different populations.

In the present study we intend to establish reference values for amino acids among healthy adult Sudanese from both sexes. To our knowledge, no such study has been conducted in Sudan or in neighbouring countries in Africa.

This study population were all well-nourished and there was no significant variation in food habits. Of notice, out of the 22 studied amino acids, males showed significantly higher levels in 12 amino acids including all LNAA. These results are in agreement with previous reports from Western population (Caballero *et al*., 1991; Armstrong and Stave, 1973; Gregory *et al*., 1986). Several factors may play a role in determining the lower plasma LNAA concentrations in females elative to males. One possible factor, as suggested by Caballero *et al*. (1991) could be differences in insulin sensitivity as young females have higher glucose-mediated insulin output and lower glucose disposal rate per unit of plasma insulin than do men during clamp studies (Hale *et al*., 1985). Such increased insulin output could tend to lower the plasma concentrations of the branched-chain amino acids (valine, leucine and isoleucine), being the most-insulin-responsive amino acids.

Studies which included children and elderly groups observed that these gender related differences in amino acids appear to develop after adolescence, because studies in preadolescent children found no major differences in plasma amino acid concentrations between males and females (Armstrong and Stave, 1973; Gregory *et al*., 1986). Interestingly, such sex related differences disappeared in the elderly subjects due to the marked rise in the plasma amino acids concentrations in elderly females whereas no significant changes were observed in elderly males (Caballero *et al*., 1991; Galante *et al*., 1978). This has been attributed to age-related decrease in insulin responsiveness (Marchesini *et al*., 1987) particularly among females.

The marked differences in amino acids levels between males and females could be also attributed, at least in part, to the monthly loss due to menstruation as the females in our study were all within the reproductive age group. Lysine is the only essential amino acid which was significantly higher among the female group and threonine does not seem to be affected by gender.

Of notice, our results showed significantly higher level of tryptophan among the males, not only compared to the female group in our study but even to that reported among other populations. Although the means of most of the amino acids in our study were either below or comparable to the means reported for Western population (Rudman *et al*., 1989) (Table 2), the tryptophan level among our male study group appear to be even higher than that reported among other populations. Tryptophan is the precursor of the neurotransmitter 5-hydroxy

Table 2: Plasma amino acids among Sudanese and Western males

Amino acid	Sudanese males (Mean±SEM)	Western males (Mean±SEM)
Tryptophan	86.6±4.40	54.9±2.4
Isoleucine	65.7±1.50	83.8±4.0
Leucine	118.2±2.60	158.9±5.5
Histidine	128.2±4.20	96.8±2.6
Methionine	30.4±1.00	34.7±1.4
Lysine	91.5±5.10	172.9±7.1
Phenylalanine	52.9±1.10	67.9±2. 1
Threonine	134.5±3.00	145±5.9
Valine	201.1±4.50	262±8.9
Alanine	389.1±10.9	372±16.2
Arginine	85.1±2.40	70.5±5.2
Cystine	46.7±1.10	-
Glutamic acid	37.1±1.90	-
Glycine	221.3±6.30	247±8.5
Proline	225.5±6.70	-
Serine	107.3±2.50	103±4.6
Tyrosine	60.5±1.60	77.5±3.4
Ornithine	34.0±2.70	68.3±5.0
Homocystin	7.8±1.10	-
α-Amino-bytric acid	9.5±0.60	-
Citrulline	25.9±0.90	-
Taurine	56.5±1.40	48.9±4.2

Amino acids not measured in the Western study are indicated by the symbol

tryptamine, also known as serotonin which plays a pivotal role in the control of arousal, sleepiness and mood (Blomstrand, 2006). It has been reported that the mean rate of synthesis of serotonin in normal males was 52% higher than in normal females (Nishizawa *et al*., 1997). This could explain the gender related difference in tryptophan observed in our study.

CONCLUSION

Present study confirmed that gender clearly affects plasma free amino acids levels among Sudanese. This should be taken into consideration when establishing base line values or interpreting results of amino acids profile. Neglecting this factor may lead to serious misinterpretation of amino acids analysis results.

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