The influence of a 3-week body mass reduction program on the metabolic parameters and free amino acid profiles in adult Polish people with obesity

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Abstract

Background. Previous studies have showed differences in the amino acid (AA) composition in the plasma of people with obesity when compared to lean individuals, but the perturbations of AA concentrations in obesity and the dynamics of AA changes after weight loss is not fully understood.

Objectives. The objective of the study was to evaluate the effect of a short-term weight reduction program on the metabolic status and plasma AA levels in individuals with obesity.

Material and methods. A total of 24 adult Polish patients with a BMI between 34 and 49 kg/m² were enrolled in a 3-week controlled body mass reduction program based on everyday physical activity and a hypocaloric diet (25–30% less than total daily energy requirements). At baseline and after the program, anthropometric measurements, biochemical parameters and free AA profiles were determined.

Results. After the weight loss program, significant changes in body mass and metabolic parameters (e.g., low-density lipoprotein, triglyceride, fasting glucose, and insulin levels) were observed. Positive changes in a homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) following the program were also found. The levels of 10 AAs (α-amino-n-butyric acid, alanine, citrulline, glutamine, glycine, hydroxyproline, isoleucine, proline, sarcosine, and threonine) had significantly increased following weight loss. Only aspartic acid was present at a significantly lower concentration after the program.

Conclusions. Using a 3-week controlled body mass reduction program based on physical activity and a hypocaloric diet, we were able to demonstrate significant changes in biochemical parameters and free AA profiles. To better understand these changes, future studies should involve a long-term program with more patients.

Key words: obesity, amino acids, metabolic profile, body mass reduction
Introduction

Obesity is a significant public health problem reaching the level of pandemic in the developed world. It not only predisposes individuals to serious chronic conditions, such as type 2 diabetes, cardiovascular diseases and certain cancers, but it is also a leading cause of premature death worldwide. The etiology of obesity is multifactorial and includes interactions between genetic and environmental factors. Obesity results from energy homeostasis disorders, caused by an excessive intake of calories in relation to energy expenditure, and other lifestyle-related factors, such as a sedentary job and inactivity.1,2

In recent years, there has been a growing interest in the study of the human metabolome in obesity. The term “metabolome” is defined as the complete set of all metabolites in the body, tissues or cells (e.g., amino acids, lipids, carbohydrates, or nucleotides).3 Free amino acids (AAs) constitute a particularly interesting group of metabolites.4,5

Previous studies showed significant differences in plasma AA composition in people with obesity compared to individuals with normal body mass.6–9 Newgard et al. observed that among a total of 16 AAs measured in the serum of adult African-American subjects, 8 amino acids (alanine, valine, leucine/isoleucine, phenylalanine, tyrosine, glutamate/glutamine, aspartate/asparagine, and arginine) were found in dramatically higher levels in obese participants (median body mass index – BMI: 36.6 kg/m²) vs lean participants (median BMI: 23.2 kg/m²).10 Only the glycine level was lower in patients with obesity.

Strong discrepancies between the branched-chain amino acid (BCAA) and AA levels in children with obesity (8–18 years old) compared to lean individuals were also described by McCormack et al.11 A report on Japanese subjects with obesity showed that plasma levels of alanine, glycine, glutamate, tryptophan, tyrosine, and BCAs were associated with high visceral fat accumulation and could be dependent on genetic and environmental factors.12 Moreover, Yamakado et al. hypothesized that the amount of visceral fat changes AA profile, and that the multivariate logistic regression model of free AAs in plasma can discern non-apparent visceral obesity in adult Asian individuals.12

The associations between the BCAA levels and tyrosine with visceral adiposity – irrespective of ethnicity, lifestyle or environmental conditions – were also studied by Marti et al.13

Despite the great interest in and development of the state of knowledge about metabolic profiles in people with obesity, the perturbations in AA concentrations in the serum of adult patients suffering from obesity after weight loss are not fully understood. The previous studies in this area focused on describing the changes in metabolic profile after weight loss achieved by bariatric surgery or long-term body mass reduction programs based on lifestyle intervention. In addition, previous studies evaluated the AA changes in groups of people of different races or with different baseline BMI levels, and used other metabolomic assay techniques. Only a few studies investigated the effect of weight reduction on the level of amino acids in time points shorter than 1 year on a Caucasian population. An intriguing issue is the dynamics of changes in the metabolome under weight loss intervention.

This is the first study which demonstrates alterations in AA profiles and a number of biochemical parameters: fasting serum glucose, fasting serum insulin and a homeostatic model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), C-reactive protein (CRP), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) after a 3-week program based on a controlled, energy-restricted diet (25–30% less than daily energy requirements) of a strictly specified macronutrient composition and physical activity. The panel of free AAs analyzed was the most comprehensive among all previously conducted studies and it covered concentrations of 42 metabolites.

Material and methods

Participants and study design

The study was conducted in the Department of Internal Diseases, Metabolism and Nutrition of the Heliodor Swiecki University Hospital in Poznań, Poland, between September 2014 and June 2015. Twenty-four Polish patients (10 men and 14 women) with a BMI between 34 and 49 kg/m² (mean BMI: 40 ± 4 kg/m²) were enrolled. Mean age was 46 ± 12 years and ranged from 24 to 66 years. Diabetes mellitus type 1 or uncontrolled type 2 diabetes, a vegetarian or any other alternative diet, cancer, a history of eating disorders, or chronic diseases related to metabolism (e.g., chronic liver, kidney or pancreatic diseases, inborn metabolic diseases, autoimmune diseases, or inflammatory bowel diseases) were considered to be exclusion criteria. The occurrence of diseases listed in the exclusion criteria was checked through the use of specific diagnostic tests and medical histories. None of the patients smoked.

The study protocol was approved by the Bioethics Committee at Poznan University of Medical Sciences (No. 333/14), and was performed in accordance with the Declaration of Helsinki. All the patients signed statements of informed consent before they participated in the study.

The subjects were hospitalized for 3 weeks and received controlled daily aerobic physical training, under the supervision of a physical therapist, and a hypocaloric diet based on a 25–30% reduction of caloric dietary intake compared to their total estimated energy requirement. The total daily energy requirement was calculated on actual body weight using the Harris-Benedict formula and the physical activity level index (PAL). To more accurately determine total energy expenditure, the energy requirements were calculated using the Harris-Benedict formula and the physical activity level index (PAL).
energy expenditure and to assess the caloric value of the diet before the program, subjects completed a 3-day intake assessment prior to the study (the 24-hour dietary assessment covers 3 days). The subjects received the same type of diet prepared by dietetic food caterers based on the planned menu. The average value of total daily energy intake before the program was 2,935.6 ±490.2 kcal (2,764.0 ±406.1 kcal for women; 3,340.5 ±284.5 for men), and the average value during the body mass reduction program was 2,016.0 ±281.7 kcal (1,880.0 ±234.0 kcal for women; 2,220.0 ±219.1 kcal for men).

Each patient received a diet with an identical composition of macronutrients (especially proteins) derived from the same products. The diet consisted of 20% of calories from protein, 25–30% from fat, and 50–55% from carbohydrates. The daily fiber intake from the diet was over 25 g. Physical training included active and passive breathing exercises for 30 min a day, cardiovascular aerobic exercise (Nordic walking or cycling) twice a day for 60 min and resistance training for 30 min daily. Each patient who participated in the study was educated on proper nutrition and nutritional recommendations for obesity by a qualified nutritionist.

Serum assays

Serum glucose (3.3–5.8 mmol/L), TC (3.4–5.2 mmol/L), HDL (>1.6 mmol/L), TG (0.5–1.9 mmol/L), AspAT (5–40 U/L), and AlAT (5–40 U/L) were determined by a fully automated Modular P-800 Roche (Diamond Diagnostic, Holliston, USA). LDL was measured indirectly using the Friedewald equation. Insulin (2.0–25.0 mU/L) was determined by a micro–particle enzyme immunoassay (Abbot, Abbot Park, USA). Serum CRP was analyzed by a commercial assay (Dade Behring, Marburg, Germany). The QUICKI was calculated using the inverse of the sum of the logarithms of fasting insulin and fasting glucose levels: \( l/[\log(\text{fasting insulin } \mu\text{U/mL}) + \log(\text{fasting glucose } \text{mg/dL})] \). The HOMA-IR was calculated according to this formula: fasting insulin \( \mu\text{U/L} \times \text{fasting glucose } \text{nmol/L}/22.5 \).

An important element of the study was the analysis of the serum profiles of 42 AAs in obese patients and their possible changes after weight loss. Fasting plasma was collected for AA analysis in the morning after 10 h of fasting and was stored in −80°C before analysis. The determination of free AA serum profiles was performed using the fully validated, highly selective liquid chromatography–tandem mass spectrometry method (LC-MS/MS). Samples were prepared using aTRAQ–based methodology (Sciex, Framingham, USA), and then analyzed by a high-performance liquid chromatograph 1260 Infinity (Agilent Technologies, Santa Clara, USA) interfaced to a triple–quadrupole mass spectrometer 4000 QTRAP (Sciex, Framingham, USA). The detailed sample preparation protocol, LC-MS/MS parameters and validation results using serum samples were presented in the previous reports.14,15

Anthropometric measures

For each patient, anthropometric measurements were carried out at baseline and after the 3-week weight reduction program. Height was measured to the nearest centimeter using a stadiometer. Weight and analysis of body composition was performed by Tanita MC 980 MA – bodyfat analyzer (TANITA, Tokio, Japan) based on the measurement of bioelectrical impedance, with a tetrapolar foodpad-style electrode arrangement. The subjects stood on the metal contacts in bare feet, as recommended in the manual. Body mass index was defined as the individual’s body mass divided by the square of their height, with the value given in kg/m². Waist–hip ratio (WHR) was the ratio of waist circumference to hip circumference, measured in cm with the use of medical measuring tape (SECA, Hamburg, Germany).

Statistical analysis

In order to examine changes in the anthropometric and biochemical parameters, as well as free AA serum profiles induced by the weight loss program, the Shapiro-Wilk test of normality was used in the first step. Variables with normal distribution were then subjected to the paired t-test. For the analysis of variables that were not normally distributed, the Wilcoxon signed-rank test was used. In all tests, a p-value of ≤0.05 was considered to be statistically significant. Additionally, in order to evaluate the influence of a reduction in body weight on the free AA levels in the serum, a principal component analysis (PCA) was conducted. PCA is used to bring out any patterns in the study dataset and to visualize the differences among groups of analyzed samples. Since PCA is conducted without any prior information on sample classification, it is defined as an unsupervised statistical method. Prior to PCA, normalization by sum and by autoscaling of the amino acid concentrations were performed. The statistical analyses were carried out using STATISTICA v. 10.0 (StatSoft, Kraków, Poland) and the MetaboAnalyst 3.0 web portal.16

Results

Anthropometric and biochemical characteristics of the study population

The differences in the anthropometric and biochemical parameters measured before and after the 3-week weight loss program are presented in Table 1.

After the applied weight loss program, significant decreases in body mass, BMI, waist circumference, and hip circumference were observed. The average reduction in body mass was 6.0 kg (5.1%), which significantly decreased the BMI level (39.7 ±4.1 kg/m² vs 37.7 ±3.9 kg/m²; p < 0.0000). The average waist and hip circumferences

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**Table 1:** Anthropometric and biochemical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After 3 weeks</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>110 ±5</td>
<td>104 ±5</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39.7 ±4.1</td>
<td>37.7 ±3.9</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92 ±10</td>
<td>88 ±9</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>100 ±12</td>
<td>96 ±11</td>
<td>&lt;0.0000</td>
</tr>
</tbody>
</table>

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changed from 121.8 ±11.7 cm to 118.0 ±11.1 cm (p = 0.0000) and from 129.0 ±9.1 cm to 126.0 ±8.3 cm (p = 0.0000), respectively. After the 3-week program, we observed an 8.7% reduction in fat mass. A reduction in muscle mass and total body water was also noticed in the study group (Table 1).

Table 1. Anthropometric characteristics, biochemical parameters and free AA concentrations of the study population before and after the weight loss program
Baseline median fasting blood glucose levels in patients with obesity were elevated compared to the values after body mass reduction (Table 1). Similarly, the fasting insulin levels in patients at baseline were significantly higher than at the end of the study. We observed positive changes in HOMA-IR after weight loss (Fig. 1). Other important changes observed after weight loss were the reductions in TG concentration (12.5%), the LDL-cholesterol level (11.4%) and CRP (3.4%); however, those differences were not statistically significant.

* obtained based on paired t-test or Wilcoxon test; ** statistically significant; BMI – body mass index; FFM – free fat mass, TBW – total body water; LDL – low-density lipoprotein; CRP – C-reactive protein; HOMA-IR – homeostatic model assessment of insulin resistance; QUICKI – quantitative insulin sensitivity check index; ALAT – alanine aminotransferase; AspAT – aspartate aminotransferase.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before the program</th>
<th>After the program</th>
<th>After – before the program</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td>% difference</td>
</tr>
<tr>
<td>Ornithine</td>
<td>65.91 ±16.94</td>
<td>71.03 ±17.76</td>
<td>5.12 ±19.05</td>
<td>7.8</td>
</tr>
<tr>
<td>Phosphoethanolamine</td>
<td>1.79 ±0.62</td>
<td>1.74 ±0.46</td>
<td>−0.04 ±0.64</td>
<td>−2.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>50.60 ±9.04</td>
<td>48.66 ±7.59</td>
<td>−1.94 ±10.24</td>
<td>−3.8</td>
</tr>
<tr>
<td>Proline</td>
<td>146.42 ±44.28</td>
<td>190.51 ±53.79</td>
<td>44.09 ±66.76</td>
<td>30.1</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>1.31 ±0.66</td>
<td>1.62 ±0.86</td>
<td>0.31 ±0.48</td>
<td>23.9</td>
</tr>
<tr>
<td>Serine</td>
<td>106.88 ±24.21</td>
<td>112.35 ±25.04</td>
<td>5.47 ±24.60</td>
<td>5.1</td>
</tr>
<tr>
<td>Taurine</td>
<td>93.74 ±25.84</td>
<td>88.95 ±21.94</td>
<td>−4.79 ±22.37</td>
<td>−5.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>91.94 ±21.87</td>
<td>109.08 ±31.94</td>
<td>7.14 ±31.85</td>
<td>18.6</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>42.41 ±10.99</td>
<td>42.16 ±7.24</td>
<td>−0.25 ±8.35</td>
<td>−0.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>42.51 ±11.20</td>
<td>46.81 ±14.16</td>
<td>4.30 ±11.54</td>
<td>10.1</td>
</tr>
<tr>
<td>Valine</td>
<td>211.71 ±42.25</td>
<td>227.04 ±37.33</td>
<td>15.33 ±42.31</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* obtained based on paired t-test or Wilcoxon test; ** statistically significant; BMI – body mass index; FFM – free fat mass, TBW – total body water; LDL – low-density lipoprotein; CRP – C-reactive protein; HOMA-IR – homeostatic model assessment of insulin resistance; QUICKI – quantitative insulin sensitivity check index; ALAT – alanine aminotransferase; AspAT – aspartate aminotransferase.

Fig. 1. Histograms of the differences in parameters associated with carbohydrate metabolism measured before and after the 3-week weight loss program. HOMA-IR – homeostatic model assessment of insulin resistance; QUICKI – quantitative insulin sensitivity check index.
AA profiles of serum

The analysis of free AA profiles in the serum samples collected before and after the 3-week weight loss program revealed a number of significant changes in the metabolic profile of the patients. Although the applied methodology allows for measurements of 42 free AAs, both proteinogenic and non-proteinogenic, not all of the metabolites occurred in measurable concentrations in the samples. For this reason, the following 9 AAs were excluded from further statistical analysis: phosphoserine, argininosuccinic acid, homocitrulline, anserine, carnosine, homocysteine, γ-amino-n-butyric acid, cystathionine, and δ-hydroxylysine. The remaining 33 metabolites, along with their concentrations as measured in the analyzed serum samples, are listed in Table 1. The levels of 10 AAs (α-amino-n-butyric acid, alanine, citrulline, glutamine, glycine, hydroxyproline, isoleucine, proline, sarcosine, and threonine) were significantly higher after weight loss compared with their values before the program. Only aspartic acid was lower in patients after the program. According to the univariate statistical analyses performed, the highest differences in serum levels were observed for the following metabolites: glycine ($p = 0.0002$), α-amino-n-butyric acid ($p = 0.0007$), proline ($p = 0.0014$), and glutamine ($p = 0.0014$).

The influence of weight loss on AA profiles was also investigated using multivariate statistical analysis – PCA – which involves a group of variables simultaneously. Free AA profiles were analyzed by PCA to determine any clusters with respect to the 2 groups of samples: before and after intervention. In Fig. 2, points corresponding to the analyzed serum samples are contained in the space spanned by the first 2 principal components (PC 1 vs PC 2). Although the 2 groups of samples did not form separate clusters, a partial separation between samples was observed (Fig. 2). The results of PCA indicate that the changes in free AA profiles were strong enough to cause grouping of the samples according to their inclusion in one of the groups studied.

Discussion

This paper presents a comprehensive picture of the metabolic changes in Polish patients with obesity after a 3-week weight loss program. Although the duration of the body mass reduction program was relatively short, it is noteworthy that the weight reduction was carried out under strictly controlled conditions. All patients were treated with the same type of diet prepared by dietetic food caterers and the level of compliance remained under supervision during the entire 3 weeks. The implemented program of physical exercise was also the same in all study participants.

The weight loss achieved in all patients during the 3-week program (based on a hypocaloric diet of a strictly defined macronutrient composition and physical activity) was very satisfactory and it affected the measured
metabolic parameters and body mass composition. It has been already demonstrated that a 5% weight loss reduces or eliminates disorders associated with obesity; it improves both TC and TG, and causes a reduction in plasma glucose level. Similar trends were observed in this study, even though the body mass reduction was achieved after a short-term program. The TG and LDL-cholesterol levels were reduced in response to weight loss, though those differences were not statistically significant. As a result of weight reduction, positive changes in carbohydrate profiles were observed (Fig. 1). We noticed positive changes in HOMA-IR and QUICKI after weight loss. The HOMA-IR and QUICKI are widely validated and applied in the estimation of insulin sensitivity. High HOMA-IR and low QUICKI were independently and significantly associated with an increased risk of impaired glucose tolerance (IGT) and type 2 diabetes. The HOMA-IR cut off value which correlates with an increased risk of metabolic and cardiovascular diseases was estimated in several studies, in which its dependence on age, gender and ethnicity was noted. In the study population, HOMA-IR was elevated, which may have influenced the observations. Moreover, Wahl et al. first observed that the initial plasma concentrations of AAs predict the success or lack thereof of a 3-month standardized diet and exercise intervention based on individual dietary guidance caused a normalization in circulating BCAA. In their study on Japanese subjects, Tochikubo et al. first observed that the initial plasma concentrations of AAs predict the success or lack thereof of a 3-month standardized diet and exercise weight loss program. The 1-year body mass reduction program based on lifestyle intervention (changes in diet and exercise habits) conducted by Reinehr et al., showed an increased level of serine, glutamine and methionine, but not proline, after the body mass reduction in a group of German children with obesity. Wahl et al. described that the proline levels were reduced in children suffering from obesity compared to children with normal weight. Moreover, Wahl et al. demonstrated that methionine and glutamine were diminished at baseline in children with obesity who reduced weight during lifestyle intervention, as compared with
children without the body mass reduction in the same kind of intervention.26 The same relationship was described by Pathmasiri et al. in a study on an adult population with obesity, which leads to the hypothesis that dysregulation in the AA levels might be a consequence of poor lifestyle habits (diet and exercise).32

Some inconsistencies between our findings and the data obtained from previous studies were found. These differences may have been caused by different duration and methods of the weight reduction program, or by additional factors, such as the ethnicity or age of the study population, baseline BMI values and the level of weight loss achieved, or the protein content of the patients’ normal diet. The current study was conducted on Polish patients (Caucasian race), while the previous study involved mainly Asian populations. In our study, the subjects were hospitalized for 3 weeks in a controlled environment. Additionally, each patient received the same type of a hypocaloric diet based on a 25–30% reduction in caloric dietary intake compared to total energy requirement. To eliminate the possible effect of diet composition on the levels of circulating AAs, a diet with an identical composition and sources of macronutrients (especially proteins) was administered to all patients. Moreover, the mean BMI in our study population after the weight loss program was lower, but still above 35 kg/m², which could account for the differences in the AA changes in our study compared to previous data.

Conclusions

This study is the first to describe the changes in 42 free AA profiles during a 3-week body mass reduction program in adult Polish patients with obesity. Our study shows that the metabolic profile of patients undergoing a strictly controlled weight reduction program were characterized by high dynamics of change and were visible after 3 weeks of body mass reduction.

The 3-week diet and physical activity program caused significant changes in body mass and biochemical parameters (e.g., the fasting glucose and insulin levels, HOMA-IR, CRP, and TG) and changes in free AA profiles. We observed that the levels of 10 out of the 42 AAs measured were significantly higher after weight loss. The highest differences in serum levels were observed for the following metabolites: glycine, α-amino-n-butyric acid, proline, and glutamine. Some variations between our findings and the data obtained from previous studies were found. These differences may have been caused by different duration and methods of the weight reduction program, or by additional factors, such as the ethnicity or age of the study population, baseline BMI values and the level of weight loss achieved, the protein content of the patients’ normal diet, or the impact of physical activity on the metabolism of AA. To better understand these changes, future studies should involve a long-term program with more patients.

References
