Elevated circulating Glutamate associates with subclinical Atherosclerosis independently of established Risk Markers: a cross-sectional study

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Abstract

Objective: Elevated plasma glutamate levels associate with an increased risk of cardiovascular disease (CVD). Because plasma glutamate levels also strongly associate with visceral adiposity, NAFLD, insulin resistance and high circulating levels of branched-chain amino acids (BCAA), it is unknown to what extent elevated circulating glutamate is an independent marker of an increased risk of atherosclerosis.

Methods: Plasma levels of glutamate and BCAA were measured in 102 subjects who were precisely phenotyped for body fat mass and distribution (MR tomography), liver fat content (^{1}H-MR spectroscopy), insulin sensitivity [oral glucose tolerance test and hyperinsulinemic, euglycemic clamp (N=57)] and carotid intima-media thickness (cIMT).

Results: Plasma glutamate levels, adjusted for age, sex, body fat mass and visceral fat mass, correlated positively with liver fat content and cIMT (all std.-ß≥0.22, all p≤0.023) and negatively with insulin sensitivity (std.-ß≤-0.31, p≤0.0019). Glutamate levels also associated with cIMT, independently of additional adjustment for liver fat content, insulin sensitivity and BCAA levels (std.-ß≥0.24, p≤0.021). Furthermore, an independent positive association of glutamate and IL-6 levels was observed (N=50; std. ß=0.39, p=0.028). While glutamate, adjusted for age, sex, body fat mass and visceral fat mass, also correlated positively with cIMT in this subgroup (std. ß=0.31, p=0.019), after additional adjustment for the parameters liver fat content, insulin sensitivity, BCAA or IL-6 levels, adjustment for IL-6 most strongly attenuated this relationship (std. ß=0.28, p=0.05).

Conclusions: Elevated plasma glutamate levels are associated with increased cIMT, independently of established CVD risk factors and this relationship may in part be explained by IL-6-associated subclinical inflammation.

Keywords: Plasma Glutamate, Non-Alcoholic Fatty Liver Disease, Visceral Obesity, Insulin Resistance, Carotid Intima-Media Thickness, Endothelial Cells

Abbreviations: BCAA, branched-chain amino acids; cIMT, carotid intima-media thickness; IL, interleukin; IS, insulin sensitivity; MR, magnetic resonance; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; TAT, total adipose tissue; VAT, visceral adipose tissue

Introduction

Subjects with increased cardiometabolic risk are often characterized by visceral obesity and ectopic accumulation of lipids in non-adipose tissue, such as the skeletal muscle and the liver, e.g. resulting in non-alcoholic fatty liver disease (NAFLD) (1-3). Research focussing on the role of organ cross-talk identified dysregulated release and signalling of lipids, adipokines, cytokines, hepatokines and myokines, mediating the effects of these phenotypes, particularly involving insulin resistance (4-6). In addition, research applying blood metabolomics identified novel pathways, such as dysregulated trimethylamine N-oxide and branched-chain amino acid (BCAA) metabolism, involved in the pathogenesis of cardiometabolic diseases (7,8).

Somewhat unnoticed in the field of cardiometabolic research, blood metabolomic studies found that increased plasma levels of glutamate associated with higher prevalence of type 2 diabetes (9,10). Furthermore, elevated plasma glutamate levels were found to associate with fasting and 2hr hyperglycemia during an oral glucose tolerance test (11). In the Framingham Heart Study and in the Malmö Diet and Cancer Study elevated glutamate levels associated with increased estimates of total and visceral adiposity, dyslipidemia and insulin resistance and with an increased risk of incident diabetes. The relationship of glutamate levels with the risk of diabetes was independent of age, sex, body-mass index and fasting glycemia (12). In addition, in fully adjusted models elevated plasma glutamate levels were found to associate with increased risk of incident cardiovascular disease (CVD) in the PREDIMED Study (13).

However, it is unknown whether these relationships are independent of precisely measured fat mass and fat distribution, NAFLD and insulin resistance, or whether elevated glutamate levels may represent a novel and independent pathomechanism for cardiometabolic diseases. Therefore, in the present study we investigated whether plasma glutamate levels associate

with hyperglycemia and insulin resistance, independently of plasma BCAAs and precise measures of body fat distribution and liver fat content. Furthermore, we investigated whether plasma glutamate levels associate with subclinical atherosclerosis, determined by increased carotid intima-media thickness (cIMT), independently of these phenotypes and of BCAAs, and whether glutamate may have pro-inflammatory effects in endothelial cells.

Materials and Methods

A total of 102 participants from the Tübingen Diabetes Family Study (14,15), in whom 1. plasma metabolomic data, 2. measurements of body fat mass and distribution and of liver fat content, using magnetic resonance (MR) tomography and ^{1}H-MR spectroscopy and 3. measurements of cIMT, were available, were included in the present analysis. As storage (-80°C), freezing and thawing of plasma samples is most critical for metabolomic analyses (16) we could only measure metabolomics in unthawed samples of 102 out of the initially phenotyped 314 subjects (15), in whom plasma samples were still available. Individuals fulfilled at least one of the following criteria: a family history of type 2 diabetes, a BMI > 27 kg/m^2, previous diagnosis of impaired glucose tolerance or gestational diabetes. Subjects were considered healthy according to a physical examination and routine laboratory tests. The subject's highest education (lower secondary, higher secondary, baccalaureate, other) was obtained from questionnaires. Informed written consent was obtained from all participants and the Ethics Committee of the University of Tübingen had approved the protocol.

Oral glucose tolerance test, simple anthropometrics and blood pressure

Subjects underwent a frequently sampled 2hr, 75g oral glucose tolerance test (OGTT). Venous plasma samples were obtained at 0, 30, 60, 90, and 120 minutes for determination of plasma glucose and insulin levels. Whole body insulin sensitivity was calculated from glucose and insulin values during the OGTT as proposed by Matsuda and DeFronzo (17). BMI was calculated as weight divided by the square of height (kg/m^2). Waist circumference was measured in the upright position at the midpoint between the lateral iliac crest and the lowest rib. The blood pressure measurement was performed at the dominant arm, unless there was a deformity, using a sphygmomanometer and a stethoscope to auscultate the Korotkoff sounds. Blood pressure was measured in duplicate, and the mean value of both measurements was calculated.

Euglycemic, hyperinsulinemic clamp

In a subgroup of 57 subjects insulin sensitivity was also determined during a euglycemic, hyperinsulinemic clamp with a primed insulin infusion at a rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$ for 2 hours. The insulin sensitivity index measured during the clamp (in $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$) was calculated as the mean infusion rate of glucose (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) necessary to maintain euglycemia during the last 40 minutes of the euglycemic hyperinsulinemic clamp divided by the steady state plasma insulin concentration.

Total body fat mass, body fat distribution and liver fat content

Measurements of total body- and visceral fat and mass were performed by an axial T1-weighted fast spin echo technique with a 1.5 T whole-body magnetic resonance imager. Liver fat content was measured by localized proton magnetic resonance (^{1}H-MR) spectroscopy as previously described (14).

Carotid intima-media thickness

The cIMT intima-media thickness was measured in the fasting state using a high-resolution ultrasound system (AU5 idea, Esaote Biomedica, Munich, Germany) with an integrated electrocardiography package as previously described (18).

Analytical procedures

Blood glucose was determined using a bedside glucose analyzer (glucose-oxidase method; YSI, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was determined on an ADVIA Centaur XP and all other blood parameters on an ADVIA 1800 clinical chemistry system (Siemens Healthcare systems, Erlangen, Germany).

A total of 265 blood metabolites were analyzed by the targeted IDQ metabolomics platform from Biocrates (Innsbruck, Austria). This platform combines flow injection (acylcarnitines, glycerophospholipids), liquid chromatographic (amino acids, bile acids), and gas chromatographic (free fatty acids) mass spectrometric (MS) approaches. The identification of metabolites is based on exact mass, multi-reaction monitoring and comparison with standard compounds (if commercially available). For the detection of acylcarnitines, lipids, amino acids and bile acids an API 4000 QTrap (AB Sciex, Foster City, USA) was used. The plasma content of fatty acids was measured as their corresponding methyl ester derivatives using a 7890 GC and a 5795 MSD (Agilent, Waldbronn, Germany).

Statistical analyses

Values are presented as means ± SD. Data that were not normally distributed (Shapiro-Wilk *W* test) were logarithmically transformed. For statistical testing Pearson's correlations, ROC analyses and multivariable linear regression analyses were used. For statistical testing the analysis program JMP 14.2.0 of SAS was used.

Results

The characteristics of the subjects are shown in the Table 1. Subjects (58 women, 44 men) had a mean age of 50 years and a body-mass index of 30.4 kg/m^2. One participant of the study had an elevated fasting glucose level of 7.42 mM, while his 2hr glucose value during the OGTT (6.6 mM) and his HbA1c level (5.1%) indicated that he did not have diabetes.

Univariate relationships

In univariate analyses plasma levels of glutamate were higher in males compared to females (difference log data 0.58, lower/upper 95% CI=0.39/0.76, p<0.0001) and did not correlate with age (r=-0.04, lower/upper 95% CI=-0.23/0.16, p=0.69) or total body fat mass (r=0.10, lower/upper 95% CI=-0.10/0.29, p=0.31). The participant's highest education was not associated with glutamate levels (r=0.07, lower/upper 95% CI=-0.13/0.26, p=0.48). Total body fat mass, visceral fat mass and liver fat content correlated strongly and positively with each other (all r ≥0.41; all p-values <0.0001), and all these parameters correlated negatively with insulin sensitivity (all r ≤-0.40; all p-values <0.002). Plasma levels of glutamine correlated positively with liver fat content (r=0.20, p=0.047), negatively with IS_{clamp} (r=-0.27, p=0.040) and, relatively strong and positive, with BCAA levels (r=0.51, p<0.0001). BCAA levels also correlated positively with visceral fat mass (r=0.47, p<0.0001) and liver fat

content (r=0.40, p<0.0001), negatively with IS_{clamp} (r=-0.37, p=0.0047) and positively with glutamate levels (r=0.44, p<0.0001). Plasma glutamate levels did not correlate with body fat mass, but correlated positively with visceral fat mass and liver fat content (all r ≥0.40; all p-values <0.0001) and negatively with insulin sensitivity (all r ≤-0.36; all p-values <0.0003). Furthermore, a positive correlation of plasma glutamate with cIMT (r=0.30, p=0.002) was observed (Table 2).

To better estimate the strength of the relationship of plasma glutamate levels with cIMT the glutamate levels were also divided into tertiles. Thereby, cIMT was found to be highest in subjects in the upper tertile of plasma glutamate levels (p for trend = 0.0004; Figure 1).

Predictive analyses

When cIMT was divided into tertiles, in a forward stepwise regression model including the clinical parameters age, sex, systolic and diastolic blood pressure, body-mass index, and LDL-cholesterol, C-reactive protein and glutamate levels, only higher age [likelihood-ratio-test (LR)-test: 21.3] and glutamate levels (LR-test: 7.5) predicted being in the highest compared to the lowest tertile of cIMT. Using ROC curve analysis inclusion of glutamate levels, in addition to age, increased the AUC for being in the highest compared to the lowest tertile of cIMT from 0.79 to 0.84.

Multivariable relationships

Independent relationships of glutamate levels with insulin sensitivity, liver fat content and cIMT were also investigated using multivariable linear regression models. In these models glutamate levels were adjusted for sex. Furthermore, although glutamate levels did not

correlate with age or total body fat mass, adjustment for these parameters was also done, because they strongly associate with the tested cardiometabolic risk parameters. In these models (Table 3) glutamate levels, correlated negatively with insulin sensitivity (IS_{OGTT}: std. ß=-0.31, p=0.0019; IS_{Clamp}: std. ß=-0.41, p=0.0005). Furthermore, adjusted glutamate levels correlated positively with liver fat content (std. ß=0.22, p=0.02) and with cIMT (std. ß=0.25, p=0.01). In addition, glutamate levels correlated with cIMT independently of additional adjustment for liver fat content and insulin sensitivity (adjusted for IS_{OGTT}: std. ß=0.24, p=0.02; adjusted for IS_{clamp} std. ß=0.35, p=0.01).

As plasma glutamate levels also correlated strongly with insulin sensitivity in our study and also with liver fat content and BCAA levels, and because there is no evidence that glutamate may play a major role in the pathogenesis of insulin resistance, fatty liver or dysregulated BCAA levels, we additionally adjusted for these parameters, considering them possible confounders in the relationship with cIMT. In these extended multivariable models, glutamate levels, adjusted for age, sex, body fat mass, visceral fat mass, liver fat content and insulin sensitivity, correlated positively with cIMT (std. ß=0.24, p=0.02, Table 4, Model 1) and a positive relationship of glutamate levels with cIMT was also found (std. ß=0.26, p=0.01) after additional adjustment for BCAA levels (Table 4, Model 2). To test for possible over-adjustment we also determined the variance inflation factors (VIFs) in our multivariable regression models. In all multivariable regression models that were performed with the total population (N=102) studied, the VIFs were lower than 5, indicating that collinearity was not a problem.

In the search of mediators of the relationship of glutamate levels with cIMT, we focused in an exploratory approach on measures of subclinical inflammation. In this respect high-sensitivity C-reactive protein (hs-CRP) and, in a subgropup of 50 subjects, interleukin-6 (IL-6) levels, were previously measured. After adjustment for age, sex, body fat mass and

visceral fat mass, plasma glutamate levels only tended to associate positively with hs-CRP levels (std. ß=0.20, p=0.10), however, an independent positive association of circulating glutamate with IL-6 levels (std. ß=0.39, p=0.028) was observed. While circulating glutamate, adjusted for age, sex, body fat mass and visceral fat mass, also correlated positively with cIMT in this subgroup of 50 subjects (std. ß=0.31, p=0.019), after additional adjustment for the parameters liver fat content, insulin sensitivity, BCAA or IL-6 levels, adjustment for IL-6 most strongly attenuated this relationship (std. ß=0.28, p=0.05). In this small group of 50 subjects, in whom IL-6 levels were available, in the multivariable regression model including age, sex, body fat mass and visceral fat mass, only the VIF for visceral fat mass was higher than 5 (6.67). However, because glutamate and IL-6 levels also correlated positively with each other prior to adjustment for other parameters, possible over-adjustment may not be a problem in this subgroup of subjects.

Discussion

High-throughput metabolic profiling studies integrating clinical data have recently provided important information about novel pathways of cardiometabolic diseases (7,8). In this respect elevated plasma glutamate levels were found to associate with a cardiometabolic risk profile (12) and to predict incident CVD (13). However, glutamate *(i)* promotes hyperinsulinemia, *(ii)* contributes to gluconeogenesis as a glucogenic amino acid, and *(iii)* is increasingly released from hepatocytes into the circulation in NAFLD (19,20). Therefore, it cannot be excluded that the observed relationships of glutamate levels with the cardiometabolic risk profile may be confounded by insulin resistance and NAFLD. Furthermore, glutamate levels were found to strongly correlate positively with increased visceral fat mass (21,22). In addition, in insulin resistant conditions glutamate is produced in the first step of BCAA catabolism (8). Thus, on the one hand, elevated circulating glutamate levels may simply

reflect increased circulating BCAA, which are thought to be involved in the pathogenesis of type 2 diabetes (8). On the other hand, plasma glutamate may be involved in the pathogenesis of cardiometabolic diseases. Support for this hypothesis is provided by the fact that glutamate receptors are expressed in various tissues that become dysfunctional early on in the pathogenesis of CVD, such as the cardiac muscle and the endothelium. Furthermore, signalling via metabotropic glutamate receptors was found to promote lipopolysaccharide-induced liver damage and reactive oxygen species production (19).

We can provide novel information that glutamate associates with higher liver fat content, lower insulin sensitivity and increased cIMT, independently of precisely measured total body fat mass and visceral fat mass. In addition, we found that glutamate levels may help to identify subjects with elevated cIMT, an early marker of subclinical atherosclerosis. Interestingly, we found that subjects with elevated glutamate levels have higher cIMT, even after further adjustment for liver fat content and insulin sensitivity. Moreover, this relationship was present after additional adjustment for circulating BCAAs.

Although we cannot infer causal relationships from these data, the present findings indicate that it may be worthwhile investigating whether plasma glutamate levels impact on the pathogenesis of atherosclerosis. To address this hypothesis in an exploratory approach we focused on measures of subclinical inflammation. Plasma glutamate levels did not associate with hs-CRP levels. However, in a subgroup of 50 subjects, in whom plasma IL-6 measurements were available, plasma glutamate levels correlated positively with plasma IL-6 concentrations (r=0.30, p=0.036) and this relationship was also present after adjustment for age, sex, body fat mass and visceral fat mass (std. ß=0.39, p=0.028, Figure 2). Circulating glutamate, adjusted for age, sex, body fat mass and visceral fat mass, also correlated positively with cIMT in this subgroup of 50 subjects. After additional adjustment for the parameters liver fat content, insulin sensitivity, BCAA or IL-6 levels, adjustment for IL-6

most strongly attenuated this relationship. This finding supports the hypothesis that glutamate might have pro-inflammatory effects and thereby be involved in the pathogenesis of atherosclerosis.

A possible pro-inflammatory effect of glutamate is supported by the observation that in synovial fluid of patients with rheumatoid arthritis glutamate levels were found to strongly correlate positively with RANTES and IL-8 levels, and that glutamate induced TNF-alpha production, which was followed by further upregulation of chemokine and cytokine production in synovial cell cultures (23). As metabotropic glutamate receptors were also found in the lining of blood vessels, both, in rat and macaque hearts (18), it is plausible that such pro-inflammatory effects of glutamate are also present in endothelial cells. If these findings can be replicated in other studies, there is also support that the observed relationship of increased intake of glutamate with increased cardiovascular mortality (24), which was not only explained by a more unhealthy diet, particularly one including higher intake of red meat and lower intake of whole grains, is important, as glutamate may be involved in the pathogenesis of atherosclerosis.

On the other hand, a study using a Mendelian randomization approach did not find support that genetically determined glutamate levels are involved in the pathogenesis of ischemic heart disease (25). However, in that study only one single nucleotide polymorphism (SNP, rs239614) that reached genome-wide significance for glutamate levels could be tested. Furthermore, the effect size of the SNP on circulating glutamate was relatively small. As circulating glutamate is considered to strongly increase in pathologies associated with increased BCAA metabolism (8), and is increasingly released from hepatocytes into the circulation in nonalcoholic steatohepatitis (26), these pathologies may stronger impact on circulating glutamate than genetic variability. In that case measurement of plasma glutamate levels may help to identify subjects with an increased risk of CVD, which may particularly

relate to the presence of visceral obesity, NAFLD and insulin resistance. Furthermore, it may be warranted to put more focus on reducing the dietary intake of the umami taste receptor-activating glutamate, which is being widely found as a flavour enhancer (27), particularly in processed food.

Our study has the limitation that we could only study a relatively small population that had precise measurements of glucose and lipid metabolism, body fat distribution, liver fat content and cIMT. Furthermore, we cannot draw definite conclusions about causal relationships from our cross-sectional analyses. For this predictive analyses in large cohorts having precise measurements of the confounding parameters, such as precisely measured insulin sensitivity and visceral fat mass and animal studies investigating mechanisms of glutamate action, are necessary.

In conclusion, we found that elevated plasma glutamate levels associate with higher liver fat content, lower insulin sensitivity and increased cIMT, independently of precisely measured total body fat mass and visceral fat mass. Furthermore, the relationship of elevated plasma glutamate levels with increased cIMT was independent of liver fat content and insulin sensitivity. As our analyses revealed that plasma glutamate associates with increased plasma IL-6 levels in humans, our data suggest that it may be worthwhile studying whether elevated plasma glutamate levels play a major role in the pathophysiology of CVD.

Data Availability

Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Table 1 Subject characteristics

Parameter	**Values**
Sex (males/females)	44/58
Age (years)	50 (10)
Body weight (kg)	89.0 (17.4)
Waist circumference (cm)	98.8 (13.3)
Body mass index ($kg \cdot m^{-2}$)	30.4 (5.2)
Total body fat $_{MRT}$ (kg)	27.5 (11.6)
Visceral fat $_{MRT}$ (kg)	3.3 (1.9)
Liver fat $^{1}{}_{H\text{-}MRS}$ (%)	6.6 (5.6)
NAFLD (%)	54%
Fasting glucose (mM)	5.3 (0.5)
2h glucose (mM)	7.5 (1.6)
IS_{OGTT} (arb. units)	11.6 (6.3)
IS_{Clamp} ($\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$)*	0.059 (0.030)
Total cholesterol (mg/dl)	202 (37)
HDL cholesterol (mg/dl)	53 (13)
LDL cholesterol (mg/dl)	126 (29)
Blood pressure $_{Systolic}$	127 (18)
Blood pressure $_{Diastolic}$	78 (11)
Hs-CRP (mg/dl)	0.29 (0.48)

cIMT (mm)	0.58 (0.09)
BCAA (µM)	489 (79)
Glutamine (µM)	662 (122)
Glutamate (µM)	55 (29)

Data are means (SD). MRT, magnetic resonance tomography; ^{1}H-MRS, proton magnetic resonance spectroscopy; IS, insulin sensitivity; Hs-CRP, high-sensitivity C-reactive protein; BCAA, branched-chain amino acids. *Available in 57 subjects.

Table 2 Selected correlations

	Liver fat		IS_{OGTT}		IS_{Clamp}		cIMT		BCAA		Glutamine		Glutamate	
	r	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**	**r**	**p lower/ upper 95% CI**
Visceral fat	**0.56**	**<0.0001 0.41/0.68**	**-0.40**	**<0.0001 -0.55/-0.22**	**-0.41**	**0.0014 -0.61/-0.17**	**0.29**	**0.003 0.11/0.46**	**0.47**	**<0.0001 0.30/0.61**	0.12	0.25 -0.08/0.30	**0.54**	**<0.0001 0.39/0.67**
Liver fat	-	-	**-0.50**	**<0.0001 -0.63/-0.34**	**-0.52**	**<0.0001 -0.69/-0.30**	**0.21**	**0.04 0.01/0.39**	**0.40**	**<0.0001 0.22/0.56**	**0.20**	**0.05 0.00/0.38**	**0.40**	**<0.0001 0.22/0.55**
IS_{OGTT}	-	-	-	-	**0.73**	**<0.0001 0.58/0.83**	0.03	0.75 -0.16/0.22	-0.15	0.12 -0.34/0.04	0.03	0.80 -0.17/0.22	**-0.36**	**0.0002 -0.51/-0.18**
IS_{Clamp}	-	-	-	-	-	-	0.13	0.34 -0.14/0.38	**-0.37**	**0.0047 -0.57/-0.12**	**-0.27**	**0.04 -0.50/-0.01**	**-0.50**	**<0.0001 -0.68/-0.28**
cIMT	-	-	-	-	-	-	-	-	0.07	0.49 -0.13/0.26	0.01	0.88 -0.18/0.21	**0.30**	**0.002 0.11/0.47**
BCAA	-	-	-	-	-	-	-	-	-	-	**0.51**	**<0.0001 0.35/0.64**	**0.44**	**<0.0001 0.27/0.59**
Glutamine	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.31 -0.10/0.29

Total body- and visceral fat were measure by magnetic resonance (MR) tomography and liver fat by ^{1}HMR spectroscopy.

Table 3 Independent relationships of glutamate levels with liver fat content, insulin sensitivity and cIMT

	IS_{OGTT}			IS_{clamp}			Liver fat content			cIMT		
Parameter	***std. β***	***ß***	***P lower/ upper 95% CI***	***std. β***	***ß***	***P lower/ upper 95% CI***	***std. β***	***ß***	***P lower/ upper 95% CI***	***std. β***	***ß***	***P lower/ upper 95% CI***
Intercept	**0**	**2.39**	**0.04 0.16/4.61**	**0**	**-3.15**	**0.01 -5.55/-0.74**	0	-1.07	0.61 -5.18/3.04	**0**	**-2.47**	**<0.0001 -3.12/-1.83**
Age			**0.007 0.16/0.98**			**0.0008 0.36/1.28**			0.30 -1.16/0.36			**<0.0001 0.28/0.51**
Sex[#]			**0.01 -0.35/-0.05**			**0.048 -0.35/-0.00**			0.35 -0.15/0.42			0.29 -0.07/0.02
TAT			0.17 -0.54/0.10			**0.03 -0.74/-0.03**			0.09 -0.08/1.15			0.45 -0.06/0.13
VAT			**0.01 -0.57/-0.07**			0.14 -0.47/-0.07			**0.004 0.23/1.18**			0.90 -0.08/0.07
Glutamate	**-0.31**	**-0.31**	**0.002 -0.51/-0.12**	**-0.41**	**-0.40**	**0.0005 -0.62/-0.19**	**0.22**	**0.42**	**0.02 0.06/0.78**	**0.25**	**0.07**	**0.01 0.02/0.13**

[#]Female; IS, insulin sensitivity; TAT, total adipose tissue; VAT, visceral adipose tissue. Confounder effect estimates are not reported to avoid mistaken interpretations of these estimates.

Table 4 Independent relationships of glutamate levels with cIMT in extended models

		cIMT		
Parameter	***std. β***	***ß***	***P***	***lower/ upper 95% CI***
Model 1				
Intercept	**0**	**-2.44**	**<0.0001**	**-3.10/-1.79**
Age			**<0.0001**	**0.26/0.51**
Sex[#]			0.36	-0.07/0.02
TAT			0.67	-0.08/0.12
VAT			0.83	-0.09/0.07
IS_{OGTT}			0.70	-0.05/0.07
Liver fat content			0.15	-0.01/0.06
Glutamate	**0.24**	**0.07**	**0.02**	**0.01/0.13**
Model 2				
Intercept	**0**	**-1.78**	**0.005**	**-3.01/-0.55**
Age			**<0.0001**	**0.24/0.50**
Sex[#]			0.30	-0.07/0.02
TAT			0.79	-0.08/0.11
VAT			0.98	-0.08/0.08
IS_{OGTT}			0.61	-0.04/0.08
Liver fat content			0.09	-0.00/0.06
BCAA			0.21	-0.26/0.08
Glutamate	**0.26**	**0.08**	**0.01**	**0.02/0.14**

[#]Female; IS, insulin sensitivity; TAT, total adipose tissue; VAT, visceral adipose tissue. Confounder effect estimates are not reported to avoid mistaken interpretations of these estimates.

Figure legends

Figure 1

Plasma glutamate levels and carotid intima-media thickness (N=102).

Figure 2

Relationship of plasma glutamate levels with plasma interleukin-6 levels, adjusted for age, sex, body fat mass and visceral fat mass.

Figure 1

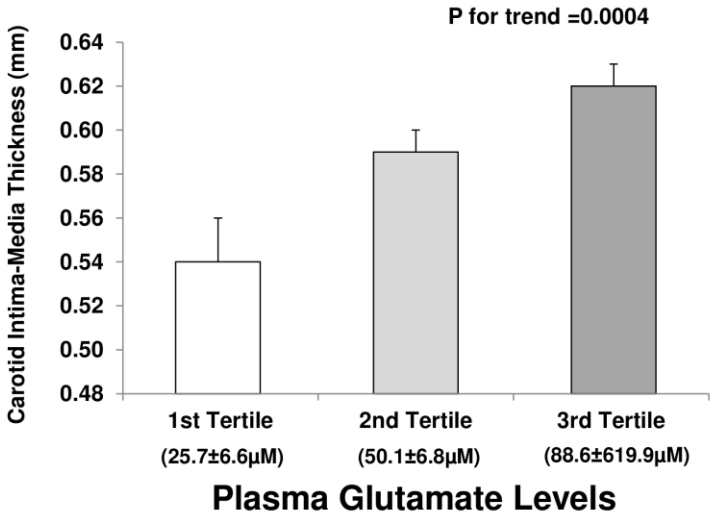

Figure 2