Full title: Large-Scale Analysis of Circulating Amino Acids and Gene Expression in Relation to Abdominal Obesity

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Abstract

Background: Circulating branched-chain amino acid (BCAA) levels are increased in obesity. One hypothesis explaining this phenomenon is downregulated catabolism in adipose tissue. Glutamate is a byproduct of this pathway and was found to be positively associated with central fat accumulation. We assessed the association between circulating glutamate and abdominal obesity, and how it relates to gene expression pathways in abdominal fat depots and cell types.

Methods: We used logistic regression to assess the odds of presenting abdominal obesity with varying circulating glutamate in 4,665 individuals from the TwinsUK cohort. We also compared glutamate levels in twin pairs discordant for abdominal obesity. We accessed gene expression profiles of adipose tissue and cells across depots derived from independent cohorts for phenotype, differential expression and pathway analysis.

Results: Individuals in the 5th quintile of circulating glutamate had a 7-fold increase in the risk of presenting abdominal obesity compared to those in the 1st quintile. This risk was greater than that associated with being in the 5th quintile of either BCAAs. Twins with abdominal obesity had significantly higher glutamate levels than their leaner co-twins. Most genes of the BCAA catabolism pathway were more expressed in the stromal vascular fraction (SVF) compared to adipocytes and associated with abdominal obesity in both men and women. Among the top pathways to be upregulated in visceral SVF was the glutamate receptor signaling pathway.

Conclusion: Glutamate is a potential biomarker of abdominal fat accumulation. Differences in gene expression across adipose tissue depots and cell types reflect this association.

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Abbreviations

BCAAs	Branched-chain amino acids
BCAT	Branched-chain aminotransferase
BCKD	Branched-chain keto-acid dehydrogenase
BCKDK	BCKD kinase
BMI	Body mass index
CI	Confidence interval
CVD	Cardiovascular diseases
DXA	Dual-energy absorptiometry
HDL-C	High-density-lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
IPA	Ingenuity pathway analysis
LDL-C	Low-density-lipoprotein cholesterol
MetS	Metabolic syndrome
OR	Odds ratio
PPM1K	Protein Phosphatase, Mg2+/Mn2+ Dependent 1K
SAT	Subcutaneous adipose tissue
SVF	Stromal vascular fraction
T2D	Type 2 diabetes
VAT	Visceral adipose tissue
WC	Waist circumference

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Introduction

In the past 10 years, studies based on metabolomics have allowed investigators to establish that obesity is associated not only with alterations in circulating carbohydrates and lipids, but also in circulating amino acids [1]. The three branched-chain amino acids (BCAAs), namely leucine, isoleucine and valine, have been the most studied. Indeed, their circulating level has been consistently and positively associated with obesity and the related metabolic alterations as well as with long-term risk of type 2 diabetes (T2D) and cardiovascular diseases (CVD) (see [1] for review).

The first step of BCAA catabolism is a transfer of the amino group from the BCAA toward alpha-ketoglutarate [2]. This reaction, catalyzed by the branched-chain aminotransferase (BCAT) enzyme, produces a keto-acid and glutamate. The second step of this pathway is performed by the branched-chain keto-acid dehydrogenase (BCKD) complex. The latter is regulated by the addition or removal of a phosphate group on its E1 subunit by a kinase (BCKDK) and a phosphatase (PPM1K) respectively. Although the pathophysiology of the association between circulating BCAAs and obesity is not yet fully understood, we know it involves reduced catabolism in peripheral tissues [3,4]. Of note, studies have shown that adipose tissue gene expression of BCAA catabolic enzymes was lower in individuals who are obese vs lean [5] and in those who are unhealthy vs healthy [3,6]. Moreover, She et al. have shown that expression of these enzymes in adipose tissue increased following bariatric surgery and was accompanied by a significant decrease in circulating BCAAs [7].

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This phenomenon has brought interest on intermediates and byproducts of the BCAA-catabolism pathway, the levels of which could also be altered in obesity. One of these byproducts, glutamate, has particularly captured our attention [8,9]. We have shown that, out of nearly 200 metabolites, glutamate was the most strongly associated with visceral adipose tissue (VAT) accumulation in a sample of 59 middle-aged women [5]. Although glutamate is much less studied than the BCAAs, studies that did measure it generally found that it is more strongly correlated with central fat accumulation than the BCAA levels, as shown in the Framingham Heart Study Generation 3 cohort for waist circumference (WC) [10] and in a sample of 1,499 Japanese participants for VAT area measured by computed tomography [11].

In the TwinsUK cohort, Menni et al. investigated whether the relationship between circulating metabolites and T2D or blood pressure was mediated by trunk fat mass [12]. They found that the strongest independent association with trunk fat, was seen for glutamate. Of note, the three BCAAs were not identified as being independently associated with trunk fat in this analysis. However, this was not the central analysis of the study and as such, those results were not further explored.

Considering the strength of the association between circulating glutamate and abdominal obesity and its consistency across cohorts as well as measurement tools of body fat distribution, we aimed to explore this relationship further. In this study, we first assess the potential of glutamate as a biomarker of abdominal obesity in comparison with that of BCAAs in a large cohort of more than 4000 deeply phenotyped twins. Second, we contrasted the expression of BCAA catabolic enzymes across adipose depots, cell types,

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sexes and abdominal obesity utilizing large population-based tissue cohorts. Finally, we performed global analysis of adipose tissue transcriptomes derived from individuals with abdominal obesity, contrasting adipose depots and cell types.

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Methods

Study population and sample

The TwinsUK cohort is a registry of volunteer twins from the United Kingdom. Its original aim was to study osteoporosis in women, which explains why most participants (83%) are females [13]. Its focus has now shifted towards the investigation of healthy aging in both sexes. One of the main strengths of this cohort is the comprehensive characterization of the participants through various high-throughput approaches such as genomics, transcriptomics and metabolomics [13]. The participants were not aware of the specific hypothesis that would later be tested, and all provided informed consent.

For the present study, we considered individuals for whom metabolomic and body composition data were available and taken within 6 months of each other. This project was approved by the ethics committee of Université Laval.

Metabolomics

Untargeted metabolomic measurements were performed by Metabolon Inc. as described elsewhere [12]. Briefly, plasma samples were analyzed by ultra-high-performance liquid chromatography-tandem mass spectrometry and metabolites were identified by automated comparison. Metabolite concentrations were divided by the median of the day and inverse normalized. Our focus was on glutamate, but we performed all analyses on BCAA levels for comparison purposes.

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Body composition

Body composition was measured by dual-energy absorptiometry (DXA, Hologic QDR, Hologic Inc.) [14] and trunk fat was expressed as the percentage of fat in the trunk region.

Because there is no formal abdominal obesity definition based on trunk fat measurement, we used the linear regression between WC and percent trunk fat to identify a threshold. A 37.7 percentage of trunk fat corresponded to 95 cm of WC [15] according to the regression and was used to define abdominal obesity.

Cardiometabolic risk parameters

Blood samples were collected while participants were in the fasted state. Only measurements for which the date of visit was within 6 months of that when the sample for metabolomics was obtained were included in this study.

Glucose was measured with an enzymatic colorimetric assay (Johnson and Johnson Clinical Diagnostic Systems) and insulin with an immunoassay (Abbott Laboratories) [16]. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as: insulin (mU/L) * glucose (mmol/L)/ 22.5 [17].

Systolic and diastolic blood pressures were taken by a trained nurse or research assistant after participants had been seated for at least 3 minutes and using an automated cuff sphygmomanometer (Omron HEM-713C) [16].

Triglycerides, low-density-lipoprotein cholesterol (LDL-C) and high-density-lipoprotein cholesterol (HDL-C) were measured with a Cobas Fara machine (Roche Diagnostics) [16].

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We identified the presence of metabolic syndrome (MetS) features as follows: high WC (>102 cm for men, >88 cm for women); high triglycerides (>1.7 mmol/L); low HDL-C (<1.1 mmol/L for men, <1.3 mmol/L for women); high fasting glucose (≥5.6 mmol/L); and high blood pressure (≥130/85 mmHg) [18].

Statistical analyses

We evaluated the association between circulating amino acid levels and trunk fat using mixed linear regressions. The models were adjusted for age, sex, metabolomic batch and twin-pair clustering. We calculated the odds ratio (OR) of presenting abdominal obesity and MetS features according to circulating amino acid quintiles using mixed logistic regressions adjusted for age, sex, metabolomic batch and twin-pair clustering. We evaluated the potential influence of genetics on the association between amino acids and trunk fat by conducting a co-twin case-control analysis. First, we identified monozygotic twin pairs discordant for trunk fat, that is, the monozygotic pairs in the top 5% of intra-pair trunk fat difference. Then we compared the circulating amino acid level between leaner and heavier (in terms of trunk fat) twins of each pair using paired and two-tailed Student' t-test. Analyses were performed using R (1.1.463). The lmer4 (1.1-20) package [19] was used to perform mixed models.

RNA Sequencing of adipose derived from obese individuals

Adipose tissue samples were collected from obese individuals undergoing bariatric surgery at the Quebec Heart and Lung Institute (IUCPQ, Laval University, Quebec City, Canada). Informed consent was obtained from each participant through the management

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framework of the IUCPQ Obesity Biobank. The protocol was approved by the Research Ethics Committee of IUCPQ (Protocol # 21320). Subcutaneous adipose tissue (SAT) and omental VAT samples from 43 individuals were included in this study with body mass index (BMI) > 35.5 kg/m^2 and age from 29 to 65 years. The majority of the participants were females (n=22). RNA sequencing was done using whole adipose tissue (n=20) as well as isolated adipocytes and stromal vascular fraction (SVF) from both adipose depots (n=13).

Adipose tissue was digested within 30 minutes of collection using collagenase adapting the Robdell method [20]. The SVF cells, adipocytes and whole tissue were cryopreserved and stored in liquid nitrogen. From the frozen samples, 0.5 to 3 million cells were re-suspended in 500 uL TRIzol Reagent and total RNA was extracted using the miRNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. RNA library preparations were carried out on 500 ng of RNA with RNA integrity number (RIN)>7 using the Illumina TruSeq Stranded Total RNA Sample preparation kit, according to manufacturer's protocol. Final libraries were analyzed on a Bioanalyzer and sequenced on the Illumina HiSeq 2500. The sequenced reads were pair-ended and 100 – 125 bp in length.

RNASeq Analysis

The sequencing reads were trimmed for quality (phred33 ≥30) and length (n≥32), and Illumina adapters were clipped off using Trimmomatic v.0.35 (Bolger, A. *et al*. 2014). Filtered reads were then aligned to the GRCh37 human reference using STAR v.2.5.3a [21].

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Raw read counts of genes were obtained using htseq-count v.0.6.0 (Anders 2015]. Differential gene expression analysis was done using DeSeq2 v.1.18.1 [22]. Ingenuity Pathway Analysis (IPA: QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis) was used for identifying enriched pathways. We also used publicly available data from MuTHER, METSIM and GTEx cohorts to validate our results.

To identify VAT specific SVF genes, we did differential gene expression analysis between visceral-specific SVF and matched adipocyte RNA-Seq data, identifying 9,023 SVF-specific genes (log2FC of >=2, Adj. p <= 0.05). The genes were further filtered by restricting to those upregulated in VAT SVF in comparisons with SAT SVF using a nominal p value of $p \leq 0.05$ and it resulted in 1,820 genes (**Supplementary Table 2**) including 1,117 (61%) protein coding genes.

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Results

Association between circulating amino acid and abdominal obesity

We included 4,665 individuals for whom both metabolites and phenotypes were available, and their characteristics can be found in **Table 1**. The vast majority (94%) of participants were females, and average BMI, WC, total cholesterol, triglycerides and HOMA-IR were within normal ranges, indicating that the sample was representing population averages as shown before [23].

We applied a linear mixed model linking circulating glutamate with measurements of obesity and found a significant and positive association with trunk fat (β: 0.23, p-value: 2.1e-70) and BMI (β: 0.27, p-value: 3e-78). We also tested the association between circulating glutamate and additional metabolic and T2D-related traits and found positive association with total triglycerides (β: 0.29, p-value: 1.2e-62) and the HOMA-IR index (β: 0.31, p-value: 5.7e-38) and an negative association with HDL-C (β: -0.23, p-value: 2.1e-32). Associations with these variables were all stronger for glutamate than for any of the three BCAAs (see **Supplemental Table 1**).

Next, we computed the odds of presenting abdominal obesity in the 2nd, 3rd, 4th and 5th circulating amino acid quintiles compared to the 1st with mixed logistic regression models. Results are presented in **Figure 1**. Compared with the 1st quintile of circulating glutamate, the OR of presenting abdominal obesity was 1.26 (95%CI: 0.97, 1.64) for the 2nd quintile, 2.37 (95%CI: 1.81, 3.10) for the 3rd, 3.37 (95%CI: 2.56, 4.44) for the 4th and 7.00 (95%CI: 5.21, 9.39) for the 5th. Participants in the 5th glutamate quintile also had a

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greater prevalence of most MetS features: high WC (OR: 9.78, 95%CI: 4.65, 20.58), high triglycerides (OR: 7.28 , 95%CI: 4.84, 10.94), low HDL-C (OR: 4.91, 95%CI: 2.94, 8.21) and high fasting glucose (OR: 4.27, 95%CI: 2.73, 6.68). Individuals in the 5th quintile of any one of the BCAAs also had higher prevalence of these metabolic dysfunction markers, but the risk was lower than for glutamate (see **Supplemental Figure 1**). The risk of having high blood pressure was not significantly higher in the 5th vs the 1st quintile of glutamate, leucine and isoleucine. It was slightly elevated for the 5th circulating valine quintile (**Supplemental Figure 1**).

Non-genetic effect of circulating amino acids and the link with abdominal obesity

We identified 54 monozygotic twin pairs discordant for trunk fat. Their characteristics are presented in **Table 2**. Mean intra-pair trunk fat difference was 14.75 %. On average, heavier twins had greater adiposity parameters, triglyceride and HOMA-IR values as well as lower HDL-C levels. Age was calculated at the time blood was drawn for metabolomic measurements, and because in some cases the twins were not tested on the same day, there is a small, non-significant difference in mean age of the 2 subgroups.

As shown in **Figure 2**, heavier twins of discordant pairs had significantly higher levels of circulating glutamate, leucine, isoleucine and valine than their leaner co-twin. This indicates that the relationship between these circulating amino acids and trunk fat is at least partially independent from genetic background.

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Adipose Tissue Expression of BCAA catabolic enzymes and association with abdominal obesity

We compared gene expression of BCAA catabolic enzymes between adipose tissue depot and cell types in patients with obesity. The characteristics of this sample are presented in **Supplemental Table 3**. Regardless of the fat depot examined, we found variable expression across cell-types (**Figure 3**) with the majority of the genes investigated having more pronounced expression in SVF or whole tissue compared to mature adipocytes. However, *BCKDHA* (SAT Adj. p=3.81E-14, VAT Adj. p=1.27E-21) and *BCKDHB* (SAT Adj. p=1.5E-54, VAT Adj. p=1.84E-60), encoding the BCKD complex E1 subunit, were upregulated in adipocytes compared with the SVF fraction (**Figure 3**). In terms of depot-specificity, *BCKDHA* was upregulated in visceral compared to subcutaneous adipocytes (Adj. p=1.1E-6, **Table 3**) whereas *BCAT2*, encoding BCAT, was upregulated in subcutaneous compared to visceral adipocytes (Adj. p=2.8E-4).

Next, we investigated the association between adipose gene expression of BCAA catabolic enzymes and adiposity measurements. We used the MuTHER resource [24] (n~800, female, SAT) and the METSIM Study [25] (n~800 male, VAT) for transcription-wide association analysis of abdominal adiposity (trunk fat and WHR respectively). In both sexes and depots, *BCKDHA*, *BCKDHB*, *BCKDK* and *BCAT2* were inversely associated with adiposity measurements (BMI and percentage trunk fat), whereas *BCAT1* was positively associated. The association with obesity-traits was the weakest for *BCKDK* (**Table 4**). Interestingly, we noted that *BCAT2* showed the strongest association with abdominal obesity in SAT, consistent with the results that the gene was also

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upregulated in SAT-derived adipocytes (**Figure 3**). On the other hand, *BCAT1* showed the most significant association with adiposity in VAT. However, this seems not to be attributed to differential expression across fat depots (**Table 3**) potentially pointing towards specific action of this enzyme across depot. We then performed high-powered sex-based analysis using GTEx data of SAT and VAT across sexes and noted that the observed effects were not due to the fact that SAT data was only available for women and VAT only for men (**Table 5**).

Glutamate signaling across adipose depots and cell populations

To further explore potentially important adipose tissue physiology pathways, we performed untargeted genome-wide differential expression analysis in our sample of obese patients. We focused on visceral-specific signatures in SVF due to: 1) our observations that the majority of BCAA catabolic enzymes were upregulated in SVF; and 2) the importance of VAT in the pathophysiology of abdominal obesity.

Interestingly, pathway analysis on these SVF - VAT genes identified glutamate receptor Signaling as one of top-three enriched pathways (p-value=2.34E-05, **Supplementary Table 4**) including 12 (out of 38) mapped genes *DLG4, GLS, GRIK1, GRIK5, GRIN1, GRIN2A, GRIN2C, GRIP1, GRM4, GRM8, HOMER1, SLC38A1* (**Figure 4**). To further validate the origin of the glutamate receptor signalling pathway in SVF as well as its depot-specificity, we utilized our recent single-cell expression atlas derived from SAT and VAT SVF [26]. Of the total of 38 genes mapping to the glutamate receptor signalling pathway, 18 (47%) were expressed in at least one adipose progenitor/stem cell from

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either depot. Twelve of these showed a differential expression (Nominal P<0.05) across depot with nine (75%) being upregulated in visceral-derived progenitor cells (**Supplementary Table 5**). The top visceral-specific genes reaching statistical significance after correction for multiple testing are shown in **Supplemental Figure 2.** We then queried the GWAS catalog (June 15, 2020) for the expanded list of visceral-specific glutamate signalling genes identified here (*DLG4*, *GLS*, *GRIK1*, *GRIK5*, *GRIN1*, *GRIN2A*, *GRIN2C*, *GRIP1*, *GRM4*, *GRM8*, *HOMER1*, *SLC38A1* and *GLUL)* and found 31% of them to be associated with a metabolic trait (**Supplementary Table 6**).

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Discussion

The first aim of this study was to assess the ability of circulating glutamate to act as a biomarker for abdominal obesity. We report that individuals with the highest levels of circulating glutamate have a 7-fold higher risk of presenting abdominal obesity than those with the lowest levels. This risk was greater than that associated with the highest quintile of any of the BCAAs. We compared circulating glutamate levels between twins from monozygotic twin pairs discordant for abdominal fat. Heavier twins had significantly higher glutamate levels than their leaner counterpart, indicating that the relationship between glutamate and abdominal fat is at least partially independent from genetic background. In the second aim, analyzing the transcriptome of adipose tissue and constituent cell types from obese patients showed that BCAA enzymes show cell type- and adipose depot-dependent variation in expression. We also found glutamate receptor signaling as one of the top enriched pathways among the genes that are upregulated in SVF from VAT confirming the role of SVF cell population in glutamate receptor signaling. Further narrowing down the individual cell types, the progenitor cell population was found to be expressing the genes involved in glutamate receptor signaling.

The positive and significant association between glutamate and trunk fat in the TwinsUK cohort was first reported by Menni et al. in 2016 [12]. The present paper explores this relationship further by focusing on the risk of presenting abdominal obesity, the role of

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genetic background and the reflection of these associations in the transcriptome of adipose tissue depots and cell types.

We previously reported, in a secondary analysis of a sample of 59 women, that circulating glutamate offered a 78% accuracy for discriminating women with and without visceral obesity (VAT area ≥ 100 cm^2) [8]. We obtained similar results in a larger cohort of 99 men and 99 women, in which WC was used to evaluate body fat distribution. Glutamate was strongly associated with WC, and provided 90% accuracy at identifying which individuals had (or did not have) abdominal obesity (WC ≥ 95 cm) [9]. The results from the present study are consistent with our previous observations that high circulating glutamate level is associated with an increased prevalence of excess central fat accumulation.

The pathophysiology of the association between circulating glutamate and abdominal obesity has never been formally investigated. As mentioned previously, glutamate is a by-product of BCAA catabolism [2], which is downregulated in adipose tissue of obese individuals [3]. Considering this, we suggest that alterations of the BCAA catabolic pathway in obesity could impact not only circulating BCAAs but also circulating glutamate levels.

The microbiota has been suggested to play a role in circulating glutamate level. Indeed, Liu et al. identified *B. thetaiotamicrion* as a bacterial species lower in obese subject and significantly associated with circulating glutamate [27]. Moreover, its level increased after bariatric surgery, concomitantly with a decrease in circulating glutamate. Differences in

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microbiota might partly explain our finding that heavier twins from monozygotic pairs discordant for abdominal obesity have significantly greater circulating glutamate than their leaner co-twin.

We recently investigated the relationship between the abundance of BCAA catabolic enzymes in adipose tissue and obesity in women and found depot- and enzyme-specific differences in the association [5]. Here, we follow-up these findings in multiple ways including not only across depots and cell types but also between sexes. We confirmed many of our previous results, including the upregulation of *BCAT1* in VAT vs SAT. We also confirmed that *BCKDHA*, *BCKDHB*, *BCKDK* and *BCAT2* were negatively associated with obesity (BMI and Percentage trunk fat), whereas *BCAT1* was positively associated with these variables. We also confirmed findings of a weak association between adiposity markers and *BCKDK*.

Our untargeted analysis showed significant adipose depot-related differential expression patterns of the key genes involved in glutamate receptor signalling. *GRIK1* was among the top candidate genes and a copy number variation in *GRIK1* was reported to be associated with severe early onset of obesity [28]. Genetic polymorphism in *GRIN1* was found to be resulting in weight loss in a study by Spraggs et al. [29]. Moreover, *GRIP1* was identified to be a major regulator of macrophage infiltration and inflammation in adipose tissue [30]. Taken together, these findings show that glutamate receptor signaling in adipose tissue is related to obesity and adipose tissue dysfunction.

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Glutamate receptor signaling was studied extensively in neuronal cells [31]. However, the pathways and functions of glutamate receptors in non-neuronal tissues are still not well understood. Our study showed enrichment of group III glutamate receptors *GRM4* and *GRM8* in SVF from VAT. Glutamate receptors were shown to modulate insulin production in islets of Langerhans [32] and in that study they also found that group III glutamate receptor agonists inhibited insulin secretion. This is consistent with the notion that the link between development of metabolic complications associates with increased VAT accumulation. However, further analysis of the progenitor population in the SVF is necessary to understand the exact process involved.

The strengths of this study include the large number of participants in the biomarker-related analysis as well as the use of different adipose tissue depots and cell types in the RNAseq analysis. Limitations are the cross-sectional nature of the analysis and the fact that samples included mostly Caucasian women, making extrapolation to other population subgroups difficult.

In conclusion, we have shown that circulating glutamate is positively associated with adiposity and that individuals with high glutamate levels are at increased risk of presenting abdominal obesity. Moreover, we report that most BCAA catabolic enzymes are more strongly expressed in SVF than adipocytes and that the glutamate signaling pathway is strongly upregulated in VAT SVF of individuals with obesity. Therefore, we suggest that adipose tissue differences in cell types and depots might play a role in the relationship between circulating glutamate and central fat accumulation, an idea worth exploring in future research.

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Disclosures

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Table 1. TwinsUK sample characteristics

Variable		**Mean ± SD**
Sex (F : M)	n=4665	4400 : 265
Zygosity (MZ : DZ)	n=4665	2247 : 2418
Age (years)	n=4665	53.02 ± 13.87
BMI (kg/m^2)	n=4596	25.98 ± 4.74
Trunk fat (%)	n=4665	35.72 ± 8.76
WC (cm)	n=1153	80.56 ± 11.71
Cholesterol (mmol/L)	n=2855	5.54 ± 1.07
HDL-C (mmol/L)	n=2855	1.83 ± 0.48
LDL-C (mmol/L)	n=2855	3.23 ± 0.98
Triglycerides (mmol/L)	n=4058	1.09 ± 0.62
SBP (mmHg)	n=1817	122.31 ± 17.29
DBP (mmHg)	n=1817	76.25 ± 11.07
HOMA-IR index[a]	n=3881	1.21 (0.67-1.75)

Results are presented as mean ± standard deviation except for HOMA-IR, which is presented as median (interquartile range).

Abbreviations: M: male, F: female, MZ: monozygotic, DZ: dizygotic, BMI: body mass index. WC: waist circumference, HDL-C: high-density-lipoprotein cholesterol, LDL-C: low-density-lipoprotein cholesterol, CRP: c-reactive protein, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment of insulin resistance.

Table 2. Characteristics of leaner and heavier (in terms of trunk fat) twins from pairs discordant for trunk fat

Variable	Leaner		Heavier	
		mean ± SD		mean ± SD
Sex (F : M)	n=54	53 :1	n=54	53 :1
Zygosity (MZ : DZ)	n=54	54 :0	n=54	54 :0
Age (years)	n=54	52.03 ± 12.89	n=54	52.79 ± 13.17
BMI (kg/m^2)	n=54	22.70 ± 3.69	n=54	29.01 ± 4.82
Trunk fat (%)	n=54	27.23 ± 5.97	n=54	41.98 ± 5.76
WC (cm)	n=10	77.80 ± 8.95	n=6	86.17 ± 9.43
Cholesterol (mmol/L)	n=35	5.23 ± 0.95	n=38	5.65 ± 1.16
HDL-C (mmol/L)	n=35	1.99 ± 0.49	n=38	1.72 ± 0.36
LDL-C (mmol/L)	n=35	2.88 ± 0.78	n=38	3.35 ± 1.09
Triglycerides (mmol/L)	n=48	0.80 ± 0.25	n=50	1.23 ± 0.69
SBP (mmHg)	n=20	117.40 ± 16.77	n=15	125.20 ± 17.11
DBP (mmHg)	n=20	73.75 ± 11.87	n=15	79.60 ± 10.62
HOMA-IR index[a]	n=43	0.63 (0.43-0.84)	n=42	1.44 (0.80-2.08)

Results are presented as mean ± standard deviation except for HOMA-IR, which is presented as median (interquartile range). Age was calculated at the time blood was drawn for metabolomic measurements, and because in some cases the two twins of a pair did not come on the same day for testing, there is a small difference between the two groups.

Abbreviations: M: male, F: female, MZ: monozygotic, DZ: dizygotic, BMI: body mass index. WC: waist circumference, HDL-C: high-density-lipoprotein cholesterol, LDL-C: low-density-lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment of insulin resistance.

Table 3. Differential gene expression analysis result between VAT and SAT samples for BCAA catabolic enzymes

Gene names	**Adipose tissue**		**Adipocytes**		**SVF**	
	Log2FC	Adj. p-value	Log2FC	Adj. p-value	Log2FC	Adj. p-value
BCKDHA	-0.01	0.98	0.74	1.05E-06	-0.07	0.88
BCKDHB	-0.04	0.85	0.24	0.35	-0.06	0.88
BCKDK	0.06	0.37	0.20	0.57	0.11	0.71
BCAT1	-0.07	0.83	0.15	0.99	-0.34	0.13
BCAT2	0.09	0.29	-0.83	2.8E-04	0.54	8.7E-06

Gene expression was measured in adipose tissue from obese individuals. n=20 for whole adipose tissue, n=12 for subcutaneous adipocytes, n=10 for visceral adipocytes and n=11 for SVF.

BCKDHA and *BCKDHB* code for the alpha and beta components of the Branched-Chain Keto Acid Dehydrogenase (BCKD) complex E1 subunit. *BCKDK* codes for the kinase inhibiting BCKD. *BCAT1* and *BCAT* code for the mitochondrial and cytoplasmic isoforms of the Branched-Chain Aminotransferase enzyme.

Abbreviations: SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue, SVF: stroma-vascular fraction.

Table 4. Association between BCAA catabolic enzymes and abdominal obesity in SAT from females and VAT from males

In SAT of females

Gene names	% Trunk Fat (adj. for BMI)			BMI		
	p-value	q-value	Beta	p-value	q-value	Beta
BCKDHA	2.6E-16	1.7E-14	-0.017	3.5E-35	2.9E-33	-0.025
BCKDHB	2.5E-19	2.4E-17	-0.023	6.9E-26	2.6E-24	-0.026
BCKDK	4.5E-04	2.5E-03	-0.006	3.7E-04	1.3E-03	-0.006
BCAT1	8.4E-07	9.6E-06	0.012	3.3E-15	4.5E-14	0.019
BCAT2	1.0E-26	3.0E-24	-0.022	1.4E-22	4.0E-21	-0.021

In VAT of males

Gene names	Waist-to-hip ratio			BMI		
	p-value	q-value	Beta	p-value	q-value	Beta
BCKDHA	8.31E-03	6.59E-02	-0.095	3.28E-10	3.78E-08	-0.224
BCKDHB	6.86E-06	2.42E-04	-0.161	8.93E-19	4.30E-16	-0.311
BCKDK	7.86E-01	7.00E-01	0.010	2.82E-04	5.16E-03	-0.131
BCAT1	3.11E-08	2.25E-06	0.198	3.30E-23	2.87E-20	0.347
BCAT2	1.37E-03	1.78E-02	-0.115	3.01E-10	3.50E-08	-0.224

Gene expression in SAT of women is from the MuTHER resource, n~800. Gene expression in VAT of men are is from the METSIM study, n~800.

BCKDHA and *BCKDHB* code for the alpha and beta components of the Branched-Chain Keto Acid Dehydrogenase (BCKD) complex E1 subunit. *BCKDK* codes for the kinase inhibiting BCKD. *BCAT1* and *BCAT* code for the mitochondrial and cytoplasmic isoforms of the Branched-Chain Aminotransferase enzyme.

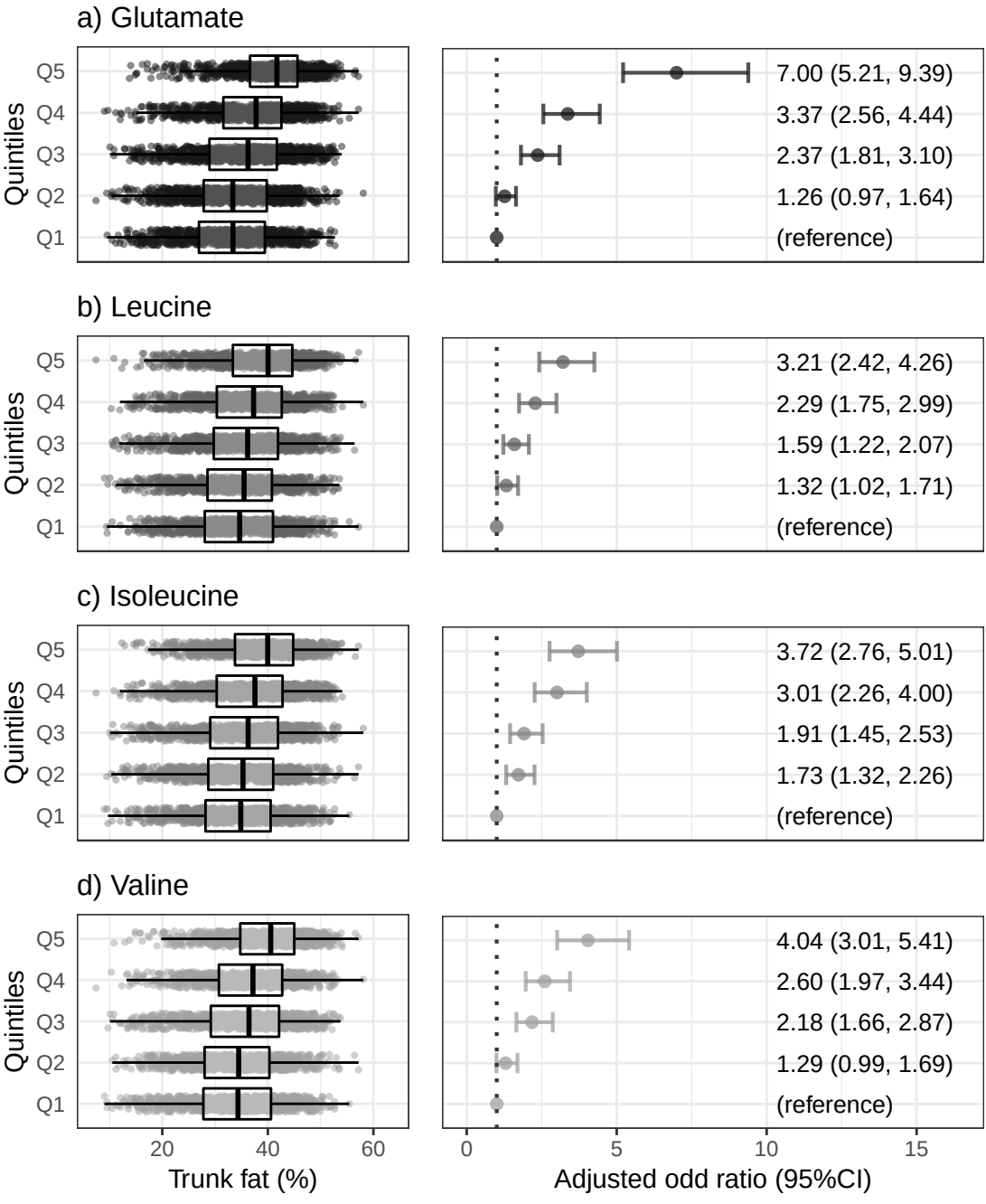
Abbreviations: BMI: body mass index, SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue.

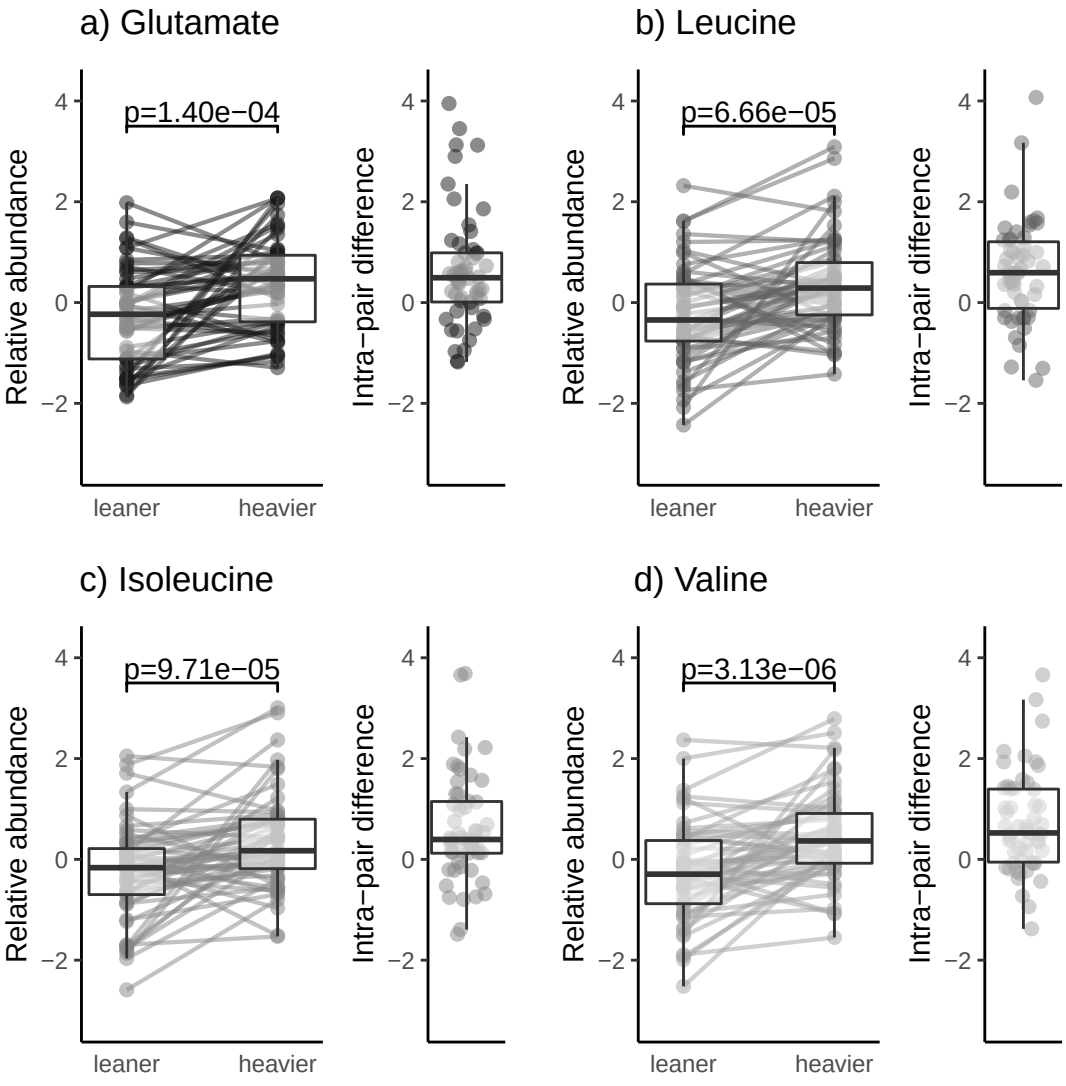
Table 5. Sex-specific analysis results for BCAA catabolic enzymes in subcutaneous and visceral adipose tissue

Gene names	**Subcutaneous**		**Visceral**	
	Log2FC	Adj. p-value	Log2FC	Adj. p-value
BCKDHA	0.04	0.71	0.21	0.24
BCKDHB	0.17	0.12	0.25	0.24
BCKDK	-0.04	0.75	0.10	0.41
BCAT1	0.19	0.35	-0.05	0.88
BCAT2	0.12	0.51	0.23	0.22

This analysis was performed using GTEx data of subcutaneous and visceral adipose tissue across sexes.

BCKDHA and *BCKDHB* code for the alpha and beta components of the Branched-Chain Keto Acid Dehydrogenase (BCKD) complex E1 subunit. *BCKDK* codes for the kinase inhibiting BCKD. *BCAT1* and *BCAT* code for the mitochondrial and cytoplasmic isoforms of the Branched-Chain Aminotransferase enzyme


a) Glutamate
Quintiles
Q5
Q4
Q3
Q2
Q1
7.00 (5.21, 9.39)
3.37 (2.56, 4.44)
2.37 (1.81, 3.10)
1.26 (0.97, 1.64)
(reference)
b) Leucine
3.21 (2.42, 4.26)
2.29 (1.75, 2.99)
1.59 (1.22, 2.07)
1.32 (1.02, 1.71)
(reference)
c) Isoleucine
3.72 (2.76, 5.01)
3.01 (2.26, 4.00)
1.91 (1.45, 2.53)
1.73 (1.32, 2.26)
(reference)
d) Valine
4.04 (3.01, 5.41)
2.60 (1.97, 3.44)
2.18 (1.66, 2.87)
1.29 (0.99, 1.69)
(reference)
20
40
60
Trunk fat (%)
0
5
10
15
Adjusted odd ratio (95%CI)


a) Glutamate
p=1.40e−04
Relative abundance
Intra−pair difference
leaner
heavier
b) Leucine
p=6.66e−05
Relative abundance
Intra−pair difference
leaner
heavier
c) Isoleucine
p=9.71e−05
Relative abundance
Intra−pair difference
leaner
heavier
d) Valine
p=3.13e−06
Relative abundance
Intra−pair difference
leaner
heavier
4
2
0
−2

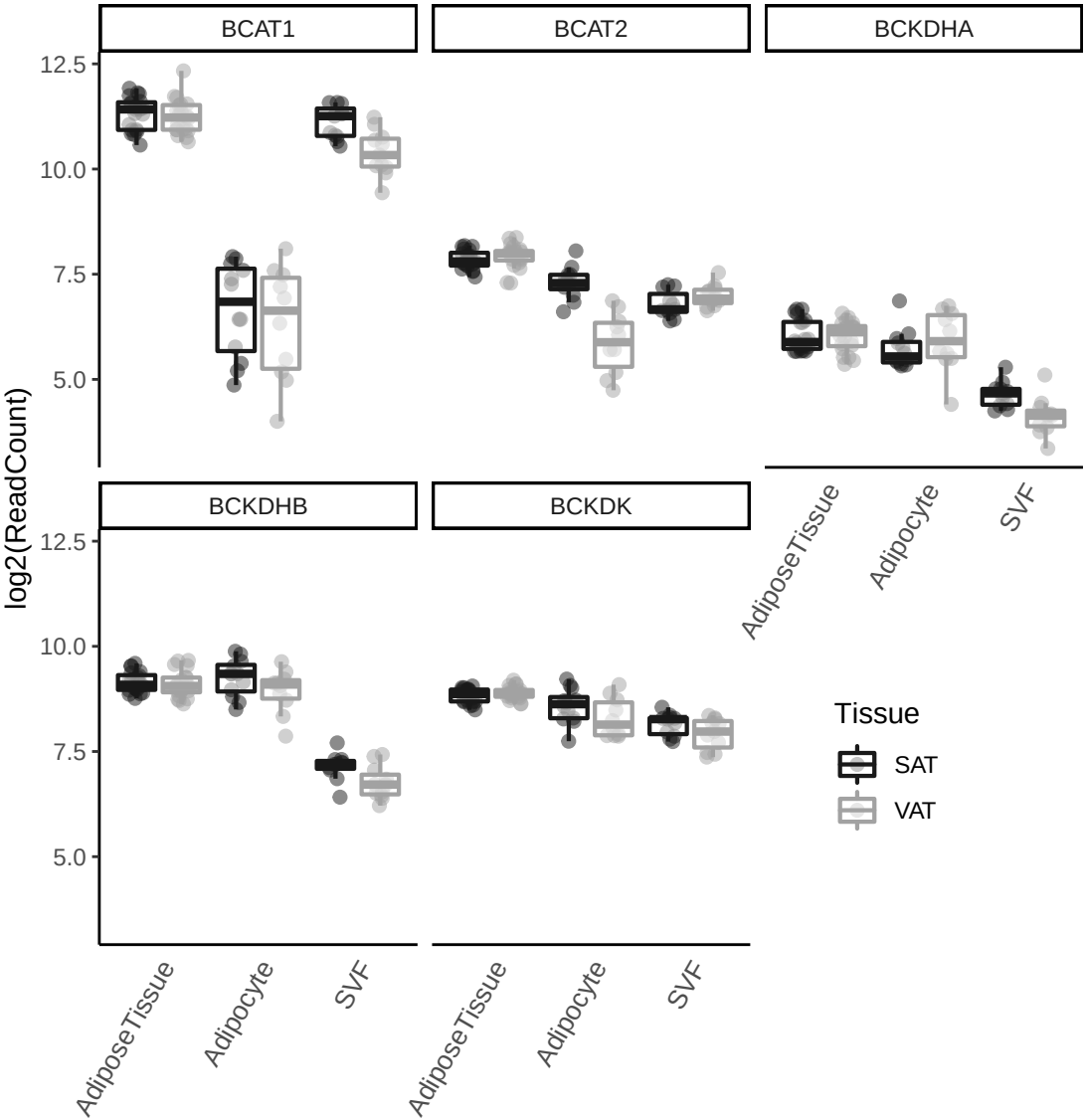
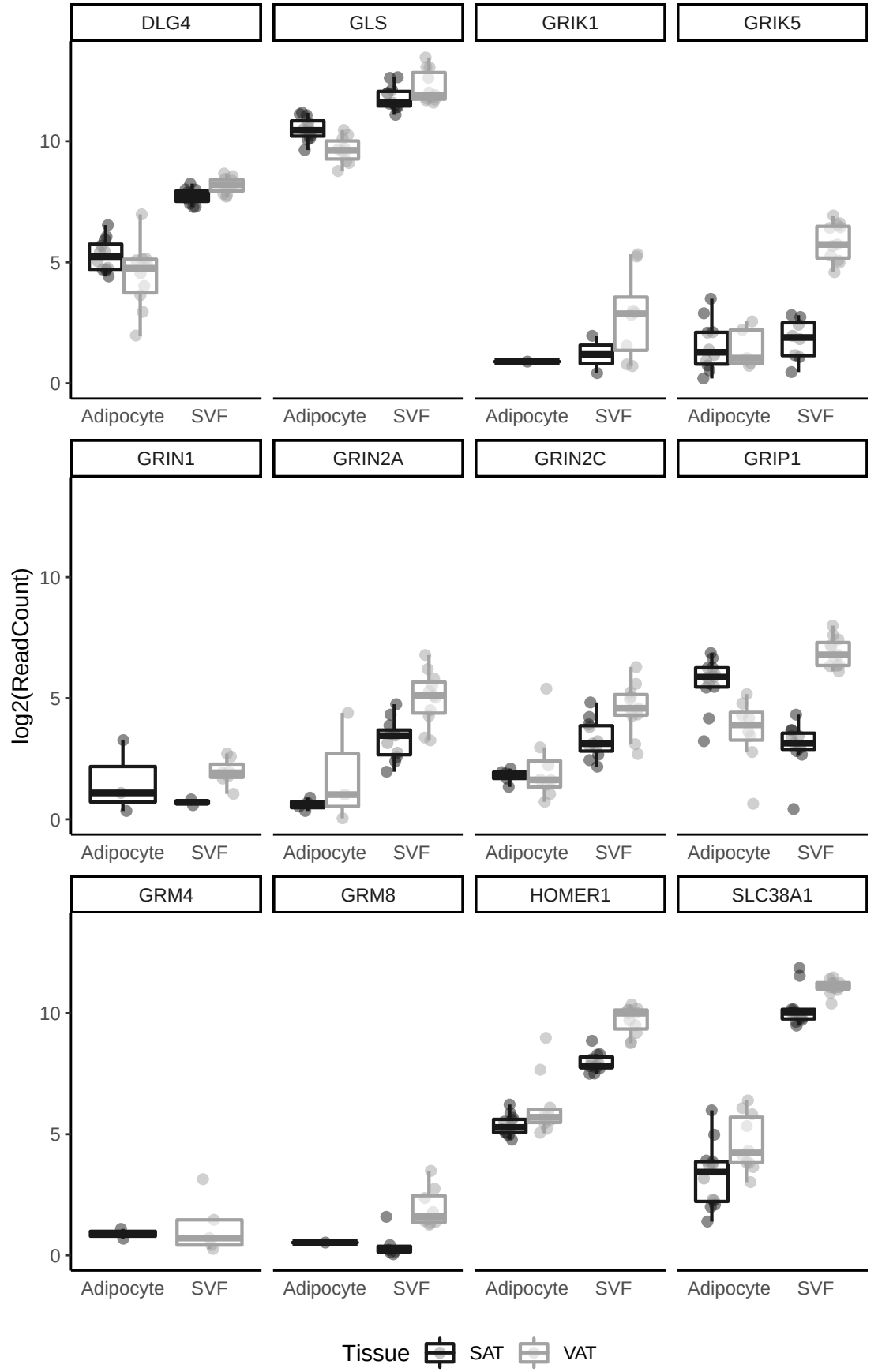

BCAT1
BCAT2
BCKDHA
BCKDHB
BCKDK
log2(ReadCount)
12.5
10.0
7.5
5.0
AdiposeTissue
Adipocyte
SVF
Tissue
SAT
VAT

Figure 1. Trunk fat and odds ratio of having abdominal obesity according to circulating amino acid quintiles. Left panels: abdominal fat for each circulating amino acid quintiles. The lower, middle and top borders of the boxplot represent the second quantile, median and third quantile, respectively. Right panel: Odds ratio of having abdominal obesity of the second, third, fourth and fifth circulating amino acid quintiles, compared to the first. Error bars represent the 95% confidence interval. Abdominal obesity was defined as having 37.7% of fat or more in the trunk region. Odds ratios were obtained for mixed logistic regression models, adjusted for age, sex, metabolomic batch and twin pair clustering.

Figure 2. Difference in circulating amino acids between heavier and leaner twins from monozygotic pairs discordant for trunk fat percentage (n=108, 54 pairs). Twin pairs were considered discordant if the intra-pair trunk fat difference was greater or equal to the 95th percentile of intra-pair trunk fat percentage difference for monozygotic twins, that is 11.61% of trunk fat difference. In each discordant pair, we identified the leaner and heavier (in terms of trunk fat) individual. Left panels show the circulating amino acid level of all individuals, according to whether they are the leaner of heavier twin of the pair. The right panels show the circulating amino acid difference between the heavier and leaner twin. P-values are from two-tailed and paired Student's t-tests.

Figure 3: BCAA catabolic enzymes show a variable gene expression pattern among the cell types of adipose tissue in individuals with obesity. *BCAT1* shown increased expression in SVF compared to adipocytes and *BCKDHA* shows the reverse pattern.

Figure 4: Expression levels of glutamate receptor genes shows increased expression in SVF derived from VAT. Adipocytes from both SAT and VAT had consistently lower expression in comparison with SVF.


DLG4
GLS
GRIK1
GRIK5
GRIN1
GRIN2A
GRIN2C
GRIP1
GRM4
GRM8
HOMER1
SLC38A1
log2(ReadCount)
0
5
10
Adipocyte
SVF
Tissue
SAT
VAT