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Alterations of plasma metabolite profiles related to adipose tissue distribution and cardiometabolic risk

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Running Head: Branched-chain amino acids and visceral obesity

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ABSTRACT

Context. Metabolomic profiling of obese individuals revealed altered concentrations of many metabolites, especially branched-chain amino acids (BCAA), possibly linked to altered adipose tissue BCAA catabolism. **Objective**. We tested the hypothesis that some features of this metabolite signature relate closely to visceral obesity and concomitant alterations in cardiometabolic risk factors. We also postulated that alterations in BCAA-catabolizing enzymes are predominant in visceral adipose tissue. **Methods**. Fifty-nine women (BMI 20-41 kg/m^2) undergoing gynecologic surgery were recruited and characterized for overall and regional adiposity, blood metabolite levels using targeted metabolomics and cardiometabolic risk factors. Adipose samples (visceral and subcutaneous) were obtained and used for gene expression and western blot analyses. **Results.** Obese women had significantly higher circulating BCAA and Kynurenine/Tryptophan (KYN/Trp) ratio than lean or overweight women ($p<0.01$). Principal component analysis confirmed that factors related to AA and the KYN/Trp ratio were positively associated with BMI, fat mass, visceral or subcutaneous adipose tissue area and subcutaneous adipocyte size ($p\leq0.05$). AA-related factor was positively associated with HOMA-IR ($p\leq0.01$). Factors reflecting glycerophospholipids and sphingolipids levels were mostly associated with altered blood lipid concentrations ($p\leq0.05$). Glutamate level was the strongest independent predictor of visceral adipose tissue area ($r=0.46$, $p<0.001$). Obese women had lower expression and protein levels of BCAA-catabolizing enzymes in visceral adipose tissue compared to overweight or lean women ($p\leq0.05$). **Conclusions**. Among metabolites altered in obesity, plasma concentrations of BCAA and the KYN/Trp ratio are closely related to increased adiposity. Alterations in expression and protein levels of BCAA-catabolizing enzymes are predominant in visceral adipose tissue.

Keywords: visceral obesity, branched-chain amino acids, cardiometabolic risk factors

INTRODUCTION

Visceral obesity is associated with accumulation of triglycerides in ectopic tissues or organs and development of comorbid conditions such as dyslipidemia, high blood pressure, type 2 diabetes and increased cardiovascular disease risk (47). Metabolomics may offer a broader insight into our understanding of the metabolic alterations related to central distribution of fat in obesity (58).

Several metabolites have been examined as potential biomarkers of obesity and cardiometabolic alterations. Phosphatidylcholine (PC) is the main circulating phospholipid and is mostly found on HDL particles; it plays a role in the regulation of circulating lipoprotein levels, especially VLDL (7). Plasma PC levels were found to be altered in the obese state and may be involved in obesity-related hepatic steatosis (49). Sun et al. observed high blood PC levels as well as elevated cholesterol and triglyceride concentrations in pigs fed a high fat diet without weight gain compared to animals fed a control diet (43). Lysophosphatidylcholines (lysoPC) are hydrolyzed derivatives of PC, and studies have demonstrated that these compounds play a role in the development of atherosclerosis and hyperlipidemia (27, 30). They are components of oxidized LDL cholesterol particles, which are related to the risk of cardiovascular disease (16, 26). Sphingolipids, particularly sphingomyelins (SM), are ceramide precursors and usually co-localize with cholesterol on cell membranes and LDL surface (21). Evidence suggests that ceramide concentrations are associated with insulin resistance (12, 21, 46). The ratio of kynurenine to tryptophan levels is an increasingly recognized marker of inflammation and metabolic alterations which has been shown to be high in adult overweight or obese individuals (24, 53), and also appears to be related to waist circumference independent of age (24).

Many studies also focused on amino acids (AA) in the context of obesity (38, 44, 50, 54). Most reported elevated plasma levels of branched-chain amino acids (BCAA, leucine, isoleucine, valine) in obese children, adolescents and adults compared to their lean counterparts (1, 5, 18, 29, 32, 33, 50, 58), and weight loss was associated with lower BCAA levels (39). Circulating alanine, phenylalanine, tyrosine,

glutamate/glutamine, aspartate/asparagine and arginine have also been shown to be increased in obese individuals (33). Significantly higher plasma BCAA, aromatic amino acids (AAA), C3 and C5 acylcarnitine levels are also found among metabolically unwell versus metabolically well individuals, independent of BMI (3). Results regarding alterations in other AA remain inconsistent (3, 18, 33).

BCAA catabolism occurring in peripheral tissues involves their initial, reversible transformation into α-ketoacids by branched-chain amino acid aminotransferase (BCATm), followed by irreversible decarboxylation of these compounds to acyl-CoA esters by the branched-chain ketoacid dehydrogenase complex (BCKDC) (15). Branched-chain ketoacid dehydrogenase kinase (BCKDK) acts as an inhibitor of BCKDC through phosphorylation (15) which can be reversed by branched-chain ketoacid dehydrogenase phosphatase (PP2Cm) (59). Obesity-related increases in circulating BCAA and other AA could be due to a defect in BCAA-catabolizing enzymes in adipose tissue (15, 19).

While many studies have focused on plasma metabolite alterations in obesity, insulin resistance and type 2 diabetes, only a few studies have considered body fat distribution, other markers of cardiometabolic risk (13, 44, 55) and possible depot-specific alterations in BCAA-catabolizing enzymes (15, 19). In addition, only one study has performed metabolomics-based AA analysis including human visceral and subcutaneous adipose tissue from non-obese and obese individuals (13). Thus, we attempted to identify which features of the plasma metabolite signature of obesity were related to visceral obesity and cardiometabolic risk factors, and assessed possible depot-specific alterations in expression and protein level of BCAA-catabolizing enzymes in visceral and subcutaneous adipose tissue. We tested the hypothesis that some features of the obesity metabolite signature relate closely to visceral obesity and concomitant alterations in cardiometabolic risk factors. We also postulated that alterations in BCAA-catabolizing enzymes are especially apparent in visceral adipose tissue.

SUBJECTS AND METHODS

Subjects

Women were recruited at the Gynecology Unit of CHU de Quebec. The project was approved by the Medical Ethics Committee of the Institution. Written, informed consent was obtained from all prospective participants before they were included in the study. None of the women quit the study. We selected patients for whom blood samples had never been thawed and for whom visceral and subcutaneous adipose tissue samples as well as axial computed tomography data were available. A sample of 59 healthy women aged 37.7 to 59.4 years was studied. Menopausal status could be determined for 56 women and was based on FSH level, reported presence/absence of menstrual bleeding and regularity of the menstrual cycle. A total of 39 women were considered pre- or perimenopausal and n=17 were considered postmenopausal. Women elected for total (n=56) or subtotal (n=1) abdominal hysterectomies, some with salpingo-oophorectomy of 1 (n=12) or 2 (n=16) ovaries. Three women underwent surgery for myomectomy by laparotomy or cauterization of endometrial lesions. Characteristics of the women included in this study are shown in **Table 1**.

Body fatness and body fat distribution measurements

These data were collected a few days prior to or on the morning of surgery. Dual-energy x-ray absorptiometry was performed with a Hologic QDR-4500A densitometer and the Whole-body fan beam software V8.269:3 (Hologic, Bedford, MA) was used to measure total body fat mass, fat percentage and lean body mass. Visceral and subcutaneous adipose tissue cross-sectional areas were measured by computed tomography with a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI). To perform the test, women had to lie in the supine position, their arms stretched above the head. To determine scanning position, a scout image of the body was used and images were obtained at the level of the L4-L5 vertebrae. To obtain visceral adipose tissue area, the intra-abdominal cavity was delineated using the ImageJ 1.33u software (National Institutes of Health, Bethesda, MD) at the internal-most aspect of the abdominal and oblique muscle walls around the cavity as well as the posterior aspect of

the vertebral body. The area of subcutaneous adipose tissue was then calculated by subtraction of measured visceral adipose tissue area from total adipose tissue area at L4-L5. Highlighting and computation of adipose tissue areas were performed within the attenuation range of −190 to −30 Hounsfield units. All images were analyzed by the same observer.

Plasma lipid and lipoprotein measurements

On the morning of surgery, 12-hour fasting blood samples were obtained. Cholesterol and triglyceride levels in plasma and lipoprotein fractions were measured with the Olympus AU400 (Beckman Coulter, Mississauga, Canada). Ultracentrifugation (14) was performed to isolate VLDLs. For the HDL fraction, heparin and $MnCl_2$ (11) were added to the infranatant to precipitate LDLs. Lipid concentration in this lipoprotein fraction was obtained by difference. Concentrations of apolipoprotein B and A1 were measured using the Siemens Healthcare Diagnostics BN ProSpec (Siemens Healthcare Diagnostics, Mississauga, Canada).

Glucose homeostasis, adipokines and inflammatory markers

The glucose oxidase method was used to assess plasma glucose. Plasma insulin levels were measured by ELISA (Millipore, Billerica, MA or ALPCO, Salem, NH). These data were then used to calculate the homeostasis model assessment (HOMA) insulin resistance index (28). Plasma IL-6 and TNF-α concentrations were measured with assay kits from R&D Systems (Minneapolis, MN); plasma adiponectin levels were assessed with an assay kit from B-Bridge International (Cupertino, CA) and leptin levels were measured with an assay kit from EMD Millipore (Billerica, MA).

Adipose tissue sampling and adipocyte isolation

Subcutaneous adipose tissue samples were collected at the site of surgical incision (lower abdomen) and visceral adipose tissue samples were taken from the distal portion of the greater omentum. They were

158 immediately carried to the laboratory and a portion of fresh tissue was kept for adipocyte isolation and
159 cell sizing as previously described (31).
160
161 ***Metabolomics analysis***
162 Targeted metabolomics analysis in plasma samples was performed at the Helmholtz Zentrum München,
163 Institute of Experimental Genetics, Genome Analysis Center in Neuherberg, Germany. Metabolites were
164 quantified in 10µL plasma using the AbsoluteIDQ^{TM} kit p180 (BIOCRATES, Innsbruck, Austria) and
165 ESI-LC-MS/MS and ESI-MS/MS measurements. Assay procedures of the kit and metabolite
166 nomenclature have been described previously (36, 37). Sample handling was performed by a Hamilton
167 Microlab STAR^{TM} robot (Hamilton Bonaduz, Bonaduz, Switzerland) and an Ultravap nitrogen evaporator
168 (Porvair Sciences, Leatherhead, UK). MS analyses were carried out on an API 4000 LC-MS/MS System
169 (AB Sciex Deutschland, Darmstadt, Germany) equipped with 1200 Series HPLC (Agilent Technologies
170 Deutschland, Boeblingen, Germany) and HTC PAL auto sampler (CTC Analytics, Zwingen, Switzerland)
171 controlled by the Analyst 1.5.1 software. Data evaluation for quantification of metabolite concentrations
172 and quality assessment were performed with the MetIDQ™ software package. Internal standards were
173 used to calculate concentrations of 188 metabolites including free carnitine, acylcarnitines, amino acids,
174 biogenic amines, lysoPCs, PCs and SMs. Concentrations were expressed as µmoles/L.
175
176 ***Gene expression analysis***
177 A subgroup of patients (n=20) covering a wide range of adiposity and visceral adipose tissue area was
178 selected. Total RNA was extracted using the RNeasy lipid tissue extraction kit and on-column DNase
179 treatment (Qiagen, Valencia, CA) from whole adipose tissues. Quantification of total RNA was
180 performed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE)
181 and total RNA quality was assayed using the Agilent BioAnalyzer (Agilent Technologies, Santa Clara,
182 CA). First-strand cDNA synthesis was accomplished using Superscript III Rnase H-RT (Invitrogen Life
183 Technologies, Burlington, Canada) and purified with PCR purification kit (Qiagen, Hilden, DE).

Oligoprimer pairs were designed by GeneTool 2.0 software (Biotools Inc, Edmonton, CA) and their specificity was verified by blast in the GenBank database. The synthesis was performed by IDT (Integrated DNA Technology, Coralville, IA). Primer sequences were:

BCKDHA, GATGACAAGCCCCAGTTCCCA/TGGGGTTGATGATCTGGCCTT;

BCKDHB, GCGGCAGGTGGCTCATTTTACT/CAGTAGGATCTTTGGCCAATGAGTTAT;

BCAT1, GGTCCCATATTCAACATCTGCTAGTCT/TCCCATCTTGCAGTCCCCAGT;

BCAT2, TTACGCGCCGCACGGATCAT/GGTCGGTAAATGTCTTCCCAAAC; and

BCKDK, CTCGGTACCTGCAGCAAGAACTT/ATCGGAGGGAAGTCTGTCAGCT.

Complementary DNA was used to perform fluorescent-based real time PCR quantification using the LightCycler 480 (Roche Diagnostics, Mannheim, DE). Calculation of the number of copies of each mRNA was performed according to Luu-The et al. (22). PCR amplification efficiency was verified. We assessed ATP synthase O subunit, hypoxanthine phosphoribosyltransferase 1 (HPRT1), glucose-6-phosphate dehydrogenase and 18S ribosomal RNA (52). Normalization was performed using HPRT1 as it showed the lowest variation among samples. Quantitative real time RT-PCR measurements were performed by the CHU de Quebec Gene Expression Platform.

Western blotting analysis

Adipose tissue samples were homogenized and equivalent amounts of proteins (20 μg) were subjected to SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes overnight. Membranes were blocked, probed with antibodies, washed, and then detected by the enhanced chemiluminescence (Millipore, Canada) method. Densitometry analysis was performed with ImageJ (National Institutes of Health, Bethesda, MD). Antibodies for p-ser239 BCKDE1α, total BCKDE1α, for BCATm and BCKD kinase and for actin were purchased from Bethyl Laboratories (TX), Abcam (Canada) and Millipore respectively. A single band was detected for each protein at the expected molecular weight: 46 kDa for p-ser239 BCKDE1α, total BCKDE1α, and BCKD; 44 kDa for BCATm, and 42 kDa for actin.

Dietary data

Women completed a 3-day food record prior to the surgery (n=46). Dietary data were not available for 13 women. A research assistant reviewed the records with the help of a registered dietician and could call back participants to obtain more details. Dietary intakes were analyzed using the Nutrition Data System for Research (NDS-R) software v.4.02 (Minneapolis, MN).

Statistical analyses

Differences among BMI categories for plasma metabolite levels, gene expression and protein level were assessed by one-way ANOVA. Principal Component Analysis (PCA) was used to reduce dimensionality of the data set. Fourteen major principal components were examined after Varimax rotation. Factors were considered relevant if they had an eigenvalue above 1. Metabolites were included in a factor if their component load was $\geq|0.5|$. Metabolites included in each factor are presented in **Table 2**. Scores were calculated for each patient based on standardized scoring coefficients previously obtained. Associations between PCA factor scores and adiposity or cardiometabolic risk factors were assessed with Spearman coefficients. Associations between blood metabolites and adiposity measurements, blood lipids and glucose homeostasis variables were tested with Pearson correlation coefficients. Covariance analysis was performed to statistically adjust for total body fat mass, visceral or subcutaneous adipose tissue areas. When non-normally distributed according to the Shapiro-Wilk test, variables were transformed with Log_{10} or Box-Cox transformations. Statistical analyses were performed with JMP software (SAS Institute, Cary, NC).

RESULTS

Plasma metabolites

From the 188 metabolites analyzed, 138 were detectable in plasma, including 20 amino acids, 7 biogenic amines, 14 SM, 16 acylcarnitines, 10 lysoPC, 36 (diacyl)-phosphatidylcholine (PCaa) and 35 (acyl-alkyl)-phosphatidylcholine (PCae) species. We performed PCA with the 138 detectable plasma metabolite concentrations and obtained 14 relevant factors. When performing univariate analysis between PCA factor scores and cardiometabolic risk factors, 5 main factors containing most of the metabolites emerged and showed relevant correlations with various adiposity or metabolic parameters. Univariate analyses between PCA factor scores and adiposity or cardiometabolic risk variables are presented in **Table 3**. Factor 1 included all amino acids except aspartate and glutamate, biogenic amines derived from amino acid metabolism, C4 acylcarnitines, C5 acylcarnitines and free carnitines. This factor explained 50% of the variance in our data set ($p<0.0001$).

Adiposity markers

Factor 1 was positively correlated with several adiposity markers including BMI, total body fat mass, visceral adipose tissue area and subcutaneous adipose tissue area, subcutaneous cell size. A factor related to the KYN/Trp ratio also correlated with all adiposity markers. Factor 8, reflecting 5 of the 10 lyso PCs, was negatively correlated with most adiposity variables such as BMI, body fat mass, visceral and subcutaneous adipose tissue areas. Factor 11 (reflecting glutamate and octadecanoyl carnitine, C18) was positively associated with visceral adipose tissue area. Consistent with results obtained in PCA, obese women (BMI$\geq$30 kg/m^2) had significantly higher levels of plasma BCAA and increased KYN/Trp ratio (**Figure 1**). Conversely, no difference was found for total lysoPC, PCae and aa as wells as total SM and SM(OH) among BMI categories.

We tested associations between levels of individual AA and adiposity measurements (**Table 4**). Concentrations of alanine, BCAA, tyrosine and lysine were all positively correlated with several adiposity

257 indices (BMI, total body fat mass, subcutaneous and/or visceral adipose tissue areas). Concentrations of
258 alanine, BCAA, glutamate and tyrosine were positively associated with visceral adipose tissue area. The
259 same variables were also related to subcutaneous adipose tissue area. Levels of isoleucine, leucine and
260 tyrosine were positively and significantly correlated to mean adipocyte diameter in both fat
261 compartments. Glutamate level was the strongest independent predictor of visceral adipose tissue area
262 (adjustment for total body fat mass) and was also positively associated with mean adipocyte size in
263 visceral tissue only. Plasma alanine and arginine concentrations were related to larger subcutaneous
264 adipocytes.
265
266 ***Lipid profile***
267 The AA-related factor (Factor 1) was negatively associated with levels of HDL-cholesterol, HDL-
268 triglyceride and HDL-Apo A1. Positive associations were also observed between Factor 2 (reflecting
269 almost all SM and 19 of the 35 PCae), and total, LDL- and HDL-cholesterol as well as LDL-apo B levels.
270 Negative correlations were observed between Factor 2 and VLDL-cholesterol, total and VLDL-
271 triglyceride. Positive associations were found between Factor 3 (mainly PCaa and 2 lysoPC) and total,
272 VLDL- and HDL-cholesterol concentrations. Factor 3 was also positively associated with total, VLDL-
273 and HDL-triglyceride as well as HDL-Apo A1 levels. The glutamate-C18-related factor correlated
274 negatively with the total/HDL-cholesterol ratio and positively with total and VLDL-triglyceride levels.
275 Factor 14 (KYN/Trp ratio) was negatively associated with concentrations of HDL-cholesterol and HDL-
276 Apo A1 and positively with the total/HDL-cholesterol ratio and LDL-triglycerides.
277
278 ***Glucose homeostasis, adipokines and inflammatory markers***
279 The AA-related Factor 1 was positively associated with fasting insulin and HOMA-IR. The glutamate-
280 C18-related factor was also positively correlated with these variables. Factor 1 was positively associated
281 with leptin and IL-6 concentrations. The factor related to the KYN/Trp ratio was also positively
282 associated with leptin levels. Negative correlations were also observed with adiponectin levels. Factor 11

was negatively associated with TNF-α level. As shown in **Table 4,** we also tested the association between plasma AA and HOMA-IR. There was a significant positive correlation between plasma leucine and HOMA-IR. **Figure 2** shows the associations between each plasma AA and BMI before and after adjustment for HOMA-IR. Plasma lysine, arginine, threonine, tyrosine, phenylalanine, valine, leucine, isoleucine and alanine were all positively associated with BMI both before and after adjustment. Plasma histidine, methionine and proline were positively correlated to BMI only after adjustment for HOMA-IR. The association between glutamate and BMI was no longer significant after adjustment.

Gene expression and protein levels

Considering the strong associations between levels of BCAA and adiposity measurements, we further investigated BCAA-catabolizing enzymes in visceral and subcutaneous adipose tissues. As shown in **Figure 3A**, gene expression of BCKDHA and B as well as BCAT2 were significantly decreased in visceral adipose tissue among women with a BMI>30 kg/m^2 compared to lean and overweight individuals ($p<0.05$ for all). BCKDE1α mass and its phosphorylation on Ser293 were also decreased in visceral adipose tissue of women with a BMI>30 kg/m^2 (**Figure 3B**). The ratio of p-BCKDE1α Ser293/BCKDE1α mass was not different among subgroups. Protein level of BCKDK slightly decreased in omental adipose tissue of obese women but did not reach statistical significance (**Figure 3B**). In the subcutaneous depot, only BCAT2 mRNA expression was decreased in obese women, and none of the proteins examined showed consistent obesity-related differences (**Figure 3C and D**).

Dietary data

We assessed the association between blood and dietary AA, mean daily energy and macronutrient intake. Mean energy intake was 2088 calories per day. Mean values for percent energy from macronutrients were 46%, 33% and 16% from carbohydrates, fat and protein, respectively. There was no association between protein or dietary amino acid intake and blood AA concentrations (data not shown).

DISCUSSION

The aim of this study was to identify plasma metabolite patterns related to visceral obesity and concomitant alterations in cardiometabolic risk factors. We also aimed to identify adipose depot-specific alterations in BCAA-catabolizing enzymes in obesity. We found that blood levels of BCAA and the KYN/Trp ratio were elevated in obese women. PCA showed that most amino acids as well as some amino acid metabolites and short chain acylcarnitines were positively associated with body fat mass, visceral and subcutaneous adipose tissue areas, subcutaneous adipocyte size and markers of insulin resistance. Levels of PCae and SM were positively associated with cholesterol-rich lipoprotein levels while PCaa were mostly related to lipid content of VLDL and HDL particles. Glutamate and C18 acylcarnitine concentrations were associated with visceral adipose tissue area, blood triglyceride and glucose homeostasis markers. The former was the best independent predictor of visceral adipose tissue area. PCR analysis and western blotting showed reduced expression and protein levels of enzymes involved in the second step of BCAA catabolism specifically in visceral adipose tissue. To our knowledge, this study is the first to focus on the metabolomics signature of visceral obesity with comprehensive characterization of body composition, fat distribution, adipocyte hypertrophy and cardiometabolic risk factors as well as gene expression and protein level of BCAA-catabolizing enzymes in the visceral and subcutaneous depot.

PCA showed that altered plasma levels of AA and some of their metabolites, such as kynurenine (KYN) and C5 acylcarnitine, are associated with many adiposity markers as reported by other groups (3, 24, 33, 42, 44). One other study had examined associations between levels of glutamine, leucine, isoleucine, phenylalanine, tyrosine and visceral obesity (25). In our analyses, plasma AA alterations other than that of glutamate seemed to be related to overall adiposity rather than with specific visceral fat accumulation and PCA confirmed this association pattern. Altered plasma AA, and AA-related catabolic products such as 2-aminoadipic acid (2-AAA), kynurenine (KYN) and C5 acylcarnitine were also related to insulin resistance. This result is consistent with previous studies reporting impaired AA, and especially BCAA metabolism in obese and insulin resistant subjects (4, 8, 33, 51). Interestingly, our results suggest that the

association between plasma BCAA and obesity is independent of insulin resistance level assessed by HOMA-IR. This finding may be reconciled with previous reports (8, 34) by considering potential sites for BCAA metabolism. In addition to impaired BCAA catabolism in adipose tissue, reduced protein synthesis by insulin-resistant muscles may also contribute to the plasma AA pool (41). Liver was also shown to be an important site of BCAA catabolism. Liver steatosis has been associated with these mechanisms and could also possibly contribute, in part, to the elevation of plasma AA observed in diabetic patients. Our results underline the fact that even in the absence of pronounced insulin resistance, adipose tissue dysfunction and obesity are sufficient to see increased AA levels in circulation. We propose that plasma AA level alterations are mostly associated with overall adiposity, but may be worsened in the presence of insulin resistance, type 2 diabetes or the metabolic syndrome.

Adipose tissue is thought to be an important regulator of whole-body BCAA homeostasis (15, 19). For example, Herman et al. demonstrated that BCAT2 knockout mice transplanted with perigonadal fat from wild-type mice had lower blood BCAA levels compared to non-transplanted knockout mice (15). Another report found that BCKDHA, BCKDHB and dihydrolipoamide dehydrogenase expression was reduced in subcutaneous adipocytes of obese individuals (19). Considering that the first step of BCAA catabolism by BCATm produces glutamate, it could be possible that the accumulation of glutamate observed in visceral obese patients is due to altered BCKDC expression or activity. We observed that expression of genes from the BCKDH complex as well as BCAT2 were decreased in visceral fat of obese individuals. These results are consistent with those of Lackey and al. (19), who showed lower expression of BCKDA and BCKDB specifically in visceral adipose tissue of obese individuals with or without metabolic syndrome. In addition, we also observed reduced expression of BCAT2 and the unaltered expression of BCKDK in the same depot. Only BCAT2 expression was reduced in the subcutaneous tissue of obese women. These results indirectly suggest that catabolism of BCAA is decreased in adipose tissue, particularly in the omental depot. Since catabolism of BCAA and β-oxidation of fatty acids occur in mitochondria, substrate abundance could overload this organelle and contribute to decrease BCAA catabolism in obese

individuals. Yehuda-Shnaidam et al. (56) showed that adipocyte mitochondrial content was similar in obese and lean. This observation supports the hypothesis that mitochondria of obese individuals could be less efficient. Accordingly, Yin et al. (57) demonstrated that mitochondrial function decreased independently of cell size and fat depot in obese individuals.

Despite growing evidence that obesity involves a defect in BCAA-catabolizing enzymes and/or gene expression (15, 19), the impact of obesity on protein abundance and their activation status remains poorly investigated. She et al. (40) have shown that elevated BCAA levels are coincident with decreased BCKDE1α and BCATm protein level in adipose tissue of two animal models of obesity in the fed state. The activation state of the enzyme, based on Ser293 phosphorylation, was not affected. This group also performed western blotting analysis on subcutaneous and visceral adipose tissue of morbidly obese individuals before and after bariatric surgery. They observed a significant increase in BCATm and BCKDE1α protein level in both visceral and subcutaneous adipose samples obtained 17 months after the surgery on average. There was no difference in BCKDK protein level after the intervention (25). These authors had not looked at the effect of obesity *per se.* Our data now provide clear evidence that obesity is associated with reduced BCKDE1α mass without any impact on Ser293 phosphorylation. These effects are observed in visceral and not subcutaneous fat, which is in contrast with the fact that both fat depots responded similarly after gastric bypass-mediated weight loss in the She et al study. Reduced visceral adipose tissue expression and protein levels of enzymes from the BCKD complex could contribute to the increase in circulating glutamate and BCAA levels of obese individuals. Further mechanisms include signaling through mTOR and eNOS induction (48). Mechanisms of improved glycemia seem to include BCAA signalling or metabolism. However improved insulin response might be due to distinct regulatory pathways in surgery- vs. dietary-induced weight loss. BCAA levels are significantly lowered by gastric bypass but not by dietary treatment (20).

We found strong positive associations between the KYN/Trp ratio and adiposity indices. Kynurenine is a metabolite of tryptophan degradation and previous studies (23, 24) suggested that the KYN/Trp ratio could be a potent indirect indicator of indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme of tryptophan catabolism. This pathway is induced by pro-inflammatory cytokines, especially interleukin-2, TNF-α and interferon-gamma (24). We did not find associations between the KYN/Trp ratio and TNF-α levels, possibly because inflammatory alterations in our sample were generally mild, the differences in TNF-α concentration among BMI groups being non-significant.

Since glycerophospholipids and sphingolipids are present in membranes and participate in lipoprotein transport and metabolism, associations between these metabolites and blood lipids were expected. We observed many significant associations between PC- and SM-related factors with variables of the blood lipid profile. Graessen et al. (12) showed a decrease in many PC species with improvement of the blood lipid profile in patients who had undergone Roux-en-Y gastric bypass surgery. In our study, we found that levels of PCaa, the most abundant phosphatidylcholine subtype, correlated with plasma cholesterol and triglyceride levels. Moreover the PCaa-related factor was a correlate of VLDL and HDL lipid content, consistent with the presence of these compounds at the surface of lipoproteins. Conversely, the PCae and SM-related factors were related to higher levels of cholesterol-rich lipoproteins. Moreover, PCA analysis showed that the factor related to LysoPC was negatively associated with most adiposity markers but not with the blood lipid profile. These results on PC and LysoPC are consistent with those of Barber et al. (2), who observed higher plasma PC species in mice fed a high fat diet and lower levels of LysoPC in mice and obese diabetic or non-diabetic humans (2). Moreover, 1-linoleoylglycerophosphocholine (L-GPC), or LysoPC C18:2, has been associated to peripheral insulin sensitivity and lower risk of developing impaired glucose tolerance in a large sample of subjects, even after adjustment for sex, age, BMI and family history (6, 9). In our sample of women, LysoPC C18:2 and other LysoPC species are included in PCA factor 8 which was exclusively correlated with adiposity and BMI instead of insulin resistance measured with HOMA-IR. Miao et al. (30) reported that some LysoPC species were increased, while others were

412 lowered in hyperlipidemic rats fed a high-fat diet for 6 weeks. Another study in mice fed a high-fat diet
413 showed elevation of PC species in blood and liver which were associated with increased fat accumulation
414 while most LysoPCs were decreased and some others were increased (17). Similar results were observed
415 in humans by Kim et al. (18), where specific LysoPC species (14:0 and 16:0) were increased in
416 overweight and obese men while LysoPC C18:1 was decreased. Other types (18:2 and 20:2) were not
417 different among BMI categories. These results emphasize the need to consider specific LysoPC
418 subspecies while correlations between PCs and parameters of the blood lipid profile or adiposity indices
419 appear to be more consistent from one study to another.
420
421 We assessed whether dietary amino acid intake was related to circulating amino acid levels, since
422 Newgard and al. (33) had observed higher plasma BCAA concentrations in rats fed a BCAA-enriched
423 diet. In humans, a study by Tai et al. (45) showed that among individuals stratified for their HOMA index
424 and having normal BMI, insulin resistance was positively correlated with plasma BCAA levels
425 independently of protein intake. We found no association between plasma amino acid levels and their
426 respective dietary intake. Our results are consistent with a study from Piccolo and al, where obese women
427 were enrolled in a weight loss trial and randomized in a 20g per day whey supplementation vs a gelatin
428 control group. They did not observe a significant increase in BCAA plasma levels in the supplementation
429 group compared to controls (34). We conclude that increases in plasma BCAA and other amino acids
430 observed in women with obesity are mostly independent from the diet. Possible bias associated with the
431 use of dietary records, especially in obese individuals (10, 35) needs to be considered as a limitation of
432 our analysis.
433
434 Additional limitations are also acknowledged. Our sample did not include men, which prevented us from
435 assessing sex differences. In addition, our cross-sectional design prevented us from making inferences on
436 potential cause and effect relationships. Finally, none of our participants were diagnosed with type 2
437 diabetes. In fact, most had fasting glycemia within the normal range and thus potential associations

between altered indices of glucose homeostasis and the metabolome may have been underestimated in these subjects. The fact that association patterns were detectable in the absence of overt type 2 diabetes may point toward subclinical mechanisms effective even in obese individuals that are relatively healthy from the metabolic standpoint.

In conclusion, our analysis supports the documented elevation of plasma AA, particularly BCAA, and some of their metabolites in obesity. Alterations in BCAA-catabolizing enzyme expression and protein levels were mostly specific to visceral fat and may contribute to their increase in in circulation.

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DISCLOSURE STATEMENT

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654 **Table 1**: Age, anthropometrics, body composition, fat distribution and metabolic characteristics of study
655 participants (n=59)
656

Characteristics	Lean n=23	Overweight n=21	Obese n=15	*p*
Age (years)	47.2 ± 5.0	47.0 ± 5.1	46.7 ± 5.1	0.96
Weight (kg)	59.8 ± 4.6	70.2 ± 5.3	87.6 ± 9.0	<.0001
BMI (kg/m^2)	23.2 ± 1.3	27.0 ± 1.3	34.0 ± 3.3	<.0001
Fat mass (kg)	19.2 ± 3.4	25.6 ± 3.3	36.1 ± 5.2	<.0001
Lean body mass (kg)	39.8 ± 3.8	43.6 ± 3.1	50.3 ± 4.6	<.0001
Fat percentage (%)	31.3 ± 4.4	35.8 ± 2.9	40.6 ± 3.0	<.0001
Menopause status (%)[a]	26.1	31.6	35.7	0.83
Adipose tissue areas (cm^2)[b]				
Total	290[c] ± 60	411 ± 85	608 ± 81	<.0001
Visceral	69 ± 27	93 ± 29	154 ± 54	<.0001
Subcutaneous	215 ± 47	323 ± 66	447 ± 65	<.0001
Adipocyte diameter (µm)				
Visceral	78 ± 12	79 ± 15	93 ± 13	0.08
Subcutaneous	87 ± 13	97 ± 18	106 ± 11	0.005
Glucose homeostasis				
Insulin (µU/ml)	7.7[c] ± 5.2	6.8 ± 3.5	10.4[e] ± 3.63	0.05
Fasting glucose (mmol/L)	5.4 ± 0.63	5.5 ± 0.5	5.6 ± 0.6	0.42
HOMA-IR	1.9[c] ± 1.4	1.7 ± 0.9	2.6[e] ± 1.0	0.06
Plasma cholesterol (mmol/L)				
Total	5.10 ± 0.89	4.87 ± 0.74	5.27 ± 1.02	0.39
VLDL	0.34 ± 0.18	0.40 ± 0.34	0.57 ± 0.31	0.95
LDL	3.15 ± 0.85	3.06 ± 0.68	3.46 ± 0.87	0.30
HDL	1.62 ± 0.40	1.41 ± 0.34	1.24 ± 0.24	0.10
Total/HDL	3.31 ± 0.88	3.57 ± 0.78	4.36 ± 0.96	0.05
Plasma triglyceride (mmol/L)				
Total	1.09 ± 0.41	1.18 ± 0.70	1.50 ± 0.58	0.06
VLDL	0.59 ± 0.38	0.71 ± 0.65	0.97 ± 0.51	0.95
LDL	0.23 ± 0.07	0.22 ± 0.07	0.27 ± 0.08	0.16
HDL	0.27 ± 0.05	0.25 ± 0.07	0.27 ± 0.08	0.42
Plasma apolipoproteins (g/L)				
Total Apo B	0.91 ± 0.22	0.87 ± 0.16	1.06 ± 0.22	0.44
VLDL-Apo B	0.09 ± 0.04	0.10 ± 0.05	0.12 ± 0.05	0.20
LDL-Apo B	0.81 ± 0.21	0.77 ± 0.13	0.94 ± 0.20	0.85
HDL-Apo A1	1.63 ± 0.25	1.49 ± 0.23	1.42 ± 0.15	0.40
Plasma adipokines and inflammatory markers[f]				
Il-6 (pg/mL)	0.86 ± 0.19	0.93 ± 0.19	1.38 ± 0.24	0.39
TNF-α (pg/mL)	1.57 ± 0.55	0.73 ± 0.53	0.63 ± 0.67	0.83
Leptin (ng/mL)	9.6 ± 1.1	12.5 ± 1.1	22.4 ± 1.3	<0.0001
Adiponectin (µg/mL)	11.4 ± 1.2	11.4 ± 1.2	9.3 ± 1.5	0.46

657 Data are presented as means ± standard deviations; Lean: BMI<25kg/m^2, Overweight: BMI ≥25 and <30kg/m^2, Obese:
658 BMI≥30kg/m^2; [a]n=56, [b]n=58, [c]n=22, [d]n=57, [e]n=14, [f]n=49 (lean: n=18, overweight: n=19 and obese: n=12)

659 **Table 2:** PCA factors metabolite composition

Factors	Metabolites	Eigenvalue	Variance (%)
1	AA except Asp and Glu, 2-AAA, creatinine, KYN, sarcosine, SM C20:2, C0, C10:2, C4, C5	69.7	50.1
2	SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM (OH) C24:1, SM C16:0, SM C16:1, SM C18:0, SM C18:1, SM C24:0, SM C24:1, SM C26:0, SM C26:1, PC aa C28:1, PC ae C30:0, PC ae C32:1, PC ae C32:2, PC ae C34:0, PC ae C34:2, PC ae C34:3, PC ae C36:1, PC ae C36:2, PC ae C36:3, PC ae C36:5, PC ae C38:3, PC ae C38:4, PC ae C38:5, PC ae C40:2, PC ae C40:3, PC ae C40:4, PC ae C40:5, PC ae C40:6, PC ae C42:5	12.6	9.1
3	lysoPC a C16:1, lysoPC a C20:3, PC aa C30:0, PC aa C32:0, PC aa C32:1, PC aa C32:2, PC aa C32:3, PC aa C34:1, PC aa C34:2, PC aa C34:3, PC aa C34:4, PC aa C36:1, PC aa C36:2, PC aa C36:3, PC aa C36:4, PC aa C38:3, PC aa C38:4, PC aa C38:5, PC aa C40:4, PC aa C40:5, PC aa C42:5, PC ae C34:1, PC ae C38:2	9.1	6.6
4	Spermidine , lysoPC a C26:0, lysoPC a C28:0, lysoPC a C28:1, PC aa C24:0, PC aa C26:0, PC aa C40:2, PC aa C42:4, PC aa C42:6, PC ae C30:1, PC ae C42:1, PC ae C42:2, PC ae C44:3	6.8	4.9
5	C10 , C10:1, C12 , C14 , C14:1 , C14:2 , C16 , C18:1 , C18:2 , C2	4.6	3.3
6	PC aa C36:0, PC aa C36:5, PC aa C36:6, PC aa C38:0, PC aa C38:6, PC aa C40:1, PC aa C40:3, PC aa C40:6, PC aa C42:2, PC ae C38:0, PC ae C38:6, PC ae C42:0	4.1	2.9
7	PC aa C42:0, PC aa C42:1, PC ae C42:3, PC ae C42:4, PC ae C44:4, PC ae C44:5, PC ae C44:6	3.1	2.2
8	lysoPC a C17:0, lysoPC a C18:0, lysoPC a C18:1, lysoPC a C18:2, lysoPC a C20:4	2.9	2.1
9	Asp	2.4	1.7
11	Glu, C18	1.8	1.3
12	PC ae C38:1	1.7	1.2
13	C3	1.6	1.1
14	KYN/Trp	1.4	1.0
15	Serotonin	1.3	0.9

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661

662 **Table 3:** Pearson correlations between relevant metabolite PCA factors and adiposity indices or
663 cardiometabolic risk factors
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Factors Variables	**Factor 1** AA	**Factor 2** PCae and SM	**Factor 3** PCaa	**Factor 8** LysoPC	**Factor 11** Glutamate-C18	**Factor 14** KYN/Trp ratio
Adiposity indices						
BMI	0.47**	-	-	-0.32#	-	0.51***
Body fat mass	0.45**	-	-	-0.38*	-	0.51***
Visceral AT Area[a]	0.37*	-	-	-0.36*	0.38*	0.39*
Subcut. AT Area[a]	0.44**	-	-	-0.40*	-	0.52***
Adipocyte size OM	-	-	-	-	-	0.35*
Adipocyte size Sc	0.42**	-	-	-	-	0.44**
Plasma cholesterol						
Total	-	0.35*	0.36*	-	-	-
VLDL	-	-0.40*	0.36*	-	-	-
LDL	-	0.39*	-	-	-	-
HDL	-0.39*	0.32#	0.33*	-	-0.30#	-0.45**
Total/HDL	-	-	-	-	-	0.39#
Plasma triglycerides						
Total	-	-0.38*	0.43**	-	0.28#	-
VLDL	-	-0.39*	0.39*	-	0.30#	-
LDL	-	-	-	-	-	0.28#
HDL	-0.28#	-	0.38*	-	-	-
Plasma apolipoproteins						
Total Apo B	-	-	-	-	-	-
VLDL-Apo B	-	-	-	-	-	-
LDL-Apo B	-	0.28#	-	-	-	-
HDL-Apo A1	-0.37*	-	0.47**	-	-	-0.33#
Glucose homeostasis						
Fasting insulin[b]	0.37*	-	-	-	0.38*	-
HOMA-IR[b]	0.35*	-	-	-	0.36*	-
Plasma adipokines and inflammatory markers[c]						
Il-6	0.49**	-	-	-	-	-
TNF-α	-	-	-	-	-0.41*	-
Leptin	0.42*	-	-	-	-	0.41*
Adiponectin	-0.34#	-	-	-	-	-

665 Table shows factors for which significant correlation was observed with at least one variable; AT: adipose tissue; ***p≤0.0001,
666 **p≤0.001, *p≤0.01, #p≤0.05; -: non-significant p-value; a: n=58, b: n=57, c: n=49

Table 4: Pearson correlations between plasma AA levels, adiposity indices and HOMA-IR

Amino acids	BMI	Fat mass	Visceral AT area[a]	Subcutaneous AT area[a]	Omental adipocyte size	Subcutaneous adipocyte size	HOMA-IR
Monoamine/monocarboxyle							
Alanine	0.34*	0.31#	0.35*	0.28#	-	0.26#	-
Glycine	-	-	-	-	-	-	-
Isoleucine	0.40*	0.40*	0.28#	0.35*	0.27#	0.32#	-
Leucine	0.39*	0.37*	0.31#	0.33*	0.30#	0.29#	0.26#
Valine	0.34*	0.37*	0.27#	0.31#§	-	-	-
Heterocyclic							
Proline	-	-	-	-	-	-	-
Aromatic							
Phenylalanine	0.29#	0.29#	-	-	-	-	-
Tryptophan	-	-	-	-	-	-	-
Tyrosine	0.35*	0.37*	0.31#	0.34*	0.26#	0.31#	-
Thioether							
Methionine	-	-	-	-	-	-	-
Hydroxy							
Serine	-	-	-	-	-	-	-
Threonine	0.30#	-	-	-	-	-	-
Carboxamide							
Asparagine	-	-	-	-	-	-	-
Glutamine	-	-	-	-	-	-	-
Monoamine/dicarboxyle							
Aspartate	-	-	-	-	-	-	-
Glutamate	0.28#	-	0.46**§	0.33*	0.26#	-	-
Diamine/monocarboxyle							
Arginine	0.27#	0.28#	-	-	-	0.29#	-
Histidine	-	-	-	-	-	-	-
Lysine	0.26#	0.29#	-	0.27#	-	-	-
Urea cycle							
Citrulline	-	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-

***p≤0.0001, **p≤0.001, *p≤0.01, #p≤0.05; -: non-significant p-value; §: remained significant after adjustment for total body fat mass; AT: adipose tissue; a: n=58, b: n=57

672 **FIGURE LEGENDS**

673 **FIGURE 1:** Plasma metabolite levels among patients stratified for BMI. * $p<0.05$. BCAA: branched-
674 chain amino acids, AAA: aromatic amino acids, AA: amino acids, MUFA: monounsaturated fatty acids,
675 PUFA: polyunsaturated fatty acids, Lyso-PC: glycerophospholipids, PC: glycerophospholipids, SM:
676 sphingolipids, KYN: kynurenine, Trp: tryptophan, AC: acylcarnitines, C0: carnitine
677
678 **FIGURE 2:** Correlations between plasma AA and BMI before (n=59) and after (n=57) statistical
679 adjustment for HOMA-IR. Pearson correlation coefficients are shown. The significance threshold is
680 indicated by the dashed line.
681
682 **FIGURE 3:** Panel A, Gene expression of BCKDHA, BCKDHB, BCKDK, BCAT1 and BCAT2 in
683 visceral adipose tissue among patients stratified for BMI. Panel B, Representative Western blots and
684 quantification of BCKDE1α ser293, total BCKDE1α, BCKDE1α ser293/BCKDE1α, BCKDK and
685 BCATm protein levels in visceral adipose tissue among patients stratified for BMI. Panel C, Gene
686 expression of BCKDHA, BCKDHB, BCKDK, BCAT1 and BCAT2 in subcutaneous adipose tissue
687 among patients stratified for BMI. Panel D, Representative Western blots and quantification of
688 BCKDE1α ser293, total BCKDE1α, BCKDE1α ser293/BCKDE1α, BCKDK and BCATm protein levels
689 in subcutaneous adipose tissue among patients stratified for BMI. * $p<0.05$. Protein levels were
690 normalized to total actin levels. Lanes were run on the same gel but were non-contiguous. n=5-8
691

Figure 1

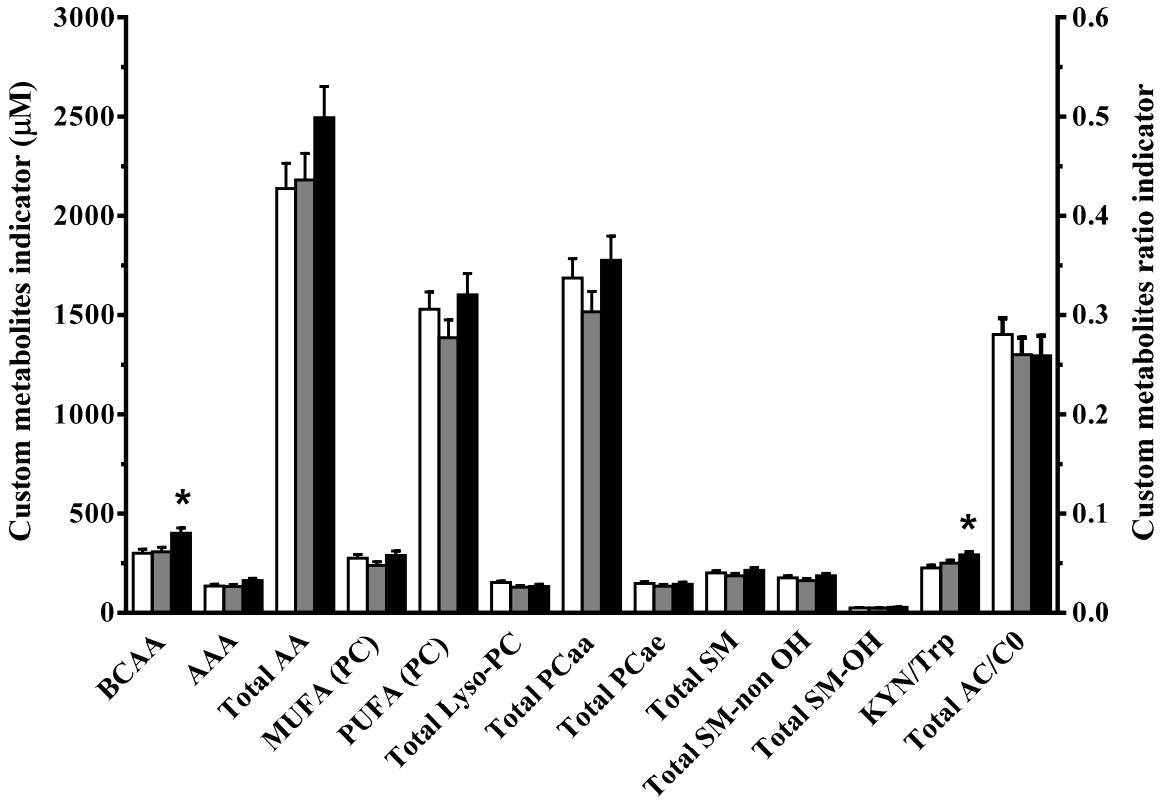

Custom metabolites indicator (μM)
Custom metabolites ratio indicator
3000
2500
2000
1500
1000
500
0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
BCAA
AAA
Total AA
MUFA (PC)
PUFA (PC)
Total Lyso-PC
Total PCaa
Total PCae
Total SM
Total SM-non OH
Total SM-OH
KYN/Trp
Total AC/C0

Figure 2

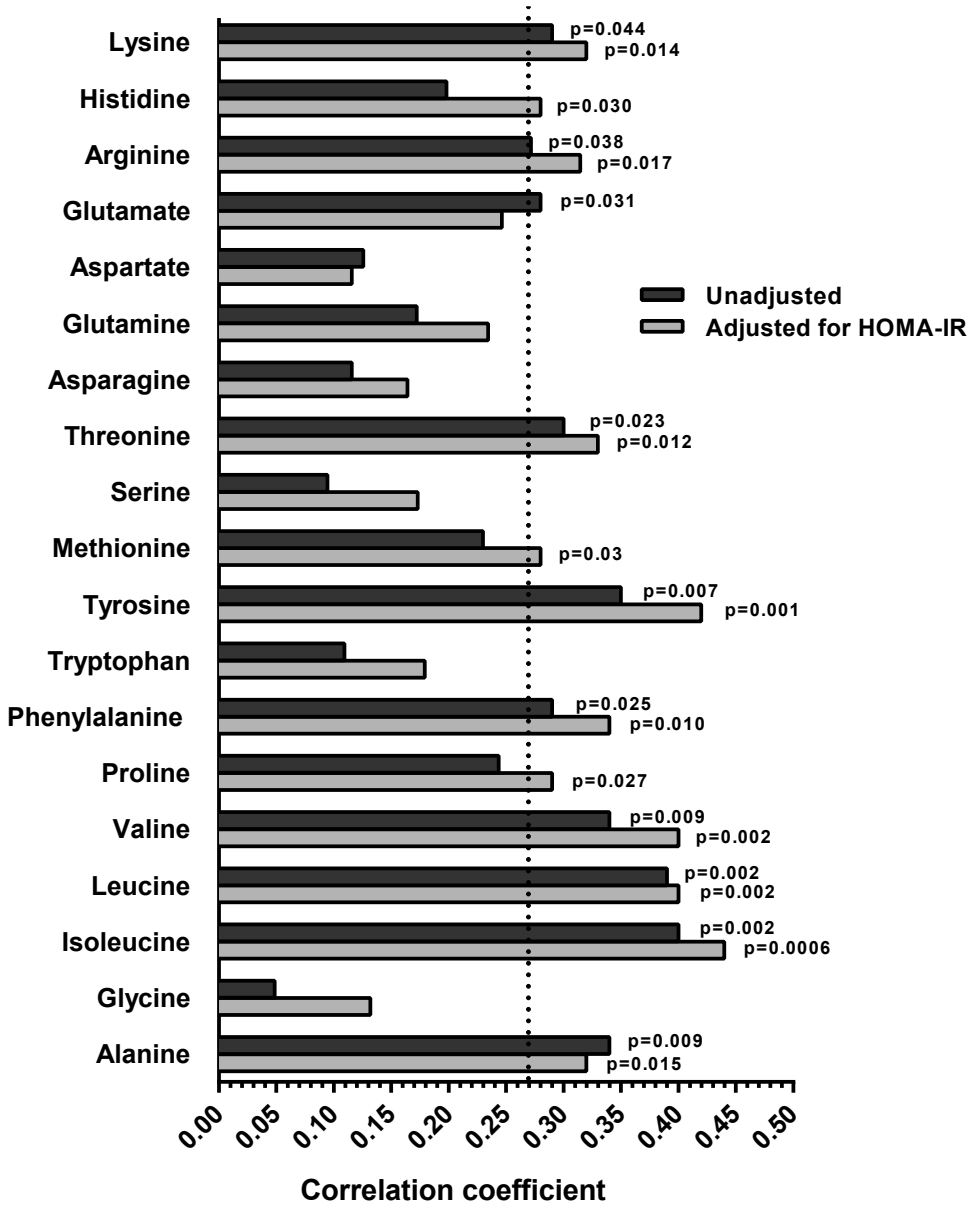
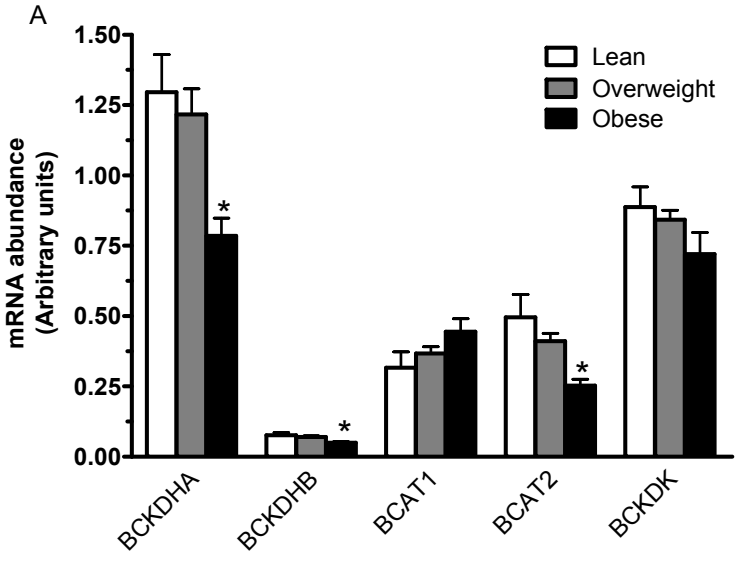
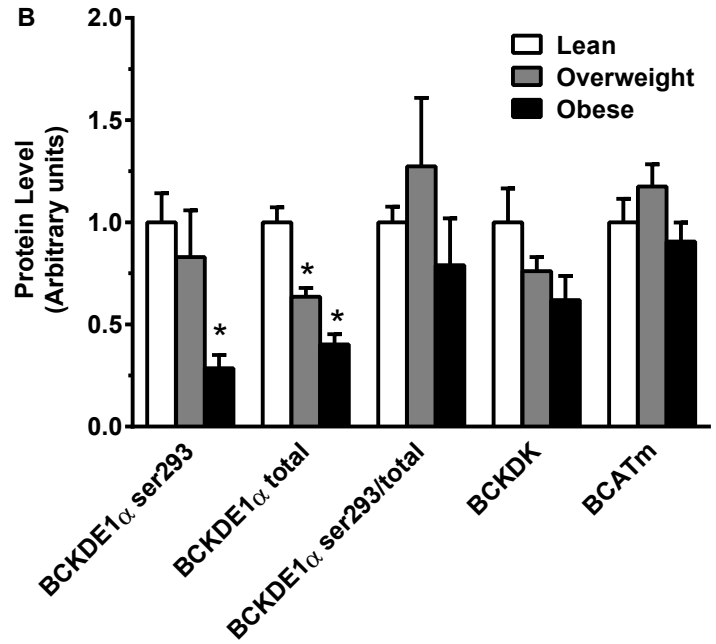
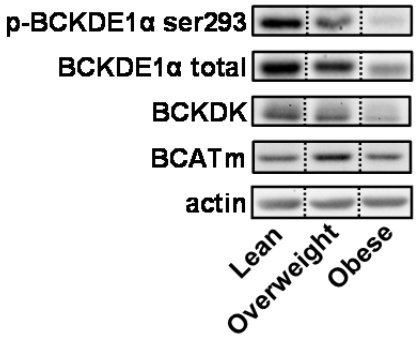
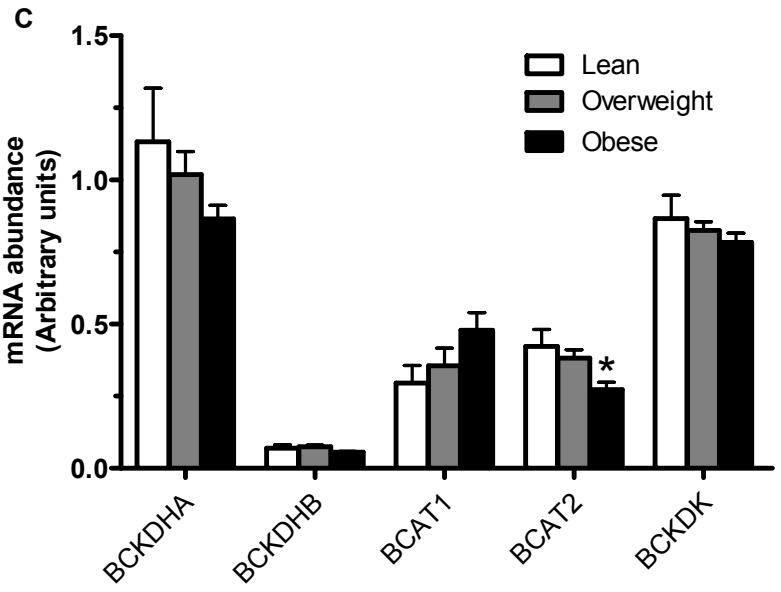

Lysine
Histidine
Arginine
Glutamate
Aspartate
Glutamine
Asparagine
Threonine
Serine
Methionine
Tyrosine
Tryptophan
Phenylalanine
Proline
Valine
Leucine
Isoleucine
Glycine
Alanine
p=0.044
p=0.014
p=0.030
p=0.038
p=0.017
p=0.031
p=0.023
p=0.012
p=0.03
p=0.007
p=0.001
p=0.025
p=0.010
p=0.027
p=0.009
p=0.002
p=0.002
p=0.002
p=0.002
p=0.0006
p=0.009
p=0.015
Unadjusted
Adjusted for HOMA-IR
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
Correlation coefficient

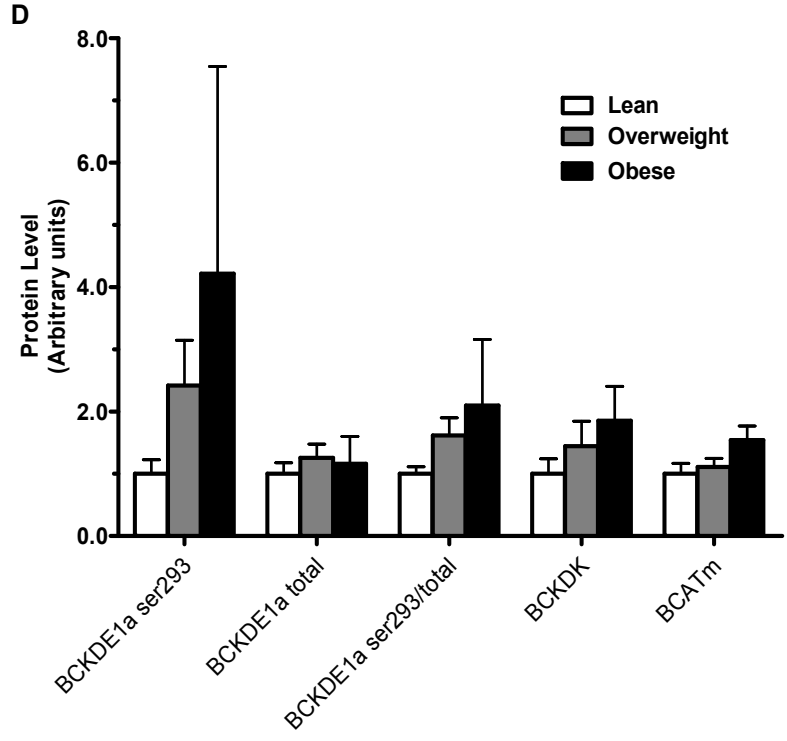
Figure 3

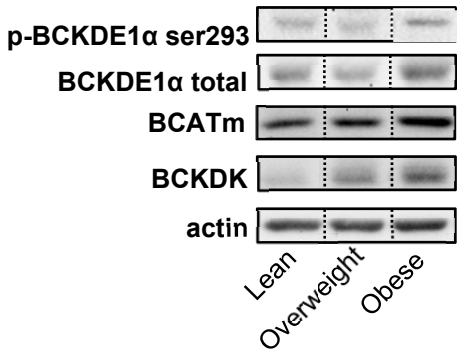

A
mRNA abundance (Arbitrary units)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Lean
Overweight
Obese
*
*
*
BCKDHA
BCKDHB
BCAT1
BCAT2
BCKDK

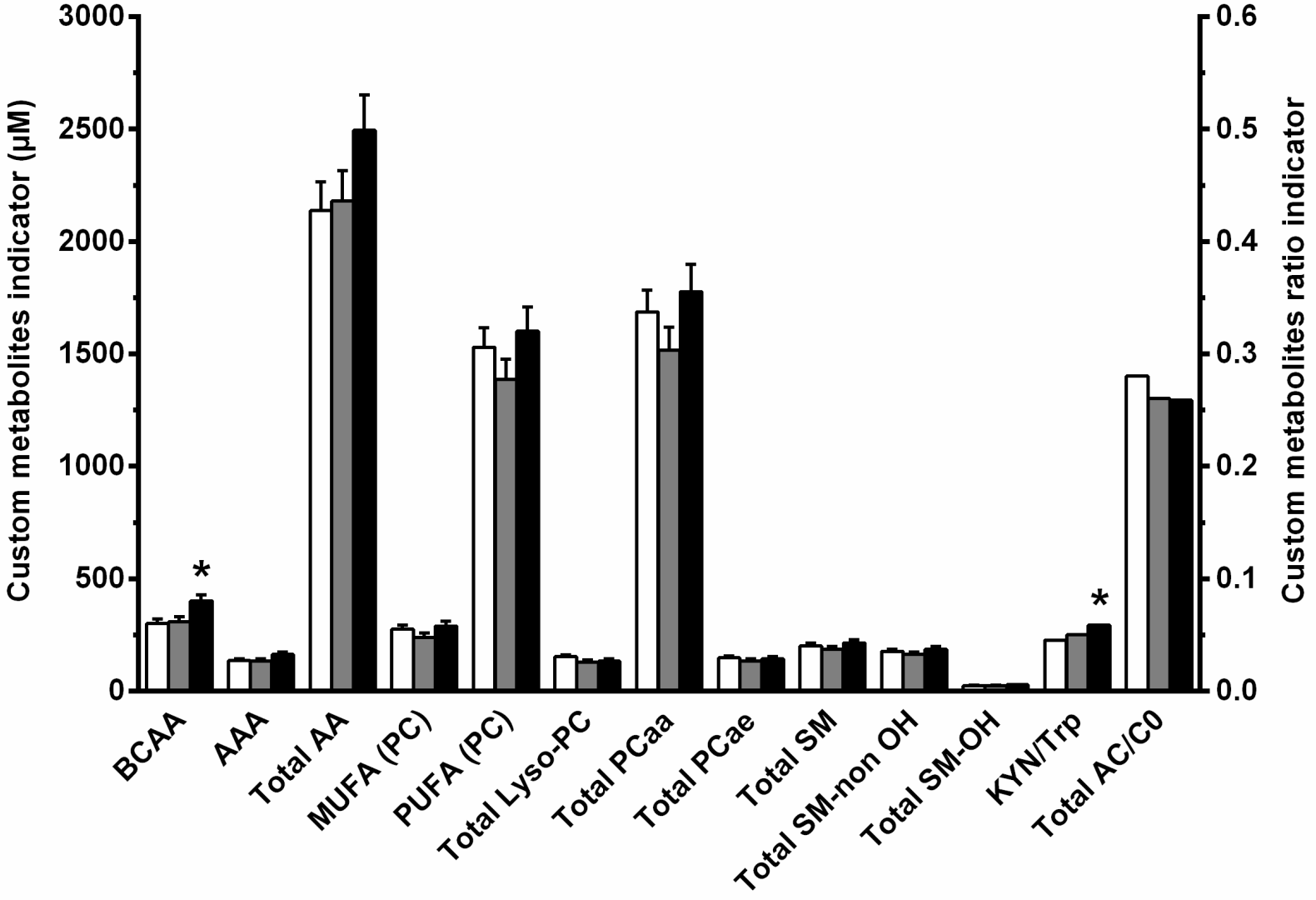

B
Protein Level (Arbitrary units)
2.0
1.5
1.0
0.5
0.0
Lean
Overweight
Obese
*
*
*
BCKDE1α ser293
BCKDE1α total
BCKDE1α ser293/total
BCKDK
BCATm

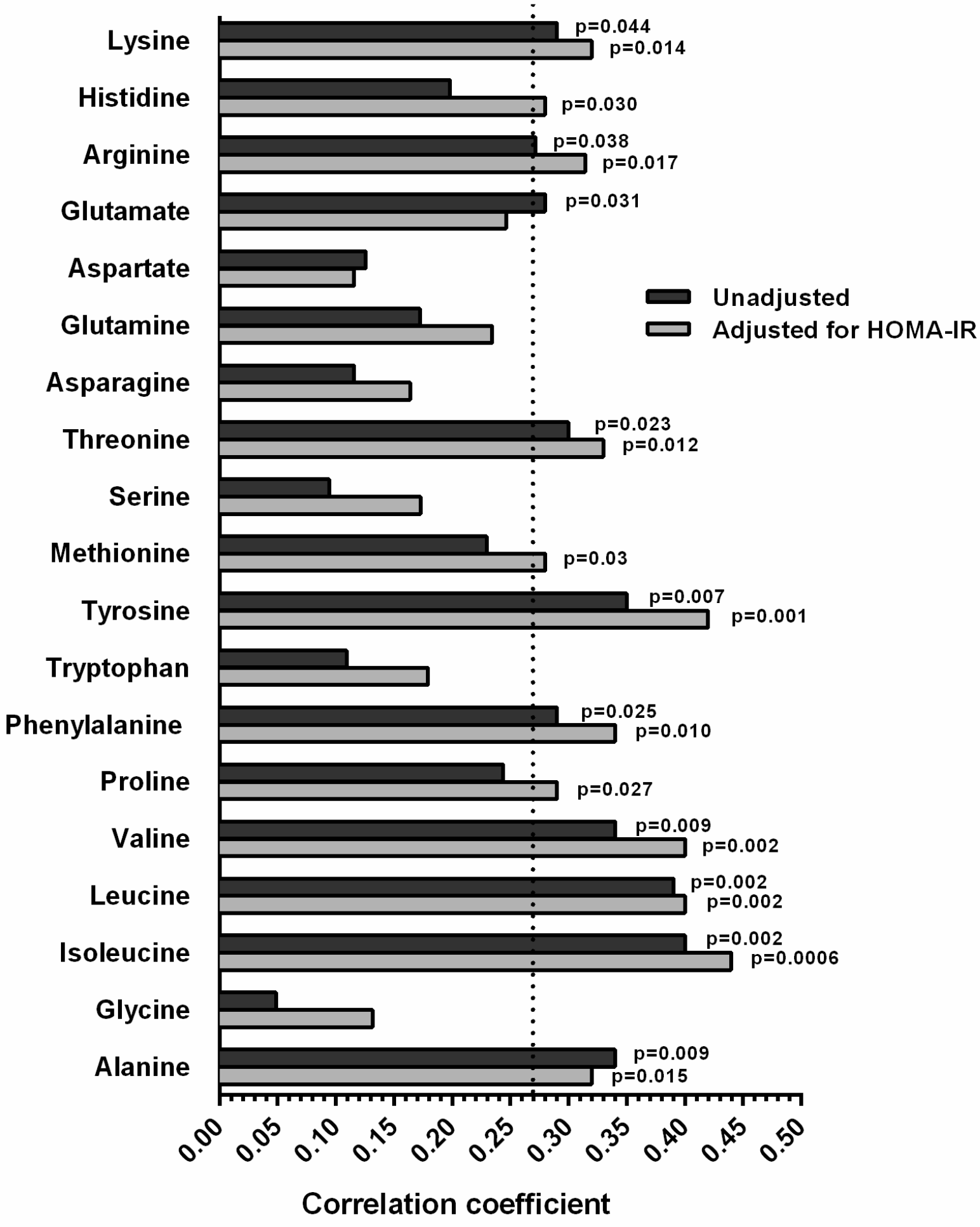

p-BCKDE1α ser293
BCKDE1α total
BCKDK
BCATm
actin
Lean
Overweight
Obese

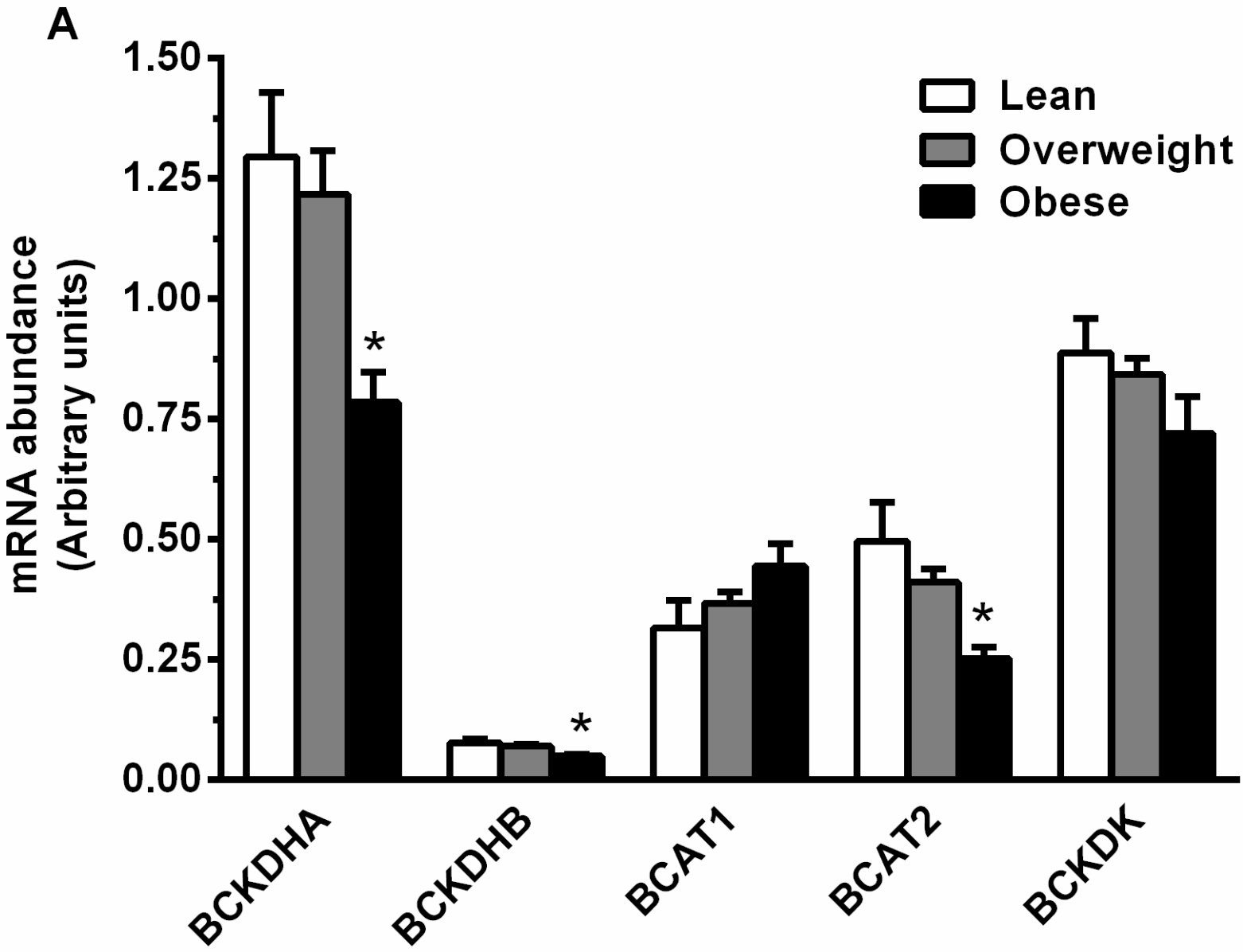

C
mRNA abundance (Arbitrary units)
1.5
1.0
0.5
0.0
Lean
Overweight
Obese
*
BCKDHA
BCKDHB
BCAT1
BCAT2
BCKDK

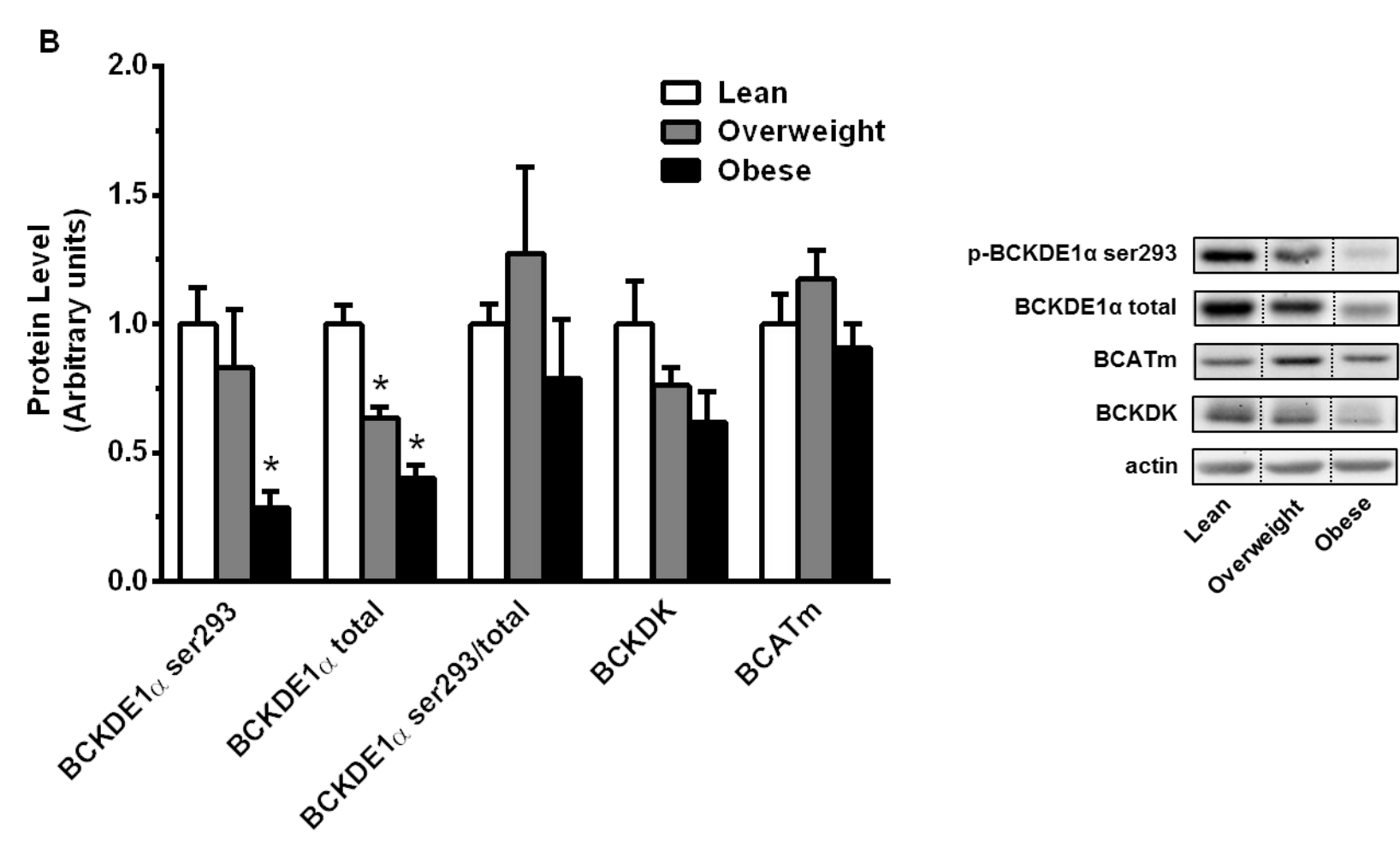

D
Protein Level (Arbitrary units)
8.0
6.0
4.0
2.0
0.0
Lean
Overweight
Obese
BCKDE1a ser293
BCKDE1a total
BCKDE1a ser293/total
BCKDK
BCATm

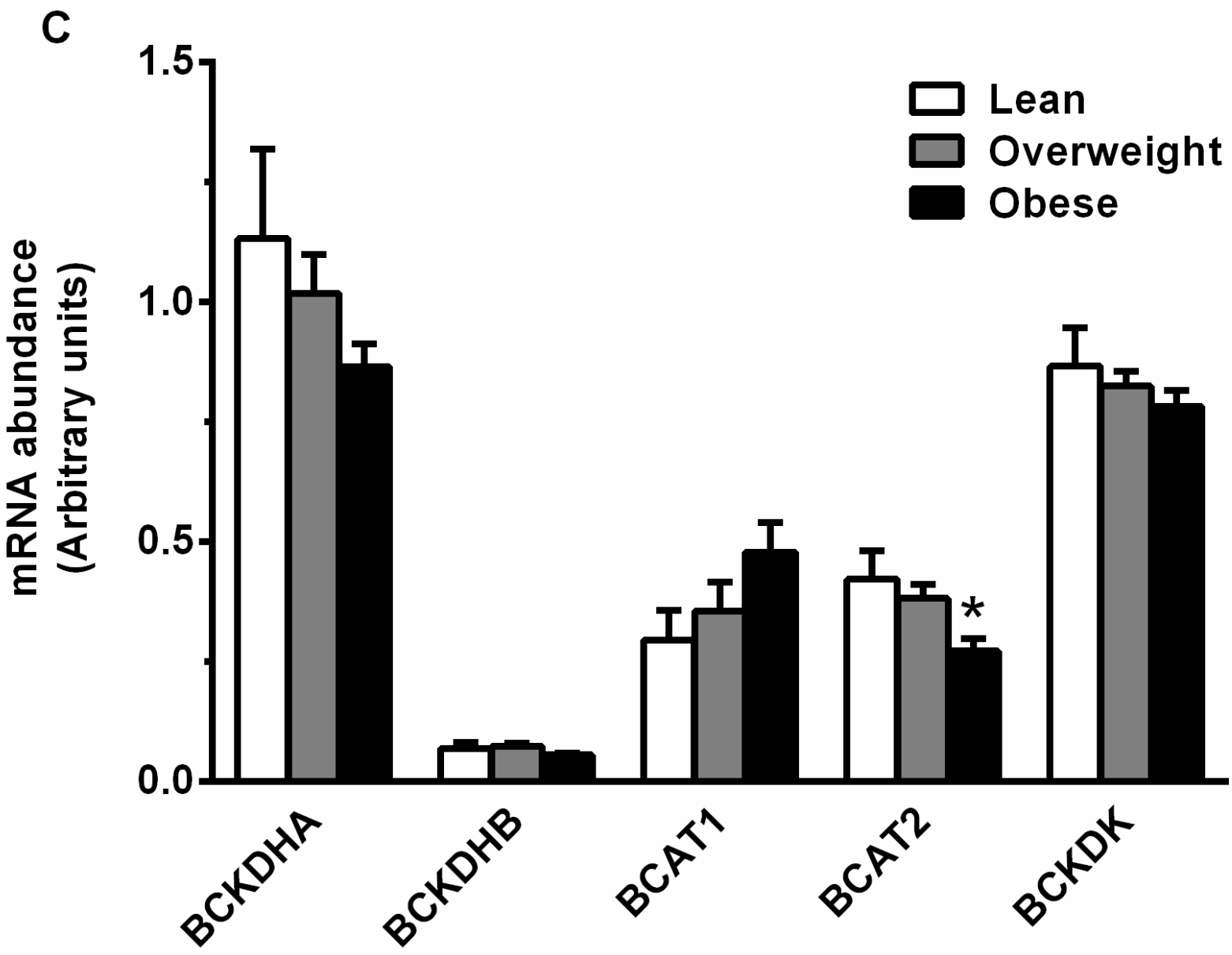

p-BCKDE1α ser293
BCKDE1α total
BCATm
BCKDK
actin
Lean
Overweight
Obese

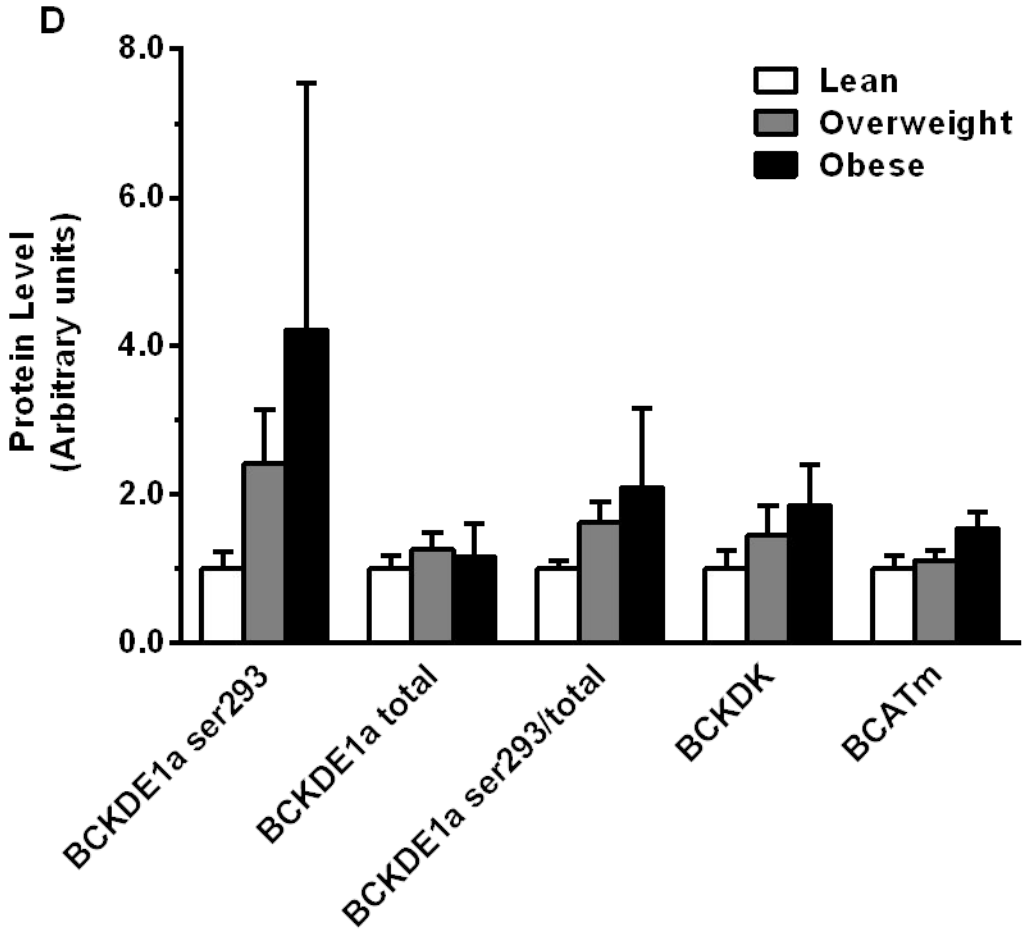

Custom metabolites indicator (µM)
Custom metabolites ratio indicator
3000
2500
2000
1500
1000
500
0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
BCAA
AAA
Total AA
MUFA (PC)
PUFA (PC)
Total Lyso-PC
Total PCaa
Total PCae
Total SM
Total SM-non OH
Total SM-OH
KYN/Trp
Total AC/C0

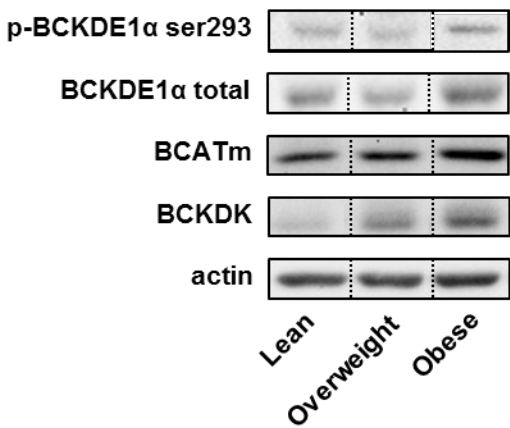

Lysine
p=0.044
p=0.014
Histidine
p=0.030
Arginine
p=0.038
p=0.017
Glutamate
p=0.031
Aspartate
Glutamine
Asparagine
Threonine
p=0.023
p=0.012
Serine
Methionine
p=0.03
Tyrosine
p=0.007
p=0.001
Tryptophan
Phenylalanine
p=0.025
p=0.010
Proline
p=0.027
Valine
p=0.009
p=0.002
Leucine
p=0.002
p=0.002
Isoleucine
p=0.002
p=0.0006
Glycine
Alanine
p=0.009
p=0.015
Unadjusted
Adjusted for HOMA-IR
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
Correlation coefficient


A
mRNA abundance
(Arbitrary units)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Lean
Overweight
Obese
*
*
*
BCKDHA
BCKDHB
BCAT1
BCAT2
BCKDK


B
Protein Level (Arbitrary units)
2.0
1.5
1.0
0.5
0.0
Lean
Overweight
Obese
*
*
*
BCKDE1α ser293
BCKDE1α total
BCKDE1α ser293/total
BCKDK
BCATm
p-BCKDE1α ser293
BCKDE1α total
BCATm
BCKDK
actin
Lean
Overweight
Obese


C
1.5
1.0
0.5
0.0
mRNA abundance
(Arbitrary units)
Lean
Overweight
Obese
*
BCKDHA
BCKDHB
BCAT1
BCAT2
BCKDK


D
8.0
6.0
4.0
2.0
0.0
Protein Level
(Arbitrary units)
Lean
Overweight
Obese
BCKDE1a ser293
BCKDE1a total
BCKDE1a ser293/total
BCKDK
BCATm


p-BCKDE1α ser293
BCKDE1α total
BCATm
BCKDK
actin
Lean
Overweight
Obese