Histidine and arginine are associated with inflammation and oxidative stress in obese women

Yu-Cun Niu[1]†, Ren-Nan Feng[1]†, Yan Hou[2], Kang Li[2], Zhen Kang[3], Jian Wang[3], Chang-Hao Sun[1]* and Ying Li[1]*

[1]*Department of Nutrition and Food Hygiene, School of Public Health, Harbin Medical University, Harbin, People's Republic of China*
[2]*Department of Biostatistics, School of Public Health, Harbin Medical University, Harbin, People's Republic of China*
[3]*Harbin Center for Disease Control and Prevention, Harbin, People's Republic of China*

Abstract

The aims of the present study were to examine the serum amino acid profiles in obese and non-obese women and investigate the relationships between the serum amino acids and inflammation and oxidative stress in a human case–control study. Serum amino acids, inflammatory biomarkers (C-reactive protein and IL-6) and oxidative biomarkers (superoxide dismutase, malondialdehyde and glutathione peroxidase) were measured and compared in 235 obese women and 217 non-obese controls. The relationships between serum amino acids and inflammatory and oxidative biomarkers were examined using multiple linear regression. Among the amino acids determined, serum histidine, arginine, threonine, glycine, lysine and serine were found to be significantly lower in obese women as compared to non-obese controls ($P < 0.001$). The difference was the greatest for histidine ($P < 0.001$). In obese women, both histidine and arginine were negatively associated with inflammation and oxidative stress. In non-obese controls, histidine was negatively associated with oxidative stress. The findings in this study indicate that the metabolism of amino acids is abnormal in obese women in whom histidine and arginine have close relationships with inflammation and oxidative stress.

Key words: Amino acids: Inflammation: Oxidative stress: Obesity

Obesity is a major risk factor for a number of chronic diseases, including type 2 diabetes, hypertension and CVD[1]. Increased levels of inflammatory and oxidative biomarkers are the main features of obesity, which is the pathological and physiological feature of many conditions[2]. Obesity could lead to metabolic disorders of blood glucose and lipids[3,4]. And numerous studies[5–8] have confirmed that the metabolic disorders of blood glucose and lipids are closely related to inflammation and oxidative stress. However, it is still unclear if amino acid metabolism is altered and if there are links between serum amino acids and inflammation and oxidative stress in obese subjects.

As carbohydrate, protein and fat are the main macronutrients, whose metabolisms are closely related with each other, we hypothesised that imbalanced amino acid metabolism might also exist in obese subjects. The aims of this study were to compare the serum amino acid profiles between obese and non-obese women, and evaluate the associations between amino acid metabolism and inflammation and oxidative stress in a human case–control study.

Methods

A total of 235 middle-aged obese women and 217 non-obese controls were recruited into the present study based on the criteria: (1) BMI of 18–24 kg/m² (non-obese control group); BMI $\geq$ 28 kg/m² (obese group)[9]; (2) fasting blood glucose < 6·1 mmol/l and 2-h postload blood glucose concentration < 7·8 mmol/l; (3) BMI fluctuation < 0·5 kg/m² in the past 6 months; (4) no disease that could influence the metabolism of nutrients, such as kidney, liver and gastrointestinal diseases; (5) no history of endocrine metabolic disorders, acute infection, injuries or chronic inflammatory diseases; (6) not being pregnant. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by

the Human Research Ethics Committee of the Harbin Medical University. Written informed consent was obtained from all subjects.

Information on age, weight, height, alcohol use, cigarette smoking, menstrual status, and physical activity at work and at leisure was obtained from questionnaires. Dietary intakes were measured with a validated semi-quantitative FFQ based on frequency[10]. Energy and nutrient intakes were calculated with the Food Nutrition Calculator (V1·60; Chinese CDC, Beijing, China).

Using a standard protocol, trained examiners measured body weight ($\pm 0·1$ kg), height ($\pm 0·1$ cm) and the blood pressure of all participants. BMI was calculated as body weight (kg) divided by the square of height (m). Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after resting for at least 10 min. The mean of the three closest readings was used in the analysis.

Subjects were instructed to fast for 12 h and blood samples were collected in the morning. After the fasting blood sample collection, each subject was given an oral glucose tolerance test and blood samples were collected in 2 h after the 75 g glucose was taken. Antecubital venous blood was collected to obtain serum and plasma, and then stored at $-80°C$.

Samples of serum (100 μl) were spiked with acetonitrile (100 μl), vortex-mixed for 30 s, immediately centrifuged at 11 000 *g* for 10 min at 4°C and the supernatant was recovered. An Alliance 2695 separation module and a 2487 UV detector from Waters were used for the HPLC analysis (CV%, 4·2–5·3%). Derivatisation and gradient elution of serum amino acids were followed according to the manufacturer's instructions (Waters Corporation, Milford, MA, USA).

Fasting blood glucose, 2-h postload blood glucose, total cholesterol, HDL-cholesterol and TAG were determined using commercial kits (Biosino Biotechnology, Beijing, China), standard enzymatic colorimetric techniques and an auto-analyser (MOL-300, Shanghai, China).

C-reactive protein (CRP) and IL-6 were assayed using ELISA with commercial kits (CRP, Biocheck, Inc., Foster City, CA, USA; IL-6, R&D Systems Europe, Abingdon, UK) according to the manufacturers' instructions. Superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase were measured with commercial kits using enzymatic methods (Jiancheng Technology, Nanjing, China).

Statistical analysis

Data were presented as means and standard deviations. Comparisons between obese and non-obese women in the population study were made by using Student's *t* test for continuous variables and χ^2 test for categorical variables. ANCOVA was used to compare serum amino acid levels between obese women and non-obese controls, adjusting for potential covariates (age, smoking, alcohol use, menopause, and physical activity at work and at leisure). Multiple linear regression (adjusted for the aforementioned variables) between serum amino acids and inflammatory and oxidative biomarkers was also calculated in overall, non-obese controls and obese women, respectively. Statistical analyses were

carried out with SAS software (version 9.1; SAS Institute, Inc., Cary, NC, USA).

Results

Clinical parameters

As shown in Table 1, BMI, systolic blood pressure, diastolic blood pressure, 2-h postload blood glucose concentration, total cholesterol, TAG, fat and energy intakes (data not shown), CRP, IL-6 and MDA were significantly higher, while HDL-cholesterol, SOD and glutathione peroxidase of obese women were significantly lower than that of non-obese controls. There were no significant differences in the other variables (smoking, alcohol drinking, menopause and physical activity; data not shown).

Amino acid profile

The serum concentrations of amino acids in obese women and non-obese controls are presented in Table 1. Accounting

Table 1. Clinical parameters and amino acid profiles of obese women and non-obese controls

(Mean values and standard deviations)

	Obese women (*n* 235)		Controls (*n* 217)	
	Mean	SD	Mean	SD
Age (years)	44·13	1·73	43·39	2·57
BMI (kg/m^2)	30·68*	2·79	21·87	1·58
SBP (mmHg)	129·09*	9·23	113·34	12·74
DBP (mmHg)	82·00*	6·98	74·01	8·16
TC (mmol/l)	5·00*	0·51	4·54	0·77
TAG (mmol/l)	1·41*	0·24	1·10	0·32
HDL-C (mmol/l)	1·33*	0·09	1·56	0·23
FBG (mmol/l)	4·54	0·61	4·52	0·63
2 h-PG (mmol/l)	5·76*	1·12	5·47	1·07
CRP (ng/ml)	2·42*	1·46	1·26	0·66
IL-6 (pg/ml)	5·57*	3·93	4·58	2·99
SOD (U/ml)	91·29*	23·49	102·61	25·2
MDA (nmol/ml)	2·96*	0·98	2·51	0·89
GSH-PX (μmol/l)	158·90*	36·43	167·39	33·01
Amino acids (mmol/l)				
His	172·47*†	41·71	202·07	54·34
Arg	98·58*†	21·84	108·62	22·24
Thr	157·69*†	40·07	174·16	39·94
Gly	113·42*†	24·23	124·25	27·69
Lys	69·91*†	18·10	79·91	22·30
Ser	84·70*†	26·63	93·67	24·53
Ile	39·75	11·16	44·32	12·04
Pro	108·42	30·70	103·28	22·30
Glu	89·71	27·56	87·75	21·61
Met	27·85	14·65	30·51	16·63
Leu	85·49	31·89	87·47	28·72
Phe	62·84	23·11	63·47	20·95
Val	110·21	45·65	111·74	50·09

SBP, systolic blood pressure, DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, HDL-cholesterol; FBG, fasting blood glucose; 2 h-PG, 2-h postload blood glucose concentration; CRP, C-reactive protein; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-PX, glutathione peroxidase.
* Mean values were significantly different from those of non-obese controls ($P < 0·001$; Student's *t* test).
† Mean values were significantly different ($P < 0·001$; analysis made by ANCOVA), adjusted for age, smoking, alcohol use, menopause and physical activity at work and at leisure.

glycine and serine are non-essential amino acids. There could be a higher level of catabolism or utilisation for the essential amino acids; and higher level of utilisation or lower for *de novo* synthesis of non-essential amino acids in obese subjects. However, as to why the lower circulating levels of these amino acids were found in obese subjects, was still unclear. Whether there are any functional interactions with these amino acids still needs more investigation. As we all know, amino acids were not only the common nutrients, but also cell signalling molecules, regulators of gene expression and the protein phosphorylation cascade[24]. Our findings suggest that more attention should be paid to the imbalanced amino acid metabolism in obese subjects.

In the present study, we found that obese subjects had the most significant histidine level and that histidine was negatively associated with inflammation and oxidative stress in obese women. In addition, there have been reports indicating that histidine could suppress appetite and influence body weight through its conversion into neuronal histamine in the hypothalamus of female subjects[25,26]. Arginine also has the effects of anti-inflammation, anti-oxidative stress and regulating of the vascular functions. Thus, it is interesting to uncover if obese subjects could benefit from histidine or arginine supplementation in their daily diet.

There are still some limitations in the present study. First, this study only included middle-aged female subjects as they have higher obesity prevalence. The findings from the sample of females alone in this case–control study may not be generalised to the total population. Second, this case–control study may not provide fully convincing evidence of the causal effects of histidine and arginine on chronic inflammation and oxidative stress in obese human subjects; hence, further animal and clinical studies are necessary to clarify these associations.

In conclusion, our study indicated that amino acid metabolic disorder might exist in obese women. Overall, six kinds of serum amino acids were significantly lower in obese women; serum amino acid profiles could discriminate between obese and non-obese women; and histidine and arginine have close relationships with inflammation and oxidative stress in obese women.

Acknowledgements

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