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Circulating glutamate level as a potential biomarker for abdominal obesity and metabolic risk

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Abstract *Background and aim:* Circulating level of glutamate, a by-product of the catabolism of branched-chain amino acids, has been positively correlated with visceral adipose tissue accumulation and waist circumference (WC). The aim of the present study was to assess the potential of using glutamate level to identify individuals with abdominal obesity and a high cardiometabolic risk.
Methods and results: The study sample included 99 men and 99 women. Fasting serum glutamate was measured using the Biocrates p180 kit. Anthropometric and metabolic variables were used to identify individuals with abdominal obesity (WC $\geq$ 95 cm in both sexes), the hypertriglyceridemic waist (HTW) phenotype and the metabolic syndrome (MetS). Mean (±SD) age was 34.1 ± 10.1 years, mean BMI was 29.0 ± 6.2 kg/m^2 and mean WC was 92.7 ± 16.5 cm. Glutamate was strongly correlated with WC ($r = 0.66$ for men; $r = 0.76$ for women, both $p < 0.0001$) and multiple markers of metabolic dysfunction, particularly fasting triglyceride level ($r = 0.59$ for men; $r = 0.57$ for women, both $p < 0.0001$), HDL-cholesterol level ($r = -0.45$, $p < 0.0001$ in both sexes) and the HOMA-IR index ($r = 0.65$ for men; $r = 0.60$ for women, both $p < 0.0001$). Logistic regressions showed that glutamate had an excellent accuracy to identify individuals with abdominal obesity (ROC_AUC: 0.90 for both sexes), a good accuracy to identify those with the HTW phenotype (ROC_AUC: 0.82 for men; 0.85 for women) and fair-to-good accuracy for the MetS (ROC_AUC: 0.78 for men; 0.89 for women).
Conclusion: Glutamate level may represent an interesting potential biomarker of abdominal obesity and metabolic risk.

Abbreviations: ANOVA, Analysis of variance; BCAA, Branched-chain amino acid; BCAT, Branched-chain aminotransferase; BCKA, Branched-chain ketoacid; BCKD, Branched-chain-ketoacid dehydrogenase; BMI, Body mass index; DBP, Diastolic blood pressure; FHS, Framingham Heart Study; HDL-C, High-density-lipoprotein cholesterol; HOMA-IR, Homeostatic model assessment of insulin resistance; HTW, Hypertriglyceridemic; LDL-C, Low-density-lipoprotein cholesterol; MetS, Metabolic syndrome; NCEP-ATPIII, National Cholesterol Education Program Adult Treatment Panel III; ROC_AUC, Area under the receiving operating characteristic (ROC) curve; SAT, Subcutaneous adipose tissue; SBP, Systolic blood pressure; SD, Standard deviation; TG, Triglycerides; VAT, Visceral adipose tissue; WC, Waist circumference.

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Introduction

Obesity, which is defined as abnormal or excessive fat accumulation, is associated with an increased prevalence of cardiovascular diseases, diabetes and some cancers [1]. One of the main determinants of metabolic health is the amount of fat accumulated around organs of the abdominal cavity, known as visceral adipose tissue (VAT) [2]. Direct quantification of VAT accumulation requires imaging methods, which are expensive, time consuming and, in some cases, involve radiation. Therefore, anthropometric measurements are often used as surrogates of VAT, the most common being the waist circumference (WC) [2].

In recent years, the 3 branched-chain amino acids (BCAAs, namely leucine, isoleucine and valine) have been extensively studied for their implication in obesity and concomitant metabolic dysfunctions. Reviews of this literature are now available, notably by Newgard who summarized the current body of evidence showing that BCAAs are associated with obesity, diabetes and cardiovascular diseases, and that they are predictive of diabetes development and treatment outcomes [3].

The amino acid glutamate, a by-product of the catabolism of all 3 BCAAs, has attracted less attention, but there is growing evidence that it could also represent a promising marker of metabolic dysfunction. Its measurement is less frequent than that of the BCAAs, but most studies that did measure it yielded consistent results. Kimberly et al. investigated the metabolite profile of non-alcoholic steatohepatitis in the Framingham Heart Study (FHS) 3rd generation ($n = 997$) [4]. They reported that glutamate was a stronger predictor of WC than the 3 BCAAs (age and sex adjusted $\beta = 0.28$ for glutamate vs 0.18 for isoleucine, 0.16 for valine and 0.13 for leucine, all $p < 0.0001$). In the study of the offspring cohort from the FHS ($n = 1015$), Cheng and collaborators [5] reported a trend towards a positive association between glutamate and WC (age and sex adjusted $r^2 = 0.05$, $p = 0.07$). In the same study, Cheng et al. reported that in the Malmo Diet and Cancer cohort ($n = 746$), the association between glutamate and WC was significant even when adjusted for age, sex and body mass index (BMI) ($r^2 = 0.17$, $p < 0.0001$). Moreover, Zhao et al. showed that glutamate was positively and significantly associated with WC in a sample of 431 normoglycemic American Indians ($\beta = 2.33$, $p = 0.0003$ when adjusted for sex, age, relatedness, site, lifestyle and socioeconomic status; $\beta = 2.31$, $p = 0.0002$ when further adjusted for dietary intake and the homeostatic model assessment of insulin resistance [HOMA-IR] index) [6].

Fewer studies investigated the relationship between amino acids and VAT specifically. The first to do so was Yamakado et al., in 2012, who studied nearly 1500 Japanese participants [7]. In the univariate correlation analysis, glutamate was the amino acid most strongly associated with VAT area ($r = 0.49$, p-value not available). This has since been confirmed by 2 other studies: by our team in 2015 [8] and by Takashina et al., in 2016 [9]. In both cases, glutamate was the strongest correlate of VAT among all the amino acid tested ($r = 0.46$, $p < 0.001$ in Boulet et al. and $r = 0.57$, $p < 0.05$ in Takashina et al.). Furthermore, in a secondary analysis of the sample from 2015, we showed that the correlation between circulating glutamate and VAT was independent of total fat mass measured by Dual Energy X-ray Absorptiometry ($r = 0.36$, $p = 0.006$) [10].

More studies are needed before concluding on the possibility of identifying high risk individuals using amino acid levels, either individually or in combinations. In the present study, we aimed to evaluate the potential of using circulating glutamate as a biomarker of abdominal obesity and metabolic risk.

Methods

Study population

The original cohort from which the present sample was drawn included 664 individuals recruited between May 2004 and April 2007 in the Quebec City metropolitan area [11]. All participants were Caucasian and were between 18 and 55 years of age. Participants were invited to come to the laboratory to fill a lifestyle and demographic questionnaire. Blood samples and anthropometric measurements were taken by a nurse and a trained research assistant, respectively. A subsample of 100 obese and 100 non-obese individuals, with 50 women in each group, were selected for metabolomics profiling [12]. Metabolites were successfully measured in 199 of these 200 participants. This project was approved by the Ethics Committee of Laval University.

We excluded 1 man from the analysis because his WC measurement was not available. The final sample consisted of 198 participants (99 men and 99 women). Three women and 1 man did not have fasting glucose level data available, therefore metabolic syndrome (MetS) status (see below) could be determined for 98 men and 96 women.

Metabolomics

Targeted metabolomics was assessed with the Biocrates Absolute IDQ p180 kit using mass spectrometry as described elsewhere [12]. Although the main focus of the present study was glutamate, we expanded our analysis to 7 other amino acids: the 3 BCAAs as well as alanine, glycine, tryptophan and tyrosine.

Abdominal obesity and metabolic risk definitions

The HOMA-IR index was calculated as (glucose * insulin)/22.5 [13]. Abdominal obesity was defined by a WC measurement $\geq$95 cm for both men and women. This WC threshold was chosen because it was identified using a VAT area threshold ($\geq$130 cm^2) rather than a BMI threshold [14]. Because the aim of the present study was to test the relationship between glutamate and body fat distribution rather than general adiposity, this choice was the most relevant.

We used 2 metabolic risk stratification tools: the hypertriglyceridemic waist (HTW) phenotype and the MetS (Table 1). Various thresholds have been reported for both the WC and the fasting triglycerides (TG) criteria of the HTW phenotype. In the present study, we decided to use those determined in samples most similar to ours, i.e. WC $\geq$ 90 cm, TG $\geq$ 2.0 mmol/L for men [15] and WC $\geq$ 85 cm, TG $\geq$ 1.5 mmol/L for women [16]. Presence of the MetS was defined using the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria from 2004 [17], i.e. 3 or more of the following: WC $>$ 102 cm for men or $>$88 cm for women, TG $\geq$ 1.7 mmol/L, high-density-lipoprotein cholesterol (HDL-C) $<$1.1 mmol/L for men or $<$1.3 mmol/L for women, fasting glucose $\geq$5.6 mmol/L and diastolic blood pressure (BP) $\geq$130 mmHg or systolic BP $\geq$ 85 mmHg.

To assess the influence of metabolic health on the association between glutamate and WC, we broke down the 2 metabolic risk algorithms (HTW phenotype and MetS) to isolate their WC criterion. First, we compared the glutamate level of individuals with and without high WC according to the WC threshold of each algorithm. Second, in each of these subgroups, we compared individuals with and without another criterion of the algorithm (high TG for the HTW and any of the MetS features for the MetS).

Statistics

Participant characteristics are presented as mean $\pm$ standard deviation (SD) unless specified otherwise. Men and women were compared using Student's t-test for continuous variables and Fisher's exact test for dichotomous variables. Univariate Pearson correlation coefficients were used to assess the association between amino acid and adiposity/metabolic variables. Variables were log-transformed when required to meet test assumptions. We compared the glutamate level of the subgroups created by the breakdown of each metabolic risk algorithm using Student's t-test.

Logistic regressions, more specifically receiving operating characteristics (ROC) curves, were used to evaluate the ability of glutamate to identify individuals with abdominal obesity, the HTW and the MetS. Glutamate's accuracy was calculated using the area under the ROC curve (ROC_AUC) and it was classified as: excellent if the ROC_AUC was above 0.9; good if it was 0.8–0.89; fair if it was 0.7–0.79; and poor if it was below 0.69 [18]. The optimal glutamate thresholds were determined using Youden's index, calculated as specificity + sensitivity - 1. Sensitivity and specificity were defined as true positive/(true positive + false negative) and true negative/(true negative + false positive) respectively. Results were considered significant when the p-value was $<$0.05. All statistical analyses were performed on JMP platform (SAS Institute, Cary, NC).

Results

Sample characteristics

The characteristics of the sample are shown in Table 2. Mean ($\pm$SD) age was 34.1 $\pm$ 10.1 years, mean BMI was 29.0 $\pm$ 6.2 kg/m^2 and mean WC was 92.7 $\pm$ 16.5 cm. Overall, 47.5% of the sample had abdominal obesity, 19.2% had the HTW phenotype and 33.0% presented the MetS. Glutamate had the lowest plasma level of all the amino acids evaluated with a mean of 42.93 $\pm$ 27.65 μmol/L (other amino acids not shown).

Although BMI did not significantly differ between sexes ($p = 0.77$), mean WC was significantly higher in men than women ($p < 0.0001$). Men had lower HDL-C levels as well as higher HOMA-IR index, systolic blood pressure (SBP), diastolic blood pressure (DBP) and glutamate level than women ($p < 0.05$ for all). The MetS was significantly more prevalent in men than in women ($p = 0.048$), but the prevalence of abdominal obesity and the HTW phenotype was not different between sexes ($p = 0.12$ and $p = 1.00$ respectively).

Associations

The univariate Pearson correlation coefficients of the association between amino acid concentrations and adiposity/metabolic variables are presented in Table 3. From the 8 amino acids examined, glutamate showed the strongest correlation with WC in both sexes ($r = 0.66$ in men and 0.76 in women, both $p < 0.0001$). When the

Table 1 Abdominal obesity and cardiometabolic risk algorithm definitions.

	Men	Women
Abdominal obesity	WC $\geq$ 95 cm	WC $\geq$ 95 cm
HTW	WC $\geq$ 90 cm and TG $\geq$ 2.0 mmol/L	WC $\geq$ 85 cm and TG $\geq$ 1.5 mmol/L
MetS	*Any 3 of the 5 below:* • WC $>$ 102 cm • TG $\geq$ 1.7 mmol/L • HDL-C $<$1.1 mmol/L • Glucose $\geq$5.6 mmol/L • BP $\geq$ 130/$\geq$85 mmHg	*Any 3 of the 5 below:* • WC $>$ 88 cm • TG $\geq$ 1.7 mmol/L • HDL-C $<$1.3 mmol/L • Glucose $\geq$5.6 mmol/L • BP $\geq$ 130/$\geq$85 mmHg

Abdominal obesity criterion is from Lemieux et al., 1996 [14]. HTW criteria are from Lemieux et al., 2000 (men) [15] and Blackburn et al., 2008 (women) [16]. MetS criteria are from the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP-III, 2004) [17].
HTW: hypertriglyceridemic waist, MetS: metabolic syndrome, WC: waist circumference, TG: triglycerides, HDL-C: high-density-lipoprotein cholesterol, BP: blood pressure.

Table 2 Sample characteristics.

Variables (unit)	Overall (n = 198)	Men (n = 99)	Women (n = 99)	Student t-test p-value
Age (years)	34.1 ± 10.1	33.9 ± 9.6	34.2 ± 10.6	0.88
BMI (kg/m^2)	29.0 ± 6.2	29.1 ± 5.9	28.9 ± 6.6	0.77
WC (cm)	92.7 ± 16.5	97.4 ± 16.4	88.0 ± 15.4	<0.0001
Cholesterol (mmol/L)	4.49 ± 1.00	4.41 ± 1.00	4.57 ± 0.99	0.25
TG (mmol/L)[a]	1.07 (0.67–1.55)	1.11 (0.68–1.66)	1.02 (0.65–1.35)	0.08
HDL-C (mmol/L)	1.33 ± 0.41	1.14 ± 0.29	1.53 ± 0.42	<0.0001
LDL-C (mmol/L)	2.76 ± 0.94	2.84 ± 1.01	2.69 ± 0.88	0.25
Glucose (mmol/L)[b]	5.65 ± 0.74	5.81 ± 0.81	5.49 ± 0.63	0.0021
Insulin (μU/mL)[a]	10.37 (6.52–14.51)	10.44 (6.34–15.55)	10.30 (6.88–14.29)	0.18
HOMA-IR[a,b]	2.43 (1.69–3.61)	2.49 (1.69–3.95)	2.41 (1.67–3.53)	0.0461
SBP (mmHg)	121 ± 11	123 ± 11	119 ± 11	0.0089
DBP (mmHg)	78 ± 9	79 ± 9	76 ± 10	0.0310
Glutamate (μmol/L)	42.93 ± 27.65	52.54 ± 29.74	33.33 ± 21.58	<0.0001
Metabolic risk algorithms	**Overall (n = 198)**	**Men (n = 99)**	**Women (n = 99)**	**Fisher's exact test p-value**
Abdominal obesity	47.5%	53.5%	41.4%	0.12
HTW	19.2%	19.2%	19.2%	1.00
MetS[b]	33.0%	39.8%	26.0%	0.0478

Results are presented as mean ± standard deviation (SD) unless stated otherwise. Men and women were compared using two-tailed Student t-test for continuous variables and Fisher's exact test for dichotomous variables.
BMI: body mass index, WC: waist circumference, TG: triglycerides, HDL-C: high-density-lipoprotein cholesterol, LDL-C: low-density-lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, SBP: systolic blood pressure, DBP: diastolic blood pressure.
[a] Median (interquartile range).
[b] Overall n = 194 (98 men and 96 women).

association was adjusted for BMI, it remained significant in women (r = 0.22, p = 0.03) but not in men (r = 0.08, p = 0.45).

Glutamate was also correlated with age (r = 0.28, p = 0.006 in men; r = 0.41, p < 0.0001 in women), BMI (r = 0.68 in men; r = 0.73 in women, both p < 0.0001) and metabolic risk variables, including TG (r = 0.59 in men; r = 0.57 in women, both p < 0.0001), HDL-C (r = −0.45, p < 0.0001 in both sexes) and the HOMA-IR index (r = 0.65 in men; r = 0.60 in women, both p < 0.0001). Only in women was glutamate significantly associated with total and low-density-lipoprotein cholesterol (LDL-C) levels. Glutamate was not significantly correlated with fasting glycaemia in men or women.

ROC curves

We plotted the sensitivity and specificity of all possible glutamate cut-off points to identify subjects with abdominal obesity, the HTW phenotype and the MetS. The ROC curves and their descriptive statistics can be found in Fig. 1. Of the 8 amino acids examined, glutamate had the best ability to discriminate individuals with or without the 3 metabolic dysfunction algorithms (see Supplemental Table 1).

For abdominal obesity (Fig. 1a), the ROC_AUC of glutamate was 0.90 (95%CI: 0.84–0.96) for men and 0.90 (95% CI: 0.84–0.97) for women, which indicates excellent accuracy. The optimal glutamate threshold was 41.35 μmol/L for men, with a sensitivity of 90% and a specificity of 76%. The best threshold was 30.55 μmol/L in women and it had a sensitivity of 86% and a specificity of 86%.

For the HTW phenotype (Fig. 1b), the ROC_AUC was 0.82 (95%CI: 0.73–0.92) for men and 0.85 (95%CI: 0.76–0.93) for women, suggesting good accuracy. The optimal threshold was 41.55 μmol/L for men with a sensitivity of 100% and a specificity of 52%. In women, the best threshold was 41.00 μmol/L and it had a sensitivity of 84% and a specificity of 78%.

For the MetS (Fig. 1c), the ROC_AUC of glutamate was 0.78 (95%CI: 0.69–0.87) for men and 0.89 (95%CI: 0.82–0.97) for women, which corresponds to fair and good accuracy, respectively. The optimal thresholds were identical to those of the HTW phenotype, i.e. 41.55 μmol/L for men (sensitivity 88%, specificity 60%) and 41.00 μmol/L for women (sensitivity 84%, specificity 86%).

In comparison, the accuracy of BMI to identify individuals with the HTW phenotype was 0.80 (95%CI: 0.72–0.88) for men and 0.82 (95%CI: 0.74–0.90) for women. For the MetS, it was 0.81 (95%CI: 0.72–0.90) for men and 0.89 (95%CI: 0.83–0.95) for women.

Metabolic risk algorithm components

To assess the influence of metabolic health on the association between glutamate and WC, we broke down the 2 metabolic risk algorithms (HTW phenotype and MetS) to isolate their WC component (Fig. 2).

In the component analysis of the HTW phenotype (Fig. 2a), individuals categorized as having a high WC (≥90 cm for men, ≥85 cm for women) had higher circulating glutamate levels than those with a lower WC. Among the individuals with high WC, those with high TG had a higher glutamate level then those without. The small number of individuals with low WC having high TG (n = 2) made the comparison in the low-WC subgroup underpowered.

Table 3 Associations between amino acids and adiposity/metabolic variables.

	Glutamate		Leucine		Isoleucine		Valine		Alanine		Glycine		Tryptophan		Tyrosine	
Men (n = 99)																
Age	0.28	**	−0.06		−0.19		−0.01		−0.04		−0.19		−0.11		−0.02	
BMI	0.68	***	0.28	**	0.28	**	0.23	*	0.22	*	−0.56	***	−0.17		0.13	
WC	0.66	***	0.29	**	0.31	**	0.18		0.24	*	−0.54	***	−0.19		0.16	
Cholesterol	0.13		−0.14		−0.24	*	0.10		−0.05		−0.25	*	0.01		0.15	
TG	0.59	***	0.16		0.20	*	0.11		0.23	*	−0.48	***	−0.11		0.24	*
HDL-C	−0.45	***	−0.12		−0.18		−0.14		−0.12		0.37	***	0.18		−0.17	
LDL-C	−0.11		−0.21	*	−0.32	**	0.03		−0.15		−0.07		0.00		0.11	
HOMA-IR	0.65	***	0.28	**	0.37	***	0.09		0.37	***	−0.43	***	−0.24	*	0.08	
Glucose	0.15		0.09		0.16		0.03		0.01		0.08		0.08		0.09	
Insulin	0.67	***	0.28	**	0.36	***	0.09		0.39	***	−0.48	***	−0.27	**	0.07	
SBP	0.28	**	0.13		0.22	*	0.18		0.24	*	−0.26	**	−0.09		0.04	
DBP	0.25	*	0.14		0.12		0.03		0.04		−0.11		−0.10		−0.03	
Women (n = 99)																
Age	0.41	***	0.09		0.04		0.15		0.40	***	−0.11		0.07		0.11	
BMI	0.73	***	0.38	***	0.38	***	0.44	***	0.42	***	−0.39	***	0.07		0.29	**
WC	0.76	***	0.36	***	0.33	***	0.37	***	0.39	***	−0.41	***	0.03		0.25	*
Cholesterol	0.35	***	0.32	**	0.20	*	−0.01		0.23	*	−0.40	***	0.02		0.02	
TG	0.57	***	0.41	***	0.43	***	0.10		0.43	***	−0.53	***	−0.02		0.10	
HDL-C	−0.45	***	−0.09		−0.11		−0.21	*	−0.26	*	0.17		0.04		−0.11	
LDL-C	0.22	*	0.19		0.07		−0.06		0.11		−0.30	**	−0.03		−0.04	
Glucose	−0.09		−0.03		0.01		−0.10		0.04		0.10		0.01		−0.16	
Insulin	0.65	***	0.33	**	0.36	***	0.22	*	0.41	***	−0.27	**	0.05		0.22	*
HOMA-IR	0.60	***	0.31	**	0.34	***	0.21	*	0.38	***	−0.23	*	0.05		0.17	
SBP	0.44	***	0.25	*	0.26	*	0.15		0.20		−0.30	**	0.18		0.17	
DBP	0.47	***	0.27	**	0.26	*	0.14		0.13		−0.33	***	0.05		0.14	

Results are Pearson correlation coefficients.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
BMI: body mass index, WC: waist circumference, TG: triglycerides, HDL-C: high-density-lipoprotein cholesterol, LDL-C: low-density-lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, SBP: systolic blood pressure, DBP: diastolic blood pressure.
The following variables were log-transformed to comply with the statistical test assumptions: TG, insulin and HOMA-IR.

In the component analysis of the MetS (Fig. 2b), once again, individuals in the high WC subgroup (≥102 cm for men, ≥88 cm for women) had higher glutamate levels compared to those with lower WC. Moreover, individuals with high WC and at least 1 other MetS component had a higher glutamate level than those who only had high WC. However, in the low WC group, there was no glutamate level difference between those without any MetS component and those with at least 1 feature (other than WC).

Discussion

We aimed to evaluate the potential of circulating glutamate as a biomarker of abdominal obesity and metabolic risk. Among those selected, we report that glutamate was the amino acid most strongly correlated with WC and multiple markers of metabolic dysfunction, particularly total TG level, HDL-C level and the HOMA-IR index. We demonstrate that glutamate concentration used as a single variable has an excellent ability to identify individuals with abdominal obesity and a fair-to-good ability to identify those with the HTW phenotype or the MetS.

The pathophysiological mechanism underlying the association between glutamate and abdominal or visceral obesity is not yet clear. Glutamate is present in food, both naturally and by adding monosodium glutamate. Epidemiological studies have shown that dietary glutamate intake is positively associated with body weight. However, as discussed in a review on this topic [19], these observations are not supported by experimental studies and are likely due to the nutrition transition in developing Asian countries rather than to a causal relationship.

Endogenous glutamate is a by-product of the catabolism of all 3 BCAAs, which takes place mostly in skeletal muscle (~50%) and adipose tissue (~15%) [20]. The first 2 steps of BCAA catabolism are common to all 3 BCAAs, the first being a transamination by branched-chain aminotransferase (BCAT) [21]. In this reaction, an α-ketoglutarate accepts the amino group of a BCAA to produce glutamate and a branched-chain ketoacid (BCKA). The BCKA is then dehydrogenated by branched-chain-ketoacid dehydrogenase (BCKD) [21].

We previously reported that, compared to lean controls, obese women had significantly lower BCAT gene expression in subcutaneous adipose tissue (SAT) and in VAT, whereas BCKD was lower only in VAT [8]. In 2013, Lackey et al. reported that metabolically unhealthy obese women had lower mRNA transcript abundance for BCKD and BCAT than their metabolically healthy obese counterparts, but interestingly, the differences were only significant in VAT and not in SAT [22]. She et al. showed that bariatric surgery significantly increased BCKD and BCAT protein expression in both SAT and VAT. These

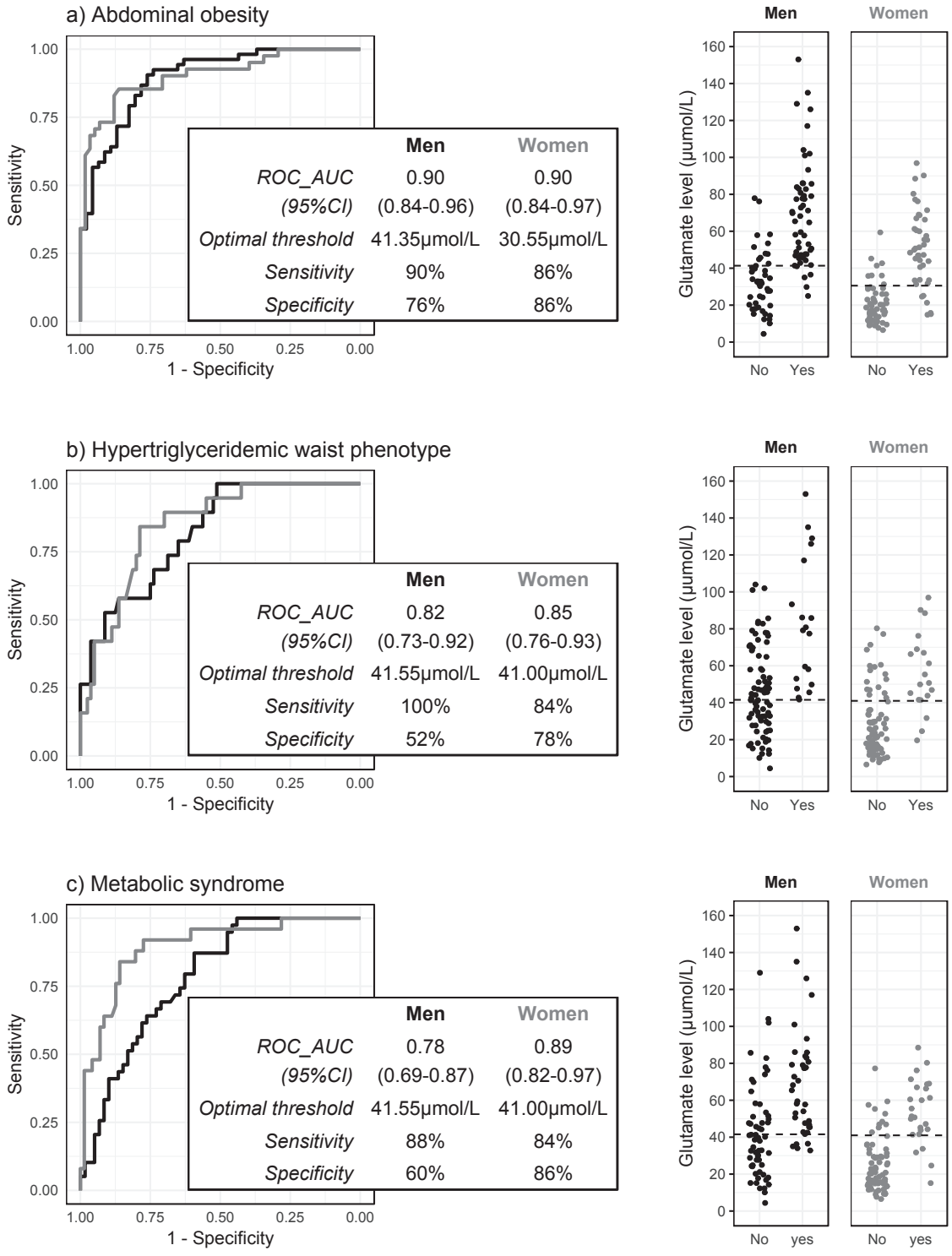

Figure 1 ROC curves of the ability of glutamate to identify individuals with (a) abdominal obesity, (b) the HTW phenotype and (c) the MetS. The best glutamate thresholds are represented by a dotted line in the scatter plots. They were determined using Youden's Index. ROC_AUC: area under ROC curve, WC: waist circumference.

changes were associated with a ~35% decrease in BCAA level [23]. Nagao et al. used a metabolic turnover analysis to demonstrate that more glutamate was dynamically produced by the adipose tissues from obese mice than by that of lean controls [24]. Interestingly, the amount of glutamate produced by skeletal muscles and the liver was not different between obese and lean mice. Overall, available evidence suggests that altered BCAA

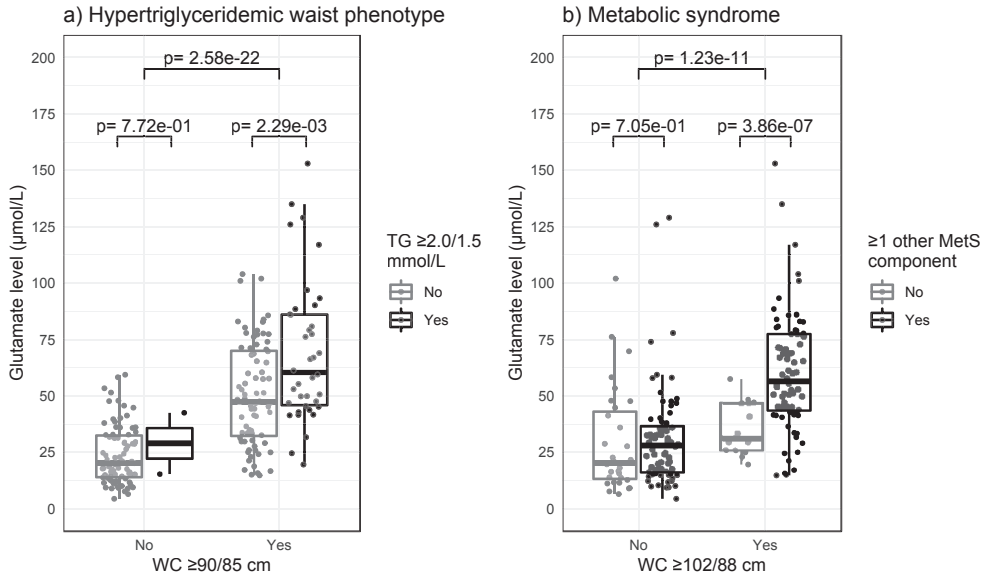

Figure 2 Glutamate level of individuals with or without high WC and metabolic dysfunction according to (a) the HTW phenotype or (b) the MetS. In the HTW phenotype analysis, 82 individuals had low WC and low TG, 2 had low WC and high TG, 76 had high WC and low TG and finally 38 had high WC and high TG. In the MetS analysis, 31 individuals had low WC and no MetS feature, 71 had low WC with at least one MetS feature, 15 had high WC and no other MetS feature and finally 76 had high WC and at least one other MetS feature. Subgroups were compared using Student's t-test. TG: triglycerides, WC: waist circumference, MetS: metabolic syndrome.

catabolism contributes to the high plasma BCAA and glutamate levels observed in obesity and metabolic dysfunction.

Although WC is often used as a surrogate of VAT measurement, it is important to keep in mind that it does not discriminate subcutaneous and visceral abdominal fat. Some individuals with a high WC have little VAT accumulation and an unaltered metabolic profile [2]. In the present analysis, we report that glutamate level of individuals with both high WC and an altered metabolic profile (either TG $>$ 2.0/1.5 mmol/L or at least 1 more MetS component) was significantly higher than those with a high WC only. Moreover, previous studies have shown that glutamate level was significantly associated with both VAT and SAT, but more strongly with VAT [7–9]. We realize that situations where measurement of circulating glutamate would be easier than that of WC are very few. However, the evidence stated above suggests that circulating glutamate level could potentially be used in combination with WC measurements to estimate VAT accumulation. Large studies with specific VAT quantification are needed to test this hypothesis.

This study has limitations. First, body fat distribution was assessed using WC, which does not discriminate visceral from subcutaneous abdominal adipose tissue. Second, the sample does not allow generalization of our results, or formal identification of glutamate thresholds for the general population. Finally, the cross-sectional design of the study does not allow to assess causality of the relationship between glutamate level and disease development.

The present study shows that glutamate is a potential biomarker of abdominal obesity and metabolic risk. We encourage other investigators to take interest in this amino acid, to include it in their assays and their analyses and to examine its potential as a biomarker. We also suggest that it is of great interest to investigate further the relation between adipose tissue function, BCAA catabolism and circulating glutamate level.

Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.08.015.

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