Associations of fat and lean body mass with circulating amino acids in older men and women

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Abstract

Circulating amino acids are potential markers of body composition. Previous studies are mainly limited to middle age and focus on either fat or lean mass, thereby ignoring overall body composition. We investigated the associations of fat and lean body mass with circulating amino acids in older men and women.

We studied 594 women and 476 men from the Helsinki Birth Cohort Study (age 62-74 years). Bioelectrical impedance analysis was used to indicate two main body compartments by fat (fat mass/height^2) and lean mass indices (lean mass/height^2), dichotomized based on sex-specific medians. Eight serum amino acids were quantified using nuclear magnetic resonance spectroscopy. General linear models were adjusted for age, smoking and fasting glucose.

Higher lean mass index was associated with higher concentrations of branched-chain amino acids in both sexes (p≤0.001). In men, lean mass index was also positively associated with tyrosine (p=0.006) and inversely with glycine (p<0.001). Higher fat mass index was associated with higher concentrations of all branched-chain amino acids, aromatic amino acids (phenylalanine and tyrosine), and alanine in both sexes (p≤0.008).

Associations between body composition and amino acids are largely similar in older men and women. The associations are largely similar to those previously observed in younger adults.

Key words: Metabolomics, Obesity, Muscles, Amino Acids

Introduction

Human body composition undergoes substantial changes with age. With aging, lean body mass, largely composed of muscle mass, is progressively lost (1,2). Concomitantly, the total amount of fat mass increases (1), and adipose tissue undergoes changes in distribution and function (3). Body composition has an important influence on physical functioning in older adults. Maintaining muscle mass is vital in order to manage daily activities and maintain mobility (4,5). Low muscle mass may even increase mortality (6). Excess adipose tissue is also a risk factor for reduced physical capacity (7,8) and functioning (9). The effect can be direct or indirect through elevated risks for e.g. osteoarthritis, type 2 diabetes, and cardiovascular diseases (3).

Metabolomics, i.e. determination of small particles in serum related to metabolism, have been applied in search for biomarkers for clinical conditions and in uncovering biological mechanisms contributing to non-communicable diseases and their risk factors. Concentrations of circulating amino acids, especially branched-chain amino acids (BCAAs), have been linked to obesity, insulin resistance, and type 2 diabetes in young and middle-aged adults (10–12). However, although body composition typically differs between older and younger adults, only few studies have investigated the relationships between body composition and amino acids in older adults. Most of the prior studies in older adults have had small sample sizes or focused on selective groups of older adults, such as functionally-limited older adults (13,14). These studies have suggested that higher levels of circulating BCAAs and aromatic amino acids (AAAs) are associated with obesity/adiposity but also with lean body mass (13,15–19). Prior studies have also focused on either fat mass or lean body mass and have not taken into account the remaining component of the overall body. This may be problematic as the amount of lean and fat mass are closely coupled; individuals with high fat mass

also tend to have high lean mass. Hence, associations found between amino acids and fat or lean mass may partly be confounded by the remaining component of the body. Consequently, it could be that part of the associations reported between fat mass or BMI and amino acids are, in fact, due to lean mass. Therefore, it is critical to study these associations by taking into account both main body compartments simultaneously. Furthermore, to the best of our knowledge, associations between amino acids and body composition have not been reported separately in older men and older women despite the fact that men and women differ in their body composition. Better knowledge on the associations between body composition and circulating amino acids could be a first step in identification of the cellular pathways, which lead to low muscle mass or high fat mass.

The purpose of this study was to examine the associations of lean and fat mass with serum amino acid concentrations separately in older men and women. We controlled for mutual confounding by fat and lean mass by using a 2x2 matrix derived from dichotomized fat and lean mass indices.

Methods

The Helsinki Birth Cohort Study (HBCS) consists of 13,345 singletons, who were born in Helsinki in 1934–1944 and who were still alive in 1971, when all residents of Finland received a unique personal identification number. A total of 8760 individuals were born in the Helsinki University Central Hospital, and of these people, 2902 individuals were randomly selected to participate in a clinical examination in 2001–2004 to reach a target of 2000 participants. In total, 2003 individuals participated in the clinical study. Follow-up examinations were performed in 2007 (n=1083) at the age of 62 to 74 years (mean 66 SD 2.8 years) and in 2011-13 at a mean age 71 years. The study design and assessments during the clinical visits have been described in details previously (20–22).

The study was approved by the Ethics Committee of Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa and that of the National Public Health Institute, Helsinki. The present analysis uses the data from the 2007 follow-up examinations and includes participants with data on body composition (n=1070 in total, 594 women, 476 men) and metabolomics. All measurements were performed by trained study nurses. Blood pressure was measured from the right arm while the subject was in a sitting position and was recorded as the mean of 2 successive readings from a mercury sphygmomanometer. Information on smoking was obtained from questionnaires administered in 2001- 04 and in 2011-13. Those who reported being current smokers either in 2001-04 or in 2011-13 were considered smokers, others were considered non-smokers.

Body composition and anthropometry

Height was measured, without shoes on, to the nearest 0.1 cm, and weight was measured in light indoor clothing to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Body composition was assessed by bioelectrical impedance analysis using the InBody 3.0 eight-polar tactile electrode system (Biospace Co, Ltd, Seoul, Korea) (23). The instrument estimates lean body mass and body fat mass by segmental multi-frequency (5, 50, 250, and 500 kHz) analyses. The measurements were made with the subject standing in light clothing on the 4-foot electrodes on the platform of the analyzer and gripping the two palm and thumb electrodes. Fat and lean mass indices were calculated: fat mass index (FMI)=fat mass/height[in meters]^2 and lean mass index (LMI)=lean mass/height[in meters]^2. Fat and lean mass indices were divided according to median values, separately for men and women, after which four body composition categories were created: 1) low FMI and low LMI 2) low FMI and high LMI 3) high FMI and low LMI 4) high FMI and LMI. By dividing participants into these categories, we aimed at reducing confounding between fat and lean mass indices. Supplemental

Figure 1 shows the relationships between lean mass index and fat mass index and the median-split categories of body composition in women and men.

Metabolomics

Blood samples were drawn after overnight fasting. A high-throughput nuclear magnetic resonance metabolomics platform was applied for the quantification of lipid and abundant metabolite measures from serum samples in the HBCS during the 2007 clinical visit. The methods that were applied have been described in greater detail previously (12,24). In this study, we focused on amino acids, namely alanine, glycine, histidine, phenylalanine, tyrosine, leucine, isoleucine, and valine. Sum scores were created for glucogenic (alanine and glycine), aromatic (histidine, phenylalanine, and tyrosine), and branched-chain (leucine, isoleucine, and valine) amino acids by computing z-scores for each amino acid and summing them up. Fasting plasma glucose was analyzed using a hexokinase method.

Statistical analysis

Relationships between fat mass index and lean mass index were derived from linear regression models. Differences in amino acid profiles between the sexes were determined using multivariate approach by using bootstrap type multivariate Hotelling T-squared test; it is a method to compare means of all variables of interest simultaneously (in the present analysis the amino acid profiles) while maintaining the chosen magnitude of Type I error. The relationships of body composition categories with background characteristics and amino acids were analyzed using general linear models. The main and interactive effects of fat and lean mass indices were analyzed by entering dichotomized lean mass index and fat mass index variables and their interaction as independent variables into the models. Age, fasting glucose concentration and smoking were introduced into the

models as covariates when appropriate. In the case of violation of the assumptions (e.g. non-normality), a bootstrap-type test was used. The normality of variables was evaluated by the Shapiro-Wilk W test. Cohen's effect-size statistics (d) was used as an indicator of strength of body compositions' effects on amino acids. Cohen's standard for effect size values above 0.20, 0.50 and 0.80 represent small, moderate and large effect sizes, respectively (25). Partial correlations (adjusted for age, fasting glucose concentration, and smoking) between percent body fat and amino acids were calculated with Sidak-adjusted (multiplicity) probabilities. All analyses were performed using STATA 15.0 (StataCorp, College Station, TX, USA).

Results

Characteristics of study participants are presented in Table 1 (women) and Table 2 (men). Women had higher mean fat mass index than men (9.4 SD 3.6 vs. 6.1 SD 2.4, t-test $p<0.001$) but lower mean lean mass index than men (17.8 SD 1.6 vs. 20.6 SD 1.7, $p<0.001$). The proportions of smokers among women were 16% (low FMI low LMI), 18% (low FMI high LMI), 29% (high FMI low LMI), and 24% (high FMI high LMI) (FMI $p=0.022$, LMI $p=0.78$, interaction $p=0.40$) and among men 25% (low FMI low LMI), 34% (low FMI high LMI), 20% (high FMI low LMI), and 20% (high FMI high LMI) (FMI $p=0.029$, LMI $p=0.42$, interaction $p=0.31$), respectively. The amino acid profiles differed between men and women ($p<0.001$) (Supplemental Table 1). Men had higher concentrations of histidine, tyrosine, leucine, isoleucine, and valine than women but lower glycine concentrations. Alanine or phenylalanine concentrations did not differ between men and women.

There were no interactions between fat and lean mass on amino acid scores (Fig. 1). Of the individual amino acids, only valine was affected by the interaction of fat and lean mass index in women (p=0.049) (Fig. 2).

Glucogenic amino acid score, adjusted for age, smoking, and fasting glucose concentration, was associated with fat mass index in women (Cohen's d=0.26, 95% CI 0.10 to 0.42, p=0.017) (Fig. 1). Of the glucogenic amino acids, alanine was positively associated with fat mass index in both women (d=0.40, 95% CI 0.24 to 0.56, p<0.001) (Fig. 2) and men (d=0.39, 95% CI 0.21 to 0.57, p<0.001) (Fig. 3). Glycine, in turn, was inversely associated with lean mass in men (d=0.40, 95% CI 0.22 to 0.59, p<0.001) (Fig. 3). Aromatic amino acid score was positively associated with fat mass index in women (d=0.54, 95% CI 0.37 to 0.70, p=0.006) and men (d=0.59, 95% CI, 0.41 to 0.78, p<0.001) (Fig.1). Of the individual AAAs, phenylalanine and tyrosine were associated with fat mass index in women (phenylalanine d=0.64, 95% CI 0.48 to 0.81, p<0.001; tyrosine d=0.48, 95% CI 0.32 to 0.64, p<0.001) and men (phenylalanine d=0.67, 95% CI 0.49 to 0.86, p<0.001; tyrosine d=0.56, 95% CI 0.37 to 0.74) (Fig. 2 and 3), and tyrosine was positively associated with lean mass index in men (d=0.45, 95% CI 0.27 to 0.63, p=0.003) (Fig. 3). Branched-chain amino acid score was positively associated with both fat and lean mass indices in both women (FMI d= 0.67, 95% CI 0.50 to 0.83, p<0.001; LMI d=0.60, 95% CI 0.44 to 0.76, p<0.001) and men (FMI d=0.55, 95% CI 0.37 to 0.74, p<0.001; LMI d=0.54, 95% CI 0.36 to 0.72, p<0.001) (Fig. 1). All individual BCAAs (leucine, isoleucine, valine) were positively associated with lean and fat mass indices in both sexes (p<0.008) (Fig. 2 and 3). The strength of effect (d) between individual BCAAs and fat mass index was 0.45 (95% CI 0.29 to 0.62) for leucine, 0.51 (95% CI 0.34 to 0.67) for isoleucine, and 0.54 (95% CI 0.38 to 0.70) for valine in women. In men, the corresponding figures were 0.54 (95% CI 0.36 to 0.73) for leucine, 0.61 (95% CI 0.43 to 0.80) for isoleucine, and 0.38 (95% CI 0.20 to 0.56) for valine. The strength of effect between individual BCAAs and lean mass index was 0.43 (95% CI 0.27 to 0.60)

for leucine, 0.44 (95% CI 0.28 to 0.61) for isoleucine, and 0.47 (95% CI 0.31 to 0.63) for valine in women. In men, the corresponding figures were 0.51 (95% CI 0.33 to 0.69) for leucine, 0.53 (95% CI 0.35 to 0.71) for isoleucine, and 0.45 (95% CI 0.26 to 0.63) for valine.

Correlations of percent body fat with amino acids and amino acid scores are shown in Supplemental Table 2.

Discussion

This study investigated the associations of body composition with serum amino acid concentrations among older men and women. As fat and lean mass are correlated, we controlled for the mutual confounding by fat and lean mass by using a 2x2 matrix in categorizing the subjects. The associations were largely similar in men and women. Higher fat mass was associated with higher aromatic and branched-chain amino acid scores in both sexes and also with higher glucogenic amino acid score in older women. Higher lean body mass was associated with higher branched-chain amino acid score in both sexes. A novel finding was an inverse association between lean body mass and glycine among men.

The findings on the associations between lean body mass and serum branched-chain amino acids are in line with those of previous studies. Jourdan and colleagues reported that fat free mass index, measured using bioelectrical impedance analysis, was positively associated with the sum of BCAA concentrations in men and women aged 54-75 years (15). Another study in women, with a mean age of 47 years, reported that BCAA concentrations were positively associated with absolute lean mass

and lean mass index, determined using dual energy x-ray absorptiometry (DXA) (17). BCAAs were also associated with fat free mass index and thigh muscle cross-sectional area in a small sample of functionally limited older men and women (13) and with appendicular lean mass index (lean mass of the lower limbs/h^2) in older black men (16). Positive associations between indicators of lean mass and serum AAA concentrations have also been reported in older adults (15,16). In the present study, higher levels of tyrosine, an aromatic amino acid, were associated with higher lean mass index but only in men.

Possible mechanisms linking circulating branched-chain amino acid concentrations and lean mass include the stimulating effect of BCAAs on protein synthesis in muscle tissue (26). BCAAs can stimulate protein synthesis through mTOR signaling pathway (26). Of the BCAAs, leucine is particularly effective in upregulating protein synthesis (27,28). It has been suggested that leucine intake is particularly important for older adults in preventing muscle loss (29).

Glycine, a glucogenic amino acid, was inversely associated with lean mass in men. To the best of our knowledge, associations between glycine and lean mass have not previously been reported in older adults. Men had lower glycine levels than women in the present study, which is in line with sex differences reported in prior studies (12,30). Glycine has also been found to be lower in those with higher BMI (30). Further, glycine levels have been shown to increase after bariatric surgery in younger adults (31,32). However, it is unclear whether this increase in glycine level is due to loss of fat mass, loss of lean mass or some other metabolic change following substantial weight loss. In the light of our results, the rise in glycine levels accompanying weight loss might be related to loss of lean mass, which typically occurs with fat loss (33). The association between lean mass and glycine is also supported by prior findings, which link sarcopenia, i.e. reduced muscle mass and function,

with mitochondrial dysfunction and the glycine pathway. It has been hypothesized that sarcopenia has its origin in mitochondrial dysfunction (34). Mitochondrial dysfunction, in turn, has been reported to lead to upregulation of serine/1-carbon/glycine pathway in skeletal muscle of mice (35). This upregulation of serine/1-carbon/glycine pathway is plausibly a part of a compensatory response against increased mitochondrial stress to preserve cellular function (36). Hence, it could be speculated that rising serum glycine levels might serve as a marker of sarcopenia among older men, but more research is needed to confirm this.

Fat mass index was positively associated with AAAs, namely with tyrosine and phenylalanine, and BCAAs in both men and women. These associations were largely independent of those with lean mass index. In line with our findings, higher body mass index was associated with higher levels of tyrosine and phenylalanine and BCAAs in the Framingham Offspring cohort studied at a mean age of 55 years (18). Further, a study on 2400 women aged 40-80 years reported positive associations between BCAAs and visceral fat mass determined using DXA (19). Our findings are also supported by dietary weight-loss interventions, in which weight loss was directly related to the decline of circulating BCAAs, tyrosine, and phenylalanine (37).

Many studies in younger adults have linked elevated BCAA levels to obesity, insulin resistance and type 2 diabetes (10,38,39). Obesity and disturbances in glucose metabolism are intertwined as obesity is an important risk factor for insulin resistance and type 2 diabetes. Hence, disturbances in glucose metabolism in a part of the subjects with high adiposity in our cohort might explain some of the association observed between fat mass and BCAAs. Some studies reported an amino acid score including tyrosine, phenylalanine, and isoleucine to strongly predict future diabetes (38) and cardiovascular disease (40). BCAAs have been suggested to be indicators of metabolic disturbances

rather than that of obesity (10,41). BCAA overload may even have a causal role in developing

insulin resistance and T2DM (10,29). It has been suggested that high BCAA levels, when combined

with low IGF-1, activate the mTOR/S6K1 kinase pathway and phosphorylation of IRS1 on multiple

serines, leading to insulin resistance (10). According to another theory, individuals with impaired

BCAA metabolism could develop β-cell mitochondrial dysfunction through accumulation of toxic

BCAA metabolites (11). On the other hand, BCAAs could be just a side product of the process of

worsening metabolism or related to a possible compensatory mechanism in response to obesity

(11,29). Nevertheless, we observed associations between fat mass and BCAAs regardless of

adjusting the present analyses for fasting glucose concentration.

The levels of BCAAs may also be affected by diet (42). Vegetarian and vegan diets have been found

to be associated with lower levels of BCAAs, and higher levels of BCAAs may indicate an

obesitogenic dietary pattern containing high amounts of animal and milk proteins (43). Further, a

weight-loss intervention study suggested that the decline in AA concentrations during weight loss

varies according to the protein content of the diet (37). However, some researchers have argued that

BCAA intake is only weakly associated with fasting BCAA levels (44).

The present findings also suggest that concentrations of alanine, a glucogenic amino acid, are higher

in those with high fat mass. Alanine was also found to be associated with BMI in a sample consisting

of younger and older adults (18). Alanine is an important substrate and regulator for glucose

metabolism. Decline in circulating alanine has been frequently reported after weight-loss

interventions (45). Alanine is also related to BCAA catabolic pathway (46). Hence, elevated alanine

levels can simply reflect the higher BCAA catabolic activity in individuals with high fat mass.

A strength in this study is that we took into account both fat and lean mass, which may have mutual confounding effect on amino acids. Furthermore, we had a relatively large community sample including both men and women. We also analyzed several (groups of) amino acids. This study has also some limitations. The cross-sectional design limits drawing conclusions on causality. Compared to dual-energy x-ray absorptiometry, the accuracy of bioelectrical impedance analysis in determining fat and lean mass is more limited (47). We had no information on insulin resistance of the participants, which could have helped in dissecting the roles of adiposity and glucose/insulin metabolism in more detail.

Conclusions

The present study suggests that the associations between body composition and serum amino acids are largely similar in older community-dwelling men and older women. Both lean and fat mass are positively associated with branched-chain amino acids. In addition, fat mass is associated with aromatic amino acid concentrations. Despite marked differences in body composition between younger and older adults, the present findings suggest that the circulating amino acid profile is largely similarly linked to obesity in older adults than in younger adults. The present study identified potential candidate molecules to be focused on in future studies investigating cellular pathways leading to low lean mass or high fat mass in older adults. Especially, the role of glycine in development of sarcopenia and low muscle mass needs to be focused upon.

study, interpretation of the data and revised the draft critically for important intellectual content. HK analyzed the data, participated in the interpretation of the results and revised the draft critically for important intellectual content. JGE participated in the conception and design of the study, collection of the data, interpretation of the data and revised the draft critically for important intellectual content. All authors accepted the final version of the manuscript.

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Conflict of Interest

None.

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Table 1. Characteristics of women of the Helsinki Birth Cohort Study according to fat mass index (FMI) and lean mass index (LMI) in 2007.

	FMI low		FMI high		P-values		
	LMI low (n=220)	LMI high (n=77)	LMI low (n=77)	LMI high (n=220)			
					FMI	LMI	Interaction
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
Age, years	66 (3)	66 (3)	66 (3)	66 (3)	0.31	0.69	0.74
BMI, kg/m^2	23.0 (1.9)	25.5 (1.5)	27.3 (1.6)	32.1 (4.2)	<0.001	0.001	<0.001
Fat mass, kg	17.6 (4.1)	19.5 (4.1)	26.3 (3.0)	33.6 (8.5)	<0.001	0.001	<0.001
Lean mass, kg	43.9 (4.0)	50.7 (4.0)	44.0 (3.6)	50.9 (4.7)	0.61	<0.001	0.88
Weight, kg	61.5 (6.8)	70.2 (6.4)	70.3 (5.4)	84.6 (11.8)	<0.001	0.001	<0.001
Height, cm	163 (5)	166 (6)	160 (6)	162 (6)	<0.001	0.001	0.47
Body fat, %	28 (5)	28 (4)	37 (3)	39 (5)	0.001	0.15	0.002
Glucose fasting, mmol/L	6.3 (0.6)	6.4 (0.6)	6.4 (0.6)	6.6 (0.7)	0.006	0.010	0.21
Glucose 2-h , mmol/L	7.2 (1.8)	7.3 (2.0)	7.8 (2.2)	8.4 (2.5)	<0.001	0.072	0.16
Systolic BP, mmHg	141 (20)	142 (20)	143 (21)	147 (20)	0.065	0.10	0.46
Diastolic BP, mmHg	87 (11)	88 (11)	90 (12)	92 (11)	<0.001	0.082	0.87

Note. BMI, body mass index; High lean mass index >17.7 kg/m^2, low lean mass index ≤17.7 kg/m^2, high fat mass index>8.9 kg/m^2, low fat mass index≤8.9 kg/m^2

Table 2. Characteristics of men of the Helsinki Birth Cohort Study according to fat mass index (FMI) and lean mass index (LMI) in 2007.

	FMI low		FMI high		P-values		
	LMI low (n=164)	LMI high (n=74)	LMI low (n=74)	LMI high (n=164)			
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	FMI	LMI	Interaction
Age, years	65.4 (2.2)	65.6 (2.6)	65.7 (2.9)	66.4 (2.9)	0.11	0.062	0.35
BMI, kg/m2	26.1 (1.2)	30.5 (2.9)	23.3 (1.6)	26.6 (1.5)	<0.001	<0.001	0.012
Fat mass, kg	15.0 (2.6)	25.7 (7)	13.4 (3.1)	21.4 (3.6)	<0.001	<0.001	0.004
Lean mass, kg	69.0 (4.7)	69.0 (6.7)	60.1 (5.1)	58.3 (4.2)	0.11	<0.001	0.091
Weight, kg	83.9 (5.9)	94.8 (10.7)	73.4 (6.8)	79.6 (6.1)	<0.001	<0.001	0.004
Height, cm	179 (5.1)	176 (6.2)	177 (5.7)	173 (5.4)	<0.001	<0.001	0.18
Body fat, %	17.8 (2.5)	26.9 (4.8)	18.1 (3.3)	26.8 (3.2)	<0.001	0.83	0.56
Glucose fasting, mmol/L	6.6 (0.6)	6.9 (0.7)	6.7 (0.6)	6.8 (0.6)	0.023	0.009	0.31
Glucose 2-h , mmol/L	7.3 (2.0)	7.3 (2.4)	8.6 (2.5)	8.2 (2.6)	<0.001	0.40	0.57
Systolic blood pressure, mmHg	143 (21)	144 (17)	148 (22)	149 (18)	0.011	0.62	0.93
Diastolic blood pressure, mmHg	88 (11)	88 (10)	91 (12)	93 (10)	<0.001	0.40	0.30

Note. BMI, body mass index; High lean mass index >20.5 kg/m^2, low lean mass index ≤20.5 kg/m^2, high fat mass index>5.7 kg/m^2, low fat mass index≤5.7 kg/m^2

Fig 1. Adjusted (age, fasting glucose and smoking) amino acid scores across body composition categories in women and men. 'LMI low' refers to lean mass index (lean body mass/h^2) equal or below the sex-specific median and 'LMI high' to lean mass index above the sex-specific median. 'FMI low' and 'FMI high' are defined correspondingly. Amino acid scores are calculated by summing up the z-scores of the respective amino acid concentrations.

Fig. 2 Adjusted (age, fasting glucose and smoking) amino acid concentrations across body composition categories in women. 'LMI low' refers to lean mass index (lean body mass/h^2) equal or below the sex-specific median and 'LMI high' to lean mass index above the sex-specific median. 'FMI low' and 'FMI high' are defined correspondingly.

Fig. 3 Adjusted (age, fasting glucose and smoking) amino acid concentrations across body composition categories in men. 'LMI low' refers to lean mass index (lean body mass/h^2) equal or below the sex-specific median and 'LMI high' to lean mass index above the sex-specific median. 'FMI low' and 'FMI high' are defined correspondingly.
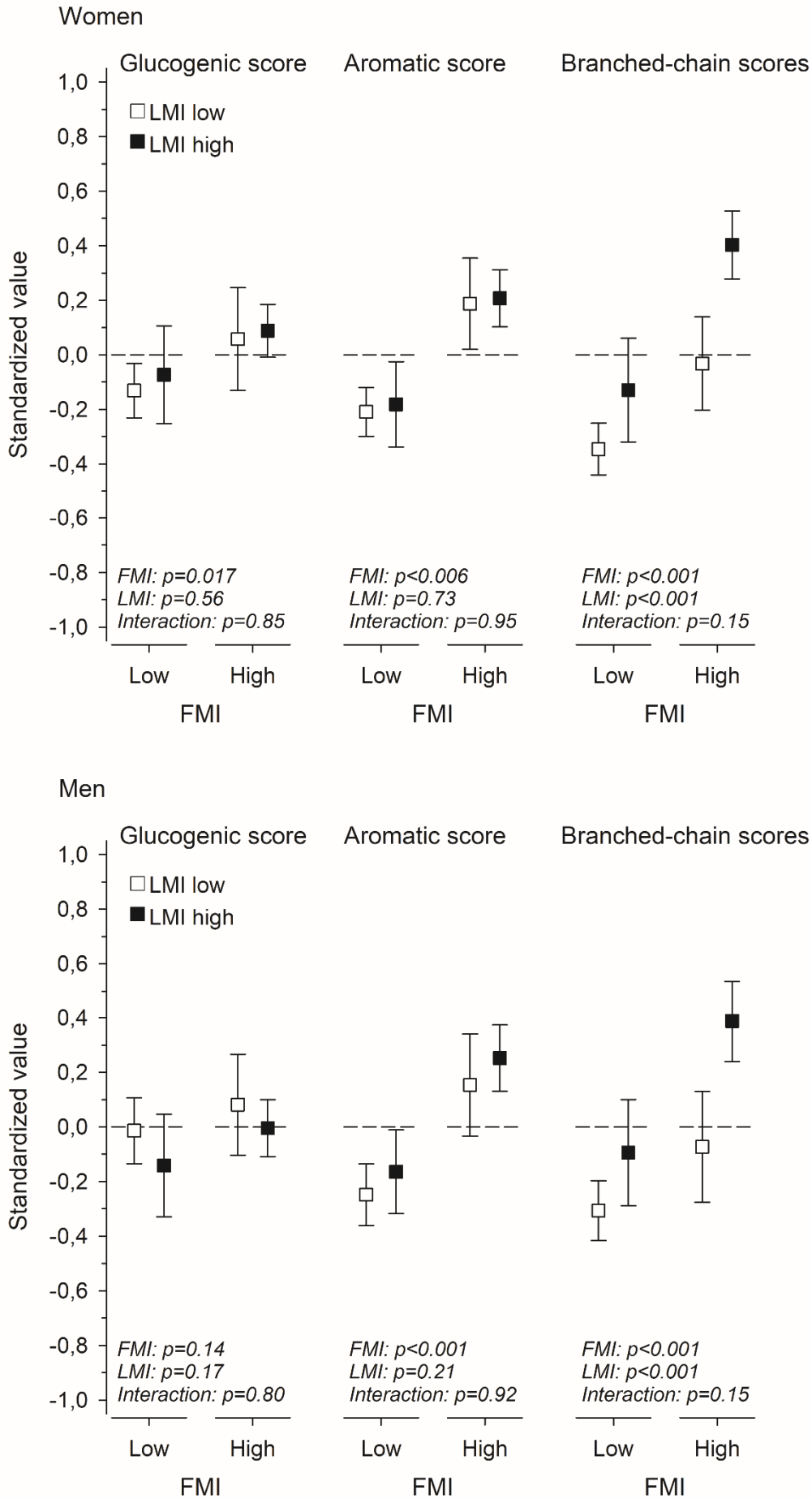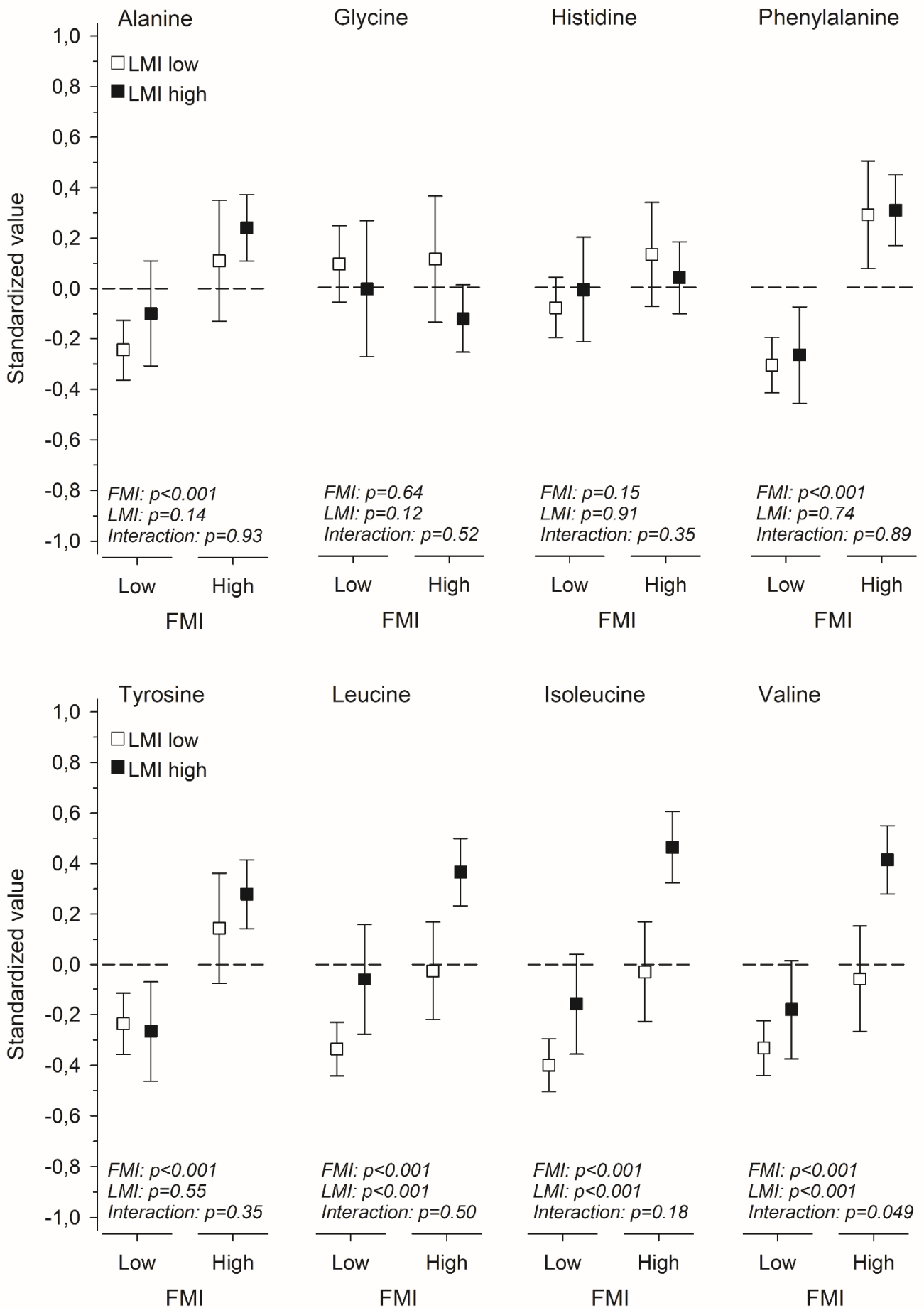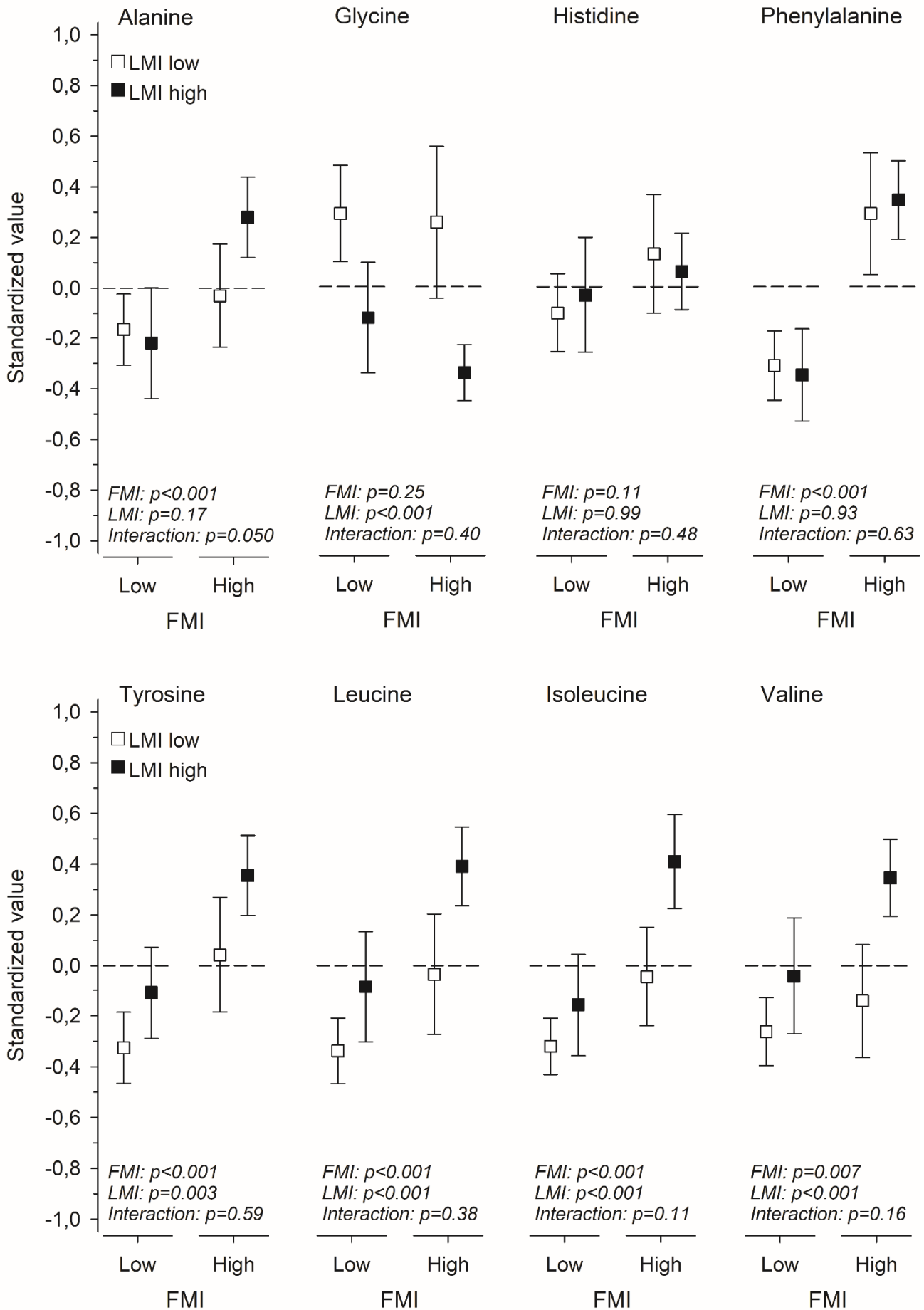
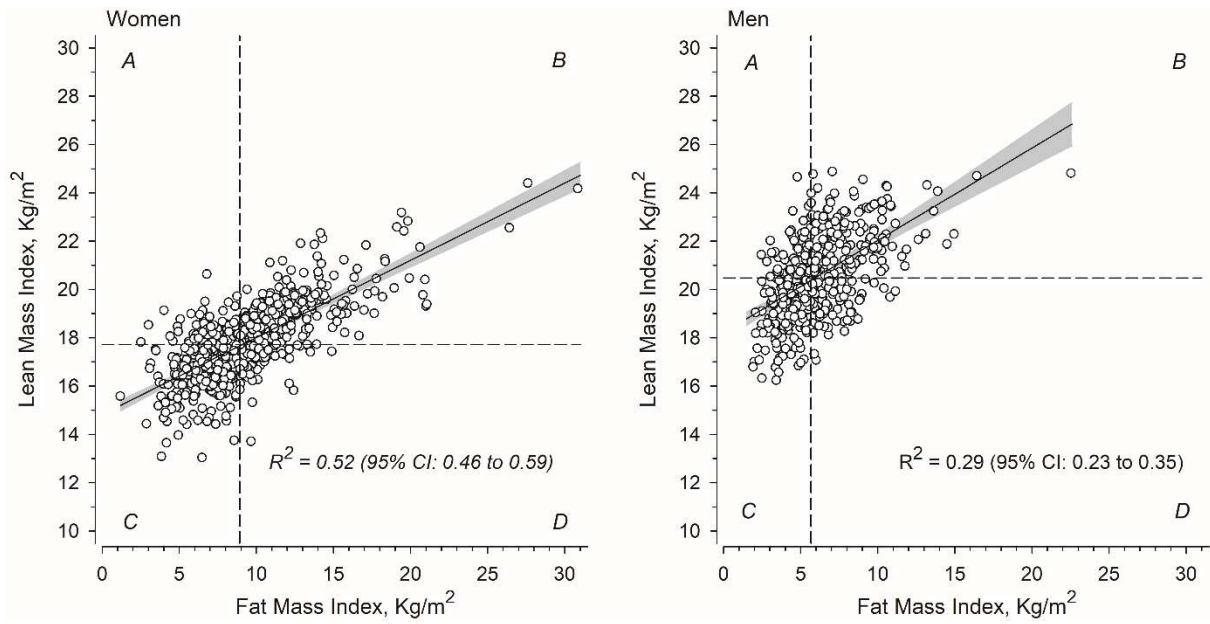Women

Men

Fig. 1

Fig. 2

Fig. 3

Supplemental Figure 1. Relationships between fat mass index and lean mass index in women (left panel) and men (right panel). Fat and lean mass indices were each divided according to sex-specific median values into two categories and based on them, four categories were created: C) lean low fat low A) lean high fat low D) lean low fat high B) lean high fat high.

Supplemental Table 1. Amino acid concentrations in women and men
from the Helsinki Birth Cohort Study in 2007.

Amino acid, mmol/L	Women N=594 Mean (SD)	Men N=476 Mean (SD)	P-value[a]
Alanine	0.457 (0.053)	0.452 (0.059)	0.17
Glycine	0.324 (0.069)	0.285 (0.047)	<0.001
Histidine	0.072 (0.011)	0.074 (0.011)	0.007
Phenylalanine	0.086 (0.012)	0.086 (0.011)	0.86
Tyrosine	0.058 (0.012)	0.060 (0.013)	<0.001
Leucine	0.082 (0.013)	0.094 (0.016)	<0.001
Isoleucine	0.052 (0.013)	0.063 (0.017)	<0.001
Valine	0.189 (0.034)	0.218 (0.037)	<0.001

Note. Bootstrap type multivariate Hotelling's T-squared test: p<0.001

[a]Adjusted using Hommel's method

Supplemental Table 2. Partial correlations of percent fat mass with amino acid concentrations and amino acid scores adjusted for age, smoking and fasting glucose concentration in the Helsinki Birth Cohort Study in 2007.

	Percent fat mass	
	Women r (95% CI)	Men r (95% CI)
Amino acid		
Alanine	0.20 (0.12 to 0.27)***	0.18 (0.09 to 0.27)**
Glycine	-0.05 (-0.14 to 0.04)	-0.16 (-0.26 to -0.06)*
Histidine	0.07 (-0.01 to 0.15)	0.07 (-0.02 to 0.16)
Phenylalanine	0.35 (0.28 to 0.42) ***	0.28 (0.20 to 0.36)***
Tyrosine	0.30 (0.22 to 0.37) ***	0.24 (0.16 to 0.33)***
Leucine	0.31 (0.23 to 0.38) ***	0.20 (0.11 to 0.28)**
Isoleucine	0.35 (0.28 to 0.42) ***	0.22 (0.14 to 0.31)***
Valine	0.31 (0.23 to 0.38) ***	0.16 (0.07 to 0.25)**
Amino acid scores		
Glucogenic score	0.13 (0.05 to 0.21)*	0.05 (-0.04 to 0.14)
Aromatic score	0.31 (0.24 to 0.38) ***	0.25 (0.16 to 0.33)***
Branched-chain score	0.36 (0.29 to 0.43) ***	0.21 (0.13 to 0.30)***

*p< 0.05, **p< 0.01, ***p< 0.001; Sidak-adjusted probabilities