Plasma amino acid profile is altered by visceral fat accumulation and is a predictor of visceral obesity in humans

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Abstract

Background: The plasma amino acid profile can be a useful indicator in clinical settings because it changes in response to various metabolic alternations. However, the association between the plasma amino acid profile and body fat accumulation has not been evaluated in humans.

Objective: This study aimed to relate plasma amino acids to visceral fat accumulation in humans because excess visceral fat raises the odds ratio of developing metabolic syndrome.

Design: A total of 1,449 subjects (985 males and 464 females) who had undergone a comprehensive health screening were enrolled in this study. The visceral fat area (VFA) was determined in each subject using CT imaging. Subjects were then divided into two groups according to VFA: high-VFA ($\geq$100 cm^2, n=867) and low-VFA ($<$100 cm^2, n=582). The plasma amino acid profile was analyzed together with other metabolic valuables and then compared between the two groups using uni- and multivariate analyses.

Results: As the degree of visceral fat accumulation increased, plasma concentrations of several amino acids changed significantly. Glu, Val, Leu, Ile, Tyr, Ala, Phe, Pro, Lys, Orn, Trp, Met, His and alpha-aminobutyric acid (ABA) levels were significantly higher in the high-VFA group compared to the low-VFA group, whereas the levels of Gly, Ser, Gln and Asn were significantly lower. To evaluate the potential of using amino acids as an indicator of VFA, a discriminant analysis was conducted with the multivariate logistic regression analysis “AminoIndex”, and the ROC curve was calculated. The resulting “AminoIndex” exhibited an area under the ROC curve of 0.81 (95% confidence interval; 0.78 to 0.83), with higher sensitivity and specificity by 80% and

65%, respectively.

Conclusions: The plasma amino acid profile changes depending on visceral fat content and can be used as a marker for diagnosing elevated visceral obesity in humans.

Introduction

Recent progress in metabolomics has enables the high throughput measurement of diverse amino acids [1-3] and has shown the new possibility of using amino acid analysis of biological samples as a biomarker discovery tool by generating diagnostic indices through systematic multivariate regression models[4]. Published studies have shown that amino acids in biological fluids change in response to metabolic alternations during the courses of various diseases, such as renal failure[5], cancer[6], atherosclerosis[7], and insulin resistance[8]. The balance between branched amino acids (Leu, Val and Ile; BCAA) and aromatic amino acids (Phe and Tyr; AAA) is known as Fischer's ratio and is one of a few classical indicators used to monitor hepatic encephalopathy[9]. Recently, a novel multivariate logistic regression model of plasma free amino acids ("AminoIndex") was reported for the discrimination of various disease states in rat models of type-1 and type-2 diabetes [10]and the progression of liver fibrosis in chronic hepatitis C in humans[11]. Furthermore, the usefulness of amino acid profiles in various tissues in combination with other "-omics" datasets has been reported for investigating metabolic and regulatory networks in animals[12, 13].

Recent studies have suggested that there may be an association between plasma amino acid levels and obesity in both animals and humans. For instance, elevated plasma BCAAs and glutamate were observed in obese rodents [14, 15] and humans[16]. The plasma ratio of Trp/large neutral amino acids was also lower in obese subjects[16, 17]. Furthermore, a reduction in the levels of Gly, Trp, Thr, His, taurine, citrulline and cystine has been reported in obese subjects[16]. These studies strongly indicate that the volume of adipose tissue or its dysfunctions could affect amino acid metabolism and the levels of amino acids in peripheral circulation. However, the relationship between

plasma amino acid levels and fat content, particularly in a specific body fat deposit, has not been evaluated in humans.

Visceral obesity has been reported to represent a clinical intermediate phenotype reflecting the relative incapability of subcutaneous adipose tissue to act as a protective energy depot, leading to ectopic fat deposition in visceral adipose, skeletal muscle, liver, heart and other tissues[18, 19]. Thus, visceral obesity may be both a marker of a dysmetabolic state and a cause of metabolic syndrome[20]. Waist circumference and bioelectrical impedance have been reported to be better markers of visceral fat accumulation than body mass index. However, these approaches are insufficient to diagnose visceral obesity[21-23]. Although both computed tomography (CT) and magnetic resonance imaging (MRI) achieve a reliable prediction of visceral fat[24, 25], such high-cost and low-throughput measurements are unsuitable for primary diagnosis.

In this study, we first determined the plasma amino acid profile of human subjects who underwent CT scans for estimation of visceral fat area (VFA). Next, each plasma amino acid level was compared between low- and high-VFA groups to assess its discriminant power. Finally, a discriminant analysis with selected amino acids was examined with the multivariate logistic regression analysis “AminoIndex” and an additional assessment was made with ROC curve analysis.

Subjects and Methods

Subjects

A total of 1,449 subjects (985 males and 464 females) who had undergone comprehensive health screening tests between January 2008 and June 2009 at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, and the Kameda Medical Center Makuhari were enrolled. Subjects were divided into two groups according to visceral fat area (VFA) volumes calculated from CT images: high-VFA ($\geq$100 cm^2, n=867) and low-VFA (<100 cm^2, n=582). These VFA areas were chosen because previous studies have reported that the mean number of metabolic risk factors in Japanese subjects with VFA$\geq$100 cm^2 is significantly higher than in those with VFA<100 cm^2, irrespective of BMI[26]. Subjects were provided with no medical treatments before examination and blood sampling. The protocol was approved by the Ethical Committees of Mitsui Memorial Hospital and Kameda Medical Center Makuhari.

In Japan, regular health check-ups for employees are legally mandated; thus, the majority of the subjects enrolled in the study did not have serious health problems. In addition, most or all of the costs of the health screenings are paid by the company for which the individual works or by each individual. In addition, there are several options to choose from in the health screening program. The option chosen is up to each individual, not to the physicians or the company for which the individual works. Therefore, the study population was thought to not be enriched for any particular disease condition.

Analyses of metabolic parameters

Blood samples were taken from the subjects after an overnight fast. Serum levels of

total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Plasma glucose was measured by the hexokinase method, and hemoglobin A_{1c} (HbA1c) was determined using the latex agglutination immunoassay. Systolic and diastolic blood pressures were measured twice on the same day, and the mean value was used in the analysis. BMI was calculated as weight in kilograms divided by height in meters squared.

Measurement of abdominal fat area by CT scan

Subcutaneous and visceral fat areas visualized on a CT scan at the level of the umbilicus were measured using Fat Scan software (N2 System Co., Osaka, Japan). All CT scans were performed in the supine posture using a CT scanner (SOMATOM Sensation Cardiac 64, Siemens, Germany). The VFA was defined as the intraperitoneal fat bound by the parietal peritoneum or transversalis fascia, excluding the vertebral column and paraspinal muscles. The SFA was defined as the fat superficial to the abdominal and back muscles. A region of interest drawn around the external margin of the dermis was used to calculate the total abdominal fat (TAF) area. The SFA was obtained by subtracting the VFA from the TAF.

Plasma amino acid profiling

Blood samples (5 ml) were taken from forearm veins after an overnight fast in collection tubes containing EDTA-2Na (Terumo, Tokyo, Japan) and immediately cooled on ice. Plasma was obtained by centrifugation at 3000 rpm for 15 min at 4°C and stored at −80°C until analysis. Before analysis, a 50 μL portion of the plasma sample was added to 50 μL of the internal standard solution and 100 μL of acetonitrile, and the solution was mixed with a vortex-mixer as described previously[2, 3]. After mixing, the

precipitate was removed by centrifugation at 15,000 rpm for 10 min at 4°C and the supernatant was used for further analysis.

Plasma amino acid analysis was carried out with HPLC-ESI-MS following derivatization. A MSQ Plus LC/MS system (Thermo Fischer Scientific, Waltham, MA, USA) equipped with an electrospray ionization source was used in the positive ionization mode for selected ion monitoring (SIM). Xcalibur (TM) version 1.4 SR1 software was used for data collection and processing. The HPLC separation system consisted of an L-2100 (pump), L-2200 (autosampler), and L-2300 (column oven) (Hitachi High-Technologies Corporation, Tokyo, Japan). A Wakosil-II 3C8-100HG column (100 mm × 2.1 mm, 3 μm) (Wako Pure Chemical Industries, Osaka, Japan) was used for the separation. The mobile phase consisted of eluent A (25 mM ammonium formate in water) and eluent B (water:acetonitrile=40:60). In this study, twenty-four compounds were measured as described previously[2, 3]: alanine (Ala), alpha-aminobutyric acid (ABA), arginine (Arg), asparagine (Asn), citrulline (Cit), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val).

Statistical analysis

A Welch t-test was used to analyze differences in demographic variables, biochemical variables, and the plasma amino acid concentration between the high- and low-VFA groups. Significance was set at $p<0.05$. The discriminative power of the amino acids for the high- and low-VFA groups was evaluated by the area under curve of the receiver operating characteristics[27]. To evaluate the correlation among the variables, VFA, and

subcutaneous fat area (SFA), the Pearson correlation coefficient was calculated.

Multivariate logistic regression analysis for diagnosing VFA

In this study, we used the multivariate logistic regression analysis "AminoIndex" for diagnosing high-VFA subjects. The search for an optimal "AminoIndex" was performed using a previously described algorithm [10, 11, 28], based on a multivariate logistic regression model with amino acid concentrations as variables. All possible combinations of variables were investigated with cross-validation and an assessment of discriminatory power with the area under the ROC curve (AUC). In this study, the maximum number of variables for each regression formulation was restricted to six to limit the degrees of freedom and avoid overfitting. In addition, the variance inflation factor (VIF) was calculated to determine the degree of multicollinearity when its cutoff value was set at 10. Next, the best model was defined as the one with the minimum AIC (Akaike's information criterion)[29]. All of the statistical and multivariate analyses were performed with MATLAB and GraphPad Prism.

Results

Characteristics of study subjects

Age and body weight profiles for the high-VFA (≥100 cm^2; n=867) and low-VFA groups (<100 cm^2; n=582) are summarized in Table 1. Body weight was significantly higher in the high-VFA group than in the low-VFA group. Although the mean age was significantly different between the two groups, it was a negligibly small difference of 4.1 years.

The metabolic variables in visceral fat accumulation

General metabolic variables for the high- and low-VFA groups are summarized in Table 2. Waist circumference, body mass index (BMI), triglyceride levels, plasma glucose level, systolic blood pressure, diastolic blood pressure, HbA_{1c} and LDL cholesterol showed significant increases in the high-VFA group compared to the low-VFA group, with positive Pearson correlations with VFA value (n=1449). In contrast, only HDL cholesterol was significantly lower in the high-VFA group compared to the low-VFA group. Next, the discriminative performance of each parameter was evaluated by ROC analysis (ROC_AUC in Table 2). As expected, there were close relationships between the Welch t-test and ROC_AUC because of the binominal distribution in the two groups[30]. Waist circumference and BMI gave higher Pearson correlations, greater than 0.6 (n=1449), not only for visceral fat area (VFA) but also for subcutaneous fat area (SFA), without specificity between these different fat types (Table 2 and Figure 1).

Plasma amino acid profile in visceral fat accumulation

Plasma amino acid profiles are shown in Table 2. The Welch t-test indicated that the mean values of Glu, Val, Leu, Ile, Tyr, Ala, Phe, Pro, Lys, Orn, Trp, Met, His and ABA were significantly higher in the high-VFA group, with a positive correlation with VFA. By contrast, Gly, Ser, Gln and Asn were significantly lower in the high-VFA group, with a negative correlation with VFA.

The discriminative characteristics of each amino acid were also evaluated with ROC_AUC values (Table 2). Glu, Val, Leu, Ile, Tyr, Ala, Phe, Pro, Lys, Orn, Trp, Met, His and ABA were higher in the high-VFA group with statistical significance ($p<0.001$), whereas Gly, Ser, Gln and Asn were lower. Glu and Val gave higher Pearson correlations, more than 0.4, specifically for VFA, while lower correlation coefficients

were found for SFA (Table 2 & Figure 1). Other BCAAs, Tyr and Ala, showed similar specificity and higher levels of correlation with VFA.

Multivariate logistic regression analysis for discrimination between high-VFA and low-VFA groups

According to the results described above, it was suggested that further improvement in discrimination capability could be achieved using a multivariate function using plasma amino acid profiles. A multivariate logistic regression analysis, "AminoIndex", was performed with selected variables. For discrimination between the high- and low-VFA groups, a formula incorporating six amino acids (Ala, Gly, Glu, Trp, Tyr, BCAA) was developed, and the formula [-3.5250]+[0.0379]Glu+[-0.0070]Gly+[0.0034]Ala+[0.0196]Tyr+[-0.0216]Trp+[0.0054]BCAA was modeled, based on the procedure described in the Subjects and Methods. Figure 2 shows the ROC curve and the distribution plot of the obtained index, where it exhibits an area under ROC curve of 0.81 (95% confidence interval; 0.78 to 0.83), with higher sensitivity and specificity by 80% and 65%, respectively (a cutoff value 0.05).

Discussion

Physical inactivity, diet, and inherited predisposition are the predominant causes of excessive fat accumulation in adipose tissue. Obesity has become a major health concern worldwide and is related to a number of cardiovascular and metabolic disorders including insulin resistance, hyperlipidemia, and non-alcoholic fatty liver disease (NAFLD)[31-33]. Recent studies have shown that visceral fat tissue, rather than subcutaneous fat tissue, secretes adipokines such as TNF-α and IL-6 and that the levels

of these adipokines in the peripheral circulation are strongly correlated with the development of insulin resistance[34] and the occurrence of cardiovascular disease[35]. Using CT imaging, visceral fat area (VFA) is generally defined as the sum of the intraperitoneal fat area, while subcutaneous fat area (SFA) is defined as the sum of the extraperitoneal fat area between the skin and muscle[24]. Previous studies have found that if the visceral fat level is high, the risk of myocardial infarction and cerebral infarction drastically increases, even if the subject does not have hypertension, diabetes or hyperlipidemia[20, 36]. In addition, visceral fat has been reported to be associated with NAFLD, and an increase in its content is considered a potential therapeutic target in the treatment of NAFLD[37]. Thus, visceral fat accumulation is a potential risk factor and an early diagnostic marker for obesity-related diseases. Therefore, there is a critical need to elucidate the molecular pathogenesis of visceral obesity so that strategies can be developed for its prevention and treatment.

In this study, we conducted plasma free amino acid profiling in 1449 human subjects who underwent comprehensive health check-ups and CT scans for VFA and SFA measurements. We found that the majority of plasma amino acids were specifically altered with increased VFA, but not with SFA. Abdominal CT scans and waist circumferences are most frequently used for VFA prediction in clinical settings[22, 24]. However, CT scanners frequently have mechanical problems that require money, time and labor to repair, and these dysfunctions can even present radiation threats. Waist circumference is a noninvasive method to measure VFA, but it is subject to variation depending on the person who takes the measurement and the measurement site used. Additionally, the waist circumference combines subcutaneous fat and visceral fat leading to an underestimation of VFA[38]. In fact, waist circumference in this study gave

relatively higher Pearson correlations for both SFA and VFA, but did not have any specificity for either fat type. Bioelectrical impedance has been reported as a simple predictor of VFA, but a recent study showed that it provides an approximation of the total abdominal adipose fat measured by MRI, not VFA[21]. Thus, the amino acid profile and regression index presented here could be an approach for making primary measurements for specific VFA prediction.

Our results showed significant differences in plasma amino acids in accordance with the degree of VFA. We found that there were significantly higher levels of Glu, BCAAs, Tyr, Ala, Phe, Pro, Lys, Orn, Trp, Met, His and ABA in the high-VFA group, while there were lower levels of Gly, Ser, Gln and Asn. The underlying mechanism by which these amino acids change in response to VFA level is unclear; however, metabolic alternations, such as lowered insulin sensitivity, may contribute to altered amino acid levels. In the present study, BCAAs were significantly correlated with VFA, but not SFA. Elevation of plasma BCAAs has been reported in both humans and animal models of obesity[14-16]. Rosenthal et al. estimated that adipose tissue is second only to skeletal muscle in its capacity to catabolize BCAAs and that the capacities of skeletal muscle and adipose tissue are 6–7-fold larger than that of liver[39]. A previous study in mice showed that tissue-specific alterations in BCAA metabolism in liver and adipose tissue, but not in skeletal muscle, can contribute to an elevation in plasma BCAA levels in obese individuals[15]. Furthermore, a recent study demonstrated the importance of adipose BCAA enzymes and the BCAA catabolizing capacity of adipose tissue in determining circulating BCAA levels in obese mice and in possibly influencing the development of associated insulin resistance[40]. Thus, our data support previously reported findings and provide strong evidence for the importance of plasma BCAAs as a

potential marker for visceral fat specific metabolic changes. It has been suggested that BCAAs may be responsible for some of the beneficial effects of high-protein diets, including improved body weight control[41, 42], and may decrease adiposity and hepatic steatosis[12]. Similarly, BCAAs improve muscle glucose uptake, whole body glucose metabolism, and oxidation[43]. Therefore, the plasma amino acid profiling technique presented here could be a potential tool for nutritional treatments for obesity and metabolic syndrome.

Our data showed significant changes in glucogenic amino acids, such as Gly, Ala and Gly; Gly and Ser are two amino acids that had negative correlations with VFA. The reason for the reduction of Gly in the plasma of the high-VFA group is unclear. However, it has been reported that in hepatocytes glucose production from both Gly and Ser is increased in diabetic individuals, while this type of glucose production is a low under healthy conditions[44]. Gly and Ser are synthesized from glycolytic intermediates *via* 3-phosphoglycerate dehydrogenase, which is an NAD-linked enzyme that converts 3-phosphoglycerate (3-PG) to 3-phosphohydroxypyruvate and is the rate-limiting step of *de novo* serine biosynthesis[45]. Glyceroneogenesis can also produce 3-PG, so it is possible that serine can be synthesized in the liver by phosphoenolpyruvate carboxykinase (PEPCK). Thus, changes in glyceroneogenesis in adipose tissue and liver due to increased demand for glycerol and glyceride for triglyceride synthesis may affect serine biosynthesis and the subsequent Ser and Gly levels in the peripheral circulation[46]. We tested another AminoIndex with Gly in addition to BCAAs as explanatory variables, and we confirmed that it has enough discrimination power to distinguish between the high- and low-VFA groups (data not shown). Although the levels of several other amino acids, including Tyr and Phe, change depending on VFA levels, the reasons are unclear.

Therefore, further studies elucidating the mechanisms behind alternations in plasma amino acid levels are needed.

In conclusion, the results suggest that measurement of the levels of different amino acids in plasma samples is a useful approach for understanding the metabolic implications of obesity and can be used as a predictor of elevated visceral obesity in humans.

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Figure legends

Figure 1. Scatter plots of waist circumference (cm), BMI (kg/m^2), Glu (μmol/l), Val (μmol/l), Leu (μmol/l), Ile (μmol/l), Tyr (μmol/l), Ala (μmol/l) and Trp (μmol/l) versus VFA (cm^2) and SFA (cm^2); these plots have positive Pearson correlation coefficients, whereas the plots of Gly (μmol/l) versus VFA (cm^2) and SFA (cm^2) have negative Pearson correlation coefficients. Representative images (bottom) of visceral fat obesity (left) and subcutaneous fat obesity (right) are shown where the distribution of abdominal fat was measured by FatScan software based on CT scans at the level of the umbilicus. The visceral fat area is represented in red, and the subcutaneous fat area is pink.

Figure 2. Performance of the multivariate regression model "AminoIndex" for discrimination between high-VFA and low-VFA groups. (A) ROC curves for the high-VFA group versus the low-VFA group (dotted). (B) Distribution plots of AminoIndex values for high-VFA and low-VFA groups.

Table 1. Characteristics of study subjects

Significant differences between the high-VFA (≥100 cm^2) group and the low-VFA group (<100 cm^2) were evaluated by the Welch t-test: *** represents p<0.001.

	VFA<100 cm^2 means±SD	**VFA≥100 cm^2 means±SD**	***p* value**
n (male, female)	582 (304, 278)	867 (681, 186)	
Age (years)	55.7±12.6	59.8±10.9	***
Body weight (kg)	58.0±10.1	69.5±11.0	***

Table 2. The discriminative capacity for the high-VFA group (≥100 cm^2) vs. the low-VFA group (<100 cm^2) and the Pearson correlation coefficients with VFA and SFA as variables.

Numbers are means ± SD. The significance of the differences between the high-VFA group (≥100 cm^2) and the low-VFA group (<100 cm^2) were evaluated by the Welch t-test: * p<0.05, ** p<0.01, and *** p<0.001. The areas under the curve of receiver-operating characteristic curves (ROC_AUC) were evaluated with 95% CI and P-values: * p<0.05, ** p<0.01, and *** p<0.001. Variables were ordered by ROC_AUC. For evaluation of the correlations among metabolic variables, visceral fat area (VFA), and subcutaneous fat area (SFA), the Pearson correlation coefficient was used.

	VFA<100 cm^2 means±SD n=582	VFA≥100 cm^2 means±SD n=867	ROC_AUC (95% CI)	Coefficient of correlation VFA	SFA
Metabolic variables					
Waist circumference (cm)	79.1±7.6	89.6±7.5 ***	0.84 (0.82 - 0.86) ***	0.66	0.71
Body mass index (kg/m^2)	21.7±2.6	25.2±3.0 ***	0.82 (0.80 - 0.84) ***	0.61	0.72
Triglycerides (mg/dl)	94.1±52.8	146.4±117.0 ***	0.71 (0.68 - 0.74) ***	0.30	0.08
Plasma glucose (mg/dl)	94.0±14.0	104.8±22.3 ***	0.69 (0.66 - 0.71) ***	0.31	0.09
HDL cholesterol (mg/dl)	65.1±16.2	55.2±13.4 ***	0.68 (0.66 - 0.71) ***	-0.33	-0.15
Systolic blood pressure (mmHg)	118.6±18.4	130.0±17.7 ***	0.68 (0.66 - 0.71) ***	0.33	0.17
Diastolic blood pressure (mmHg)	74.3±11.1	81.4±10.7 ***	0.68 (0.65 - 0.71) ***	0.33	0.14
HbA1c (%)	5.3±0.6	5.6±0.7 ***	0.64 (0.61 - 0.67) ***	0.24	0.07
LDL cholesterol (mg/dl)	122±27.7	127.2±31.2 **	0.55 (0.52 - 0.58) **	0.07	0.14
Plasma amino acids (μmol/l)					
Glutamate	37.3±16.1	54.6±21.3 ***	0.75 (0.73 - 0.78) ***	0.49	0.21
Valine	224.5±38.0	256.5±43.7 ***	0.71 (0.68 - 0.74) ***	0.42	0.08
Leucine	114.1±22.2	132.0±25.3 ***	0.70 (0.68 - 0.73) ***	0.39	0.04
Isoleucine	66.1±14.8	77.5±17.7 ***	0.69 (0.66 - 0.72) ***	0.39	0.06
Tyrosine	73.6±14.8	83.8±16.3 ***	0.68 (0.65 - 0.71) ***	0.40	0.18
Alanine	364.8±79.2	415.2±87.3 ***	0.67 (0.64 - 0.70) ***	0.33	0.15
Glycine	258.4±63.2	227.6±48.0 ***	0.66 (0.63 - 0.69) ***	-0.31	-0.12
Phenylalanine	62.0±10.8	66.5±10.9 ***	0.63 (0.61 - 0.66) ***	0.27	0.07
Proline	142.3±40.4	159.4±40.5 ***	0.63 (0.60 - 0.66) ***	0.24	0.05

Lysine	198.2±33.2	211.5±30.9 ***	0.62 (0.59 - 0.65) ***	0.23	0.02
Ornithine	54.0±13.4	59.3±13.9 ***	0.61 (0.59 - 0.64) ***	0.20	0.02
Tryptophan	58.0±10.0	61.3±10.3 ***	0.60 (0.57 - 0.63) ***	0.20	0.02
Serine	115.1±18.3	109.4±17.7 ***	0.59 (0.56 - 0.62) ***	-0.17	-0.04
Methionine	27.1±6.1	28.4±4.9 ***	0.58 (0.55 - 0.61) ***	0.16	-0.01
Histidine	79.9±10.5	82.6±11.1 ***	0.57 (0.54 - 0.60) ***	0.14	0.06
α-Aminobutyric acid	22.0±6.5	23.7±6.9 ***	0.57 (0.54 - 0.60) ***	0.11	0.06
Glutamine	591.7±79.8	579.2±75.7 **	0.55 (0.52 - 0.58) **	-0.09	-0.05
Asparagine	45.2±7.1	44.4±6.8 *	0.53 (0.50 - 0.56) *	-0.08	-0.15
Threonine	115.8±25.0	117.6±25.2	0.52 (0.49 - 0.55)	0.05	0.00
Arginine	96.9±19.0	97.6±17.1	0.51 (0.48 - 0.54)	0.01	-0.08
Citrulline	33.5±8.2	33.9±8.5	0.51 (0.48 - 0.54)	0.01	-0.12

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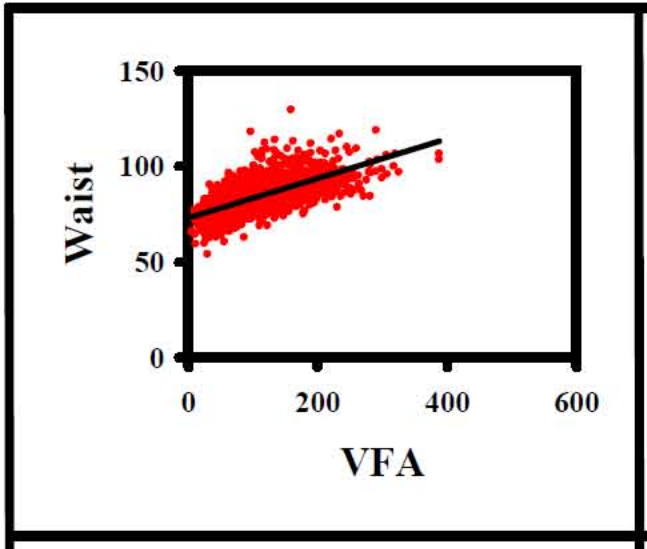
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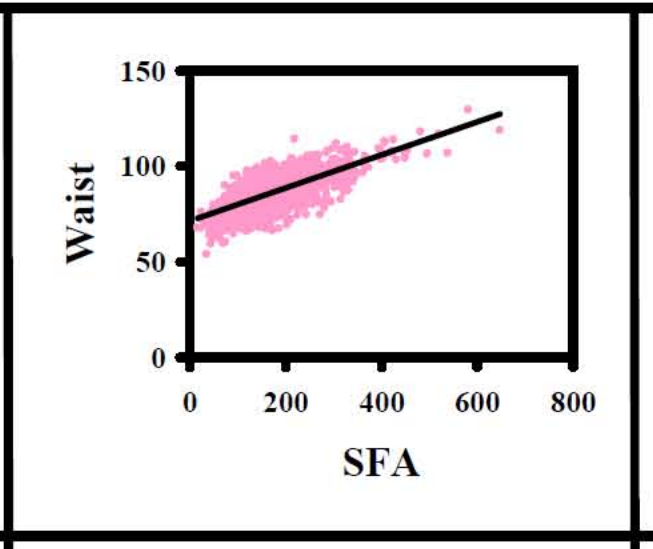
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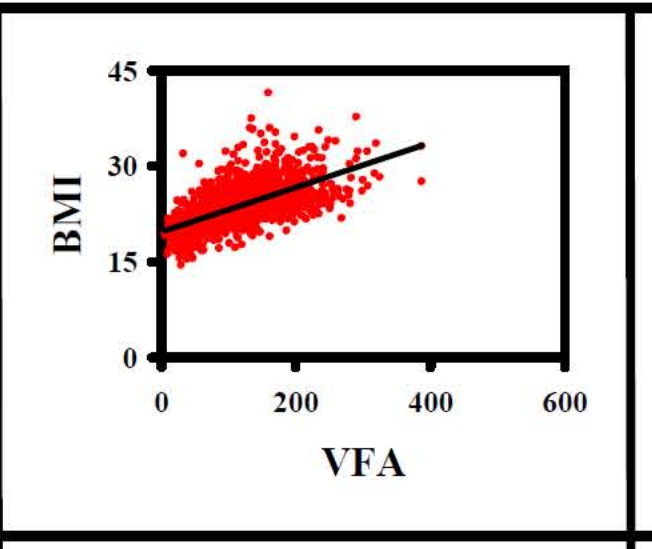
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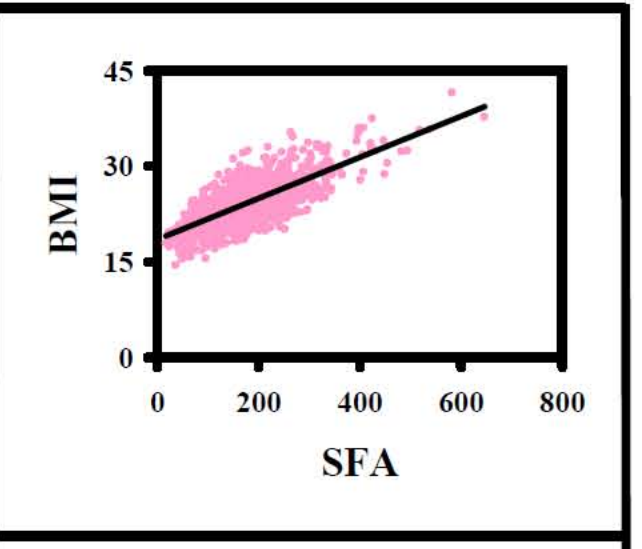
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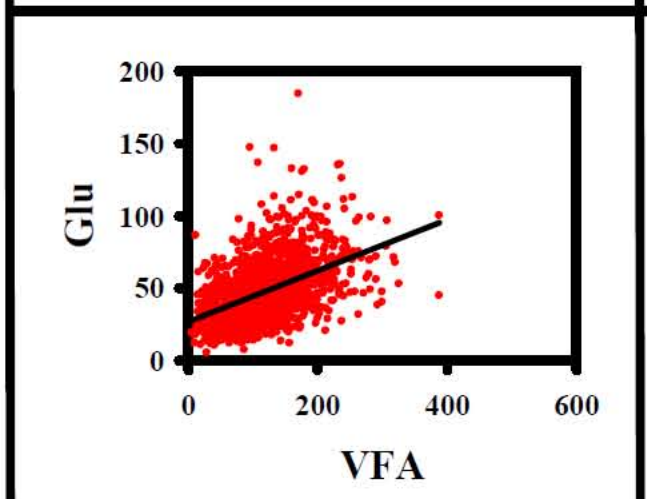
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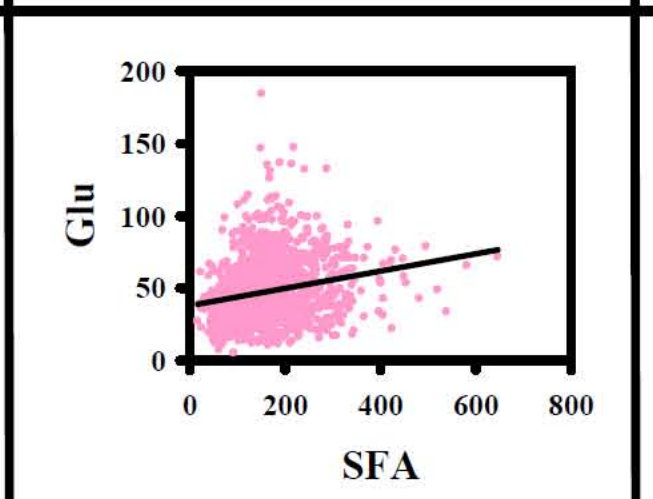
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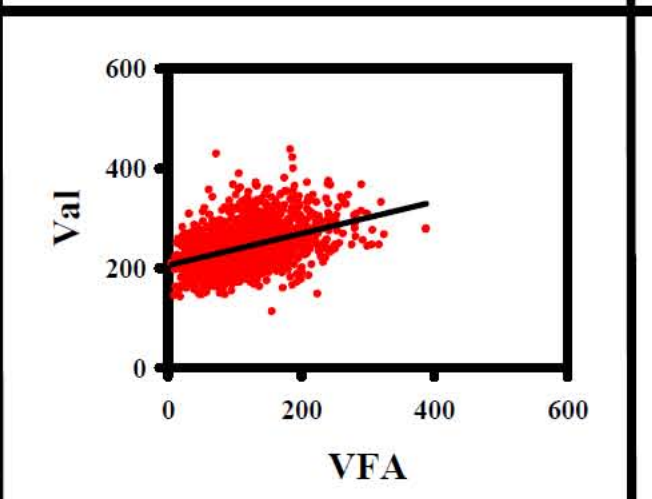
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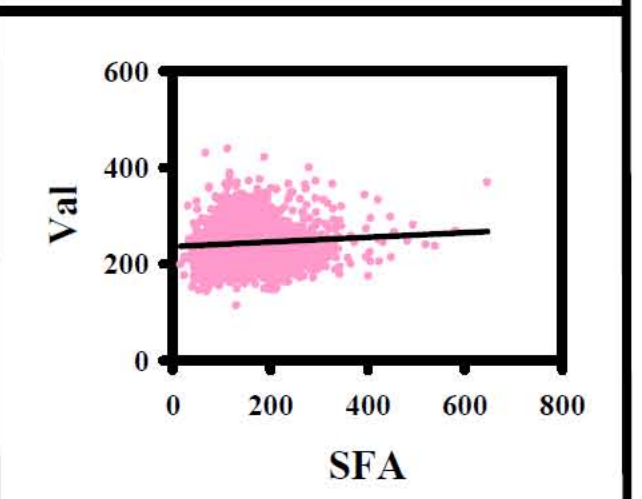
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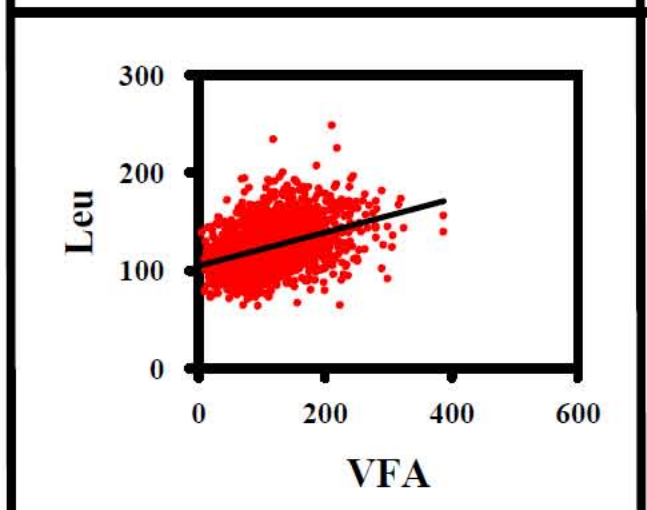
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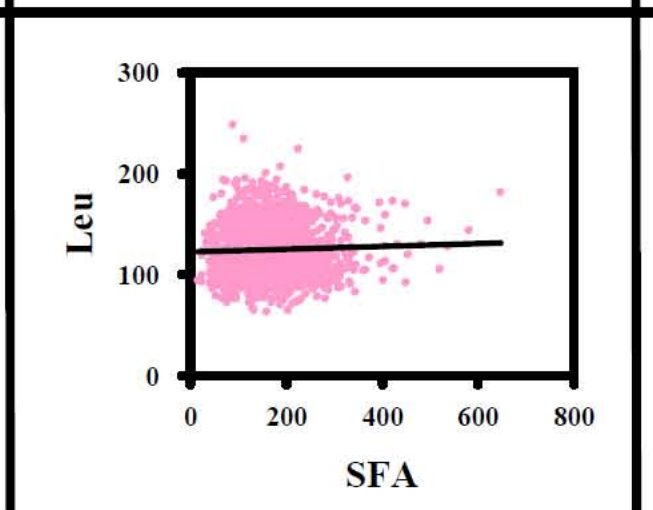
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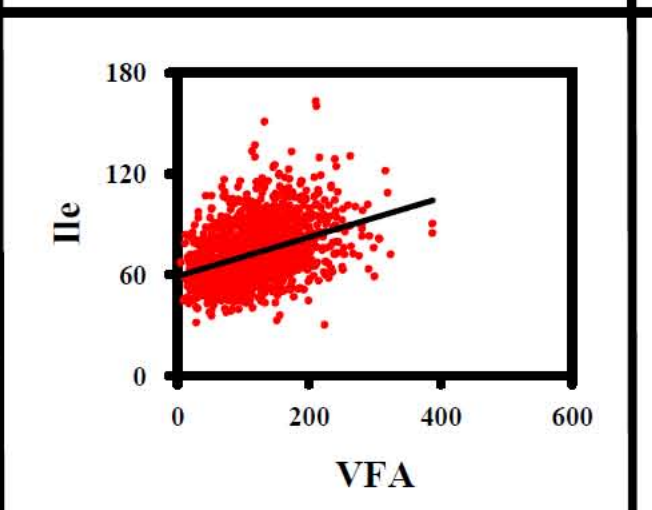
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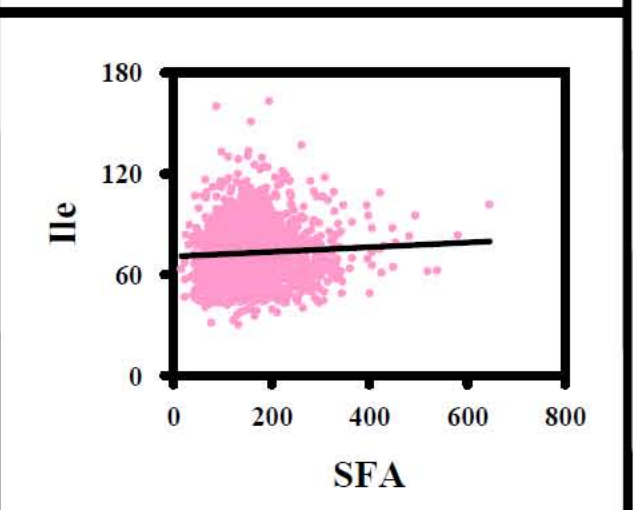
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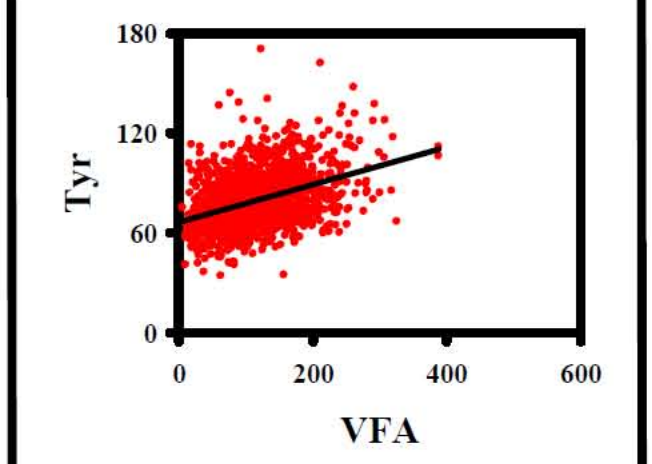
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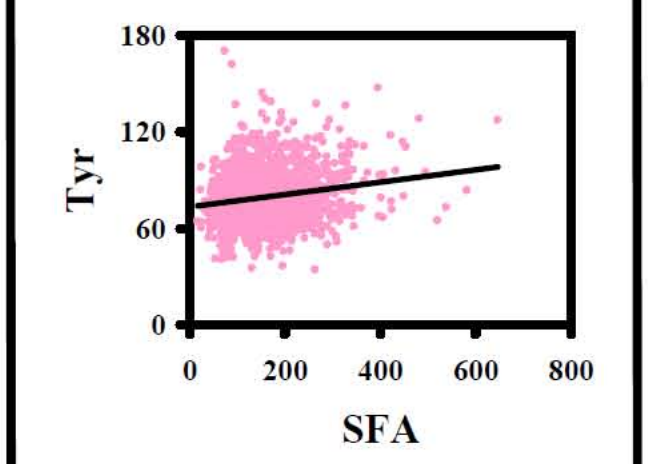
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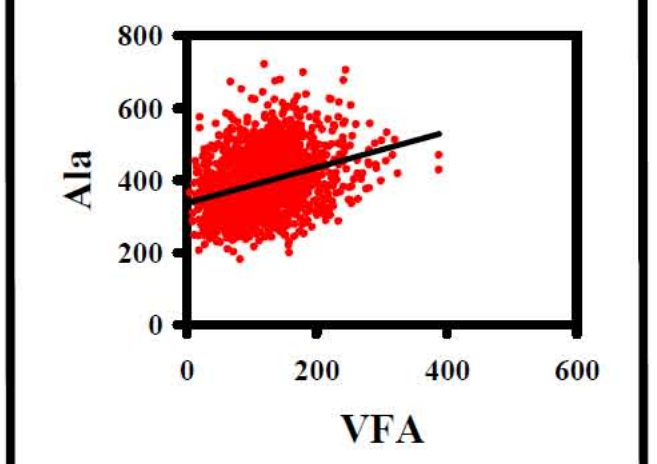
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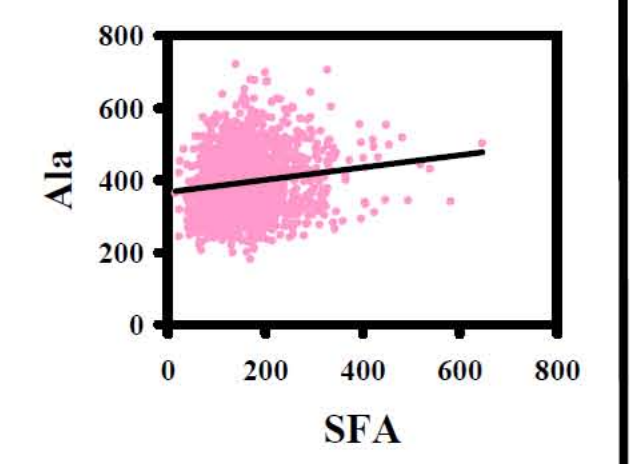
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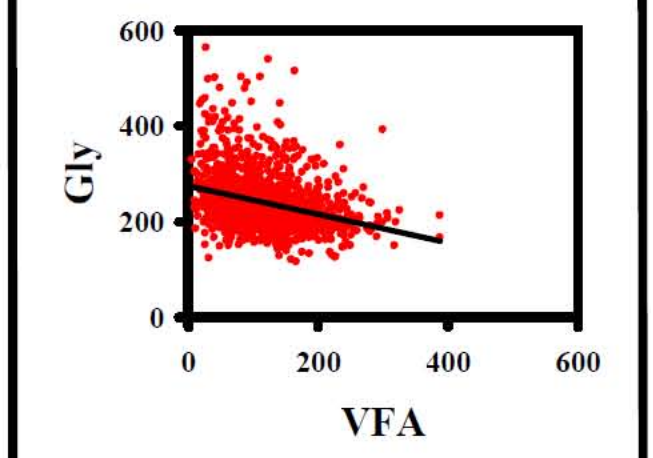
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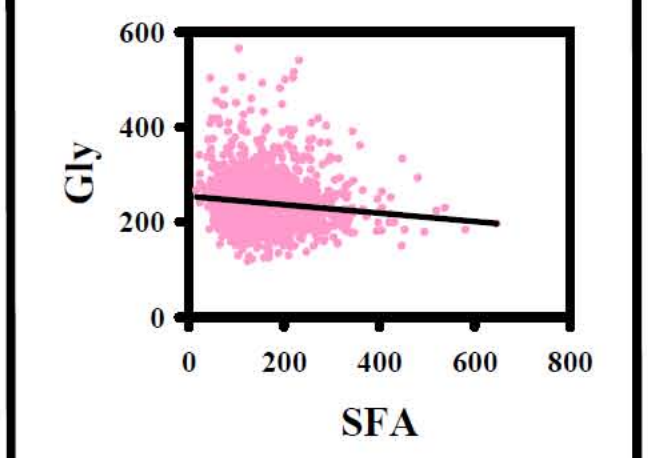
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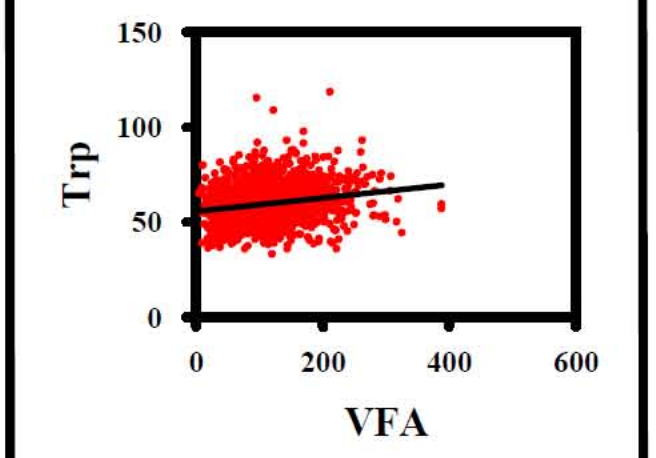
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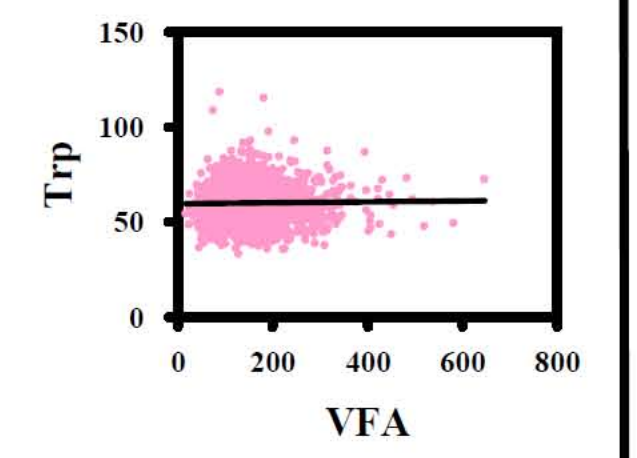
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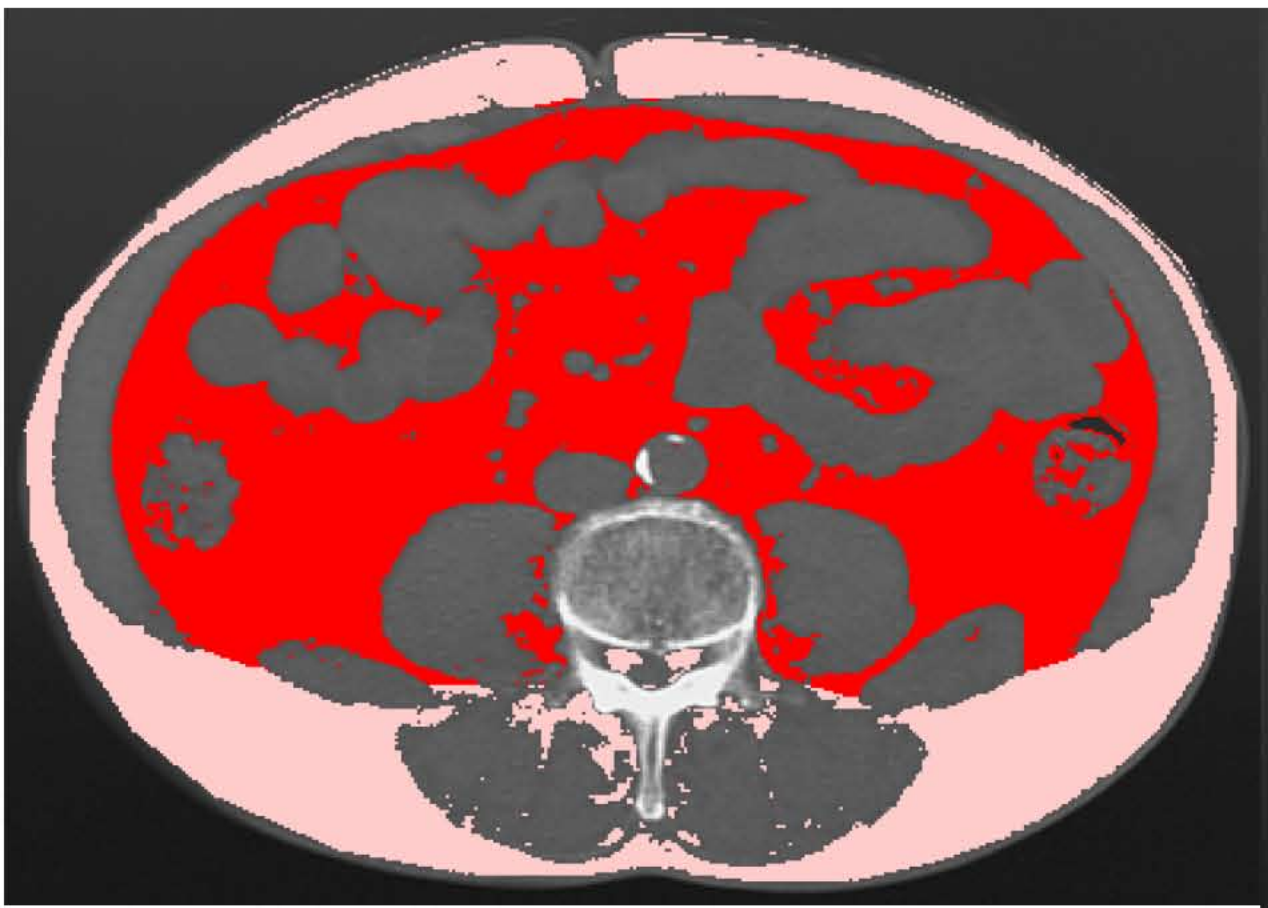

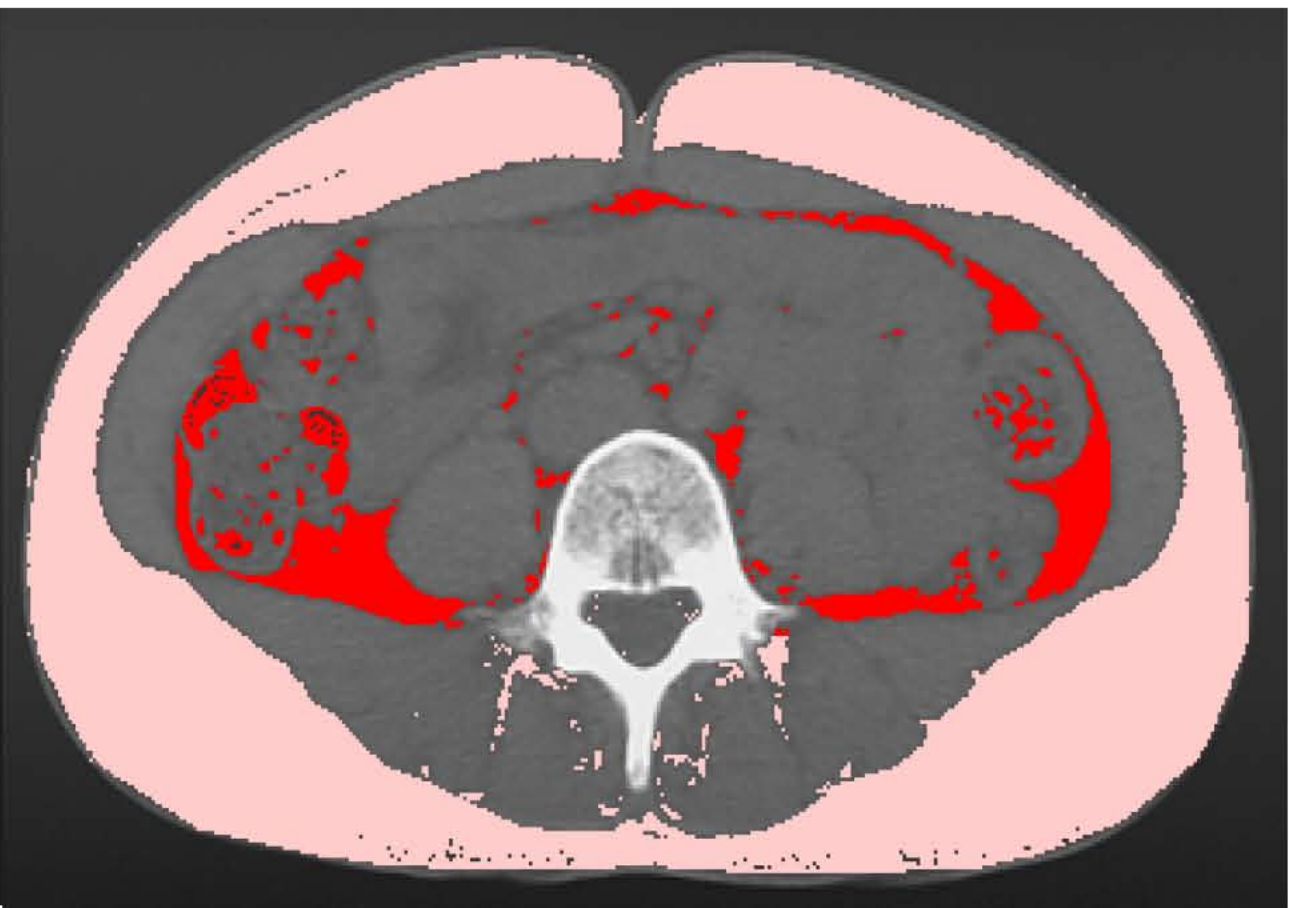

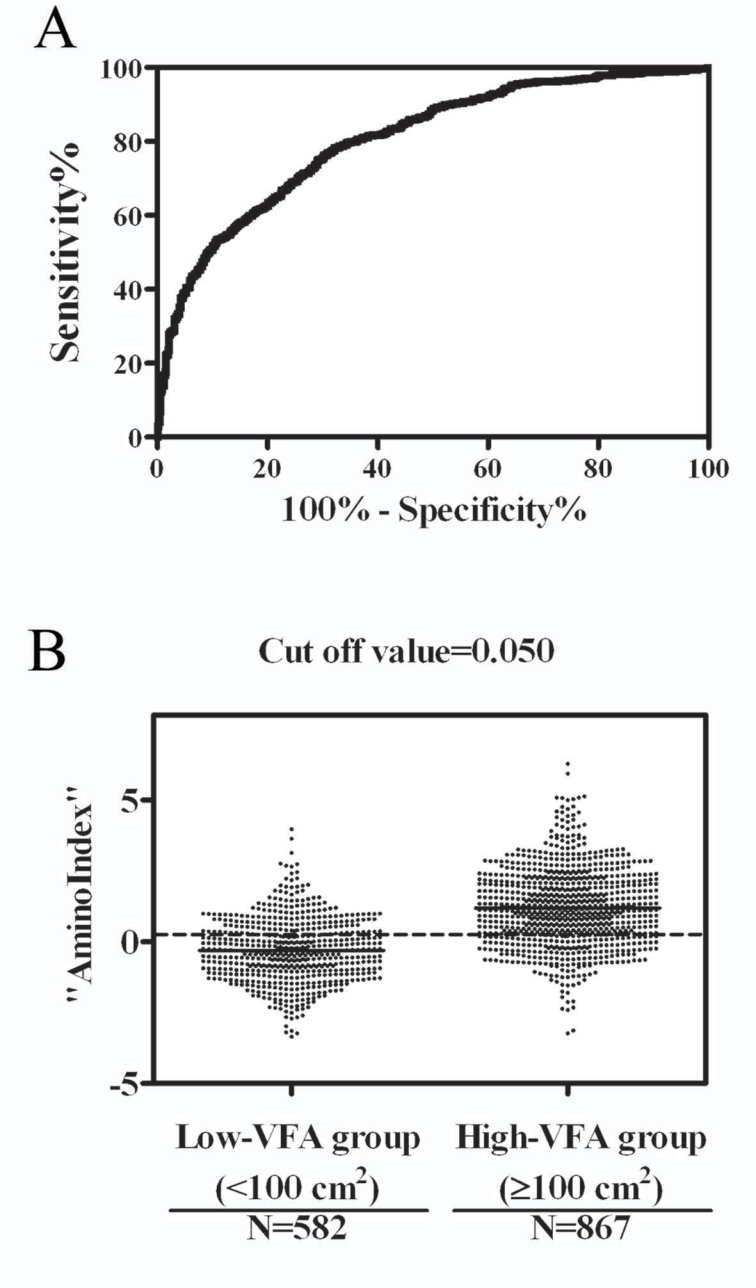
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"Amino Index"
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(<100 cm2)
N=582
High-VFA group
(≥100 cm2)
N=867