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# Food Overconsumption in Healthy Adults Triggers Early and Sustained Increases in Serum Branched-Chain Amino Acids and Changes in Cysteine Linked to Fat Gain

Amany K Elshorbagy,[1] Dorit Samocha-Bonet,[2,3] Fredrik Jernerén,[4,5] Cheryl Turner,[4] Helga Refsum,[4,6] and Leonie K Heilbronn[3,7]

[1]Department of Physiology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt; [2]Faculty of Medicine, University of New South Wales, Sydney, New South Wales, Australia; [3]Diabetes and Metabolism Division, Garvan Institute of Medical Research, Sydney, Australia; [4]Department of Pharmacology, University of Oxford, Oxford, United Kingdom; [5]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; [6]Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, Norway; and [7]Discipline of Medicine, University of Adelaide, Adelaide, South Australia, Australia

## Abstract

**Background:** Plasma concentrations of branched-chain amino acids (BCAAs) and the sulfur-containing amino acid cysteine are associated with obesity and insulin resistance. BCAAs predict future diabetes.

**Objective:** We investigated amino acid changes during food overconsumption.

**Methods:** Forty healthy men and women with a body mass index (mean ± SEM) of 25.6 ± 0.6 were overfed by 1250 kcal/d for 28 d, increasing consumption of all macronutrients. Insulin sensitivity and body composition were assessed at baseline (day 0) and day 28. Fasting serum amino acids were measured at days 0, 3, and 28. Linear mixed-effects models evaluated the effect of time in the total group and separately in those with low and high body fat gain (below compared with at or above median fat gain, 1.95 kg). At days 0 and 28, insulin-induced suppression of serum amino acids during a hyperinsulinemic-euglycemic clamp test and, in a subset ($n = 20$), adipose tissue mRNA expression of selected amino acid metabolizing enzymes were assessed.

**Results:** Weight increased by 2.8 kg. High fat gainers gained 2.6 kg fat mass compared with 1.1 kg in low fat gainers. Valine and isoleucine increased at day 3 (+17% and +22%, respectively; $P \leq 0.002$) and remained elevated at day 28, despite a decline in valine ($P = 0.019$) from day 3 values. Methionine, cystathionine, and taurine were unaffected. Serum total cysteine (tCys) transiently increased at day 3 (+11%; $P = 0.022$) only in high fat gainers ($P$-interaction = 0.043), in whom the cysteine catabolic enzyme cysteine dioxygenase (*CDO1*) was induced (+26%; $P = 0.025$) in adipose tissue ($P$-interaction = 0.045). Overconsumption did not alter adipose tissue mRNA expression of the BCAA-metabolizing enzymes branched-chain keto acid dehydrogenase E1$\alpha$ polypeptide (*BCKDHA*) or branched-chain amino transferase 1 (*BCAT1*). In the total population at day 0, insulin infusion decreased all serum amino acids (−11% to −47%; $P < 0.01$), except for homocysteine and tCys, which were unchanged, and glutathione, which was increased by 54%. At day 28, insulin increased tCys (+8%), and the insulin-induced suppression of taurine and phenylalanine observed at day 0, but not that of BCAAs, was significantly impaired.

**Conclusions:** These findings highlight the role of nutrient oversupply in increasing fasting BCAA concentrations in healthy adults. The link between cysteine availability, *CDO1* expression, and fat gain deserves investigation. This trial was registered at www.clinicaltrials.gov as NCT00562393. *J Nutr* 2018;148:1073–1080.

**Keywords:** tandem mass spectrometry, DXA, BCAT, BCKD, aromatic amino acids, fat gain, high caloric intake

## Introduction

There is increasing consensus that amino acid metabolism, in particular that of sulfur-containing amino acids (SAAs) and BCAAs, is linked to energy and glucose metabolism. The BCAAs leucine, isoleucine, and valine are elevated in obesity (1) and predict future insulin resistance (2) and diabetes (3). The reason for this elevation is unclear. BCAAs are catabolized by sequential action of branched-chain aminotransferase (BCAT) and branched-chain keto acid dehydrogenase (BCKD) enzymes. Adipose tissue is estimated to account for 15% of BCAA catabolism, with skeletal muscle being the single major


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contributor (4). Impaired catabolism of BCAAs occurs in adipose tissue from individuals with obesity (5), particularly metabolically unhealthy obesity (6).

Multidirectional associations exist between diet, BCAA concentrations, obesity, and insulin resistance. Circulating concentrations of BCAAs and aromatic amino acids are positively influenced by the intake of animal-derived protein (7, 8), which is, in turn, associated with future weight gain, insulin resistance, and diabetes (9–11). On the other hand, insulin plays a key role in regulating plasma amino acid uptake and metabolism. Plasma BCAAs are decreased in patients with functioning insulinoma (12), and insulin infusion triggers a dose-dependent decrease in plasma amino acids (13). Findings of cross-sectional studies investigating the effect of obesity on insulin-induced suppression of plasma amino acids have shown inconsistent results (14–16), possibly due to differences in the duration of obesity and degree of insulin resistance.

Insulin resistance is also linked to reduced expression and action of BCAA catabolic enzymes [reviewed elsewhere (17, 18)]. Insulin-sensitizing therapy in subjects with type 2 diabetes lowered plasma concentrations of several BCAAs (19). Bariatric surgery–induced weight loss was associated with upregulation of BCAA catabolic enzymes in adipose tissue and lowered plasma BCAAs (5). Together, these observations suggest that obesity and insulin resistance produce a reversible impairment in BCAA catabolism. However, weight loss and enhanced insulin sensitivity brought about by calorie restriction often fails to lower plasma BCAAs (20, 21).

SAAs are also linked to fat mass and energy metabolism. Methionine is the only essential SAA, which, via several intermediates, including homocysteine and cystathionine, can be converted to cysteine. Cysteine is the precursor of glutathione and of taurine via catabolism by cysteine dioxygenase enzyme (CDO). Total cysteine (tCys) in plasma and whole blood correlates with fat mass, independent of other SAAs (22–24). In 950 children, plasma tCys and insulin were positively correlated, and those in the upper tCys quartile had a doubled risk of being insulin resistant, after adjustment for body fat. Unlike BCAAs (5), plasma tCys does not change appreciably after major weight loss (25). This suggests that tCys elevation is not a consequence of the obese state. In fact, transgenic (26) and dietary data in animals, as well as in vitro data, collectively suggest that increased cysteine availability promotes fat gain (27, 28). Rats fed a diet deficient in methionine and cysteine were lean and insulin sensitive, an effect that was reversed by cysteine supplementation (29).

Animal and in vitro studies showed that CDO is strongly induced in liver and adipose tissue by increases in cysteine availability or cysteine or protein intake (30). To gain insight into the dynamics of SAA and BCAA changes during weight gain, we investigated the effect of increased energy intake in adults on serum BCAAs and SAAs, on insulin-induced suppression of amino acids, and on adipose tissue expression of selected enzymes.

## Methods

*Participants.* The study was conducted in 40 healthy, sedentary, nonsmoking men and women. Details on recruitment and exclusion criteria have been published previously (31). Participants provided written informed consent, and the study protocol was approved by the Human Research and Ethics Committee at St. Vincent's Hospital, Sydney, Australia. The participants consented to undergoing a 28-d food-overconsumption regimen that contained ~1250 kcal/d above their baseline energy requirements, as detailed previously (31). From days 0–3 and 25–28, all foods were provided at baseline energy requirements plus 1250 kcal/d, with a nutrient composition of 45% fat, 15% protein, and 40% carbohydrate. Fat intake was approximately doubled by providing 3 high-energy, high-fat snacks/d, each providing ~240 kcal (e.g., potato crisps, chocolate bars, cheesecake) and a liquid oil–based supplement (Benecalorie; Novartis; ~340 kcal) mixed in a dairy dessert (~200 kcal). On days 3–25 of the overconsumption regimen, participants were instructed to consume their regular diets and were provided with the above snacks and supplement to achieve an intake of 1250 kcal/d above their baseline energy requirement. Dietary energy and macronutrient intakes at day 0 and day 28 were calculated from 3-d diet diaries with the use of FoodWorks 2007 based on the Australian foods database (Xyris Software). The study was registered at www.clinicaltrials.gov (NCT00562393). Body weight and blood pressure were assessed and overnight fasted serum samples were obtained on 3 visits, at days 0, 3, and 28 relative to the start of overconsumption (on day 1).

*Additional assessments at days 0 and 28.* Insulin sensitivity was measured by using a 2-h hyperinsulinemic-euglycemic clamp ($60\ mU \cdot m^{-2} \cdot min^{-1}$), as described (31). Glucose was infused at a variable rate to maintain glucose at 5.0 mmol/L, and the steady-state glucose infusion rate was calculated between 90 and 120 min. Body composition (fat mass, fat-free mass, and central abdominal fat) was assessed by DXA (Lunar DPX-Lunar Radiation), as described previously (31). Body-composition data at day 28 were not available for 3 participants. Three cross-sectional computed tomography (CT) scans (Gemini GXL; Philips) were also performed to assess abdominal adipose tissue distribution as described previously (31). CT images were analyzed by using Gemini (GXL Host System; Philips). Two participants did not undergo CT scans.

*Biochemical analysis.* Blood glucose and serum insulin were measured as previously described (31). LDL cholesterol was calculated by the Friedewald equation from total and HDL cholesterol, and TGs were measured by enzymatic colorimetry (Roche). For the present study, serum amino acids and total GSH (tGSH) were assayed in stored samples by using LC–tandem MS using a modified version of a previously described method (32). Serum methionine, tCys, total homocysteine (tHcy), cystathionine, and tGSH were analyzed in a single assay. The protocol was modified to include valine, leucine, isoleucine, phenylalanine, tyrosine, and tryptophan. Briefly, deuterium-labeled isotopes were added to serum as internal standards, followed by reduction of disulfides with the use of DTT and then protein precipitation by using perchloric acid. The acid supernatant was diluted with a solution containing heptane sulfonic acid as an ion pair reagent. Taurine was extracted and assayed separately, by adding deuterium-labeled taurine to serum as an internal standard, followed by protein precipitation with the use of methanol. LC–tandem MS of all extracts was carried out by using a Shimadzu LC-20AD$_{XR}$ Prominence LC system coupled to a QTRAP5500 mass spectrometer with a Turbo V ion source (Sciex, Framingham, MA, US). Chromatographic separation was achieved on a Phenomenex Kinetex Core Shell C18 (100 × 4.6 mm, 2.6 $\mu$m) LC column with aqueous formic acid (0.05%) and methanol gradient mobile phase. For taurine

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Address correspondence to AKE (e-mail: amany.elshorbagy@alexmed.edu.eg).
Abbreviations used: BCAT, branched-chain aminotransferase; BCKD, branched-chain keto acid dehydrogenase; BCKDHA, branched-chain keto acid dehydrogenase E1$\alpha$ polypeptide; CDO, cysteine dioxygenase; CT, computed tomography; SAA, sulfur-containing amino acid; tCys, total cysteine; tGSH, total glutathione; tHcy, total homocysteine.

analysis, the mobile phase was a gradient of water with formic acid (0.5%) and heptafluorobutyric acid (0.3%), and acetonitrile. Positive-mode multiple reaction monitoring was used for detection. Linear calibration curves of the peak area ratios of analytes and internal standards were used for quantification. CVs for all amino acids were ≤5%, except for taurine, tryptophan, tGSH, and cystathionine (<10%).

*Adipose tissue mRNA expression of selected amino acid metabolic enzymes.* Subcutaneous needle adipose tissue biopsy samples were collected after an overnight fast, and mRNA was extracted as described (33). The expressions of 3 enzymes involved in SAA and BCAA catabolism, namely *CDO1*, *BCAT1*, and BCKD E1$\alpha$ polypeptide (*BCKDHA*), were evaluated in a subset of the study population with adipose tissue samples available at day 0 and day 28 ($n = 20$). Total RNA was extracted from 100–150 mg adipose tissue with the use of TRIzol reagent (Invitrogen). The integrity and concentration of RNA were assessed by spectrophotometry (Nanodrop 2000; Thermo Fisher Scientific). cDNA was synthesized with the use of an Omniscript RT kit (Qiagen), according to kit instructions. We used gene-specific primer probes from Taqman and Taqman universal FAST PCR master mix (Applied Biosystems). The samples were run in duplicate on an ABI Prism 7500 system with internal negative controls, and the threshold cycle (Ct) value for each sample was normalized to the Ct value of $\beta$-actin, which was not different between day 0 and day 28.

*Statistical methods.* Spearman correlations were used to examine relations between amino acids and body-composition variables at days 0, 3, and 28 and to correlate the day 28 changes in insulin and HOMA-IR with amino acid changes. Linear mixed-effects models were used to evaluate the effect of time on anthropometric variables and amino acid concentrations. We separately tested possible interactions by sex and by the magnitude of fat gain at day 28. Low fat gainers were defined as those with fat gain at day 28 relative to baseline below the median value of 1.95 kg, whereas high fat gainers had median or higher fat gain. When a significant interaction (defined as a $P$ value for time × group interaction <0.05) or a trend for interaction (defined as a $P$ value for time × group interaction of 0.05 to <0.1) was observed for amino acid changes, both the results for the total population and those stratified by the relevant interaction factor are shown. Sex distribution and age at baseline were compared in low and high fat gainers by using Fisher's exact test and independent-samples $t$ test, respectively.

For the purpose of evaluating the effect of food overconsumption on adipose tissue enzyme expression, the expression data were multiplied by 100,000 and log-transformed before analysis. We tested for possible interactions by fat gain (median split) on the enzyme mRNA signal by linear mixed modeling as for the amino acid changes. To maximize the use of mRNA data, 1 participant with missing fat mass data at day 28 was allocated to the high fat-gain group on the basis of being in the upper quartile for both weight gain and central fat mass gain. To compare the effect of insulin infusion on serum amino acid concentrations before and after overconsumption, we compared the percentage change in the respective amino acids in response to insulin at day 0 compared with day 28 with the use of a paired-samples $t$ test. All of the analyses were conducted by using PASW Statistics for Mac (23.0; SPSS, Inc.), and $P < 0.05$ was considered significant.

## Results

*Change in body composition and insulin sensitivity.* Body-composition and related data at day 0 and day 28 in the total population and stratified by magnitude of fat gain are presented in Table 1. The study population comprised 40 participants (20 women), with a mean ± SEM age of 36.7 ± 1.9 y. Mean baseline BMI (kg/m$^2$) was 25.6 ± 0.6 (Table 1), with 21 normal-weight participants, 14 overweight participants, and 5 obese participants. During the overconsumption regimen, absolute intakes of protein and carbohydrate increased by approximately one-third, whereas fat intake was doubled. At day 28, participants had gained an average of 2.8 kg, which included 1.8 kg fat mass (Table 1). High fat gainers ($n = 19$) showed an increase of 2.6 kg fat mass compared with 1.1 kg in low gainers ($n = 18$), and also gained more lean mass. At baseline, low and high fat gainers did not significantly differ in their sex distribution, BMI, body fat percentage, lean mass, protein intake, blood glucose, or HOMA-IR, but high gainers had higher intakes of carbohydrates, fat, and total energy (Table 1).

**TABLE 1** Characteristics at baseline (day 0) and after overconsumption of food for 28 d in the total study population and subgroups with low or high fat gain[1]

| | Total population ($n = 40$) | | Low fat gain ($n = 18$) | | High fat gain ($n = 19$) | | $P$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 | Time | Group | Time × group |
| Age, y | 36.7 ± 1.9 | — | 37.5 ± 3.2 | — | 36.8 ± 2.5 | — | — | 0.87[2] | — |
| Female, *n* | 20 | — | 10 | — | 10 | — | — | 0.86[3] | — |
| Weight, kg | 75.3 ± 1.9 | 78.1 ± 1.9 | 75.0 ± 2.7 | 76.6 ± 2.8 | 75.6 ± 2.7 | 80.0 ± 2.8 | <0.001 | 0.64 | <0.001 |
| BMI, kg/m$^2$ | 25.6 ± 0.57 | 26.6 ± 0.58 | 25.9 ± 0.80 | 26.5 ± 0.82 | 25.3 ± 0.80 | 26.6 ± 0.82 | <0.001 | 0.84 | <0.001 |
| Energy intake, kcal/d | 1978 ± 104 | 3084 ± 137 | 1827 | 2754 | 2128 | 3414 | <0.001 | 0.027 | 0.068 |
| Protein intake, g/d | 90 ± 6 | 120 ± 6 | 86 ± 9 | 110 ± 7 | 95 ± 8 | 131 ± 9 | <0.001 | 0.17 | 0.14 |
| Fat intake, g/d | 77 ± 5 | 155 ± 6 | 69 ± 7 | 140 ± 8 | 86 ± 7 | 171 ± 8 | <0.001 | 0.017 | 0.12 |
| Carbohydrate intake, g/d | 210 ± 11 | 282 ± 17 | 195 ± 15 | 245 ± 23 | 226 ± 15 | 320 ± 23 | <0.001 | 0.029 | 0.15 |
| Fat mass, kg | 25.5 ± 1.5 | 27.3 ± 1.5 | 25.6 ± 2.1 | 26.7 ± 2.1 | 25.3 ± 2.1 | 27.9 ± 2.1 | <0.001 | 0.88 | 0.001 |
| Lean mass, kg | 48.1 ± 1.4 | 48.8 ± 1.4 | 47.8 ± 2.1 | 48.1 ± 2.0 | 48.4 ± 2.1 | 49.5 ± 2.0 | 0.001 | 0.73 | 0.049 |
| Central fat, kg | 1.9 ± 0.1 | 2.1 ± 0.1 | 2.0 ± 0.18 | 2.1 ± 0.2 | 1.9 ± 0.2 | 2.1 ± 0.2 | <0.001 | 0.99 | 0.024 |
| Body fat, % | 33.4 ± 1.5 | 34.8 ± 1.4 | 33.9 ± 2.1 | 34.7 ± 2.0 | 32.9 ± 2.1 | 34.6 ± 2.0 | <0.001 | 0.86 | 0.061 |
| Visceral fat area, cm$^2$ | 79 ± 8 | 88 ± 8 | 77 ± 11 | 85 ± 11 | 81 ± 11 | 91 ± 11 | 0.001 | 0.76 | 0.75 |
| Liver density, Hounsfield units | 55.0 ± 1.8 | 52.6 ± 1.8 | 52.2 ± 2.5 | 50.9 ± 2.5 | 57.9 ± 2.5 | 54.3 ± 2.5 | <0.001 | 0.24 | 0.059 |
| Serum LDL cholesterol, mmol/L | 2.8 ± 0.1 | 2.8 ± 0.1 | 2.8 ± 0.2 | 2.7 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.2 | 0.94 | 0.58 | 0.76 |
| Blood glucose, mmol/L | 4.5 ± 0.1 | 4.6 ± 0.1 | 4.4 ± 0.1 | 4.5 ± 0.1 | 4.5 ± 0.1 | 4.6 ± 0.1 | 0.029 | 0.27 | 0.87 |
| Serum insulin, pmol/L | 69.1 ± 3.8 | 78.5 ± 3.5 | 70.6 ± 5.4 | 82.5 ± 5.0 | 67.6 ± 5.4 | 74.5 ± 5.0 | 0.008 | 0.41 | 0.46 |

[1] Values are means ± SEMs unless otherwise indicated. Low fat gainers were defined as those with fat gain (at day 28) below the median value of 1.95 kg, whereas high fat gainers had a median or higher fat gain. Means and $P$ values are from linear mixed models unless otherwise stated.
[2] Independent-samples $t$ test
[3] Fisher's exact test.

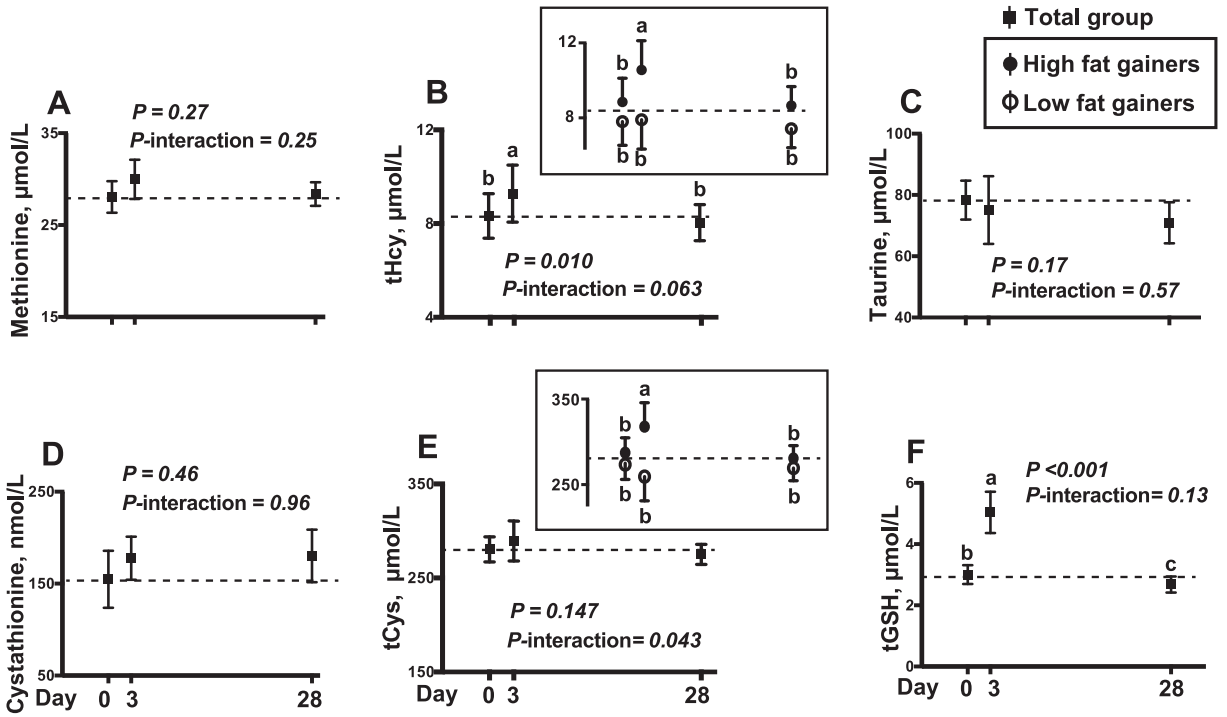


**FIGURE 1** Fasting serum sulfur-containing amino acids (A–E) and tGSH (F) at baseline (day 0) and after 3 and 28 d of food overconsumption in healthy adults. Values are means ± SEMs. *P* values are for the effect of time in the total population ($n = 40$) and the time × fat gain interaction. Where *P*-interaction is <0.1, insets depict amino acid changes separately for low fat gainers ($n = 18$; fat gain <1.95 kg/28 d) and high fat gainers ($n = 19$; fat gain ≥1.95 kg/28 d). Labeled means without a common letter differ, $P < 0.05$. tCys, total cysteine; tGSH, total glutathione; tHcy, total homocysteine.

During the overconsumption period, high fat gainers had a marginally higher increase in energy intake (359 kcal greater increase; *P*-interaction = 0.068), but the interaction for the different macronutrient intakes was not significant (Table 1).

Fasting serum insulin and HOMA-IR increased at day 3 and remained elevated at day 28 (**Supplemental Figure 1**), despite a decline ($P = 0.051$ and 0.036, respectively) relative to day 3 values. These insulin and HOMA-IR changes were similar in high and low fat gainers. As reported previously (31), peripheral insulin sensitivity assessed by the hyperinsulinemic-euglycemic clamp was also decreased at day 28 (−8%; $P = 0.03$), with no difference between high and low fat gainers (*P*-interaction = 0.29).

*Changes in SAAs.* Figure 1 shows the SAA changes in response to food overconsumption. Methionine, cystathionine, and taurine did not change in response to overconsumption either in the total group or in low or high fat gainers (Figure 1A, C, D). Fasting serum tCys showed a transient 11% increase at day 3 of the overconsumption regimen in high but not low fat gainers (*P*-interaction = 0.043), and returned to basal concentrations by day 28. tHcy transiently increased in the total population, with a trend for interaction by fat gain (*P*-interaction = 0.063) (Figure 1B, E). tGSH increased at day 3, but by day 28 had declined to below baseline concentrations (Figure 1F).

*Changes in BCAAs and aromatic amino acids.* The BCAAs isoleucine and valine acutely increased at day 3 (by 17% and 22%, respectively; $P \leq 0.002$ for both) and remained significantly elevated at day 28 (both $P < 0.001$) despite a decline ($P = 0.019$) in valine from day 3 concentrations (**Figure 2**A, C). The changes in leucine (Figure 2B) were modest ($P = 0.088$). Total BCAAs in serum (the sum of leucine, isoleucine, and valine) increased from 397 ± 10 µmol/L at day 0, to 456 ± 21 µmol/L at day 3, and 428 ± 9 µmol/L at day 28 ($P = 0.004$ and $P < 0.001$, respectively, relative to day 0). No significant difference was observed between high and low fat gainers in the changes in individual (Figure 2) or total (data not shown) BCAAs.

Among the aromatic amino acids, both tyrosine and tryptophan were transiently increased at day 3 in the total population (Figure 2E, F). Serum tryptophan additionally showed a significant interaction according to fat gain, increasing at day 3 (+17%; $P < 0.001$) only in high but not low fat gainers. Phenylalanine was transiently decreased at day 3 (Figure 2D).

*Correlations of amino acids with metabolic and anthropometric variables.* The correlations between serum amino acids and available body-composition variables at days 0, 3, and 28 are shown in **Supplemental Tables 1–3**. At day 0, fasting serum tCys, tHcy, and cystathionine correlated with adiposity and insulin resistance markers. The correlations were weaker for BCAAs and, with a few exceptions, did not reach significance. Tryptophan uniquely showed a strong positive correlation with lean mass (Spearman's $\rho = 0.60$, $P < 0.001$), and an inverse correlation with body fat percentage. At day 3, however, no significant associations were noted for any amino acid with BMI or HOMA-IR, except for cystathionine with BMI (Supplemental Table 2). At day 28, tCys correlated with adiposity variables (Supplemental Table 3). In addition, isoleucine, leucine, valine, phenylalanine, and tyrosine correlated with waist circumference, and tyrosine, valine, and tHcy correlated with insulin resistance measures.

No significant correlations were noted at day 3 or day 28 between Δ amino acids (relative to day 0) and Δ insulin or HOMA-IR (**Supplemental Table 4**). There were similarly no

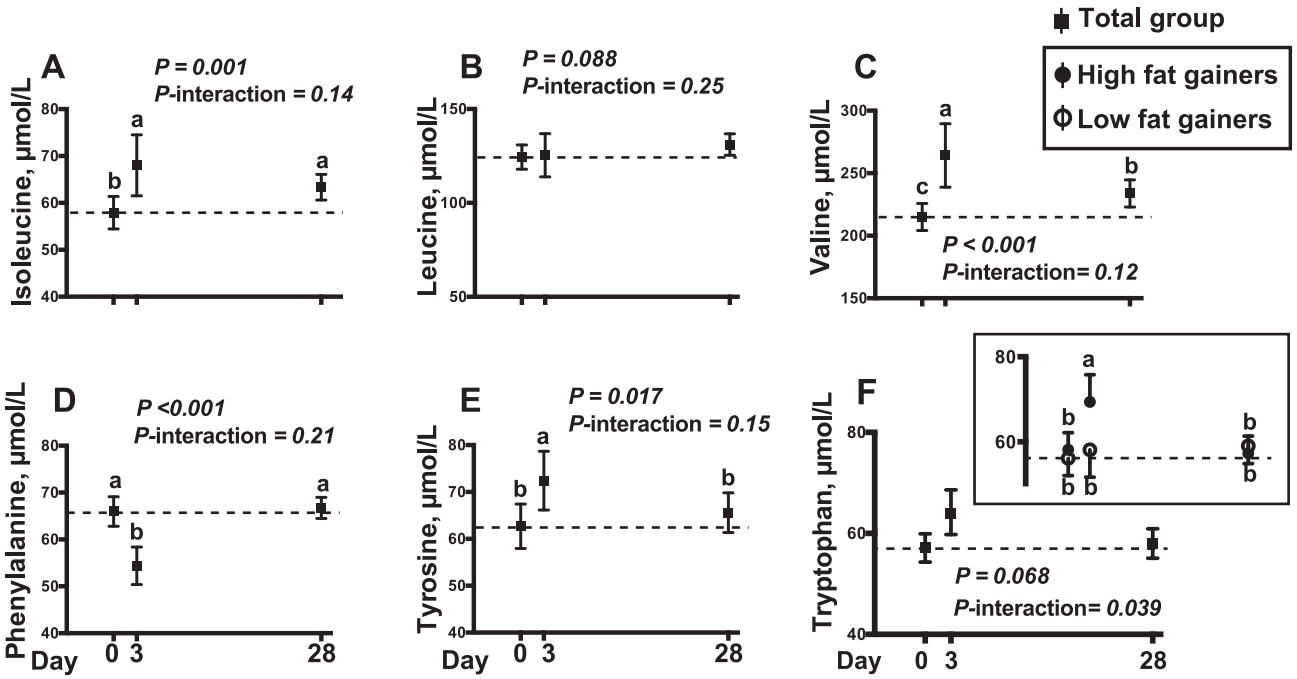


**FIGURE 2** Fasting serum BCAAs (A–C) and aromatic amino acids (D–F) at baseline (day 0) and after 3 and 28 d of food overconsumption in healthy adults. Values are means ± SEMs. *P* values are for the effect of time in the total population ($n = 40$) and for the time × fat gain interaction. Where *P*-interaction is <0.1, insets depict amino acid changes separately for low fat gainers ($n = 18$; fat gain <1.95 kg/28 d) and high fat gainers ($n = 19$; fat gain ≥1.95 kg/28 d). Labeled means without a common letter differ, $P < 0.05$.

significant correlations of Δ amino acids at day 28 (relative to day 0) with Δ fat mass or Δ protein intake, apart from a positive correlation of Δ isoleucine with Δ protein intake.

*Adipose tissue mRNA expression of selected amino acid metabolic enzymes.* We assayed subcutaneous adipose tissue mRNA expression of the cysteine catabolic enzyme CDO and the BCAA catabolic enzymes BCKD and BCAT at day 0 and day 28 of the overconsumption regimen. There was a trend ($P = 0.054$) toward induction of adipose tissue *CDO1* mRNA in the total population (**Figure 3**A). In analysis stratified by fat gain, a 26% increase in *CDO1* expression was observed in high fat gainers ($P = 0.025$) but not in low fat gainers (*P*-interaction = 0.045). Overconsumption did not alter the expression of *BCKDHA* or *BCAT1* (Figure 3B, C).

*Effect of insulin infusion on serum amino acid concentrations before and after the overconsumption regimen.* At day 0, insulin infusion lowered the serum concentrations of all amino acids (−11% to −47%), except for tCys and tHcy, which did not change, and tGSH, which increased by 53% ($P < 0.001$). The largest insulin-induced decline was observed for leucine and isoleucine, which were decreased by >45% ($P < 0.001$).

At day 28, insulin triggered elevation of serum tCys (+8%; $P = 0.014$ compared with day 0; **Figure 4**). Overconsumption did not significantly impair insulin-induced suppression of the remaining amino acids, except for phenylalanine and taurine. Serum tGSH was elevated similar to before overconsumption (Figure 4).

## Discussion

BCAAs, aromatic amino acids, and cysteine are elevated in individuals with obesity and insulin resistance, but our understanding of the time course of this change is limited. In the present study, short-term food overconsumption triggered rapid and sustained elevation of BCAAs in healthy adults, without impairing insulin suppression of BCAAs. tCys and tHcy also showed transient elevations in those who ultimately had higher fat gain, along with induction of adipose tissue expression of the cysteine catabolic enzyme CDO. This study suggests that elevations in BCAAs are an early event during increased energy intake that occurs before significant impairment of insulin-induced suppression of amino acids. The study also extends epidemiologic evidence linking plasma tCys to human obesity.

*BCAAs and aromatic amino acids.* Plasma BCAAs, and to a lesser extent aromatic amino acids, predict the risk of future diabetes (3, 34), metabolic syndrome (35), and cardiovascular disease (36). Yet, acute studies suggest that BCAA infusion does not impair glucose homeostasis in humans (37). Furthermore, chronic oral BCAA supplementation did not alter HOMA-IR, despite increasing plasma BCAAs (38). These studies suggest that BCAAs are not causal in the development of insulin resistance. However, these studies did not involve a key factor underlying obesity, namely nutrient overconsumption.

In the present study, energy overconsumption including a 33% increase in protein intake was associated with a rapid and sustained increase in BCAAs and induction of insulin resistance by HOMA-IR and by hyperinsulinemic-euglycemic clamp. Moreover, the concentrations of isoleucine, valine, and tyrosine appeared to track changes in HOMA-IR, increasing at day 3 and partly declining by day 28. One factor that could argue against the increase in BCAAs and aromatic amino acids being caused by insulin resistance is the effect of insulin infusion on the amino acid concentrations. Although food overconsumption significantly impaired peripheral insulin sensitivity during the hyperinsulinemic-euglycemic clamp, it did not impair insulin-induced suppression of BCAAs. The

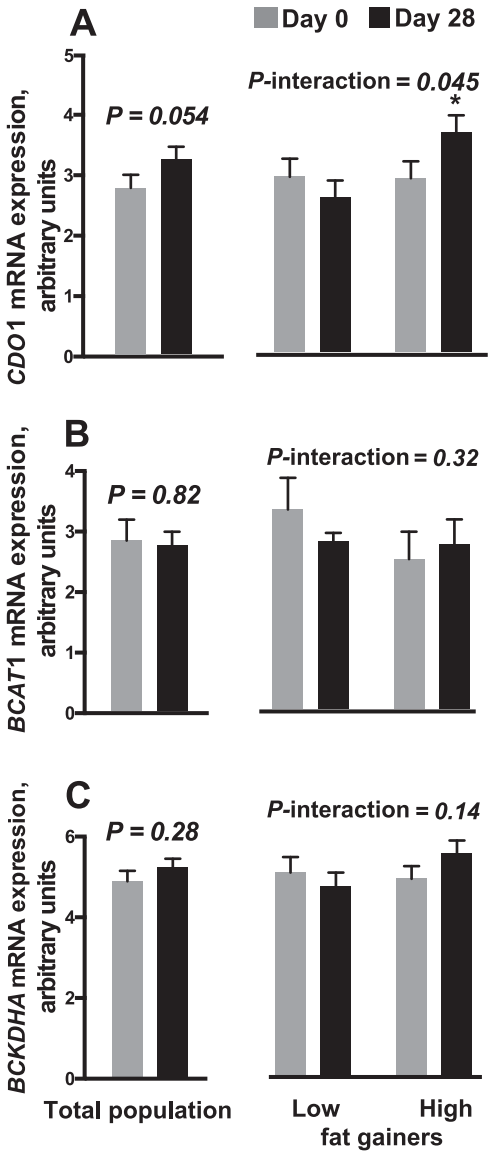


**FIGURE 3** Adipose tissue mRNA expression of *CDO1* (A), *BCAT1* (B), and *BCKDHA* (C) in a subset ($n = 20$) of the total population (left side of panel) and separately (right side of panel) in low fat gainers ($n = 10$; fat gain <1.95 kg/28 d) compared with high fat gainers ($n = 10$; fat gain ≥1.95 kg/28 d) at baseline (day 0) and after 28 d of food overconsumption. Values are means ± SEMs, calculated by linear mixed models with the use of log-transformed data. *P* values are for the effect of time in the total population and for the time × fat gain interaction. *Different from day 0, $P < 0.05$. *BCAT1*, branched-chain aminotransferase-1; *BCKDHA*, branched-chain keto acid dehydrogenase E1$\alpha$ polypeptide; *CDO1*, cysteine dioxygenase-1.

diet-induced elevation in BCAAs, or their metabolites, such as 3-hydroxyisobutyrate, may have contributed to the development of insulin resistance. 3-Hydroxyisobutyrate is a product of valine catabolism that has been recently implicated in the pathogenesis of insulin resistance triggered by high protein intake (39) via increasing FA uptake by skeletal muscle (40).

BCAA elevation in longstanding obesity is believed to result from decreased oxidation of BCAAs due to a proinflammatory shift, and increased muscle protein breakdown, secondary to insulin resistance (41). A vicious circle whereby BCAAs decrease insulin sensitivity, which, in turn, impairs BCAA catabolism cannot be excluded. Adipose tissue *BCAT1* and *BCKDHA* expressions were not altered by overconsumption. Although this implies that the impairment of BCAA catabolism only occurs late in established obesity, muscle is a quantitatively greater contributor to BCAA catabolism (4) and was not assessed. Overall, this study shows that BCAA changes are an early event during nutrient overload and weight gain, before significant impairment of insulin-induced suppression of amino acids, and hence are unlikely to be triggered solely by insulin resistance.

Phenylalanine decreased at day 3, concomitant with the increase in tyrosine. This likely reflects induction of the phenylalanine hydroxylase enzyme responsible for conversion of phenylalanine to tyrosine, as occurs in animals in response to increased dietary protein (42, 43). These findings show the importance of diet in determining circulating BCAA and aromatic amino acid concentrations, at least partly independently of obesity and insulin resistance.

*SAAs.* In contrast to the rapid increase in BCAAs and aromatic amino acids, serum methionine, cystathionine, and taurine were not altered by overconsumption, despite an average increase in protein intake from 90 g to 120 g/d. The stability of methionine may result from conversion to *S*-adenosylmethionine, which did increase in response to overconsumption (44). tGSH increased acutely at day 3, but decreased below baseline values at day 28, as is often reported in individuals with obesity (24, 45).

tCys and tHcy increased transiently at day 3 in high fat gainers, who showed a 26% increase in adipose tissue *CDO1* expression at day 28. CDO oxidizes cysteine to cysteine-sulfinic acid, which ultimately forms either taurine or pyruvate+sulfate. In mouse tissues, CDO expression is highest in liver and adipose tissue (30, 46) and is dramatically upregulated in both tissues in response to increased dietary protein or SAA intake (30). The acute *CDO1* induction observed in overfed participants at day 3 may explain the return of tCys to baseline values by day 28 via enhanced cysteine catabolism. In this case, taurine may have been expected to increase, which did not occur. However, taurine is mainly an intracellular amino acid and it is questionable how far the serum concentrations reflect whole-body taurine status (47).

*CDO1* induction specifically in high fat gainers is interesting in view of the relation of CDO with adipogenesis. In 3T3-L1 cells, *Cdo1* expression increased by 6- to 9-fold during differentiation from preadipocytes to adipocytes (46, 48) and is required for adipogenesis and lipid accumulation via interaction with PPAR-$\gamma$ (49). Recent data show that cysteine concentrations positively affect preadipocyte differentiation and lipid accumulation (50) and *Cdo1* mRNA expression in 3T3-L1 cells (N Haj-Yasein, Institute of Basic Medical Sciences, University of Oslo; personal communication, 2018) in a dose-dependent manner. An effect of CDO on fat cell size is yet to be shown. Thus, a possible interpretation of our findings is that *CDO1* induction in participants with acute tCys elevation is causally linked to higher fat gain via enhancing adipogenesis. Adipogenesis was not assessed in the current study, but the previously reported lack of detectable increase in fat cell size (33) suggests that the fat mass expansion after overnutrition is at least partly explained by adipogenesis and increased adipocyte number. Given the additional limitations of the small sample size of the present study, and the lack of a control group, the finding of *CDO1* induction with fat gain warrants replication and dissection of the mechanisms involved.

Given the transient increase in tCys, these results cannot explain the longstanding elevation of tCys observed in individuals with obesity (12–14). We speculate that long-term obesity

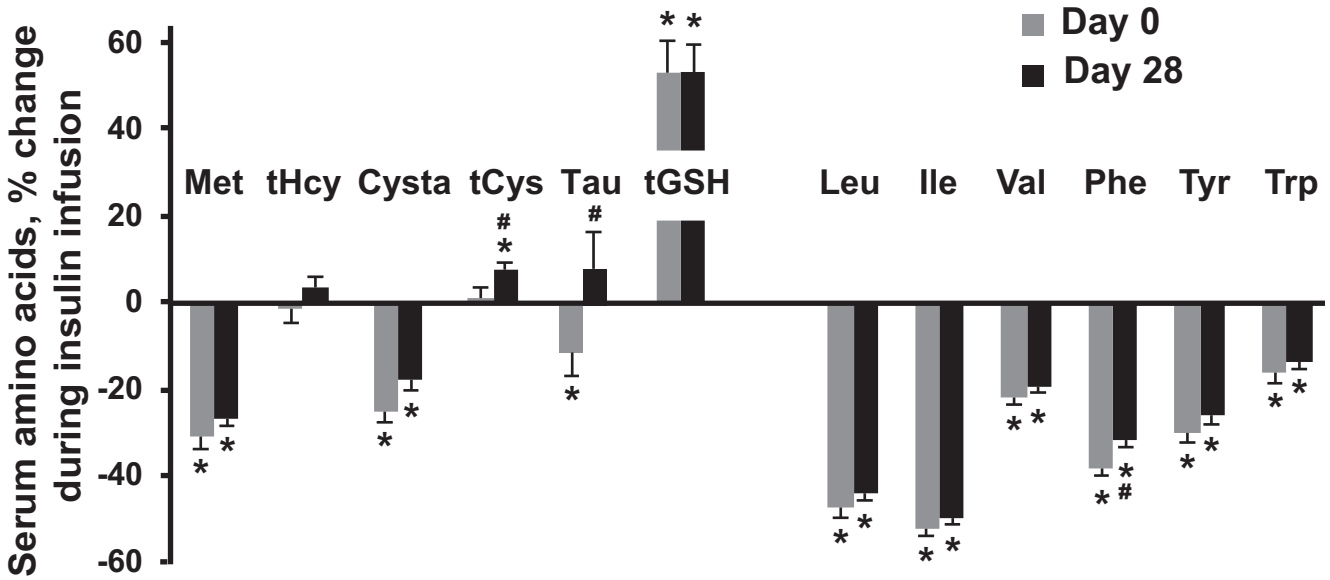


**FIGURE 4** Percentage change in serum amino acid concentrations in the total population in response to insulin infusion at baseline (day 0) and after 28 d of food overconsumption. Values are means ± SEMs, $n = 40$. *Pre- and postinsulin amino acid concentrations at the specified time point (day 0 or 28) differ by paired-samples $t$ test, $P < 0.05$; #amino acid percentage change differs from day 0 by paired-samples $t$ test, $P < 0.05$. Cysta, cystathionine; tCys, total cysteine; tGSH, total glutathione; tHcy, total homocysteine.

is characterized by a return of CDO concentrations to baseline and hence a rebound increase in tCys. Alternatively, high tCys in obese individuals may be linked to hyperinsulinemia. Circulating tCys shows strong positive correlations with serum insulin in this and other (24) populations. In the present study, supraphysiologic infusion of insulin did not affect serum tCys at baseline, but increased tCys by ~8% after overconsumption. These data raise the possibility that hyperinsulinemia and nutrient oversupply, which coexist in the obese, insulin-resistant state, act in concert via an unknown mechanism to increase serum tCys.

*Conclusions.* In summary, a 28-d food-overconsumption regimen in healthy adults produced rapid and sustained elevation of fasting serum BCAAs and induced insulin resistance. Transient elevations in tCys were observed in high fat gainers, coupled with adipose tissue CDO induction. Overconsumption also altered the response of serum tCys to insulin infusion. Our data provide insight into the role played by overnutrition in modulating the metabolism and serum concentrations of amino acids that are frequently elevated in obesity.


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## References


1. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9(4):311–26.
2. Wurtz P, Soininen P, Kangas AJ, Ronnemaa T, Lehtimaki T, Kahonen M, Viikari JS, Raitakari OT, Ala-Korpela M. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes Care 2013;36(3):648–55.
3. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011;17(4):448–53.
4. Brosnan JT, Brosnan ME. Branched-chain amino acids: enzyme and substrate regulation. J Nutr 2006;136(1 Suppl):207S–11S.
5. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol Endocrinol Metab 2007;293(6):E1552–63.
6. Naukkarinen J, Heinonen S, Hakkarainen A, Lundbom J, Vuolteenaho K, Saarinen L, Hautaniemi S, Rodriguez A, Fruhbeck G, Pajunen P, et al. Characterising metabolically healthy obesity in weight-discordant monozygotic twins. Diabetologia 2014;57(1):167–76.
7. Schmidt JA, Rinaldi S, Scalbert A, Ferrari P, Achaintre D, Gunter MJ, Appleby PN, Key TJ, Travis RC. Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. Eur J Clin Nutr 2016;70(3):306–12.
8. Elshorbagy A, Jerneren F, Basta M, Basta C, Turner C, Khaled M, Refsum H. Amino acid changes during transition to a vegan diet supplemented with fish in healthy humans. Eur J Nutr 2017;56(5):1953–62.
9. Rosell M, Appleby P, Spencer E, Key T. Weight gain over 5 years in 21,966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. Int J Obes (Lond) 2006;30(9):1389–96.
10. Bujnowski D, Xun P, Daviglus ML, Van Horn L, He K, Stamler J. Longitudinal association between animal and vegetable protein intake and obesity among men in the United States: the Chicago Western Electric Study. J Am Diet Assoc 2011;111(8):1150–5, e1.
11. Sluijs I, Beulens JW, van der, D, Spijkerman AM, Grobbee DE, van der Schouw YT. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. Diabetes Care 2010;33(1):43–8.
12. Berger M, Zimmermann-Telschow H, Berchtold P, Drost H, Muller WA, Gries FA, Zimmermann H. Blood amine acid levels in patients with

insulin excess (functioning insulinoma) and insulin deficiency (diabetic ketosis). Metabolism 1978;27(7):793–9.
13. Fukagawa NK, Minaker KL, Young VR, Rowe JW. Insulin dose-dependent reductions in plasma amino acids in man. Am J Physiol 1986;250(1 Part 1):E13–7.
14. Marchesini G, Bianchi G, Rossi B, Muggeo M, Bonora E. Effects of hyperglycaemia and hyperinsulinaemia on plasma amino acid levels in obese subjects with normal glucose tolerance. Int J Obes Relat Metab Disord 2000;24(5):552–8.
15. Forlani G, Vannini P, Marchesini G, Zoli M, Ciavarella A, Pisi E. Insulin-dependent metabolism of branched-chain amino acids in obesity. Metabolism 1984;33(2):147–50.
16. Bianchi G, Marchesini G, Brunetti N, Manicardi E, Montuschi F, Chianese R, Zoli M. Impaired insulin-mediated amino acid plasma disappearance in non-alcoholic fatty liver disease: a feature of insulin resistance. Dig Liver Dis 2003;35(10):722–7.
17. Adams SH. Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. Adv Nutr 2011;2(6): 445–56.
18. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 2014;10(12):723–36.
19. Irving BA, Carter RE, Soop M, Weymiller A, Syed H, Karakelides H, Bhagra S, Short KR, Tatpati L, Barazzoni R, et al. Effect of insulin sensitizer therapy on amino acids and their metabolites. Metabolism 2015;64(6):720–8.
20. Lips MA, Van Klinken JB, van Harmelen V, Dharuri HK, t Hoen PA, Laros JF, van Ommen GJ, Janssen IM, Van Ramshorst B, Van Wagensveld BA, et al. Roux-en-Y gastric bypass surgery, but not calorie restriction, reduces plasma branched-chain amino acids in obese women independent of weight loss or the presence of type 2 diabetes. Diabetes Care 2014;37(12):3150–6.
21. Laferrere B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B, Bose M, Teixeira J, Stevens RD, Wenner BR, et al. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. Sci Transl Med 2011;3(80):80re2.
22. Elshorbagy AK, Nurk E, Gjesdal CG, Tell GS, Ueland PM, Nygard O, Tverdal A, Vollset SE, Refsum H. Homocysteine, cysteine, and body composition in the Hordaland Homocysteine Study: does cysteine link amino acid and lipid metabolism? Am J Clin Nutr 2008;88(3):738–46.
23. Elshorbagy AK, Refsum H, Smith AD, Graham IM. The association of plasma cysteine and gamma-glutamyltransferase with BMI and obesity. Obesity (Silver Spring) 2009;17(7):1435–40.
24. Elshorbagy AK, Valdivia-Garcia M, Refsum H, Butte N. The association of cysteine with obesity, inflammatory cytokines and insulin resistance in Hispanic children and adolescents. PLoS One 2012;7(9):e44166.
25. Aasheim ET, Elshorbagy AK, Diep LM, Sovik TT, Mala T, Valdivia-Garcia M, Olbers T, Bohmer T, Birkeland KI, Refsum H. Effect of bariatric surgery on sulphur amino acids and glutamate. Br J Nutr 2011;106(3):432–40.
26. Elshorbagy AK. Body composition in gene knockouts of sulfur amino acid-metabolizing enzymes. Mamm Genome 2014;25(9):255–63.
27. Elshorbagy AK, Smith AD, Kozich V, Refsum H. Cysteine and obesity. Obesity (Silver Spring) 2012;20(3):473–81.
28. Elshorbagy AK, Kozich V, Smith AD, Refsum H. Cysteine and obesity: consistency of the evidence across epidemiologic, animal and cellular studies. Curr Opin Clin Nutr Metab Care 2012;15(1):49–57.
29. Elshorbagy AK, Valdivia-Garcia M, Mattocks DA, Plummer JD, Smith AD, Drevon CA, Refsum H, Perrone CE. Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase. J Lipid Res 2011;52(1):104–12.
30. Stipanuk MH, Ueki I, Dominy JE Jr., Simmons CR, Hirschberger LL. Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. Amino Acids 2009;37(1):55–63.
31. Samocha-Bonet D, Campbell LV, Viardot A, Freund J, Tam CS, Greenfield JR, Heilbronn LK. A family history of type 2 diabetes increases risk factors associated with overfeeding. Diabetologia 2010;53(8):1700–8.
32. Antoniades C, Shirodaria C, Leeson P, Baarholm OA, Van-Assche T, Cunnington C, Pillai R, Ratnatunga C, Tousoulis D, Stefanadis C, et al. MTHFR 677 C>T polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. Circulation 2009;119(18):2507–15.
33. Tam CS, Viardot A, Clement K, Tordjman J, Tonks K, Greenfield JR, Campbell LV, Samocha-Bonet D, Heilbronn LK. Short-term overfeeding may induce peripheral insulin resistance without altering subcutaneous adipose tissue macrophages in humans. Diabetes 2010;59(9):2164–70.
34. Tillin T, Hughes AD, Wang Q, Wurtz P, Ala-Korpela M, Sattar N, Forouhi NG, Godsland IF, Eastwood SV, McKeigue PM, et al. Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. Diabetologia 2015;58(5):968–79.
35. Yamakado M, Nagao K, Imaizumi A, Tani M, Toda A, Tanaka T, Jinzu H, Miyano H, Yamamoto H, Daimon T, et al. Plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population. Sci Rep 2015;5:11918.
36. Magnusson M, Lewis GD, Ericson U, Orho-Melander M, Hedblad B, Engstrom G, Ostling G, Clish C, Wang TJ, Gerszten RE, et al. A diabetes-predictive amino acid score and future cardiovascular disease. Eur Heart J 2013;34(26):1982–9.
37. Everman S, Mandarino LJ, Carroll CC, Katsanos CS. Effects of acute exposure to increased plasma branched-chain amino acid concentrations on insulin-mediated plasma glucose turnover in healthy young subjects. PLoS One 2015;10(3):e0120049.
38. Kitajima Y, Takahashi H, Akiyama T, Murayama K, Iwane S, Kuwashiro T, Tanaka K, Kawazoe S, Ono N, Eguchi T, et al. Supplementation with branched-chain amino acids ameliorates hypoalbuminemia, prevents sarcopenia, and reduces fat accumulation in the skeletal muscles of patients with liver cirrhosis. J Gastroenterol 2018;53(3):427–37.
39. Harris LLS, Smith GI, Patterson BW, Ramaswamy RS, Okunade AL, Kelly SC, Porter LC, Klein S, Yoshino J, Mittendorfer B. Alterations in 3-hydroxyisobutyrate and FGF21 metabolism are associated with protein ingestion-induced insulin resistance. Diabetes 2017;66(7): 1871–8.
40. Jang C, Oh SF, Wada S, Rowe GC, Liu L, Chan MC, Rhee J, Hoshino A, Kim B, Ibrahim A, et al. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. Nat Med 2016;22(4):421–6.
41. Nagao K, Yamakado M. The role of amino acid profiles in diabetes risk assessment. Curr Opin Clin Nutr Metab Care 2016;19(5):328–35.
42. Donlon J, Beirne E. Modulations of rat hepatic phenylalanine hydroxylase due to induced diabetes or high-protein diet. Biochem Biophys Res Commun 1982;108(2):746–51.
43. Crawford RD, Layne EC, Bessman SP. Phenylalanine hydroxylase isozymes in regenerating liver: effects of diet and age. Biochem Med 1985;33(1):77–89.
44. Elshorbagy AK, Jerneren F, Samocha-Bonet D, Refsum H, Heilbronn LK. Serum S-adenosylmethionine, but not methionine, increases in response to overfeeding in humans. Nutr Diabetes 2016;6:e192.
45. Di Renzo L, Galvano F, Orlandi C, Bianchi A, Di Giacomo C, La Fauci L, Acquaviva R, De Lorenzo A. Oxidative stress in normal-weight obese syndrome. Obesity (Silver Spring) 2010;18(11):2125–30.
46. Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, Ezaki O. Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 2006;147(7):3276–84.
47. Rakotoambinina B, Marks L, Badran AM, Igliki F, Thuillier F, Crenn P, Messing B, Darmaun D. Taurine kinetics assessed using [1,2-13C2]taurine in healthy adult humans. Am J Physiol Endocrinol Metab 2004;287(2):E255–62.
48. Ueki I, Stipanuk MH. 3T3-L1 adipocytes and rat adipose tissue have a high capacity for taurine synthesis by the cysteine dioxygenase/cysteinesulfinate decarboxylase and cysteamine dioxygenase pathways. J Nutr 2009;139(2):207–14.
49. Deng P, Chen Y, Ji N, Lin Y, Yuan Q, Ye L, Chen Q. Cysteine dioxygenase type 1 promotes adipogenesis via interaction with peroxisome proliferator-activated receptor gamma. Biochem Biophys Res Commun 2015;458(1):123–7.
50. Haj-Yasein NN, Berg O, Jerneren F, Refsum H, Nebb HI, Dalen KT. Cysteine deprivation prevents induction of peroxisome proliferator-activated receptor gamma-2 and adipose differentiation of 3T3-L1 cells. Biochim Biophys Acta 2017;1862(6):623–35.