Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp. 1121-1137.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

DISTURBANCES IN PLASMA ALIPHATIC AND AROMATIC AMINO ACIDS METABOLOMIC PROFILES IN EGYPTIAN OBESE WOMEN

Tahia H. Saleem[1], Ragaa H M Salama[1], Ghada A Mohamed[2], Abdelrahman H Abdel Qawy[3*], Eman Radwan[1,4]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt*
[2]*Department of Internal Medicine, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt*
[3]*Department of Biochemistry, Faculty of Pharmacy, Assiut University, Assiut, 71515, Egypt*
[4]*Department of Biochemistry, Sphinx University, New Assiut City, Assiut, Egypt*

Background: *According to the World Health Organization (WHO), Egypt ranks 18th with the highest prevalence of obesity worldwide. The impact of obesity is a result of its comorbidities rather than a direct effect. Therefore, it is of utmost importance to investigate its risk factors to establish its potential preventive strategies for future complications. This study aimed at investigating the disturbances in aliphatic and aromatic amino acids profiles in Egyptian obese women.* ***Methods:*** *The study comprised 85 women that were classified into 5 groups (17 each):1- healthy, 2-overweights, 3-moderate obese, 4-severe obese, 5-morbid obese. Plasma metabolomic profile of the previous amino acids was evaluated using an amino acid analyzer.* ***Results:*** *Compared with controls, obese subgroups had significantly stepwise higher levels in the mean plasma levels of tyrosine, total aromatic amino acids (AAA) and alanine together with significantly lower levels of leucine, isoleucine, total branched chain amino acids (BCAA), BCAA/AAA ratio and glycine.* ***Conclusion:*** *Multivariate regression analysis indicated that the significantly elevated circulating alanine levels were good independent predictors for identifying obese patients at risk of associated metabolic disorders. Moreover, the study suggested that AA metabolomic profile can be used as useful tools for early prediction of overweight women. Amino acids-directed regimens intervention could serve as a specific potential targeting strategy for management of obesity.*

Keywords: *Overweight; Obesity-subgroups; Aliphatic and aromatic AA profile; prediction; chromatography*

INTRODUCTION

Obesity is a multifactorial disease affecting over a third of the world's population[1-4]. In Egypt, the prevalence of obesity has increased in adults to reach about 40% of its population[5].

Obesity causes a huge clinical, humanistic, and economic burden to Egyptians as individuals and as a society[5].

Metabolomics has emerged as a powerful tool to identify novel risk factors for early detection of various health related diseases[6]. The plasma amino acid profile can be a useful indicator in various clinical settings because it changes in response to various metabolic alternation,[7-9] hypertension[10] and diabetes mellitous[11].

The major groups of amino acids metabolites that were reported previously to be dysregulated in obesity are the branched chain amino acids (BCAAs) and aromatic amino acids (AAAs)[12].

In the present study, we aim to examine the alteration of plasma aliphatic and aromatic amino acids profiles in Egyptian obese women

Received in 6/5/2023 & Accepted in 29/7/2023

*Corresponding author: Abdel rahman H Abdel Qawy, E-mail: abdelrahmanhassan18@yahoo.com or abdelrahmanhassan1811@gmail.com

in order to establish potential preventive strategies for obesity and its future complications.

MATERIALS AND METHODS

Patients

The present study is a case control study which is an extension to our previous study[13]**.** It was conducted in both the Biochemistry Department, Faculty of Medicine, Assiut university & Internal Medicine Department, Assiut University Hospital, Assiut, during the period from August 2021 to August 2022.

A total of 85 Egyptian women that were family unrelated and were recruited randomly from the attendants of the out-patients clinic of Internal Medicine Department. The study included 17 age matched, completely healthy control women, with no apparent evidence of any medical disorders. The age of participants ranged between 18-53 years. Their height ranged between 130-185 cm and their weight ranged between 51-135 kg.

The study participants were subdivided into 5 groups according to BMI as follows[14].

- **Group (1):** included 17 healthy controls (normal), with a BMI of 18.5-24.9 kg/m^2.
- **Group (2):** included 17 overweight women, with a BMI of 25-29.9 kg/m^2.
- **Group (3):** included 17 moderate obese women (class I), with a BMI of 30-34.9 kg/m^2.
- **Group (4):** included 17 severe obese women (class II), with a BMI of 35-39.9 kg/m^2.
- **Group (5):** included 17 morbid obese women (class III), with a BMI of ≥40 kg/m^2.

An informed consent was obtained from each patient and control and all study procedures were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (IRB no: 17200758). All participants were subjected to a full history taking; in addition to a detailed clinical and menstrual history. Personal and relevant data were collected by a questionnaire designed to ask questions about risk factors and co-morbidities of obesity.

All participants were subjected to a thorough clinical examination in the form of complete physical examinations of the chest, heart and abdomen, measurement of blood pressure, body weight, height, waist circumference and hip circumference. Routine laboratory investigations were performed as blood picture, liver functions and kidney functions.

Exclusion criteria included patients with co-morbidities (diabetes, hypertension or insulin resistance (IR), any chronic illness (liver, renal, heart, gastrointestinal, endocrine disorders or thyroid disease), coronary artery diseases, cerebral vascular accidents, active smoking, patients who received any previous treatment for about 6 months later, patients with malignancies and hematologic disorders in addition to lactating and pregnant women.

Obesity indices calculations

1. Body mass index (BMI): was calculated by dividing the weight in kg by square the height in m^2.
2. Body fat percentage (BFP) was calculated according to P. Deurenberg et al., and D. Gallagher et al., [15-16] as follows:
3. BFP = (1.2 x BMI) + (0.23 x age in years) – (10.8 x sex) – 5.4, sex is set for zero in women.
4. Fat mass index (FMI): was calculated by dividing the fat mass (FM) in kg by square the height in m^2 where FM = BFP x body weight (Kg) / 100[17-18]
5. Waist circumference/hip circumference ratio (WHR): was calculated.

Sample collection and handling

Four milliliters of antecubital venous fasting blood sample were withdrawn from each patient and control and divided into 3 tubes. Two milliliters of blood were collected in a tube containing heparin for amino acid profile assessment. One milliliter was taken on fluoride for estimation of blood glucose. One milliliter of blood were put in a Wassermann test tube, left to clot at room temperature for 10-20 min, then was centrifuged at 3000 rpm for 20 min. Sera were separated and used for estimating the routine laboratory tests and lipid profile.

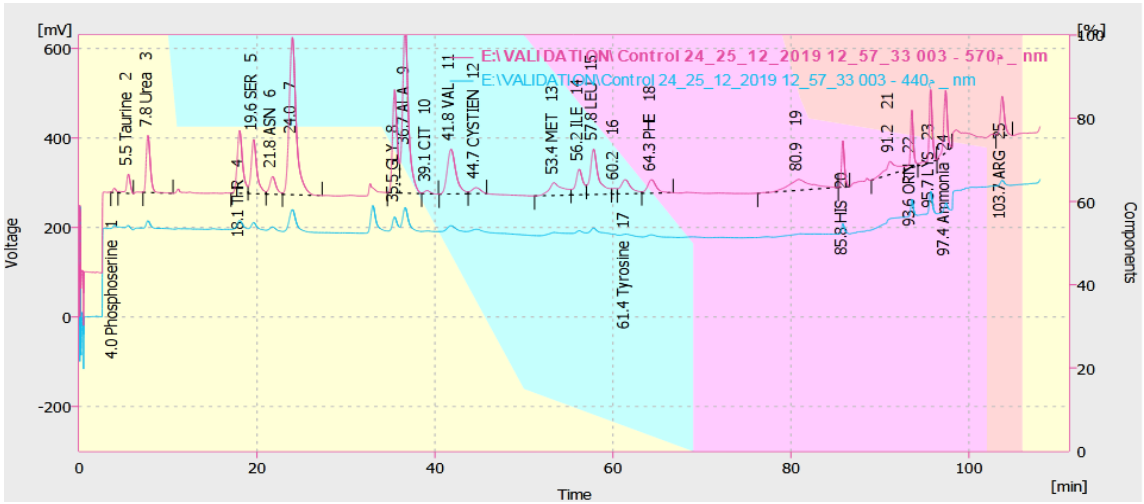
Lipid profile

Total blood cholesterol, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C) and triglycerides were assayed using colorimetric kits, supplied by spectrum diagnostics, Egypt.

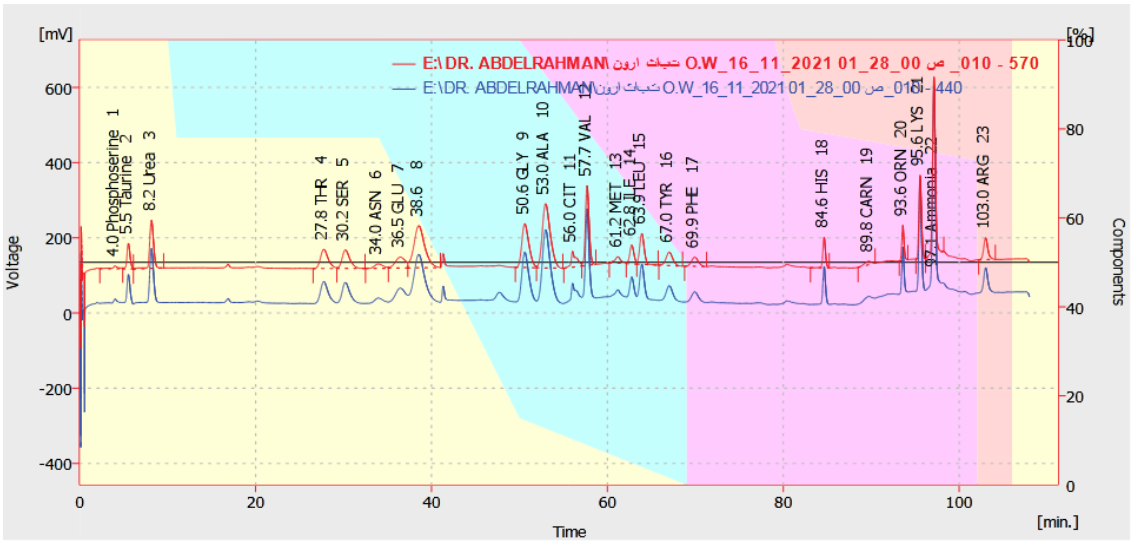
Assay of amino acids profile

Assay of amino acids was performed by the ion exchange separation method through high performance liquid chromatography using a Sykam Automatic Amino Acid Analyzer S433 supplied by Sykam GmbH, Germany (catalog no. 1120001). Free amino acid samples were prepared from plasma by acidic protein precipitation, where 200 µl of 10% sulfosalicylic acid solution was added to 800 µl plasma, mixed by vortex, then allowed to cool down at about 4 °C for 30 min. It was then centrifuged for 10 min at 14000 rpm. Supernatant liquor was diluted with same amount of sample dilution buffer (catalog no. S000015). One hundred µl of each of prepared samples and ready to use amino acid physiological standard (Catalog no. 6006005) were injected directly. A cation separation column LCAK06/Li was used (catalog no. 5112008) with the following specifications: size: 150 mm× 4.6 mm, specification range: met efficiency: > 48000, asymmetry: 0.8–1.5, resolution THR/SER: > 1700, and column pressure: 45–80bar. Buffer: Sykam LiA- 1, LiB-1, LiC-4. The ready to use ninhydrin reagent (catalog no. S000025) and citrate buffers in different pH (2.9, 4.2, and 8.0) were used and the analysis was performed at wavelength 440 nm: 570 nm. The sample chromatogram was compared to the standard measurements curve to obtain various amino acid values and then results were multiplied by a dilution factor of 2.5 **(Fig. 1).**

a)



b)



c)

d)

e)

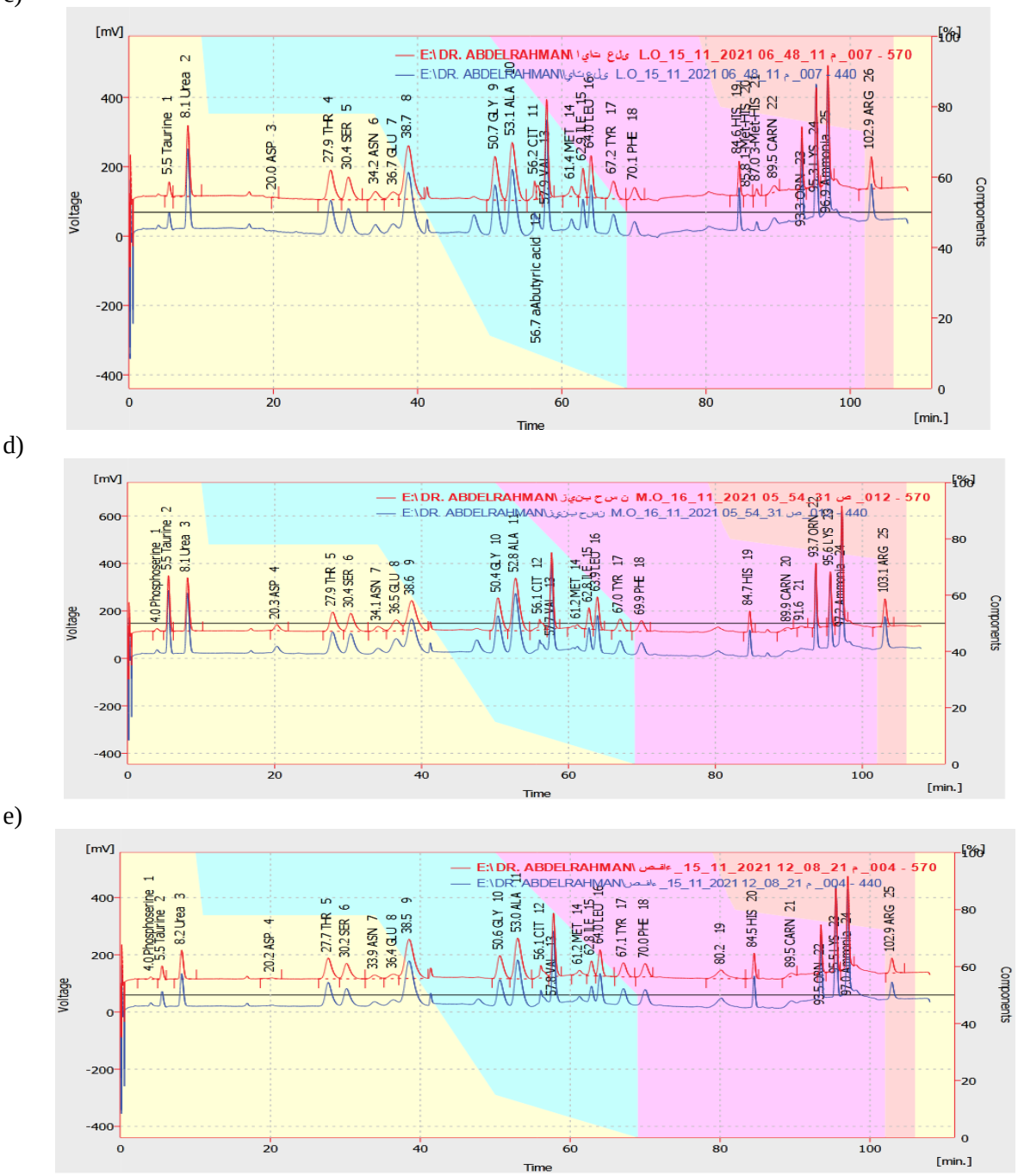


Fig. 1: AA chromatogram of each of the patients subgroups: a) controls b) overweight, c) moderate, d) severe and e) morbid obese women.

Statistical analysis

Data entry and analyses were performed using IBM-SPSS version 26 (Statistical Package for Social Science) for windows software. Continuous data were expressed as number, mean ± standard deviation (Mean ± SD) or median and Interquartile range (Median (IQ)). The normal distribution of the data was determined using the Shapiro-Wilk test. Statistical comparison of differences between test groups was evaluated by one-way ANOVA for parametric data, or Kruskal Wallis test for non-parametric data. Correlation coefficients analysis was performed using Pearson's coefficients. Univariate and multivariate regression analysis were performed to assess the effect of individual amino acids as independent risk factors for obesity. A two-tailed P-value was considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Results

Anthropometric data of subjects

The anthropometric data in the four subgroups of patients (overweight and obese classes I, II & III) are shown in **table 1** compared to controls (group 1). There was no statistical significance regarding age between the five groups. There were gradual increases in weight, waist and hip circumferences of obese women when compared to the controls.

Table 1: Anthropometric data of different study groups.

	Controls (n=17)	**Overweight (n=17)**	**Moderate Obese (n=17)**	**Severe Obese (n=17)**	**Morbid Obese (n=17)**
Age (Year)					
Mean±SD	31.41±8.11	34.71±6.3	31.41±7.92	33±9.63	37.76±8.07
P1		NS	NS	NS	NS
P2			NS	NS	NS
P3				NS	NS
P4					NS
Weight (Kg)					
Mean±SD	69.24±12.79	79.06±8.71	81.94±14.45	92.35±10.39	105.71±17.35
P1		NS	NS	<0.001	<0.001
P2			NS	<0.001	<0.001
P3				<0.01	<0.001
P4					<0.05
Height (Cm)					
Mean±SD	170.41±13.09	165.82±9.17	156.59±8.85	156.35±8.43	151.06±10.19
P1		NS	<0.01	<0.01	<0.001
P2			<0.01	<0.01	<0.001
P3				NS	NS
P4					NS
Waist Circum.(Cm)					
Mean±SD	84.82±5.58	101.82±11.48	101.76±11.12	112.76±16.1	130.41±13.99
P1		<0.001	<0.001	<0.001	<0.001
P2			NS	<0.05	<0.001
P3				<0.05	<0.001
P4					<0.01
Hip Circum.(Cm)					
Mean±SD	109.88±8.26	123.65±14.14	121.88±10.4	129.59±16.92	143.82±14.31
P1		<0.01	<0.01	<0.001	<0.001
P2			NS	NS	<0.001
P3				NS	<0.001
P4					<0.05

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups.

P3: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Obesity indices

The present study shows the levels of BMI, waist/hip ratio (WHR), fat mass index (FMI) and body fat percent (BFP) in obese subgroups and controls. Highly significant differences were found between controls and other obese subgroups regarding all parameters and the morbid obese group exhibited the highest values. **Table 2**

Biochemical investigations

The present data shows the mean levels of glucose where no significant differences were detected between any of the groups. In addition, Hb. and kidney function values were normal in all groups (data not shown). Regarding liver function tests, despite their normal reference ranges, however, some differences were observed between controls and other obese subgroups. Also, remarkably significantly higher mean values of serum total cholesterol, LDL-C, triglycerides and significantly lower HDL- C levels were shown in obese subgroups than that of controls in **Table (3)**.

Table 2: Obesity indices among different study groups.

	Controls (n=17)	**Overweight (n=17)**	**Moderate Obese (n=17)**	**Severe Obese (n=17)**	**Morbid Obese (n=17)**
BMI (Kg/m^2)					
Mean±SD	23.56±1.44	28.48±1.32	33.32±5	37.72±1.75	46.08±4.74
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001
WHR(W/H)					
Mean±SD	0.77±0.03	0.82±0.02	0.83±0.02	0.86±0.02	0.9±0.04
P1		<0.001	<0.001	<0.001	<0.001
P2			NS	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.01
BFP(BF%)					
Mean±SD	30.09±3.19	36.76±2.16	41.81±6.78	48.89±2.09	58.58±5.09
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001
FMI (kg/m^2)					
Mean±SD	7.14±1.16	10.58±1.1	14.26±5.38	18.82±1.23	27.25±5.57
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups.

P3: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Table 3: Biochemical data of different study groups.

	Controls (n=17)	Overweight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
Glucose (mmol/L)					
Mean±SD	5.37±0.87	5.49±1.08	5.08±0.9	5.31±1.11	5.6±1.17
P1		NS	NS	NS	NS
P2			NS	NS	NS
P3				NS	NS
P4					NS
AST (IU/L)					
Mean±SD	12.59±2.24	15.29±1.76	14.94±1.25	12.94±1.71	14.53±1.12
P1		<0.01	<0.01	NS	<0.01
P2			NS	<0.001	NS
P3				<0.001	NS
P4					<0.01
ALT (IU/L)					
Mean±SD	8.53±2.12	18.47±1.81	14.06±0.83	23.53±3.02	21.88±2.39
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					NS
ALP (IU/L)					
Mean±SD	61.71±5.67	44.59±3.83	64.18±2.92	64.35±4.11	82.24±2.97
P1		<0.001	NS	NS	<0.001
P2			<0.001	<0.001	<0.001
P3				NS	<0.001
P4					<0.001
Serum Cholesterol (mg/dl)					
Mean±SD	145.31±16.64	157.01±16.76	176.9±16.23	182.35±15.62	227.05±23.98
P1		NS	<0.001	<0.001	<0.001
P2			<0.01	<0.001	<0.001
P3				NS	<0.001
P4					<0.001
HDL-C (mg/dl)					
Mean±SD	52.44±2.74	42.11±3.11	33.26±3.76	27.76±1.66	24.21±5.01
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.05
LDL-C (mg/dl)					
Mean±SD	75.23±15.14	90.96±14.43	112.87±16.86	115.65±16.86	159.08±24.82
P1		<0.05	<0.001	<0.001	<0.001
P2			<0.01	<0.001	<0.001
P3				NS	<0.001
P4					<0.001
Serum TAG (mg/dl)					
Mean±SD	88.24±8.83	119.71±9.6	153.82±9.93	194.71±8.19	218.82±11.53
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups.
P3: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese

Plasma concentrations of amino acids in different study groups

Plasma concentrations of branched chain amino acids (BCAA)

Tables (4-6) represent the comparison between overweight, obese subgroups and controls regarding the plasma metabolomic profile of AAs. The overweight and obese subgroups had significantly lower levels of leucine and isoleucine when compared to controls. In addition, significantly a higher level of alanine was found in obese and overweight subgroups versus controls. Significant changes were also observed between overweight and obese groups. There was a decrease in valine level in all groups compared to controls with the overweight group possessing the least value. There was no significant difference between the three obese groups when compared to controls as regards to valine level **(Table 4)**. Moreover, there was a significant decrease in the total branched chain amino acid levels BCAA in overweight group and the two severe obese groups compared to that of controls **(Table 6)**.

Table 4: Profiles of the amino acids that were reduced in overweight and obese groups compared to controls.

(µmol/L)	Controls (n=17)	over weight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
Valine	316.79±384.3	177.78±22.21	248.98±49.62	218.18±67.35	240.27±12.86
P1		<0.001	NS	NS	NS
P2			<0.001	<0.001	<0.001
P3				<0.05	NS
P4					<0.05
Leucine	155.72±16.62	87.12±7.61	132.71±29.01	107.65±33.6	114.85±4.86
P1		<0.001	<0.05	<0.001	<0.001
P2			<0.001	NS	<0.001
P3				<0.01	NS
P4					<0.05
Iso-leucine	92.29±11.36	68±3.68	60.94±1.06	58.03±0.72	48.83±5.77
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001
Glycine	315.06±51.74	194.68±6.47	196.09±0.84	152.41±34.97	116.53±24.28
P1		<0.001	<0.001	<0.001	<0.001
P2			NS	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups.
P3: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Table 5: Profiles of the amino acids that were increased in overweight and obese groups compared to controls.

(µmol/L)	Controls (n=17)	Overweight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
Tyrosine	63.31±13.04	84.66±2.75	91.64±3.37	102.34±1.46	126.63±2.88
		<0.001	<0.001	<0.001	<0.001
			<0.001	<0.001	<0.001
				<0.001	<0.001
					<0.001
Phenylalanine	58.67±9.02	35.53±5.78	56.75±16.42	60.24±12.49	62.29±13.47
		<0.001	NS	NS	NS
			<0.001	<0.001	<0.001
				NS	NS
					NS
Alanine	243.55±35.7	229.17±22.57	363.18±49.46	381.37±8.09	557.03±103.3
		<0.001	<0.001	<0.001	<0.001
			<0.001	<0.001	<0.001
				<0.001	<0.001
					<0.001

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Table 6: Plasma concentrations of total BCAA, total AAA and BCAA/AAA ratio in different study groups.

(µmol/L)	Controls (n=17)	Overweight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
Total BCAA (µmol/L)	564.8±383.38	332.9±28.46	442.63±78.61	383.85±99.65	403.95±22.89
P1		<0.001	NS	<0.01	<0.001
P2			<0.001	NS	<0.001
P3				<0.05	NS
P4					<0.05
Total AAA (µmol/L)	121.98±12.46	120.19±8.22	148.39±19.21	162.58±12.12	188.91±11.35
P1		NS	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.01	<0.001
P4					<0.001
BCAA/ AAA	4.64±3.02	2.77±0.07	2.97±0.23	2.33±0.42	2.15±0.24
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.01	<0.05	<0.001
P3				<0.001	<0.001
P4					NS

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Plasma concentrations of aromatic amino acids (AAA)

A significant gradual increase in plasma levels of tyrosine was observed in all obese patients compared to controls and between groups. Phenylalanine level didn't change significantly in obese groups compared to controls except for overweight group. However, significant differences were observed when comparing the overweight group to obese subgroups **(Table 5)**. In total, there was a significant increase in the total AAA in obese subgroups and a significant decrease in the ratio of total BCAA to total AAA, where the least ratio was exhibited by the morbid obese group **(Table 6)**.

Plasma concentrations of non-polar simple amino acids

Regarding glycine, a significant decrease in its plasma level was observed in all obese patients as compared to controls and between all groups, except for moderate obese that was not significant **(Table 4)**. For alanine, although a significant increase in its plasma level was observed in an ascending order in the three obese patients as compared to that of controls, however, a significant marked lower concentration was noticed in the overweight group **(Table 5)**.

Table 7 shows the univariate and multivariate regression analysis of the studied amino acid profile for evaluation of the independent risk factors for obesity cases. The left side of the table shows the univariate regression analysis to assess the effect of individual amino acids. Analysis revealed that all amino acids are significant independent risk factors for obesity. At the right side, multivariate analysis is presented where odds ratio is adjusted to BMI. The study demonstrated that only alanine is significantly an independent risk factor for obesity cases ($p < 0.04$) but BCAA, AAA & their ratio were excluded from regression analysis obesity risk evaluation because of their minimal values.

Table 7: Univariate & Multivariate regression analysis of aliphatic and aromatic amino acid profile (µmol/L) parameters as independent risk factors for obesity cases.

	Univariate				Multivariate			
	OR	95.0% CI		P. value	OR	95.0% CI		P. value
		Lower	Upper			Lower	Upper	
Iso-leucine	-0.789	-0.490	-0.347	0.000	-	-	-	-
Leucine	-0.207	-0.113	0.002	0.057	-	-	-	-
Tyrosine	0.873	0.296	0.378	0.000	-	-	-	-
Phenyl-Alanine	0.320	0.062	0.290	0.003	-	-	-	-
Glycine	-0.766	-0.104	-0.072	0.000	-	-	-	-
Alanine	**0.816**	**0.045**	**0.061**	**0.000**	**-0.805**	**-0.102**	**-0.002**	**0.042**
AA	0.318	0.002	0.011	0.003	-	-	-	-

Excluded Variables: (Valine, BCAA, AAA and BCAA/ AAA (µmol/L) for their minimal values).
Dependent Variable: BMI (Kg/m²).

Discussion

Obesity is considered a pandemic with dramatic increases in its prevalence all over the world[19&20]. Obesity is a driver of many subsequent diseases such as diabetes, cardiovascular diseases, and cancer leading to major public health challenges[21&22]**.** So, it is necessary to study the role of potential strategies for obesity prevention and management[23].

It has been shown that the dysregulated amino acid metabolomic signature has a close relationship with future metabolic complications as insulin resistance or type 2 diabetes and cardiovascular diseases associated with obesity[6& 24-26].

Branched chain amino acids (BCAAs) are critical nutrient signals that received considerable attention over the past decade, because of their ability to promote protein synthesis and to affect metabolism[27, 23]**.**

In the present study, there was a decrease in individual & total BCAA levels in obese women when compared to those of controls.

Conversely, several previous studies reported increased levels of circulating BCAAs in obese persons and those with poor metabolic health. High proportion of BCAAs and their metabolic products was reported in obese subjects with insulin resistance which resulted from partial catabolism[28- 32].

Newgard, CB. and Lake, AD. et al.[33- 34] suggested that the reason for raised BCAAs level associated with insulin resistance is the decline in their catabolism in adipose tissue. This decline may be due to the down regulation of enzymes that catabolize BCAAs as a result of the repression of peroxisome proliferator-activated receptor-γ (PPAR-γ)[35- 36]**.** Also, it was found that BCAAs may have a potential to predict the development of steatohepatitis [34]; T2DM in individuals with obesity and impaired fasting glucose[37]**.** Similarly, Iwasa, M et al.[38], proposed that the increased BCAA may serve as a biomarker for increased risk of metabolic syndrome in obese subjects.

In agreement with our results, Prio, MC. et al.,[25] reported a significant down regulation of BCAA and up regulation of their metabolic derivatives as short chain acyl carnitine in visceral adipose tissue of obese and pathologically obese patients. They suggested an enhanced BCAA catabolic flux in adipose tissue of these patients, besides the tissue proteolysis and protein synthesis. Another two studies on obese adolescents found lower levels of BCAAs compared to their normal weight peers[39- 40]. Furthermore, Piccolo, BD. et al.,[41] found that BCAA levels were not elevated in a UC Davis – T2DM rat model until six months post onset of diabetes. Moreover, Xie, G et al.,[28] reported lower BCAA levels in obese women than in obese men, whereas, Stroeve, HM. et al.,[42] reported significantly lower BCAA levels in women with moderate to severe obesity (≤ 27 - < 40 BMI) than in morbidly obese one.

It has been shown that, the (mTOR) signaling pathways of BCAA and its metabolism could be therapeutic targets for treatment of insulin resistance[43], diabetes mellitus [44] and obesity [45]**.** The decreased BCAA levels of the present study obese group, in contradiction of some previous studies may be explained by our study group differences regarding their present metabolic condition, where they were metabolically healthy, and didn't suffer from diabetes or insulin resistance. Bagheri et al.[24] suggested that, elevated plasma BCAA concentrations have been shown to differentiate between metabolically healthy and unhealthy obese subjects. Moreover, it has been known that insulin in its normal level acts as a regulator of branched chain alpha keto acid dehydrogenase complex, the enzyme involved in branched chain amino acid catabolism[46-47].

On the other hand, a significant increase was observed in tyrosine and total aromatic amino acids (AAA) together with a significant decrease in BCAA/ AAA ratio in obese groups of the current study, besides the significant differences in between obese subgroups. Tyrosine and phenylalanine were found to be positively correlated with each of BMI, WHR, and FMI.

In agreement with our results, Short, K.R. et al.[48] observed that AAAs were positively correlated with adiposity. Also, Hellmuth C et al. and Chiara R et al.[49,&50] reported significant positive correlation of tyrosine with obesity and fat accumulation. Thus, these AA could be used as predictors of those raised indices and risk assessment tool for future metabolic complications. Furthermore, Butte NF. et al. [29] observed that, tyrosine was the highest ranked

metabolite contributed with obesity classification. This may be explained either by the stimulating effect of tyrosine on insulin secretion or by insulin actions on tyrosine metabolism[48].

It has been suggested that BCAAs influence brain function by competing with the uptake of the amino acid precursors of dopamine and 5-hydroxytryptamine in the brain[51]. Large neutral amino acid transport is shared by BCAAs and AAA, leading them to compete with each other. So, in our study, the reduction in the level of BCAAs in obese women lead to elevation of tyrosine amino acid level.

In the current study, obese women showed significantly lower levels of glycine with negative significant correlations with obesity indices (BMI, WHR & FMI). This is in accordance with the results of Takashina C. et al., Gaggini, M., Simmons, RM, et al.[8&52-53].

Simmons, RM et al.,[53] indicated that the reduced quantities of glycine observed in obese subjects results from an upregulation of the hepatic glycine cleavage system & that the dietary glycine supplementation potentially reduces obesity in Zucker diabetic fatty (ZDF) rats. A previous animal study showed that, glycine supplementation reduces visceral fat through the oxidation of free fatty acids in adipose tissue[54].

Glycine is synthesized from the glycolytic intermediates via 3-phosphoglycerate dehydrogenase that converts 3 PG to 3P-hydroxy pyruvate, the rate limiting step of de novo serine biosynthesis[55]. Thus, the changes in glyceroneogenesis in adipose tissue and liver due to the increased demand for glycerol and glyceride for triacylglycerols synthesis may affect the subsequent glycine level in circulation[56].

Furthermore, the levels of metabolic products of glycine that are important for metabolism as glutathione and betaine will be also affected in the obese persons according to the previous studies.

The present study demonstrated a significantly increased plasma alanine level in all obese subgroups in an ascending order as compared to those of controls. It showed a highly positive correlation with obesity indices in obese patients. These results are consistent with those of Takashina C. et al.[8]. A possible interpretation for alanine increase may be due to the hyperinsulinemia development[57]. The increased alanine level in our morbid obese group may be attributed to their insulin level which was in the upper limit of normal level (data not shown) and attempts of their body to attain the normal homeostasis. Interestingly, alanine is found to be one of the best independent predictors of the current study for recognizing the obese patients at risk of developing associated disorders (by multivariate regression analysis). Alanine AA has scarcely been studied in obesity women previously.

Conclusion

The results of AA metabolomics profile confirmed the previous notion that AA can be used as predictors for elevated obesity indices and can be used as important biomarkers for early prediction of overweight and obesity. Our results indicated that the significantly elevated circulating alanine levels were the best independent predictors for identifying obese patients at risk of associated metabolic disorders and that, the metabolomic profile of all AA could have a significant impact on obesity associated future complications.

Limitations

Further studies with a larger sample size are warranted to extend these results and to clarify the impact of plasma AA metabolomic and other different metabolomics profile on obesity complications and management.

Ethical approval

All study procedures were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (IRB no: 17200758).

A written informed consent was obtained from each participant.

Acknowledgments

The authors acknowledge the technical help of Metabolic and Genetic disorders Unit, Assiut University.

REFERENCES

1. A.M.A. Adopts, "American Medical Association", ***New Policies on Second***

Day of Voting at Annual Meeting, (2013).

2. M. Ng , T. Fleming , M. Robinson , B. Thomson, N. Graetz, C. Margono , *et al.*, "Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study", ***Lancet***, 384(9945),766-781 (2013).
3. L. Abarca-Gómez, Z. A. Abdeen, Z. A. Hamid, N. M. Abu-Rmeileh, B. Acosta-Cazares, C. Acuin, *et al.*, "Worldwide Trends in Body-Mass Index, Underweight, Overweight, and Obesity from 1975 to 2016: A Pooled Analysis of 2416 Population-Based Measurement Studies in 128.9 Million Children, Adolescents, and Adults", ***Lancet***, 390, 2627-2642 (2017).
4. T. Hamano, Y. Shiotani, M. Takeda, T. Abe, K Sundquist and T. Nabika, "Is the effect of body mass index on hypertension modified by the elevation? A cross-sectional study of rural areas in Japan", ***Int J Environ Res Public Health***, 14(9),1022 (2017).
5. A. Mohamed, E. Aliaa, E. Ibrahim, E. Nabil, E. Galal, A. Ehab, B. Engy, T. Dalia, E. Baher, F. Ahmad, A. Sherif, and V. Zoltán, "The Burden of Obesity in Egypt", ***Front Public Health***, 9,718978 (2021).
6. S. Morán-Ramos, B.E. López-Contreras and S. Canizales-Quinteros, "Gut Microbiota in Obesity and Metabolic Abnormalities: A Matter of Composition or Functionality?", ***Arch Med Res***, 48(8), 735-753, (2017).
7. T. Tanaka, Y. Ishizaka, , T. Mitushima, *et al.*, "Plasma amino acid profile is altered by visceral fat accumulation and is a predictor of visceral obesity in humans", ***Nat Prec***, (2011).
8. C. Takashina, I. Tsujino, T. Watanabe, S. Sakaue, D. Ikeda, A. Yamada, *et al.*, "Associations among the plasma amino acid profile, obesity, and glucose metabolism in Japanese adults with normal glucose tolerance", ***Nutr Metab (Lond),*** 13, 5 (2016).
9. K. Myoenzono, T. Yoshikawa , H. Kumagai, A. Zempo-Miyaki, R. So, T. Tsujimoto, Y. Choi , K. Tanaka and S. Maeda, "Changes in plasma amino acid concentrations in overweight and obese men after weight loss program including dietary modification and aerobic exercise", ***J Phys Fitness Sports Med***, 9(2),43-51 (2020).
10. H.S. Tahia, H.A. Hosam, F. Ahmed, A.A. Sara, F.M Maher and E.F. Michel, "Amino Acids Profile and Vitamin D Measurement in Hypertension in Egyptian Population", ***International Journal of Biochemistry Research & Review***, 29(9), 131-148 (2020).
11. H.S. Tahia, D. Marwa, E. Ghada, A. Ghada, A. Essam and F. Rania, "The profile of plasma free amino acids in type 2 diabetes mellitus with insulin resistance: Association with microalbuminuria and macroalbuminuria", ***Appl Biochem Biotechnol***, 188(3), 854-867 (2019).
12. C. Fattuoni, C. Mandò, F. Palmas, G.M. Anelli, C. Novielli, *et al.*, "Preliminary metabolomics analysis of placenta in maternal obesity", ***Placenta,*** 61, 89-95(2018).
13. H. S. Tahia, H. M. Ragaa, A. M. Ghada, H. A. Abdelrahman and R. Eman, "Obesity is associated with Autophagy dysregulation in Egyptian women", ***Bull Pharm Sci***, in press, (2022).
14. WHO Expert Consultation, Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies, ***Lancet,*** 363, 9403, 157-63 (2004).
15. P. Deurenberg, J.A. Weststrate and J.C. Seidell, "Body mass index as a measure of body fatness: age- and sex-specific prediction formulas", ***Br J Nutr***, 65(2), 105-114 (1991).
16. D. Gallagher, S. B Heymsfield, M. Heo, *et al.*, "Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index", ***Am J Clin Nutr,*** 72(3), 691-701 (2000).
17. P.M. De Oliveira, F. Almeida, R. Maria, S. Oliveira, L.L. Mendes, M.P. Netto and A.P.C. Cândido, "Association between fat mass index and fat-free mass index values and cardiovascular risk in

adolescents", ***Rev Paul Pediatr***, 34(1), 30–37 (2016).
18. R. Ramírez-Vélez, H.A. Carrillo, J.E. Correa-Bautista, J. Schmidt-RioValle, E. González-Jiménez, *et al.,* "Fat-to-muscle ratio: A new anthropometric indicator as a screening tool for metabolic syndrome in young Colombian people", ***Nutrients***, 10(8), 1027 (2018).
19. L. Abarca-Gómez, Z. A. Abdeen, Z. A. Hamid, N. M. Abu-Rmeileh, B. Acosta-Cazares, C. Acuin, *et al.,* "Worldwide Trends in Body-Mass Index, Underweight, Overweight, and Obesity from 1975 to 2016: A Pooled Analysis of 2416 Population-Based Measurement Studies in 128.9 Million Children, Adolescents, and Adults", ***Lancet***, 390, 2627-2642 (2017).
20. T. Hamano, Y. Shiotani, M. Takeda, T. Abe, K. Sundquist and T. Nabika, "Is the effect of body mass index on hypertension modified by the elevation? A cross-sectional study of rural areas in Japan", ***Int J Environ Res Public Health***, 14(9), 1022 (2017).
21. D. Ryan, S. Barquera, O. Barata Cavalcanti and J. Ralston, "The Global Pandemic of Overweight and Obesity. In: I. Kickbusch, D. Ganten, M. Moeti, (eds) Handbook of Global Health", ***Springer***, Cham, 2063–2098 (2021).
22. A. Mohamed, E. Aliaa, E. Ibrahim, E. Nabil, E. Galal, A. Ehab, B. Engy, T. Dalia, E. Baher, F. Ahmad, A. Sherif, and V. Zoltán, The Burden of Obesity in Egypt, ***Front Public Health***, 27;9:718978 (2021).
23. F. Xiao and F. Guo, "Impacts of essential amino acids on energy balance", ***Mol Metab***, 57, 101393 (2022).
24. M. Bagheri, F. Farzadfar, L. Qi, M. S. Yekaninejad, M. Chamari, O. A. Zeleznik, *et al.,* "Obesity-related metabolomic profiles and discrimination of metabolically unhealthy obesity", ***J Proteome Res***, 17, 1452– 1462 (2018).
25. M. C. Piro, M. Tesauro, A. M. Lena, P. Gentileschi, G. Sica, *et al.,* "Free amino acid metabolic profiling of visceral adipose tissue from obese subjects", ***Nature,*** 52(8),1125-1137 (2020).
26. G. Rocío, R. T. Armando, G. P. Omar, P. O. Edgar, F. L. Adriana, *et al.,* "Serum amino acid concentrations are modified by age, insulin resistance, and BCAT2 rs11548193 and BCKDH rs45500792 polymorphisms in subjects with obesity", ***Clin Nutr***, 40(6), 4209-4215 (2021).
27. C. Nie, T. He, W. Zhang, G. Zhang and X. Ma, "Branched Chain Amino Acids: Beyond Nutrition Metabolism", ***Int J Mol Sci***, 19(4), 954 (2018).
28. G. Xie, X. Ma, A. Zhao, C. Wang, Y. Zhang, D. Nieman, *et al.*, The metabolite profiles of the obese population are gender-dependent, ***J Proteome Res***, 13(9), 4062–4073 (2014).
29. N. F. Butte, Y. Liu, I. F. Zakeri, R. P. Mohney, N. Mehta, V. S. Voruganti, H. G¨oring, S. A. Cole and A. G. Comuzzie, "Global metabolomic profiling targeting childhood obesity in the Hispanic population", ***Am J Clin Nutr***, 102(2), 256–267(2015).
30. V. H. Wang, J. Min, H. Xue, *et al.*, "What factors may contribute to sex differences in childhood obesity prevalence in China?", ***Public Health Nutr***, 21, 2056–2064 (2018).
31. N. Houttu, K. Mokkala and K. Laitinen, "Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles", ***Clin Nutr***, 37(6),1955–1966 (2018).
32. H.T. Yu, X.Y. Fu, B. Xu, L.L. Zuo, H.B. Ma and S.R. Wang, "Untargeted metabolomics approach (UPLC-Q-TOF-MS) explores the biomarkers of serum and urine in overweight/obese young men", ***Asia Pac J Clin Nutr***, 27(5),1067–1076 (2018).
33. C. B. Newgard, "Interplay between lipids and branched-chain amino acids in development of insulin resistance", ***Cell Metab***, 15, 606–614 (2012).
34. A. D. Lake, P. Novak, P. Shipkova, N. Aranibar, D G. Robertson, M. D. Reily, L. D. Lehman-McKeeman, R. R Vaillancourt and N.J. Cherrington, "Branched chain amino acid metabolism profiles in progressive human nonalcoholic fatty liver

disease", ***Amino Acids,*** 47(3), 603-615 (2015).
35. S. H. Adams, "Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state", ***Adv Nutr,*** 2, 445–456 (2011).
36. M. M. Boulet, G. Chevrier, T. Grenier-Larouche *et al.,* "Alterations of plasma metabolite profiles related to adipose tissue distribution and cardiometabolic risk", ***Am J Physiol Endocrinol Metab***, 309(8), E736–E746 (2015).
37. F. Xu, S. Tavintharan, C. F. Sum, K. Woon, S. C. Lim, and C. N. Ong, "Metabolic signature shift in type 2 diabetes mellitus revealed by mass spectrometry-based metabolomics", ***J Clin Endocrinol Metab***, 98(6), E1060–E1065 (2013).
38. M. Iwasa, T. Ishihara, R. Mifuji-Moroka, N. Fujita, Y. Kobayashi, H. Hasegawa, *et al.*, "Elevation of branched-chain amino acid levels in diabetes and NAFL and changes with antidiabetic drug treatment", ***Obes Res Clin Pract***, 9(3),293–297(2015).
39. S.J. Mihalik, S.F. Michaliszyn, J. de las Heras, F. Bacha, S. Lee, D.H. Chace, V.R. DeJesus, J. Vockley and S.A. Arslanian, "Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation", ***Diabetes Care***, 35(3), 605-611(2012).
40. S. F. Michaliszyn, L.A. Sjaarda, SJ. Mihalik, S. Lee, F. Bacha, D. H. Chace, V. R. De Jesus, J. Vockley and S.A. Arslanian, "Metabolomic profiling of amino acids and β-cell function relative to insulin sensitivity in youth, ***J Clin Endocrinol Metab,*** 97(11), E2119-E2124 (2012).
41. B.D. Piccolo, J.L. Graham, K.L. Stanhope, O. Fiehn, P.J. Havel and S.H. Adams, "Plasma amino acid and metabolite signatures tracking diabetes progression in the UCD-T2DM rat model of type 2 diabetes", ***Am J Physiol Endocrinol Metab***, (2016).
42. J.H.M. Stroeve, E. Saccenti, J. Bouwman, A. Dane, K. Strassburg, J. Vervoort, *et al.*, "Weight loss predictability by plasma metabolic signatures in adults with obesity and morbid obesity of the DiOGenes study", ***Obesity***, 24(2),379–388 (2016).
43. K. Tajiri and Y. Shimizu, "Branched-chain amino acids in liver diseases", ***Transl Gastroenterol Hepatol,*** 3, 47(2018).
44. M.S. Yoon, "The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism", ***Nutrients,*** 8(7), 405 (2016).
45. M. Holeček, "Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements", ***Nutrition & Metabolism***, 15, 33(2018).
46. N. Rahimi, F. Razi, E. Nasli-Esfahani, M. Qorbani, N. Shirzad and B. Larijani, "Amino acid profiling in the gestational diabetes mellitus", ***J Diabetes Metab Disord,*** 16(1), 13 (2017).
47. X. Bi and CJ. Henry, "Plasma-free amino acid profiles are predictors of cancer and diabetes development", ***Nutr Diabetes***, 7(3),e249 (2017).
48. K.R. Short, J.Q. Chadwick, A.M. Teague, M. A. Tullier, L. Wolbert, C. Coleman and K.C. Copeland, "Effect of Obesity and Exercise Training on Plasma Amino Acids and Amino Metabolites in American Indian Adolescents", ***J Clin Endocrinol Metab***, 104(8), 3249-3261(2019).
49. C. Hellmuth, , F.F. Kirchberg, S. Brandt, *et al.*, "An individual participant data meta-analysis on metabolomics profiles for obesity and insulin resistance in European children", ***Sci Rep***, 9(1), 5053(2019).
50. R. Chiara, R. Maurizio, R. Fabio, C. Pierluigi, G. Veronica, *et al.*, "Manipulation of Dietary Amino Acids Prevents and Reverses Obesity in Mice Through Multiple Mechanisms That Modulate Energy Homeostasi", ***Diabetes,*** 69(11), 2324–2339 (2020).
51. J.D. Fernstrom, "Branched-chain amino acids and brain function", ***J Nutr***., 135(6), 1539S–1546S (2005).
52. M. Gaggini, F. Carli, C. Rosso, E. Buzzigoli, M. Marietti, V. Della Latta, D. Ciociaro, M.L. Abate, R. Gambino, M. Cassader, E. Bugianesi and A. Gastaldelli, "Altered amino acid concentrations in NAFLD: impact of obesity and insulin

resistance", ***Hepatology***, 67(1), 145–158 (2018).

53. R.M. Simmons, S.M. McKnight, A.K. Edwards, *et al.*, "Obesity increases hepatic glycine dehydrogenase and aminomethyltransferase expression while dietary glycine supplementation reduces white adipose tissue in Zucker diabetic fatty rats", ***Amino Acids***, 52(10), 1413–1423 (2020).
54. M. El Hafidi, I. Perez, J. Zamora, V. Soto, G. Carvajal-Sandoval and G. Banos, "Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats", ***Am J Physiol Regul Integr Comp Physiol,*** 287(6), R1387–1393(2004).
55. Y. Noguchi, N. Shikata, Y. Furuhata, T. Kimura and M. Takahashi, "Characterization of dietary protein-dependent amino acid metabolism by linking free amino acidswith transcriptional profiles through analysis of correlation", ***Physiol Genomics***, 34(3), 315-326 (2008).
56. J. Yang, S.C. Kalhan and R.W. Hanson, "What is the metabolic role of phosphoenolpyruvate carboxykinase?", ***J Biol Chem***, 284(40), 27025-2729 (2009).
57. O. D. Rangel-Huerta, B. Pastor-Villaescusa and A. Gil, "Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies", ***Metabolomics***, 15(6), 93(2019).

Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp. 1121-1137.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

الاضطرابات في ملامح التمثيل الغذائي للأحماض الأمينية الأليفاتية والأروماتيه في البلازما في السيدات المصريات البدينات

تحية سليم[١] – رجاء سلامة[١] – غادة محمد[٢] – عبد الرحمن عبد القوي[٣*] – إيمان رضوان[١،٤]

[١]قسم الكيمياء الحيوية الطبية، كلية الطب، جامعة اسيوط، اسيوط، ٧١٥١٥، مصر

[٢]قسم الأمراض الباطنة، كلية الطب، جامعة اسيوط، اسيوط، ٧١٥١٥، مصر

[٣]قسم الكيمياء الحيوية، كلية الصيدلة، جامعة اسيوط، اسيوط، ٧١٥١٥، مصر

[٤]قسم الكيمياء الحيوية ، جامعة سفنكس ، مدينة أسيوط الجديدة ، أسيوط ، مصر

تكمن خطورة السمنة في تأثيرها على كثير من الأمراض المصاحبة لها. لذلك من الأهمية بمكان التحقيق في عوامل الخطورة لوضع الاستراتيجيات الوقائية المحتملة للمضاعفات المستقبلية.

تهدف هذه الدراسة إلى البحث في الاضطرابات في ملامح الأحماض الأمينية الأليفاتية والأروماتية لدى السيدات المصريات البدينات.

شملت الدراسة ٨٥ امرأة تم تصنيفهن إلى ٥ مجموعات (١٧ امرأة لكل مجموعة):

١- أصحاء ، ٢- وزن زائد ، ٣- متوسطي السمنة ، ٤- سمنة شديدة ، ٥- سمنة مفرطة.

تم تقييم النمط الأيضي في البلازما للأحماض الأمينية السابق ذكرها باستخدام جهاز تحليل الأحماض الأمينية.

كشفت نتائج الدراسة أنه مقارنة بالمجموعة الضابطة ، كان لدى المجموعات الفرعية التي تعاني من السمنة مستويات أعلى بشكل تدريجي في متوسط مستويات البلازما من التيروزين و اجمالي الأحماض الأمينية الأروماتية الكلية والألانين جنبًا إلى جنب مع مستويات أقل بكثير من الليوسين ، والإيزولوسين ، وإجمالي الأحماض الأمينية الاليفاتية ونسبة إجمالي الأحماض الأمينية الاليفاتية الى اجمالي الأحماض الأمينية الأروماتية الكلية والجلايسين.

وتخلص الدراسة الى أن تحليل الانحدار متعدد المتغيرات يشير إلى أن مستويات الألانين المرتفعة بشكل كبير كانت مؤشر تنبؤي مستقل جيد لتحديد المرضى الذين يعانون من السمنة المفرطة المعرضين لخطر الاضطرابات الأيضية المرتبطة بها. علاوة على ذلك ، فإن نمط الأحماض الأمينية يعتبر وسيلة ضرورية للتنبؤ المبكر ومتابعة مرضى السمنة.