Published in final edited form as:

J Proteome Res. 2014 September 05; 13(9): 4062–4073. doi:10.1021/pr500434s.

The Metabolite Profiles of the Obese Population Are Gender-Dependent

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Abstract

Recent studies have identified that several amino acids, in particular, branched-chain amino acids (BCAAs), increased significantly in obese compared with lean individuals. Additionally, these metabolites were strongly associated with future diabetes, rendering them prognostic markers suitable for obese populations. Here we report a metabonomic study that reveals new findings on the role of these amino acid markers, particularly BCAAs, in a Chinese cohort including 106 healthy obese and 105 healthy lean participants. We found that the BCAAs were correlated with insulin resistance and differentially expressed in obese men, but not in obese women. The results were verified with two independent groups of participants (Chinese, n=105 and American, n=72), demonstrating that the serum metabolite profiles of the obese population are gender-dependent. The study supports the previous findings of a panel of several key metabolites as prognostic markers of obese population, and highlights the need to take into account gender differences when using these markers for risk assessment.

Graphical Abstract

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Supporting Information

Supplementary tables and figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Obesity co-segregates with metabolic abnormalities, including dyslipidemia and glucose intolerance.¹² However, obesity-induced metabolic perturbations have not been clearly determined. The strong relationship between obesity and diabetes and the rapidly increasing incidence of type 2 diabetes mellitus (T2DM) in Asia¹³ underscore the need for diabetes risk assessment and understanding of how metabolite profiles are altered.

Here we applied a metabolite profiling approach to determine metabolic differences between Chinese obese and lean human participants in a group of 106 healthy obese [median body mass index (BMI): 26.7 kg/m²] and 105 healthy lean (median BMI: 20.6 kg/m²) participants. The metabolite markers identified in this group of participants were verified in an independent group of 50 healthy Chinese overweight/obese and 55 healthy Chinese lean participants as well as a group of 55 American overweight/obese and 17 American lean participants. The association between IR and the differential metabolites was investigated between male and female participants.

Materials and Methods

Study Populations

Three cohorts of participants were recruited in the study using the same sample collection protocol. The first cohort consisting of 106 “healthy” obese (aged 23–64 years, median BMI of 26.7 kg/m²) and 105 healthy lean (aged 20–64 years, median BMI of 20.6 kg/m²) participants was recruited during routine checkups at the 6th People’s Hospital, which is affiliated with Shanghai Jiao Tong University, Shanghai, China. The healthy obese means they are obese subjects but without metabolic syndrome (MS). MS diagnostic criteria are in accordance with the proposed standard by the Chinese Diabetes Society (CDS).¹⁴ The healthy obese need to meet the following four criteria: obesity, namely BMI ≥ 25 kg/m²; FPG ≥ 6.1 mmol/L, and no previous history of diabetes, no hypoglycemic drugs used; SBP/DBP < 140/90 mmHg, and no previous high blood pressure history, no antihypertensive drugs used; fasting plasma TG < 1.7 mmol/L, and fasting plasma HDL-c ≥ 0.9 mmol/L (men) or ≥ 1.0 mmol/L (women), and no previous history of high cholesterol, no lipid-lowering drugs used. Participants in the second cohort were also recruited at the 6th People’s Hospital. Participants in the third cohort were American Caucasians and recruited by mass advertising throughout the community surrounding the Appalachian State University. The second and third cohorts were used to validate the results obtained from the first cohort.

Both obese and lean individuals were excluded from participation if they had significant cardiopulmonary, renal, or liver disease, active malignancy, or were taking diabetes medication, systemic corticosteroids, or weight loss medication.

All study measurements were obtained before 10 a.m. after an overnight fast. Serum samples were collected in the morning before breakfast and kept at –80 °C until analysis. The demographic information and clinical characteristics of all participants are shown in Table 1. The classification criteria of lean, overweight, and obese based on BMI are different in Chinese population (lean, BMI < 23.0; overweight, 23.0 < BMI < 25.0; obese, BMI ≥ 25.0)¹⁵ and American population (lean, BMI < 25.0; overweight, 25.0 < BMI < 30.0; obese,

BMI > 30.0).¹⁶ The distribution of BMI, age, and HOMA-IR of the population in each cohort are shown in Supplementary Figure S1.

This study was approved by the Institutional Review Board of the 6th People's Hospital and the Appalachian State University. All participants provided written informed consent. Figure 1 schematically shows the design and the data flow for discovery and validation of metabolite markers.

Anthropometric Measurements

Body weight and height were measured using standard methods for the calculation of BMI (kg/m²). Hip and waist circumferences were measured for the calculation of the WHR. Blood pressure, including 1st and 2nd systolic pressure and diastolic pressure, were measured twice with standard mercury sphygmomanometers for each participant while seated after 5 minutes of rest, and the average of two readings was taken.

Glucose, Insulin, and HOMA-IR

All participants underwent a 75-g OGTT in the morning after 10-h overnight fasting. Their venous blood samples were drawn at 0 (fasting state), 30 and 120 minutes. Plasma glucose concentrations (fasting glucose, 30 min postprandial plasma glucose, and 2 h postprandial plasma glucose) were measured by the glucose oxidase-peroxidase method using commercial kits (Shanghai Biological Products Institution, Shanghai, China) according to the manufacturer's instructions. Serum insulin concentrations were measured using the radioimmunoassay method (Linco Research, St Charles, MO, USA). Insulin sensitivity was measured by HOMA, using the following formula: $\text{HOMA} = (\text{fasting insulin in mU/mL} \times \text{fasting glucose in mM})/22.5$.

Biochemical Measurements

Blood and urine samples were obtained after an overnight fast to measure the analytes described below. Serum lipid profiles, including total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), blood urea, blood creatinine, blood uric acid, blood urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transSupeptidase (γ -GT), and urine creatinine were determined with a type 7600-020 automated analyzer (Hitachi, Tokyo, Japan).

Metabonomic Profiling

The metabonomic profiling analysis by ultraperformance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QTOFMS) and gas chromatography-time of flight mass spectrometry (GC-TOFMS) including sample preparation, metabolite separation and detection, metabonomic data preprocessing (e.g., metabolite feature extraction, chromatographic peak alignment, data reduction), and, finally, statistical analysis was performed following our previously published protocols.^{17, 18} The acquired MS data from UPLC-QTOFMS and GC-TOFMS was analyzed respectively by the MarkerLynx applications manager (v4.1 Waters, Manchester, UK) and ChromaTOF software (v3.30, Leco Co., CA, USA) using parameters reported in our previous work.¹⁷⁻¹⁹ Compound annotation was performed using our in-house library containing ~800 mammalian

metabolite standards. For UPLC-QTOFMS generated data, identification was performed by comparing the accurate mass (m/z) and retention time (R_t) of reference standards in our in-house library and the accurate mass of compounds obtained from the web-based resources such as the Human Metabolome Database (www.hmdb.ca). For GC-TOFMS generated data, identification was processed by comparing the mass fragments and R_t with our in-house library or mass fragments with NIST 05 Standard mass spectral databases in NIST MS search 2.0 (NIST, Gaithersburg, MD) software using a similarity of more than 70%. The two data sets obtained from UPLC-QTOFMS and GC-TOFMS were combined into a new data set and imported into SIMCA-P+12.0 software package (Umetrics, Umeå, Sweden). Principle component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were carried out to visualize the metabolic alterations between each group.^{18, 19} In addition to the multivariate statistical method, the Student's t -test was also applied to measure the significance of each metabolite. The resultant p values for all metabolites were subsequently adjusted to account for multiple testing by false discovery rate (FDR) method of Pike.²⁰ Metabolites with both multivariate and univariate statistical significance (variable importance in the projection (VIP) > 1 and $p < 0.05$) were considered potential markers responsible for the differentiation of overweight/obese from lean controls. In addition, Pearson correlations were calculated using SPSS software (IBM SPSS Statistics 19, USA). Spearman correlation coefficients were utilized to understand the relationship of the BCAA-related metabolite component with HOMA-IR. A partial Spearman correlation, adjusting for obesity status, was utilized to understand the independent relationship of the component with HOMA-IR. The significance of the correlation was evaluated by P value, and the strength of the correlation was evaluated by r . Bar plots of the metabolites were constructed using the R software package (<http://www.r-project.org>).

Results

Demographics and Clinical Characteristics

As shown in Table 1, the obese group was comprised of 63.2% women, whereas the lean participant group was comprised of 63.8% women in the first cohort. No significant difference was observed in age between the two groups (median age: 47 vs. 46 y, $P = 0.768$). As expected, obese participants were heavier and had higher waist/hip ratio (WHR) than lean participants. The median BMI was 26.7 kg/m² for the obese group, and was 20.6 kg/m² for the lean counterparts.

Obese participants had higher levels of insulin ($P < 0.001$), homeostatic model assessment of insulin resistance (HOMA-IR) ($P < 0.001$), triglyceride (TG) ($P < 0.001$), fasting blood glucose ($P < 0.05$), low-density lipoprotein (LDL) ($P < 0.001$), and serum uric acid ($P < 0.05$) than lean controls. Serum high-density lipoprotein (HDL) levels were decreased in obese participants compared to lean participants (1.38 vs. 1.60 mM; $p < 0.001$) (Table 1). However, no difference in the levels of total cholesterol ($P = 0.96$) was found between the two groups.

The demographics and clinical characteristics of the second and third cohorts are also shown in Table 1. The differences of biochemical parameters between lean and overweight/obese participants exhibited the similar trend as in the first cohort.

Serum Metabolite Profile of Obese and Lean Participants

A serum metabolite profile including 179 metabolites showed no significant difference between obese and lean participants in the first cohort. However, a clear separation was observed between male obese participants and male lean participants with an OPLS-DA model (Figure 2A, $R^2X = 0.709$, $R^2Y = 0.376$, $Q^2(\text{cum}) = 0.262$), while no separation between female obese and female lean participants occurred.

Univariate analysis of the metabolites revealed that 12 metabolites were significantly altered in male obese participants compared to male lean participants (adjusted $P < 0.05$; Table 2) after correction to account for multiple hypotheses testing. These metabolites represent key metabolic pathways involving BCAAs metabolism, fatty acid metabolism, tryptophan metabolism, bile acid metabolism and TCA cycle.

These 12 metabolite markers altered differently in male and female participants (Table 2). The serum concentrations of BCAAs, including leucine ($P = 2.27\text{e-}3$), isoleucine ($P = 9.55\text{e-}4$), and valine ($P = 6.74\text{e-}5$) were higher in obese men than in lean men, whereas no significant difference in BCAAs levels ($P > 0.2$) was observed between obese and lean women (Table 2).

Serum Metabolic Profile of Male Participants Compared to Female Participants

As shown in the OPLS-DA scores plot (Figure 2B, $R^2X = 0.183$, $R^2Y = 0.637$, $Q^2(\text{cum}) = 0.535$), a clear separation between male and female participants was achieved and a total of 39 differentially expressed serum metabolites were identified after multiple testing (Supplementary Table S1 and S2; $P < 0.05$).

Figure 3 and supplementary Figure S2 show an explicit difference between the two genders based on the serum metabolite concentrations. Among the differential metabolites, BCAAs (leucine, isoleucine, valine), creatine, uric acid, palmitic acid, myristic acid, LysoPC(18:2(9Z;12Z)), LysoPC(20:5(5Z;8Z;11Z;14Z;17Z)), n-dodecanoic acid, LysoPC(20:4(8Z;11Z;14Z;17Z)), cis-11,14-eicosadienoic acid, linoleic acid, and 12 α -hydroxy-3-oxocholadienic acid are of particular interest because they are involved in important metabolic pathways, such as fatty acid metabolism, BCAA metabolism, or bile acid metabolism.

A correlation analysis was performed among the 39 differential metabolites (Figure 4 and supplementary Figure S3), which revealed correlation coefficients among the metabolites, ranging from 1.0 (maximum positive correlation) to -0.5 (maximum anticorrelation) and 0 (no correlation, see color bar scale in Figure 4). A detailed analysis of the metabolite correlation matrices (Figure 4) in combination with their altered metabolite levels (Figure 3) indicated significant differences in BCAA metabolism, fatty acid metabolism, and bile acid metabolism between male and female. Figure 4 illustrates that several high positive (dark red and red regions) or negative (blue regions) correlations were observed among several metabolites among male and female participants. From the correlation difference matrix, palmitic acid, cis-11,14-eicosadienoic acid, linoleic acid, and alpha-palmitin were positively correlated with creatinine in males, but negatively correlated with creatinine in females. Similarly, cis-11,14-eicosadienoic acid, alpha-palmitin, myristic acid, and linoleic acid were

positively correlated with BCAAs in female participants, but this correlation became negative in males. Propionylcarnitine was positively correlated with BCAAs in females, but became more positive in males. Separate correlation matrices were created for lean female participants, lean male participants, obese female participants, and obese male participants (Supplementary Figure S3).

Additionally, as shown in the orthogonal partial least squares-discriminant analysis (OPLS-DA) scores plot of supplementary Figure S4, the metabolite profile of male participants were separated from female participants in both the lean and obese groups. The differential metabolites associated with gender are listed in supplementary Table S2. A venn diagram exhibiting the overlaps between gender differences and lean and obese phenotypes are provided in Supplementary Figure S5.

The BCAA 'Signature' in Obese Participants

The relationship between insulin sensitivity (HOMA-IR) and a principal component comprised of three BCAAs was evaluated in all participants, which revealed a significant and strong linear relationship (Figure 5 and supplementary Figure S6; $r = 0.335$; $P = 6.20 \times 10^{-7}$), even after adjusting for obese versus lean status using a partial Spearman correlation coefficient ($r = 0.299$; $P = 9.91 \times 10^{-6}$). To further ensure the independent relationship of the BCAAs component with HOMA-IR, we performed correlation analyses stratified by obese/lean status, which revealed a significant and strong linear relationship in obese males ($r = 0.476$; $P = 9.11 \times 10^{-6}$) and a significant linear relationship in obese females ($r = 0.277$; $P = 0.001$), with no significant relationship in lean subjects ($r = 0.132$; $P = 0.430$ for males and $r = 0.063$; $P = 0.610$ for females).

BCAAs in a Second Cohort of 50 Lean and 55 Overweight/Obese Participants

We recruited a group of Chinese participants comprising 50 lean and 55 overweight/obese participants. In this cohort, we identified a significant linear relationship between HOMA-IR and a principal component comprised of the three BCAAs (Figure 5 and supplementary Figure S6; $r = 0.301$; $P = 0.002$), even after adjusting for obese versus lean status using a partial Spearman correlation coefficient ($r = 0.283$; $P = 0.003$) in all the subjects. In similar way, we performed correlation analyses stratified by obese/lean status, which revealed a significant linear relationship in overweight/obese males ($r = 0.387$; $P = 0.014$) and females ($r = 0.249$; $P = 0.05$), with no significant relationship in lean subjects ($r = -0.116$; $P = 0.658$ for males and $r = 0.126$; $P = 0.538$ for females).

Similarly, the levels of the three BCAAs, leucine (adjusted $P = 0.0294$), isoleucine (adjusted $P = 0.015$), and valine (adjusted $P = 0.0294$), were significantly increased in male overweight/obese participants compared to lean participants, whereas no significant difference was observed in these BCAAs levels ($P > 0.2$) between female overweight/obese participants and lean participants (Table 3).

BCAAs in a Third Cohort of 17 American Lean and 55 American Overweight/Obese Participants

We found that the concentrations of BCAAs, including leucine (FC=1.36, $P = 5.28 \times 10^{-3}$), isoleucine (FC=1.31, $P = 2.90 \times 10^{-2}$), and valine (FC=1.26, $P = 2.60 \times 10^{-2}$) were higher in obese men than in lean men, whereas no significant difference in BCAAs levels ($P > 0.1$) was observed between obese and lean women. In addition, the levels of the three BCAAs, leucine (FC = 0.79; $P = 0.016$), isoleucine (FC = 0.75; $P = 0.0025$), and valine (FC = 0.84; $P = 0.046$), were significantly decreased in female participants compared to male participants, even after correction to account for multiple hypotheses testing (adjusted $P < 0.05$; supplementary Table S3). Principal component analysis (PCA) of the data for the 72 serum samples revealed a separation between male and female participants (Supplementary Figure S7).

Age Difference

Since the ages of the individuals in the data set ranged from 18 to 83 years, we investigated the effect of age on the serum metabolite profile. We set the age of < 40 as young and > 55 as old, and as a result, PCA scores plot showed no separation between the two groups (Supplementary Figure S8). We also compared the levels of the three BCAAs between young and old men and women in the obese and lean groups, respectively, which showed no significant difference between the two age groups ($p > 0.10$).

Discussion

In the present study, we applied a comprehensive metabonomics approach to understand the metabolic differences between healthy obese and lean humans as described in the Materials and Methods Section. Several obesity-related changes described herein confirm prior studies, including the higher levels of insulin, HOMA-IR, LDL-C and urine creatinine as well as the lower level of serum HDL-C in obese compared to lean participants. However, in contrast to comparisons of obese and lean participants previously reported in the literature, which generally focused on a small group of experimental variables or a small number of participants and often in a single gender, we found a gender-dependent metabolic profile that was consistent among three different groups of obese and lean participants, including both Chinese and U.S. male and female participants.

Metabolic Profiles of Lean and Obese Participants are Different

The metabonomics analysis of lean and obese participants revealed that male obese participants had a clearly distinct metabolic profile from male lean participants. Several metabolites associated with BCAA metabolism, fatty acid metabolism, and bile acid metabolism were altered, and changes in these metabolic profiles contributed to the difference between lean and obese participants (Table 2), which were consistent with reported findings associated with the obese phenotype.^{4, 5} We observed that levels of the BCAAs valine, leucine, and isoleucine were 12%, 17%, and 19% higher, respectively, in obese participants compared to lean participants ($P < 0.01$), reflecting an overload of BCAA catabolism in obese participants.⁴ Furthermore, the higher levels of propionyl-carnitine, C3 acylcarnitine, which reflects the propionyl CoA pool, in obese men are also in agreement

with the findings of Newgard et al.⁴ showing that byproducts or intermediates of BCAA catabolism increase specific C3 acylcarnitine levels.

BCAAs contribute to glucose recycling through the glucose-alanine cycle.²¹ There is a continuous flux of BCAAs from visceral tissues through the blood to skeletal muscle where transamination of the BCAAs takes place, thereby providing the amino group for production of alanine from pyruvate in muscle to liver to support hepatic gluconeogenesis.²² Our findings are consistent with the former reports showing that the serum levels of BCAAs were significantly higher in obese participants than in healthy lean participants. Moreover, plasma BCAA levels were positively correlated with IR,⁴ which indicates that BCAAs have effect on increasing the IR. Recently, a report by Wang et al. assessing a large cohort of 2422 participants underscored the potential key role of amino acid metabolism early in the pathogenesis of diabetes, suggesting that amino acid profiles could aid in diabetes risk assessment, and in particular branched-chain and aromatic amino acids (ArAAs), which could serve as predictors of the future development of diabetes.³ Besides BCAAs, the ArAAs, tryptophan and tyrosine, were also found to be significantly increased in obese participants,^{4, 5, 23} which are known to be related to functions of the gut microbiota^{24, 25} and has been reported to be related to obesity development.²⁶ The possible explanation is that the gut microbiota modulates the nutrient metabolism of the host, therefore contributing to the development of obesity.²⁷ BCAAs and ArAAs belong to the group of large neutral amino acids (LNAAs), which compete for the same saturable carrier across the blood brain barrier.²⁸ Increased BCAA levels in serum inhibit ArAA entry into the cell interior and therefore accumulate in the serum, leading to an increased serum ArAA levels. Imbalances in the serum “tryptophan ratio”, which is the molar ratio of tryptophan to its large neutral amino acid competitors (the sum of phenylalanine + leucine + isoleucine + valine + tyrosine), have been shown to be related to obesity.^{29, 30} The exaggerated insulin secretion response to glucose in obese participants may be due to potentiation of glucose-stimulated insulin secretion by BCAA.³¹ Moreover, it is well known that obesity is accompanied by an increase in circulating levels of multiple amino acids, including BCAA.^{4, 32, 33}

Fatty acids are an important energy source in the body and provide energy via β -oxidation. Research has shown that obesity and diabetes are related to the decreased fatty acid oxidation capacity, and as a result, excessive fatty acids will accumulate in the body as triglycerides, thereby causing lipotoxicity. Serum fatty acid composition may modulate insulin action, and increased serum saturated fatty acid concentrations are known to impair glucose metabolism, potentially causing diabetes³⁴. Consistent with the previous report by Kim et al.,⁵ palmitic acid, myristic acid, LysoPC(18:2(9Z;12Z)), LysoPC(20:5(5Z;8Z;11Z;14Z;17Z)), n-dodecanoic acid, LysoPC(20:4(8Z;11Z;14Z;17Z)), cis-11,14-eicosadienoic acid, and linoleic acid were found to be significantly increased in obese participants compared to lean participants of our study, representing fatty acids that circulate as triglycerides or other esterified species. These findings are consistent with the strong increase in triglyceride levels in obese participants.

Our study also found that obese participants had higher serum concentrations of metabolites related to energy metabolism. For example, elevated concentrations of creatine ($P < 0.05$) suggests extensive glycogenolysis occurring in order to accommodate the increased

demands for energy.³⁵ An elevated serum concentration of tyrosine in obese participants might reflect decreased synthesis of proteins in favor of lipogenesis, which indicates a shift in energy metabolism toward fat formation. These changes in amino acid metabolism have also been reported for obese people.^{36, 37} Of particular interest is the reported role of bile acids in regulating energy metabolism, where they act as a signal for fat and muscle cells to increase their energy expenditure through activation of thyroid hormone.³⁸ Taken together, bile acids could be playing a role in glucose homeostasis. Physiological effects of BCAA have been noted in various aspects of energy metabolism, including the stimulation of insulin secretion in pancreatic beta cells³⁹ and the control of appetite through the mTOR pathway in hypothalamic neurons.⁴⁰ It is therefore likely that in the future, BCAA will be used clinically as a marker of IR.

Studies in humans also have found that UA is a potent predictor of weight gain⁴¹ and serum UA levels are positively correlated with BMI in human participants.⁴² The serum levels of UA were significantly increased in obese participants compared to controls, which is consistent with the findings of Pacifico et al.⁴³ who previously demonstrated that the serum levels of UA were significantly higher in obese children compared to controls.

Metabolic Profile is Gender Dependent in Obese Population

Interestingly, our results showed an explicit difference between genders based on the metabolite quantities (Figure 3 and supplementary Figure S2). Compounds [leucine, isoleucine, valine, creatine, uric acid, palmitic acid, myristic acid, LysoPC(18:2(9Z;12Z)), LysoPC(20:5(5Z;8Z;11Z;14Z;17Z)), n-dodecanoic acid, LysoPC(20:4(8Z;11Z;14Z;17Z)), cis-11,14-eicosadienoic acid, linoleic acid, and 12 α -hydroxy-3-oxocholadienic acid] were significantly different between males and females. The significantly higher levels of palmitic acid, myristic acid, cis-11,14-eicosadienoic acid, and linoleic acid in females clearly suggest that fat metabolism is gender dependent, which are in good accordance with the well accepted evidence that females generally have a higher percentage of body fat than males.^{44, 45} A GC-MS and LC-MS-based metabolic study on an age- and gender-balanced cohort of 269 individuals also found that lipids (e.g., myristic acid and palmitic acid) were higher in female plasma than in male plasma⁴⁶ and an NMR-based metabolic study of rat plasma samples demonstrated that the total amounts of plasma lipoproteins were higher in males compared to their female counterparts.⁴⁷ An explanation for the differences observed in serum fat levels between men and women remains unknown, although differences in hormones, hormone receptors, and enzyme concentrations play a contributing role. In humans, serum cholesterol levels are different between sexes at matched ages, and in general, pre-menopausal females are less susceptible to high cholesterol levels than males.⁴⁸ A recent study by Szymańska et al.⁴⁹ evaluated the associations between metabolite profiles and phenotype parameters including different body fat distribution parameters such as android, gynoid, abdominal visceral (VAT), subcutaneous (SAT) fat in 215 plasma/serum samples from healthy overweight men (n = 32) and women (n = 83) with central obesity. They demonstrated that the association patterns were gender dependent that insulin, cholesterol, VLDL, and certain triacylglycerols (TG 54:1–3) correlated to VAT in women, while in men VAT was associated with TG 50:1–5, TG 55:1, phosphatidylcholine (PC 32:0), and VLDL. Moreover, their results revealed that waist circumference and total fat were

sufficient to predict VAT and SAT in women, while only VAT but not SAT could be predicted in men.

A GC-MS and ¹H NMR-based metabolomics study revealed gender differences in urinary metabolites involved in the metabolism of fatty acids, amino acids and TCA cycle among healthy male and female participants.⁵⁰ Our results are in agreement with previous observations indicating that females have lower plasma levels of BCAAs than males.^{37, 51} A previous study also showed that a gender difference exists in the regulation of BCAA catabolism and suggested that female sex hormones are most important in producing the gender difference.⁵² It has been suggested that the higher estrogen concentration in women may partly contribute to the lower oxidation of amino acids in women compared to men,⁵³ and that estrogen would be required for the maintenance of protein balance and fat-free mass in women.⁵⁴ Sex hormones contribute to differences in musculature because the higher testosterone levels in males during puberty increase muscle protein synthesis and net muscle protein balance, resulting in greater muscle mass than in females. Skeletal muscle is the major protein and free amino acid reserve of the body, and is the primary site in mammals for the catabolism of BCAAs. It was found that females had lower alkali-soluble protein (ASP)/DNA than males and that ASP/DNA was correlated with BCAA levels in plasma and muscle.⁵⁵ Moreover, the ASP/DNA ratio in muscle is probably the best quantitative index for assessing muscle protein content, since it reflects the amount of protein per cell nucleus.⁵⁵ This also suggests that the lower BCAA concentrations in females might be related to the mass of somatic (muscle) protein, which is conceivably influenced by hormonal factors.

Conclusion

In conclusion, we have demonstrated that the metabolite profile is significantly different between lean and obese participants. A cluster of obesity-associated changes in specific amino acid (BCAAs), fatty acid, acylcarnitine, and organic acid metabolites was identified in obese participants compared to lean participants. These metabolites were also associated with IR. Additionally we found that differences in serum metabolites and metabolic alterations, including BCAAs, fatty acids, uric acid, and creatinine in obese humans occur in a gender-dependent manner. Notably, three BCAAs (isoleucine, leucine, and valine) are correlated with IR and differentially expressed in obese men, but not in obese women. The results obtained in our study demonstrate that the serum metabolite profile of the obese population is gender-dependent and that BCAAs can be used to assess the risk of obesity in men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was financially supported by the Drug Innovation Program of National Science and Technology of China (No. 2011ZX09307-001-02) and Shanghai Science and Technology of China (No. 12DZ2295004).

We thank the participants and their families.

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Figure 1.
Flow chart of experimental design.

Figure 2.

(A) Metabolic profiles depicted by OPLS-DA scores plot of GC-TOFMS and UPLC-QTOFMS spectral data from the serum of male lean and obese participants in the first cohort. (B) OPLS-DA scores plot of and GC-TOFMS and UPLC-QTOFMS spectral data from male and female lean and obese participants (1, lean; 2 and 3, obese) in the first cohort.

Figure 3. Bar plot of the metabolic differences between male and female groups (A), lean male and female groups (B), and obese male and female groups (C) in the first cohort. A fold change (FC) value was calculated for each metabolite by taking the ratio of the mean intensities in the male and female groups. Each bar representing a fold change value was colored to indicate its corresponding P value and thereby specify the statistical significance for all male and female participants as well as lean and obese male and female participants, respectively (see color scale). The length of each bar in the plot with a value > 0 indicates a relatively higher concentration present in female participants and a value < 0 indicates a relatively lower concentration compared to the male participants. Each bar is ranked according to the P value for the corresponding metabolite, with the smallest P values appearing at the top of the figure.

Figure 4. Pearson's correlations of the quantities of the 39 serum differentially expressed metabolites measured by UPLC-QTOFMS and GC-TOFMS in both male and female participants in the first cohort. The correlation plots reveal a wide range of correlation coefficients among all of the measured metabolites, ranging from 1.0 (maximum positive correlation) to -0.5 (maximum anticorrelation) and 0 indicating no correlation (see color bar scale). Those metabolites are shown in Supplemental Table 2.

Figure 5.

Correlations between a BCAA-related metabolic “signature” and insulin sensitivity for all of the participants in the first cohort (A, $n = 211$, $r = 0.335$; $P = 6.20e-7$) and second cohort (B, $n = 105$, $r = 0.301$; $P = 0.002$). The figure shows the relationship between insulin sensitivity (HOMA-IR) and a principal component comprised of BCAA-related metabolites, including valine, leucine, and isoleucine (See Results for further description). Blue dots, male lean participants; Green dots, female lean participants; Blue diamonds, male obese participants; Black triangle, female obese participants.

Table 1
Demographics and clinical characteristics of lean and overweight/obese human participants

	Cohort I				Cohort II				Cohort III			
	Lean	Obese	P value	Lean	Overweight/obese	P value	Lean	Overweight/obese	P value	Lean	Overweight/obese	P value
Baseline characteristics	n = 105	n = 106		n = 55	n = 50		n = 17	n = 55				
Age (y)	45.9 (20.2, 63.9)	46.8 (23.0, 64.5)	7.78E-01	49.9 (15.0, 65.0)	47.0 (18.0, 64.0)	8.07E-01	61.6 (41, 81)	59.0 (44, 83)	4.06E-01			
Male/female	38/67	39/67		22/33	18/32		11/6	22/33				
BMI (kg/m ²)	20.6 (19.1, 22.2)	26.7 (25.0, 32.5)	5.40E-67	18.8 (17.4, 21.6)	27.1 (24.4, 31.6)	2.71E-40	23.6 (21.0, 24.8)	33.1 (27.0, 52.7)	1.19E-20			
Waist circumference (cm ²)	72 (60, 92)	87 (73, 108)	2.40E-39	67 (60, 89)	90 (79, 103)	6.07E-38						
Hip (cm)	87 (76, 99)	99 (88, 114)	2.73E-43	87 (78, 98)	101 (95, 110)	8.62E-16						
Systolic pressure (mmHg)	116.67 (89.33, 135)	117.83 (85.33, 138.67)	4.20E-01	110 (95, 126)	120 (100, 130)	6.74E-06	125.35 (100, 165)	131.07 (110, 180)	2.19E-01			
Diastolic pressure (mmHg)	72 (54, 88)	77 (47, 89)	2.47E-02	70 (60, 80)	74 (60, 88)	3.74E-05	75.53 (60, 96)	81.51 (60, 100)	3.18E-02			
Serum BUN (mM)	4.8 (2.9, 8.3)	4.8 (1.5, 8.4)	7.96E-01	4.6 (2.8, 6.9)	4.8 (3.1, 6.2)	4.58E-01	6.1 (3.9, 10.0)	6.47 (3.2, 14.6)	4.51E-01			
Creatinine (mM)	64 (37, 99)	62 (41, 103)	8.68E-01	64 (47, 92)	60.5 (41, 97)	2.52E-01	81 (62, 121)	80 (53, 169)	8.12E-01			
Uric acid (mM)	268 (147, 438)	279 (137, 509)	1.86E-02	266.2 (145.6, 412.2)	280.1 (146.6, 546.4)	8.64E-02						
AST (U/L)	14 (5, 49)	18 (3, 45)	2.10E-03	19 (7, 31)	21 (9, 149)	9.52E-02	31.65 (19, 53)	29.53 (20, 54)	3.48E-01			
ALT (U/L)	19 (12, 38)	20 (11, 37)	4.49E-01	14 (7, 25)	21 (8, 71)	2.33E-05	24.29 (8, 48)	28.34 (11, 72)	1.57E-01			
γ-GT (U/L)	17 (9, 59)	19 (10, 115)	1.87E-03	14 (8, 36)	16 (8, 73)	1.28E-01						
TC (mM)	4.25 (2.92, 5.17)	4.32 (2.54, 5.15)	9.62E-01	4.2 (2.3, 5)	4 (2.8, 5)	8.86E-01	5.33 (3.00, 8.69)	5.22 (2.79, 7.74)	7.75E-01			
TG (mM)	0.77 (0.37, 1.58)	0.935 (0.43, 1.68)	1.56E-05	0.82 (0.42, 1.56)	1.02 (0.42, 1.69)	5.14E-02	1.46 (0.35, 2.71)	1.89 (0.58, 7.60)	6.46E-02			
HDL (mM)	1.6 (1.03, 2.52)	1.38 (0.92, 2.28)	2.11E-07	1.56 (1.05, 2.43)	1.29 (1.06, 1.91)	2.46E-05	1.56 (0.87, 2.59)	1.28 (0.59, 2.49)	4.15E-02			
LDL (mM)	2.44 (1.17, 3.35)	2.69 (1.23, 3.36)	6.11E-04	2.42 (0.71, 3.31)	2.53 (1.27, 3.23)	5.11E-02	3.05 (1.26, 6.62)	3.15 (1.08, 5.54)	7.60E-01			
Urine creatinine (mM)	10943 (2018, 37812)	10536 (2805, 35168)	9.48E-01	8583 (3383, 20484)	10502.5 (2902, 24054)	7.82E-02						
Glucose (mM)	5 (4.23, 5.99)	5.16 (3.89, 5.95)	6.92E-03	4.9 (3.91, 6.04)	5.01 (4.04, 6.03)	3.69E-01	5.04 (3.72, 6.61)	5.72 (3.78, 16.17)	3.32E-02			
Glucose 30 (mM)	8.15 (4.85,)	8.47 (4.68, 12.34)	1.51E-01	8.54 (5.48, 13.02)	8.84 (5.4, 11.26)	4.42E-01						
Glucose 120 (mM)	5.51 (2.68, 7.73)	5.81 (3.72, 7.75)	9.13E-02	4.99 (2.06, 7.33)	5.38 (3.42, 7.79)	7.95E-02						
INS0 (U/L)	5.1 (0.2, 13.93)	8.4015 (2.34, 22.17)	1.04E-10	6.5 (3.48, 21.39)	11.42 (4.45, 21.21)	2.09E-06						
INS30 (U/L)	46.38 (56.99, 143.3)	60.55 (19.78, 249.30)	6.31E-04	44.24 (6.08, 185.84)	69.01 (16.8, 220.87)	2.44E-03						
INS120 (U/L)	23.54 (3.78, 97.99)	31.91 (4.67, 133.2)	3.39E-04	24.35 (2.95, 44.26)	36.29 (6.98, 236.02)	1.72E-03						
HOMA-IR0	1.11 (0.038, 3.15)	1.97 (0.49, 5.47)	1.15E-10	1.31 (0.76, 3.76)	2.20 (0.95, 5.31)	9.55E-07						
HOMA-IR30	16.43 (1.91, 56.43)	24.49 (5.75, 108.03)	8.49E-06	11.31 (4.9, 65.08)	20.63 (6.87, 82.36)	3.24E-03						

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Cohort I			Cohort II			Cohort III		
Lean	Obese	P value	Lean	Overweight/obese	P value	Lean	Overweight/obese	P value
HOMA-IR120	5.71 (0.66, 25.08)	8.19 (0.79, 37.32)	3.10E-04	4.23 (0.75, 10.97)	7.22 (1.23, 65.87)	1.46E-03		

Table 2

List of differential metabolites in obese participants relative to lean participants, male obese participants relative to male lean participants, and female obese participants relative to female lean participants from cohort I.

Compound	ALL lean vs. obese participants				male lean vs. obese participants				female lean vs. obese participants			
	FC ^a	p ^b	Adjusted p ^c	FC ^a	p ^b	Adjusted p ^c	FC ^a	p ^b	Adjusted p ^c	FC ^a	p ^b	Adjusted p ^c
Valine *	1.06	2.72E-03	1.76E-01	1.12	6.74E-05	2.14E-02	1.02	3.83E-01	9.72E-01	1.02	3.83E-01	9.72E-01
Creatine *	1.1	6.31E-02	3.65E-01	1.35	4.43E-04	2.14E-02	1.02	7.27E-01	9.72E-01	1.02	7.27E-01	9.72E-01
Beta-Tyrosine *	1.06	5.96E-02	3.65E-01	1.19	8.49E-04	2.14E-02	0.99	8.37E-01	9.89E-01	0.99	8.37E-01	9.89E-01
Isoleucine *	1.09	2.35E-02	3.23E-01	1.19	9.55E-04	2.14E-02	1.02	6.94E-01	9.72E-01	1.02	6.94E-01	9.72E-01
4-Hydroxycinnamic acid	1.07	2.99E-02	3.23E-01	1.18	1.41E-03	2.48E-02	1.01	7.21E-01	9.72E-01	1.01	7.21E-01	9.72E-01
Adenine *	1.06	5.50E-02	3.65E-01	1.17	1.59E-03	2.55E-02	1	9.62E-01	9.98E-01	1	9.62E-01	9.98E-01
Leucine *	1.07	4.74E-02	3.65E-01	1.17	2.27E-03	2.48E-02	1.01	8.04E-01	9.89E-01	1.01	8.04E-01	9.89E-01
Delta-hydroxylysine	1.08	6.36E-04	3.70E-02	1.12	2.89E-03	3.36E-02	1.06	4.34E-02	9.35E-01	1.06	4.34E-02	9.35E-01
Kynurenine *	1.02	3.64E-01	8.09E-01	1.11	3.12E-03	3.36E-02	0.97	2.57E-01	9.35E-01	0.97	2.57E-01	9.35E-01
Glutamic acid *	1.18	2.96E-02	3.23E-01	1.44	3.55E-03	3.81E-02	1.04	6.75E-01	9.72E-01	1.04	6.75E-01	9.72E-01
12a-Hydroxy-3-oxocholadienic acid	1.34	8.80E-05	1.38E-02	1.44	3.66E-03	3.36E-02	1.26	6.76E-03	6.12E-01	1.26	6.76E-03	6.12E-01
Tryptophan *	1.05	1.10E-01	4.53E-01	1.14	5.08E-03	3.81E-02	1	9.98E-01	9.98E-01	1	9.98E-01	9.98E-01
Tyrosine *	1.07	8.86E-02	4.20E-01	1.19	6.87E-03	5.42E-02	1.01	8.32E-01	9.89E-01	1.01	8.32E-01	9.89E-01
8-Hydroxy-7-methylguanine	1.05	1.55E-01	6.00E-01	1.16	7.08E-03	5.42E-02	0.99	8.37E-01	9.89E-01	0.99	8.37E-01	9.89E-01
Glycerol phosphate *	1.09	1.84E-01	6.71E-01	1.38	7.28E-03	6.41E-02	0.95	4.85E-01	9.72E-01	0.95	4.85E-01	9.72E-01
Camitine *	1.05	8.96E-03	3.03E-01	1.07	1.23E-02	1.37E-01	1.03	1.55E-01	9.35E-01	1.03	1.55E-01	9.35E-01
LysoPC(18:2(9Z;12Z))	0.85	5.80E-03	1.96E-01	0.79	1.50E-02	1.37E-01	0.9	1.02E-01	9.35E-01	0.9	1.02E-01	9.35E-01
LysoPC(20:5(5Z;8Z;11Z;14Z;17Z))	0.86	4.30E-03	1.76E-01	0.8	1.54E-02	1.37E-01	0.89	7.25E-02	9.35E-01	0.89	7.25E-02	9.35E-01
Alanine *	1.06	2.34E-02	3.23E-01	1.09	1.76E-02	1.74E-01	1.04	2.51E-01	9.35E-01	1.04	2.51E-01	9.35E-01
Propionyl/carnitine	1.1	3.44E-02	3.23E-01	1.18	1.83E-02	1.12E-01	1.05	4.16E-01	9.72E-01	1.05	4.16E-01	9.72E-01
n-Octadecanoic acid	0.93	3.22E-01	7.48E-01	0.75	2.29E-02	1.27E-01	1.03	7.26E-01	9.72E-01	1.03	7.26E-01	9.72E-01
Histidinol	0.99	6.87E-01	8.56E-01	1.06	2.49E-02	5.63E-01	0.96	7.34E-02	9.35E-01	0.96	7.34E-02	9.35E-01
Palmitoleic acid	0.83	2.01E-02	3.23E-01	0.71	2.91E-02	1.72E-01	0.9	2.46E-01	9.35E-01	0.9	2.46E-01	9.35E-01
Bilirubin	0.97	8.41E-01	9.76E-01	1.74	3.44E-02	1.74E-01	0.7	9.83E-02	9.35E-01	0.7	9.83E-02	9.35E-01

Compound	ALL lean vs. obese participants			male lean vs. obese participants			female lean vs. obese participants		
	FC ^a	p ^b	Adjusted p ^c	FC ^a	p ^b	Adjusted p ^c	FC ^a	p ^b	Adjusted p ^c
cis-11,14-eicosadienoic acid *	0.9	5.06E-02	3.65E-01	0.82	3.73E-02	1.72E-01	0.94	3.43E-01	9.51E-01
Glycolithocholic acid *	1	8.50E-01	9.22E-01	1.08	3.91E-02	4.50E-01	0.96	1.70E-01	9.35E-01
2-Hydroxyglutaric acid *	0.98	6.37E-01	8.71E-01	1.13	3.92E-02	3.20E-01	0.91	7.63E-02	9.35E-01
Camosine *	0.76	5.03E-01	8.56E-01	0.26	4.02E-02	2.35E-01	1.45	5.24E-01	9.72E-01
Myristic acid *	0.82	6.50E-04	3.70E-02	0.82	4.11E-02	1.99E-01	0.82	5.19E-03	6.12E-01
Allantoin	1.23	4.65E-01	8.48E-01	2.04	4.78E-02	2.32E-01	0.6	2.45E-01	9.35E-01
N-acetylneuraminic acid	1.1	1.78E-01	6.48E-01	1.24	4.88E-02	2.32E-01	1.04	6.77E-01	9.72E-01
Phenylalanine *	1.05	8.50E-02	4.20E-01	1.1	5.72E-02	2.76E-01	1.03	4.00E-01	9.72E-01

Only metabolites with unadjusted p values of < 0.05 are shown.

^a Fold change (FC) was obtained by comparing those metabolites in the obese group to the lean group; FC with a value > 1 indicates a relatively higher concentration present in obese group while a value < 1 means a relatively lower concentration compared to the lean group.

^b p values from Student's t-test.

^c Adjusted for multiple hypotheses testing by controlling for FDR 20.

* Identified by reference standard.

Table 3

List of differential metabolites in obese participants relative to lean participants, male obese participants relative to male lean participants, and female obese participants relative to female lean participants from the second cohort

Rt (min)	Dataset	Compound	All obese vs. lean participants				Male obese vs. lean participants				Female obese vs. lean participants			
			VIP ^a	FC ^b	p ^c	Adjusted p ^d	FC ^b	p ^c	Adjusted p ^d	FC ^b	p ^c	Adjusted p ^d	FC ^b	Adjusted p ^d
8.81	GC	Isoleucine *	2.23	1.35	1.84E-03	9.92E-03	1.82	1.11E-03	1.50E-02	1.15	2.16E-01	2.78E-01		
8.51	GC	Leucine *	1.68	1.21	2.00E-02	2.99E-02	1.46	4.51E-03	2.94E-02	1.08	4.52E-01	4.69E-01		
7.72	GC	Valine *	1.54	1.17	3.29E-02	3.56E-02	1.42	5.89E-03	2.94E-02	1.05	6.18E-01	6.18E-01		
0.67	ES-	Dihydroxyfumaric acid	2.58	1.39	3.06E-04	8.28E-03	1.22	1.01E-01	1.43E-01	1.53	1.27E-03	3.17E-02		
0.67	ES-	Glyceraldehyde *	2.42	1.39	7.23E-04	9.76E-03	1.24	1.19E-01	1.53E-01	1.53	2.35E-03	3.17E-02		
0.68	ES-	Hypoxanthine *	2.25	1.36	1.67E-03	9.92E-03	1.40	4.28E-02	7.71E-02	1.32	1.66E-02	9.33E-02		
5.45	GC	Lactic acid *	2.24	1.51	1.71E-03	9.92E-03	2.05	3.61E-04	9.76E-03	1.29	1.41E-01	2.71E-01		
9.01	GC	Glycine *	2.10	0.48	3.34E-03	1.42E-02	0.61	1.84E-01	2.11E-01	0.39	6.65E-03	5.98E-02		
1.85	ES-	p-Cresol *	2.03	0.53	4.43E-03	1.42E-02	0.30	9.80E-03	2.94E-02	0.68	1.34E-01	2.71E-01		
1.60	ES-	3-Nitrotyrosine	2.03	0.73	4.73E-03	1.42E-02	0.63	1.82E-02	3.82E-02	0.80	1.07E-01	2.71E-01		
1.84	ES-	cis-2-Methylaconitate	2.02	0.59	4.71E-03	1.42E-02	0.39	1.32E-02	3.23E-02	0.72	1.33E-01	2.71E-01		
0.65	ES-	4-Hydroxy-3-methoxymandelic acid *	1.98	1.54	5.84E-03	1.58E-02	2.10	4.38E-03	2.94E-02	1.26	2.46E-01	2.91E-01		
15.82	GC	Ascorbic acid *	1.91	0.62	7.92E-03	1.92E-02	0.73	2.15E-01	2.32E-01	0.54	1.73E-02	9.33E-02		
4.54	ES-	8,11,14-Eicosatrienoic acid	1.90	1.50	8.54E-03	1.92E-02	1.69	9.30E-03	2.94E-02	1.36	1.76E-01	2.78E-01		
0.65	ES-	L-beta-aspartyl-L-glycine	1.84	1.35	1.04E-02	2.15E-02	1.48	2.66E-02	5.13E-02	1.27	1.40E-01	2.71E-01		
16.17	GC	Arabinofuranose	1.84	3.46	1.26E-02	2.43E-02	2.40	2.62E-01	2.62E-01	4.07	2.67E-02	1.20E-01		
12.82	GC	N-Acetyl-L-aspartic acid	1.78	1.52	1.35E-02	2.43E-02	2.27	1.22E-02	3.23E-02	1.25	2.48E-01	2.91E-01		
1.65	ES-	Phenylpropiylglycine	1.73	1.76	1.67E-02	2.82E-02	1.48	2.32E-01	2.41E-01	2.00	4.06E-02	1.57E-01		
15.82	GC	Ornithine *	1.69	1.52	1.95E-02	2.99E-02	2.71	8.05E-03	2.94E-02	1.24	2.78E-01	3.12E-01		
8.52	GC	Glycerol *	1.65	1.28	2.24E-02	3.18E-02	1.24	1.62E-01	1.99E-01	1.31	7.02E-02	2.37E-01		
5.31	GC	Pyruvic acid *	1.63	1.68	2.48E-02	3.35E-02	2.36	1.84E-02	3.82E-02	1.50	1.54E-01	2.77E-01		
0.65	ES-	5-Methoxytryptophan	1.60	1.42	2.70E-02	3.41E-02	1.58	7.23E-02	1.22E-01	1.31	1.83E-01	2.78E-01		
5.53	GC	Ethanedioic acid	1.59	1.35	2.78E-02	3.41E-02	1.85	7.07E-03	2.94E-02	1.16	3.88E-01	4.19E-01		
0.65	ES-	L-beta-aspartyl-L-glutamic acid	1.56	1.40	3.10E-02	3.56E-02	1.60	7.86E-02	1.22E-01	1.28	2.10E-01	2.78E-01		

Rt (min)	Dataset	Compound	All obese vs. lean participants			Male obese vs. lean participants			Female obese vs. lean participants			
			VIP ^a	FC ^b	p ^c	Adjusted p ^d	FC ^b	p ^c	Adjusted p ^d	FC ^b	p ^c	Adjusted p ^d
0.89	ES-	2-Pyrocatechuic acid	1.56	2.21	3.18E-02	3.56E-02	2.70	8.16E-02	1.22E-01	1.90	1.97E-01	2.78E-01
1.41	ES-	2-Methylbutyrylglycine	1.49	1.58	4.00E-02	4.16E-02	1.59	1.87E-01	2.11E-01	1.58	1.26E-01	2.71E-01
0.66	ES-	3-Hexenedioic acid	1.46	1.40	4.40E-02	4.40E-02	1.61	1.07E-01	1.44E-01	1.29	2.16E-01	2.78E-01

Only metabolites with unadjusted p values of < 0.05 are shown.

^a Fold change (FC) was obtained by comparing those metabolites in the obese group to the lean group; FC with a value > 1 indicates a relatively higher concentration present in the obese group, while a value < 1 indicates a relatively lower concentration compared to the lean group.

^b p values from Student's t-test.

^c Adjusted for multiple hypotheses testing by controlling for FDR ²⁰.

* Identified by reference standard.