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RESEARCH ARTICLE

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Age-related metabolite profiles and their relation to clinical outcomes in young adults, middle-aged individuals, and older people

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Abstract

Age is a significant risk factor for common noncommunicable diseases, yet the physiological alterations of aging are poorly understood. We were interested in metabolic patterns between cross-sectional cohorts of different age ranges with particular emphasis on waist circumference. We recruited three cohorts of healthy subjects with different age ranges (adolescents 18–25 years, adults 40–65 years, and older citizens 75–85 years) and stratified these based on waist circumference. Using targeted LC-MS/MS metabolite profiling, we analyzed 112 analytes in plasma (amino acids, acylcarnitines, and derivatives). We associated age-related alterations with various anthropometric and functional parameters such as insulin sensitivity and handgrip strength. Strongest age-dependent increases were found for fatty acid-derived acylcarnitines. Amino acid-derived acylcarnitines displayed increased associations with BMI and adiposity. Some essential amino acids changed in opposite directions, being lower at increased age and higher with increasing adiposity. τ-methylhistidine was elevated in older subjects, especially on an adiposity background, suggesting an increased protein turnover. Both aging and adiposity are associated with impaired insulin sensitivity. Skeletal muscle mass decreased with age and increased with adiposity. Profound differences in the metabolite signatures during healthy aging and elevated waist circumference/body weight were found. Opposite changes in skeletal muscle mass as well as possible differences in insulin signaling (relative insulin deficiency in older subjects versus hyperinsulinemia associated with adiposity), might be underlying origins for the observed metabolite signatures. We describe novel associations between metabolites and anthropometric factors during aging which underlines the complex interplay of aging, insulin resistance, and metabolic health.

Abbreviations: (v/v), volume/volume; ALT, alanine-aminotransferase; BMI, body mass index; CRP, C-reactive protein; eWC, elevated waist circumference; HOMA-IR, homeostatic model assessment for insulin resistance; MetS, metabolic syndrome; T2D, type 2 diabetes mellitus.

Hans Hauner and Thomas Skurk shared last authorship.

Trial registration at the *German Clinical Trials Register*: DRKS00009797.

KEYWORDS
enable-cluster, metabolomics, amino acids, acylcarnitines, healthy aging, waist circumference, obesity

1 | INTRODUCTION

Aging is characterized by an alteration of homeostatic processes resulting in an increased risk for, for example, metabolic and cardiovascular diseases, neurodegenerative disorders, and cancer, all together limiting health span and life expectancy.[1,2] Changes in body composition, insulin sensitivity, secretion of hormones, and mitochondrial function accompany the aging process. Metabolic consequences of impaired insulin sensitivity include increased hepatic glucose production,[3] increased lipolysis in adipose tissue,[4] and defective glucose uptake and glycogen synthesis in skeletal muscle.[5,6] Metabolic changes associated with insulin resistance were shown to cause multiple alterations in the concentrations of a large number of plasma metabolites, including branched-chain amino acids,[7,8] aromatic amino acids,[9,10] acylcarnitines,[7] and various other lipid classes.[11] These changes may interfere with insulin signaling and, finally, increase the risk of hyperglycemia and type 2 diabetes (T2D). As previous studies have mainly focused on middle-aged adults, it is unclear whether these findings also apply to other age groups and how much the aging process per se contributes to the variation in plasma metabolite concentrations.

The present study aimed to elucidate how the aging process may affect metabolite profiles across defined age groups and to dissect if and how other factors such as an increased waist circumference and obesity may interfere with the age-dependent changes in metabolites. We used targeted metabolite profiling to find differences in basal metabolite concentrations between three healthy age groups with the aim of identifying age-related metabolite changes in plasma. In addition, we recruited individuals with an increased metabolic risk defined by an elevated waist circumference. Differences in plasma metabolite concentrations between individuals with a normal or increased waist circumference were determined to identify age- versus adiposity-related metabolite changes. In addition to age and waist circumference, other important factors contributing to metabolite profile changes such as body composition, insulin sensitivity, sex, nutritional factors, and the inflammatory state were considered.

2 | MATERIALS AND METHODS

2.1 | Study design and cohorts

We recruited volunteers for this study between February 2016 and January 2018 in a cross-sectional manner in the region of Freising and Nuremberg, Germany. Participants gave written informed consent prior to inclusion in the study. Further details on the study design and the cohorts can be found in Brandl et al.[12] For the present analysis, 86 adolescents, 205 middle-agers, and 160 older individuals were included, and we performed metabolite analysis on their plasma samples. The clinical characteristics of the study cohorts are shown in Table 1.

2.2 | Inclusion/exclusion criteria and phenotyping

A detailed list of inclusion/exclusion criteria can be found in Brandl et al.[12] In principle, we draw a random sample from the population in the area in Freising and Nuremberg, excluding only severe and chronic diseases. In total, we recruited (m/f, 50/50) 94 participants for the cohort of adolescents (18–25 years), 205 adults ("middle agers," 40–65 years), and 160 older citizens (75–85 years). Our volunteers were otherwise healthy, nonsmoking, community-dwelling Caucasians and had a body mass index (BMI) of 18.5–35.0 kg/m^2. Extensive phenotyping of the participants including body composition and physiological parameters was performed as described elsewhere.[12] We defined an increased cardiometabolic risk by a waist circumference of ≥102 cm (m) and ≥88 cm (f) (further referred to as metabolic syndrome, MetS). We collected blood samples in a fasted state (>12 h) and drew another fasting blood sample within 1–2 weeks during a second occasion when volunteers were invited for an oral glucose tolerance test.

2.3 | Targeted quantitative LC-MS/MS analysis of amino acid and acylcarnitine concentrations

Quantitative analysis of amino acid and derivatives ($n = 46$) and acyl-carnitine ($n = 66$) concentrations was performed using LC-MS/MS based on the methods described previously.[13,14] A list of all amino acids, derivatives, and acylcarnitines and their abbreviations is provided in Table S1. Briefly, 10 µL plasma was dissolved in 500 µL ice-cold methanol containing 28 isotope-labeled internal standards (13 acyl-carnitines and 15 amino acids). All internal standards were obtained from ChromSystems (Munich, Germany), except for Asparagine-$^{15}N_2$ and L-Glutamine-d_5, which were purchased from Cambridge Isotope Laboratories Samples (Tewksbury, MA, USA).

TABLE 1 Clinical parameters of the subjects in the age groups and stratified for sex.

		Normal waist circumference			Elevated waist circumference	
Parameter	**Gender**	**Young adults ($n=86$)**	**Middle agers ($n=106$)**	**Older people ($n=69$)**	**Middle agers ($n=99$)**	**Older people ($n=91$)**
Age (years)	M	22.3 ± 2.0[a]	52.3 ± 7.1[b]	78.7 ± 2.4[c]	54.9 ± 6.9	77.6 ± 2.5
	F	22.0 ± 1.9 [a]	52.0 ± 5.9[b]	77.9 ± 3.0[c]	50.9 ± 7.4	78.3 ± 2.9
Height (cm)	M	183 ± 7.5[a]	180 ± 5.5[a]	175 ± 6.6[b]	180.2 ± 5.4	176.7 ± 6.1
	F	169 ± 6.0[a]	166 ± 6.1[a,b]	163 ± 6[b]	166.8 ± 5.8	163.8 ± 6.3
Body weight (kg)	M	76.5 ± 10.6[a]	82.8 ± 8.1[b]	72.4 ± 6.4[a]	101.3 ± 8.3*	88.5 ± 8.7*
	F	60.9 ± 5.8	62.8 ± 9.7	58.2 ± 6.3	82.6 ± 9.7*	72.8 ± 9.2*
BMI (kg/m^2)	M	22.8 ± 2.7[a]	25.6 ± 2.2[b]	23.8 ± 2.3[a]	31.2 ± 2.2*	28.4 ± 2.6*
	F	21.5 ± 1.8	22.8 ± 3.1	21.9 ± 2.2	29.7 ± 3.0*	27.1 ± 3.1*
Waist circumference (cm)	M	81.0 ± 8.9[a]	91.6 ± 6.4[b]	92.6 ± 6.2[b]	110.8 ± 6.1*	109.8 ± 8.1*
	F	75.1 ± 6.1	76.1 ± 7.3	78.4 ± 9.0	99.0 ± 7.9*	98.5 ± 7.7*
Fat mass (kg)	M	12.4 ± 7.3[a]	19.8 ± 4.9[b]	19.9 ± 4.4[b]	32.6 ± 5.5*	30.1 ± 5.7*
	F	15.9 ± 3.7[a]	19.8 ± 6.3[b]	22.6 ± 5.2[b]	36.5 ± 7.0*	33.7 ± 6.7*
Fat mass (% body weight)	M	3.70 ± 1.5 [a]	6. ± 1.5[b]	1.6 ± 4.4[b]	10.1 ± 1.6*	10.0 ± 1.9*
	F	5.61 ± 1.2[a]	7.23 ± 2.3[b]	8.9 ± 2.2[b]	13.3 ± 2.4*	13.2 ± 2.8*
Visceral fat (kg)	M	0.87 ± 0.9[a]	2.32 ± 0.8[b]	2.83 ± 0.7[b]	5.02 ± 1.2*	5.06 ± 1.2*
	F	0.44 ± 0.3[a]	0.89 ± 0.3[b]	1.41 ± 0.3[c]	2.37 ± 0.8*	2.60 ± 0.7*
Fat-free mass (kg)	M	63.4 ± 6.3[a]	62.0 ± 5.2[a]	52.2 ± 4.9[b]	69.1 ± 5.5*	58.9 ± 5.0*
	F	45.2 ± 4.3[a]	42.5 ± 4.9[b]	35.5 ± 3.8[c]	47.7 ± 4.7*	40.1 ± 4.6*
Fat-free mass (% body weight)	M	19.0 ± 1.4[a]	19.3 ± 1.3[a]	17.7 ± 1.5[b]	21.4 ± 1.4*	19.5 ± 1.5*
	F	15.8 ± 0.9[a]	15.5 ± 1.3[a]	13.9 ± 1.1[b]	17.3 ± 1.2*	15.6 ± 1.3*
Hand grip strength (kg)	M	49.0 ± 8.5[a]	49.5 ± 9.3[a]	35.8 ± 5.6[b]	50 ± 9.1	34.1 ± 7.1
	F	30.9 ± 5.8[a]	29.6 ± 5.4[a]	21.7 ± 4.7[b]	30.5 ± 6.6	21.8 ± 4.2
Fasting plasma glucose (mg/dL)	M	77.5 ± 5.6[a]	84.4 ± 7.4[a,b]	88.2 ± 7.8[b]	98.4 ± 33.2*	92.9 ± 11.8
	F	75.2 ± 5.8[a]	79.7 ± 7.2[b]	83.9 ± 6.6[b]	84.2 ± 9.1*	89.5 ± 7.7*
Insulin (μU/mL)	M	2.96 ± 1.9	3.69 ± 2.5	4.06 ± 3.1	9.25 ± 4.8*	5.87 ± 4.1
	F	3.46 ± 1.7	3.32 ± 3.6	3.34 ± 1.9	7.75 ± 7.3*	6.23 ± 5.1*
HOMA-IR	M	0.54 ± 0.33	0.79 ± 0.57	0.9 ± 0.72	2.23 ± 1.37*	1.4 ± 1.12
	F	0.64 ± 0.32	0.66 ± 0.73	0.69 ± 0.4	1.66 ± 1.65*	1.43 ± 1.36*
CRP (mg/dL)	M	0.08 ± 0.2	0.14 ± 0.2	0.12 ± 0.1	0.24 ± 0.2	0.32 ± 0.5*
	F	0.25 ± 0.3	0.16 ± 0.4	0.45 ± 1.2	0.34 ± 0.3	0.68 ± 2.3

Note: Data are presented as mean ± standard deviation. p values of <.05 were regarded as statistically significant. Mean differences were assessed between young adults, middle agers, and older people with normal waist circumference based on a multi-group comparison with ANOVA and post-hoc Tukey test; Middle agers, respectively, older people with normal and elevated waist circumference were compared according to distribution by using t test or Wilcoxon-signed ranked test. Labeled means in a row with a common superscript letter do not differ, $p < .05$. *, different from normal waist circumference, $p < .05$.

Samples were centrifuged (10 min, 4°C, 10 000 × g) and supernatants were collected and dried in nitrogen gas. Amino acids and acylcarnitines were derivatized from their butyl esters as described by Gucciardi et al.[15] Briefly, a mixture of 95% *n*-butanol and 5% acetyl chloride (v/v) was added to the samples. Samples were subsequently incubated at 60°C for 15 min while shaken at 600 rpm (Eppendorf Thermomixer Comfort; Eppendorf, Hamburg, Germany). The samples were dried in nitrogen gas and reconstituted in a 300 μL mixture of methanol/water/formic acid (70/30/0.1% v/v). Chromatographic separation was achieved using a Zorbax Eclipse XDB-C18 column (length 150 mm, internal diameter 3.0 mm, particle size 3.5 μm; Agilent). The measurement was performed in positive ionization mode using scheduled multiple reaction monitoring (MRM). Amino acids and acylcarnitines were measured in two separate runs. For absolute quantification of amino acids, a 10-point calibration of amino acid concentrations between 1 μM and 500 μM was generated. Acylcarnitine concentrations were calculated based

on analyte-to-internal standard area ratios and respective concentrations of internal standards. Data analysis was done using Analyst 1.5.1® software (Sciex).

2.4 | Data mining and statistics

Evaluation of differences between the means of the age groups was done using a one-way analysis of variance combined with Tukey's range test. Evaluation of differences between healthy and increased waist circumference groups was done using a two-sided Student's *t* test. Metabolites were clustered by calculating Euclidean distances and applying hierarchical clustering with Ward's minimum variance algorithm and squaring of dissimilarities before clustering ("ward.D2"). Based on the hierarchical clustering, the tree was cut into 10 major clusters. Pairwise Pearson correlations between age and analyte concentrations or between waist circumference and analyte concentrations were calculated. Multiple linear regression was performed to estimate the influence of selected determinants of plasma analyte levels. A type III analysis of variance was chosen to determine partial Sums of Squares, as interactions exist between individual determinants. Sums of Squares were calculated as percentages to determine the contribution of individual determinants on plasma metabolite level.

All calculations and visualizations were done using *R* software. Besides the base packages, the following packages were also used: "pheatmap," "RColorBrewer," "circlize," "gplots," "ComplexHeatmap," "corrplot," "factoextra," "Anova."

3 | RESULTS

3.1 | Baseline characteristics

Table 1 presents selected anthropometric and clinical variables of the different age groups and those of middle-aged and older people with elevated waist circumference (eWC). Overall, characteristic age-related alterations in body composition were found for males and females. Middle-aged and older people showed an increased waist circumference, associated with increased visceral fat mass compared to young adults. In contrast, a decrease in fat-free mass, including skeletal muscle mass, was observed with increasing age. In turn, this is reflected by a reduced handgrip strength in the oldest cohort. As fasting plasma glucose levels increased with age, fasting insulin levels only in males displayed a gradual increase. Furthermore, there is considerable variation in CRP levels within the age groups, and significant differences between the age groups were not observed.

Focusing on the group with an elevated waist circumference, middle-aged and older subjects also presented a higher BMI, further associated with an increase in total and visceral fat mass. Both groups also showed a high fat-free mass and skeletal muscle mass compared with the normal-weight groups. Fasting plasma glucose and insulin concentrations were increased compared with their normal-weight peers, resulting in an increased HOMA-IR.

3.2 | Robustness of plasma metabolite concentrations in the fasting state

As fasting plasma samples were collected twice on two different days from every individual, we were able to examine the reproducibility of individual metabolite concentrations in fasting plasma by pairwise comparing metabolite concentrations and assessing correlations between both time points. Most analytes showed a correlation coefficient of at least 0.2, except for π-methylhistidine, anserine, choline, and carnosine (Figure S1; Table S1). The diet strongly influences these four analytes.[16] π-methylhistidine and its precursor anserine were described as markers for the intake of poultry meat. In contrast, τ-methylhistidine, an endogenously produced compound released upon protein breakdown, displayed a high correlation between the samples from both days (π-methylhistidine $p = 0.16$; R 0.07; τ-methylhistidine $p < .001$; R 0.07; Figure S2). Therefore, we excluded these four metabolites with low correlations between fasting plasma concentrations from subsequent analyses.

3.3 | Ten major analyte clusters display apparent alterations between age and waist groups

In total, we received plasma samples from 451 individuals. Plasma concentrations of 112 metabolites were determined in every sample. Figure 1A displays relative metabolite concentrations in plasma for every individual. Metabolites were sorted into ten major metabolite clusters using hierarchical clustering. A description of the composition of the clusters is given in Figure 1D. According to their origin (either amino acid- or fatty acid-derived), acylcarnitine species cluster according to chain length and chemical properties. Age-related changes were most clearly seen for acylcarnitines, derived from fatty acid oxidative pathways and pronounced in the higher waist circumference group (clusters 3, 7, 8, 9, and 10). Mean differences of clustered metabolites between-age groups and between normal and elevated waist circumference are summarized in Figure 1B (males) and 1C (females).

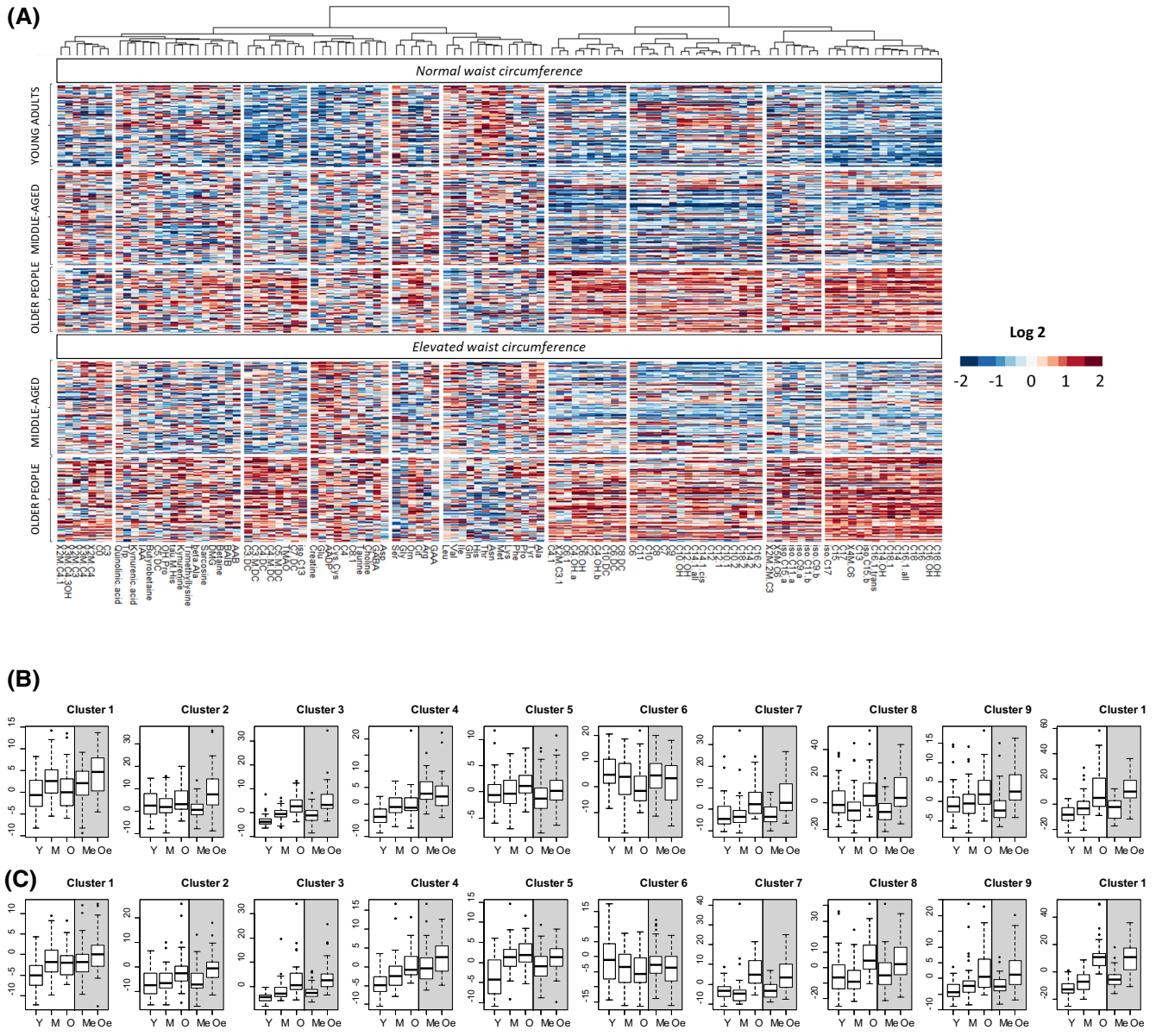

FIGURE 1 Heatmap showing relative plasma concentrations of all individuals in fasting state ($n = 451$) (A). Summed relative concentrations of analytes in individual clusters for males (B) and females (C). Y, young adults; M, middle-aged people; O, older people; Me, middle-aged people with elevated waist circumference; Oe, older people with elevated waist circumference.

3.4 | Correlations of plasma metabolite concentrations with age and waist circumference under fasting conditions

To examine metabolite changes associated with healthy aging, 261 individuals with a normal waist circumference were selected, and correlations between fasting metabolite concentrations and age were calculated (Figure 2A). Highly significant positive correlations with age were found for a large number of acylcarnitine species, with correlations between 0.4 and 0.6 for long-chain and dicarboxylic acylcarnitine species. Also, citrulline and ornithine displayed highly significant positive correlations with age. Significant correlations with age between 0.2 and 0.4 were found for medium-chain acylcarnitines and a number of non-proteinogenic amino acid intermediates. Significantly negative correlations with age were found for essential amino acids, including the branched-chain amino acids, His, Asn, Met, Thr, and Trp, as well as the non-essential amino acids Ser and hydroxyproline (OH-Pro).

To determine adiposity-related metabolite alterations independent of age, all individuals (the complete waist range) were included, but correlations between fasting metabolite concentrations and waist circumference

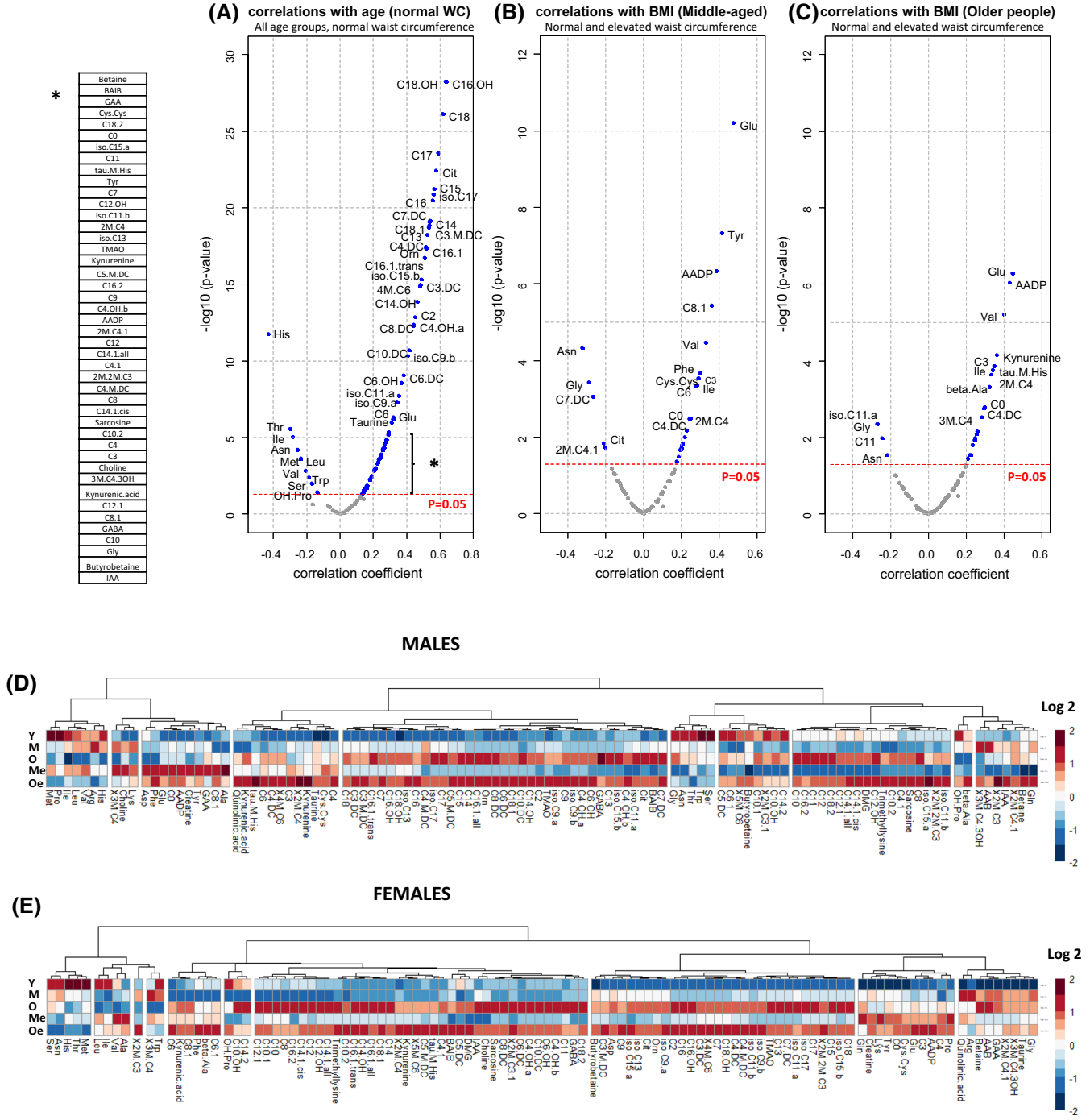

FIGURE 2 Volcano plots show the strength and significance of the correlation between fasting plasma concentrations and age (A), BMI (middle-aged) (B), and BMI (older people) (C). Relative group means of plasma concentrations in a fasting state for males (D) and females (E). Y, young adults; M, middle-aged people; O, older people; Me, middle-aged people with elevated waist circumference; Oe, Older people with elevated waist circumference.

were calculated separately for the middle-aged ($n = 205$) and older ($n = 160$) people (Figure 2B,C). With respect to adiposity-related metabolite changes, there were 18 metabolites with positive correlations between plasma concentration and waist circumference in both, the middle-aged and the group of older individuals. These included BCAAs (Val, Leu, and Ile), BCAA-derived acylcarnitines (C3, 2-M-C4, 3-M-C4, and C4-DC), aromatic amino acids (Phe and Tyr), τ-M-His, beta-Ala, Ala, AADP, cystine (Cys-Cys), kynurenine, trimethyllysine, free carnitine (C0) and malonylcarnitine (C3-DC). Common in both age groups was also the negative

correlation of Gly concentrations with waist circumference. Unique in the middle-aged group were positive correlations for Lys, Met and Pro and for long-chain fatty acylcarnitines, including long-chain hydroxyl species (C14-OH, C16-OH, C18-OH), C16 and C18:1, as well as medium-chain C10:2 and short-chain C6 and C6-DC. Furthermore, negative correlations between plasma concentration and waist circumference were found for Citrulline, Arginine, Asparagine, pimeloylcarnitine (C7-DC) and three branched acylcarnitines (C5-M-DC, iso-C17, 2-M-C4:1) in the middle-aged and for serine in the group of older citizens.

Figure 2D,E display the relative means of metabolite concentrations for males and females in the three age cohorts separated into groups with normal and elevated WC, respectively. Absolute concentration means per group and per sex are provided in Table 2 (acylcarnitines) and Tables 3 and 4 (amino acids). We observed age-related increases in acylcarnitine concentrations in both males (Figure 2D) and females (Figure 2E) for acylcarnitine species from various chemical classes and different chain lengths. In the cohort of older people, increases in long-chain acylcarnitine species were most pronounced, but also amino-acid derived and odd-numbered acylcarnitine species were higher. Furthermore, increases were observed for medium-chain dicarboxylic acid species, including adipoylcarnitine (C6-DC), pimeloylcarnitine (C7-DC), suberoylcarnitine (C8-DC), and sebacylcarnitine (C10-DC). We found only decreases for a few medium-chain acylcarnitine species, and these decreases were specific for males.

3.5 | Association of age-related metabolite concentrations with anthropometric measures

To identify potential determinants for metabolite changes associated with age, we selected anthropometric measures of metabolic health and correlated those with the metabolite concentrations in plasma. Selected anthropometric data included measures of adiposity (waist circumference, BMI, % body fat, and visceral adipose tissue), measures of insulin action (fasting insulin, HOMA-IR, and fasting plasma glucose), skeletal muscle mass and resting metabolic rate. Figure 3 shows correlations of fasting amino acid concentrations and anthropometric variables for males (A) and females (B). All 457 people with normal or elevated waist circumference were included. Amino acids were sorted according to their correlation strength in dependence on age.

In both, males and females, citrulline and ornithine had the strongest age-related increases (Figure 3A,B and Table 4) and were negatively correlated with muscle mass (Figure 3). The branched-chain amino acids (Val, Leu, and Ile) displayed significant age-related decreases (Table 3 and Figure 3A,B) and were positively correlated with muscle mass in both sexes, but most pronounced in males (Figure 3A,B). τ-methylhistidine did not correlate with muscle mass but rather showed a moderate correlation with visceral adipose tissue. Furthermore, Glu, AADP, Tyr, Kynurenine, and creatine showed positive correlations, while Asn displayed negative correlations with measures of adiposity and insulin resistance. Gly showed a strong negative association with adiposity/IR measures, specifically in males. Remarkably, for the branched-chain amino acids, no such correlations with measures of adiposity and insulin action were found in males and only for Val in females.

To decipher multiple interacting and possibly opposite effects of age and adiposity, we looked at correlations between metabolite levels and anthropometrics in individual age groups. When we selected the oldest participant group only (Figure 4A,B), we were able to find positive correlations between the concentrations of branched-chain amino acids and markers of adiposity/insulin. Other correlations like the sex-specific correlation of Gly with adiposity/IR measures in males, became more evident (Figure 4A).

3.6 | Influence of selected determinants on the variance of plasma metabolite concentrations

To assess the impact of selected determinants on the variance of plasma metabolite levels in an integrated model, we performed a multiple linear regression analysis. Besides age, we chose to include sex, BMI, visceral fat mass, skeletal muscle mass, ALT (as a measure of liver health), CRP (as a measure of the inflammatory state), and meat consumption (g/week). Figure 5 summarizes the influence of these parameters on plasma metabolites, determined by calculating the percentage of explained variation. Metabolites were sorted according to the amount of variation explained by age. It becomes obvious that age is a strong determinant of plasma acylcarnitine concentrations. Expectedly, meat intake contributes to the levels of hydroxyproline (OH-Pro), beta-alanine, and butyrobetaine. This parameter also influences aromatic and branched-chain amino acid levels as well as branched-chain amino acid-derived acylcarnitines (2-methylbutyrylcarnitine (2-M-C4), isobutyrylcarnitine (3-M-C4) and propionylcarnitine (C3)). Similarly, sex has a strong influence on branched-chain amino acids as well as on creatine and octadecadienoylcarnitine (C18:2) levels.

TABLE 2 Acylcarnitines with age-related changes in plasma in normal-weight females (F) and males (M).

			Normal waist circumference			Elevated waist circumference	
Metabolite (μM)	**Sex**	**Corr w. Age (R^2)**	**Young adults ($n=86$)**	**Middle agers ($n=106$)**	**Older people ($n=69$)**	**Middle agers ($n=99$)**	**Older people ($n=91$)**
C18.OH	F	0.72	1.97 ± 0.65[a]	2.60 ± 0.77[b]	4.31 ± 1.3[c]	2.97 ± 1.17	4.54 ± 1.38
	M	0.60	2.58 ± 0.74[a]	3.45 ± 0.98[b]	4.52 ± 1.59[c]	3.62 ± 1.3	4.67 ± 1.20
C16.OH	F	0.71	2.66 ± 0.86[a]	3.18 ± 0.93[a]	6.04 ± 1.85[b]	3.85 ± 1.3	6.01 ± 1.78
	M	0.60	3.10 ± 0.89[a]	4.05 ± 1.36[b]	6.18 ± 2.54[c]	4.35 ± 1.69	6.19 ± 1.82
C18	F	0.69	23.9 ± 8.31[a]	30.8 ± 7.3[b]	45.0 ± 10.6[c]	29.8 ± 7.07	42.3 ± 9.56
	M	0.56	28.0 ± 7.35[a]	37.9 ± 10.3[b]	45.9 ± 15.1[c]	35.4 ± 8.39	46.9 ± 11.6
C17	F	0.68	1.25 ± 0.42[a]	1.75 ± 0.49[b]	2.43 ± 0.60[c]	1.68 ± 0.31	2.35 ± 0.55
	M	0.50	1.57 ± 0.48[a]	1.97 ± 0.53[b]	2.43 ± 0.75[c]	1.74 ± 0.48	2.34 ± 0.46
C16	F	0.67	74.4 ± 16.4[a]	82.0 ± 18.8[a]	120 ± 23.8[b]	91.1 ± 14.7	120 ± 21.8
	M	0.53	85.3 ± 19.6[a]	101 ± 26.3[b]	121 ± 31.9[c]	104 ± 23.7	130 ± 28.0
C4.DC	F	0.64	16.6 ± 3.1[a]	20.5 ± 4.1[b]	26.3 ± 6.25[c]	21.54 ± 6.52	28.51 ± 8.03
	M	0.48	20.7 ± 4.9[a]	22.2 ± 3.9[a]	26.8 ± 6.33[b]	24.89 ± 6.01	30.29 ± 7.69
C15	F	0.64	0.94 ± 0.34[a]	1.24 ± 0.41[b]	1.97 ± 0.66[c]	1.24 ± 0.28	1.92 ± 0.56
	M	0.51	1.00 ± 0.26[a]	1.23 ± 0.45[a]	1.77 ± 0.76[b]	1.13 ± 0.37	1.76 ± 0.46
C14	F	0.63	19.8± 6.29[a]	20.7 ± 6.81[a]	38.3 ± 12.0[b]	23.31 ± 5.78	36.28 ± 9.37
	M	0.52	21.5 ± 6.92[a]	24.1 ± 9.30[a]	37.7 ± 16.0[b]	23.48 ± 6.87	37.52 ± 10.88
iso.C17	F	0.62	1.94 ± 0.84[a]	2.75 ± 0.89[b]	4.14 ± 1.39[c]	2.61 ± 0.69	3.93 ± 1.28
	M	0.43	1.98 ± 0.78[a]	2.63 ± 1.06[b]	3.53 ± 1.60[c]	2.31 ± 0.85	3.27 ± 1.07
C18.1	F	0.62	104 ± 29.4[a]	110 ± 27.4[a]	177 ± 38.5[b]	120 ± 26.1	171 ± 43.5
	M	0.50	117 ± 29.3[a]	129 ± 32.9[a]	164 ± 47.2[b]	123 ± 29.5	180 ± 51.2
C3.DC	F	0.62	9.52 ± 1.71	11.1 ± 1.78	13.9 ± 3.36	11.8 ± 3.29	14.3 ± 3.63
	M	0.46	12.0 ± 2.23	14.8 ± 2.81	16.7 ± 5.24	14.4 ± 3.71	18.5 ± 6.14
iso.C15.b	F	0.60	0.91 ± 0.39[a]	1.22 ± 0.43[b]	1.88 ± 0.62[c]	1.13 ± 0.34	1.85 ± 0.65
	M	0.39	1.16 ± 0.36[a]	1.24 ± 0.49[a]	1.88 ± 1.03[b]	1.10 ± 0.42	1.70 ± 0.56
C14.OH	F	0.59	6.54 ± 2.25[a]	6.32 ± 2.06[a]	11.9 ± 4.00[b]	7.67 ± 2.17	11.71 ± 3.21
	M	0.46	8.69 ± 2.50[a]	8.93 ± 3.4[a]	12.7 ± 4.52[b]	9.19 ± 3.24	13.57 ± 4.73
X4M.C6	F	0.58	2.43 ± 0.94[a]	3.11 ± 1.14[a]	4.57 ± 1.65[b]	3.48 ± 0.87	4.81 ± 1.68
	M	0.45	2.85 ± 0.84[a]	3.3 ± 1.22[a]	4.41 ± 1.77[b]	3.74 ± 1.52	5.09 ± 1.76
C16.1.all	F	0.56	23.0 ± 7.29[a]	23.1 ± 6.86[a]	41.4 ± 13.1[b]	26.9 ± 7.09	38.9 ± 12.8
	M	0.50	22.4 ± 8.26[a]	25.1 ± 8.05[a]	39.0 ± 16.7[b]	24.2 ± 6.77	38.8 ± 13.6
C13	F	0.56	0.51 ± 0.24[a]	0.64 ± 0.33[a]	1.13 ± 0.50[b]	0.61 ± 0.22	1.00 ± 0.36
	M	0.50	0.58 ± 0.21[a]	0.64 ± 0.31[a]	1.12 ± 0.46[b]	0.57 ± 0.20	1.02 ± 0.37
C3.M.DC	F	0.56	7.51 ± 1.34[a]	9.49 ± 1.64[b]	10.7 ± 3.44[b]	9.17 ± 1.87	11.7 ± 2.95
	M	0.59	8.44 ± 1.61[a]	10.6 ± 1.80[b]	11.8 ± 1.94[b]	10.2 ± 2.23	12.9 ± 3.35
C7.DC	F	0.56	5.20 ± 1.92[a]	7.79 ± 3.98[b]	13.3 ± 6.63[c]	6.43 ± 2.38	11.5 ± 6.83
	M	0.49	5.60 ± 2.11[a]	7.76 ± 3.63[a]	11.4 ± 5.53[b]	5.85 ± 2.42	11.6 ± 6.66
C16.1.trans	F	0.53	0.59 ± 0.29[a]	0.56 ± 0.26[a]	1.29 ± 0.60[b]	0.71 ± 0.36	1.22 ± 0.55
	M	0.51	0.53 ± 0.31[a]	0.72 ± 0.44[a]	1.26 ± 0.66[b]	0.75 ± 0.39	1.18 ± 0.52
C2 (mM)	F	0.51	6.68 ± 2.03[a]	7.32 ± 2.73[a]	10.7 ± 3.07[b]	7.77 ± 1.92	10.7 ± 3.24
	M	0.45	6.78 ± 2.79[a]	7.77 ± 2.59[a]	9.80 ± 2.85[b]	7.13 ± 2.05	10.6 ± 3.10
C8.DC	F	0.50	6.50 ± 3.15[a]	5.97 ± 3.58[a]	18.0 ± 9.92[b]	7.04 ± 3.65	14.44 ± 8.92
	M	0.40	6.58 ± 7.37[a]	8.27 ± 6.46[a]	15.1 ± 10.1[b]	8.1 ± 4.39	15.19 ± 9.38

TABLE 2 (Continued)

Metabolite (μM)	Sex	Corr w. Age (R^2)	Normal waist circumference			Elevated waist circumference	
			Young adults ($n = 86$)	Middle agers ($n = 106$)	Older people ($n = 69$)	Middle agers ($n = 99$)	Older people ($n = 91$)
C0 (mM)	F	0.48	2.54 ± 5.96[a]	3.19 ± 6.13[b]	3.27 ± 7.02[b]	3.59 ± 8.94	3.60 ± 8.18
	M	0.18	3.40 ± 7.76	3.70 ± 6.84	3.63 ± 8.23	4.01 ± 7.18	3.96 ± 7.52
C10.DC	F	0.47	0.84 ± 0.54[a]	0.75 ± 0.50[a]	2.38 ± 1.52[b]	0.82 ± 0.49	1.94 ± 1.36
	M	0.40	0.89 ± 1.10[a]	0.90 ± 0.79[a]	2.06 ± 1.4[b]	0.99 ± 0.55	2.12 ± 1.59
iso.C9.b	F	0.47	3.75 ± 1.25[a]	4.61 ± 2.79[a]	6.51 ± 2.81[b]	4.35 ± 1.18	6.69 ± 2.72
	M	0.45	4.80 ± 1.43[a]	4.91 ± 1.75[a]	6.78 ± 1.82[b]	4.86 ± 1.79	7.51 ± 2.76
C4.OH.a	F	0.45	11.4 ± 6.79[a]	11.3 ± 7.05[a]	27.1 ± 14.2[b]	12.2 ± 3.42	24.2 ± 12.2
	M	0.45	11.4 ± 6.80[a]	12.7 ± 5.58[a]	21.9 ± 8.44[b]	12.0 ± 4.94	25.6 ± 16.9
C6.DC	F	0.45	12.0 ± 4.92[a]	11.4 ± 5.05[a]	23.4 ± 12.7[b]	12.3 ± 5.92	21.8 ± 12.0
	M	0.40	13.1 ± 9.14[a]	14.6 ± 7.30[a]	20.9 ± 9.42[b]	15.0 ± 7.05	22.6 ± 9.95
X2M.C4	F	0.45	23.4 ± 5.15[a]	25.5 ± 6.68[a]	30.9 ± 10.1[b]	27.1 ± 7.75	35.3 ± 10.8
	M	0.29	31.8 ± 7.03	34.6 ± 7.88	34.5 ± 8.58	36.3 ± 9.43	40.4 ± 8.69
iso.C15.a	F	0.44	0.44 ± 0.22[a]	0.58 ± 0.36[a]	0.75 ± 0.26[b]	0.47 ± 0.15	0.77 ± 0.29
	M	0.25	0.61 ± 0.24	0.60 ± 0.27	0.71 ± 0.28	0.55 ± 0.42	0.87 ± 0.29
iso.C9.a	F	0.41	8.61 ± 5.16[a]	13.6 ± 11.1[a]	20.2 ± 17.6[b]	11.2 ± 4.99	20.9 ± 15.1
	M	0.32	12.4 ± 7.50	14.4 ± 12.0	18.6 ± 8.70	12.7 ± 7.95	22.0 ± 14.2
C18.2	F	0.40	34.4 ± 12.9[a]	35.0 ± 10.3[a]	50.7 ± 15.4[b]	33.6 ± 8.90	45.1 ± 11.6
	M	0.28	46.3 ± 15.6[a,b]	42.3 ± 10.9[a]	53.9 ± 18.4[b]	38.2 ± 8.87	53.2 ± 14.7
C6.OH	F	0.40	9.93 ± 3.85[a]	9.13 ± 4.14[a]	15.9 ± 6.22[b]	9.72 ± 2.85	14.9 ± 6.04
	M	0.39	10.7 ± 4.12[a]	11.2 ± 4.36[a]	15.2 ± 4.99[b]	10.6 ± 3.88	16.8 ± 7.69
iso.C11.b	F	0.38	5.09 ± 1.97[a]	5.7 ± 3.26[b]	7.73 ± 3.25[b]	5.67 ± 1.88	7.98 ± 3.46
	M	0.23	6.92 ± 2.33[a,b]	6.31 ± 2.62[a]	8.08 ± 2.49[b]	5.83 ± 2.1	9.08 ± 3.61
C3	F	0.38	165 ± 45.2	202 ± 57.5	204 ± 53.4	228 ± 65.2	251 ± 88.1
	M	0.25	238 ± 63.9	269 ± 72.8	249 ± 72.0	291 ± 66.9	332 ± 110
C6	F	0.37	37.8 ± 19.0[a]	36.6 ± 14.2[a]	52.2 ± 15.5[b]	49.1 ± 17.1*	55.5 ± 19.1
	M	0.36	36.8 ± 13.9[a]	40.2 ± 18.3[a,b]	51.8 ± 18.5[b]	50.1 ± 25.9	60.8 ± 30.1
C4.M.DC	F	0.35	4.80 ± 2.58[a]	6.54 ± 4.46[a,b]	9.01 ± 9.08[b]	6.64 ± 4.62	9.49 ± 5.66
	M	0.15	5.51 ± 11.37	7.67 ± 4.41	8.28 ± 6.44	6.02 ± 2.95	8.19 ± 4.70
C10.2	F	0.35	12.0 ± 5.90[a]	11.3 ± 5.45[a]	16.5 ± 5.50[b]	13.2 ± 3.85	17.1 ± 6.76
	M	0.17	16.3 ± 6.86[a,b]	12.9 ± 5.37[a]	19.1 ± 9.09[b]	13.9 ± 4.60	19.4 ± 8.16
C12.OH	F	0.35	10.3 ± 4.58[a,b]	8.76 ± 3.13[a]	14.5 ± 5.64[b]	10.3 ± 3.69	13.5 ± 4.12
	M	0.26	13.0 ± 5.11[a]	11.3 ± 4.92[a]	16.1 ± 6.60[b]	10.5 ± 3.64	16.2 ± 6.20
C14.1.all	F	0.34	90.6 ± 45.2[a]	74.9 ± 34.2[a]	133 ± 41.6[b]	91.5 ± 37.9	120 ± 41.7
	M	0.19	109 ± 0.64[a,b]	90.4 ± 46.7[a]	126 ± 44.6[b]	83.8 ± 26.6	133 ± 54.8
2M.2M.C3	F	0.31	1.87 ± 1.60[a]	2.82 ± 3.28[a,b]	5.06 ± 6.55[b]	2.88 ± 2.46	5.14 ± 6.61
	M	0.13	3.39 ± 3.71	3.08 ± 3.05	4.08 ± 3.15	3.03 ± 2.34	4.51 ± 3.46
C14.1.cis	F	0.30	116 ± 63.6[a]	93.7 ± 45.4[a]	169 ± 59.5[b]	112 ± 53.5	150 ± 54.0
	M	0.17	140 ± 72.4[a,b]	110 ± 61.0[a]	162 ± 59.0[b]	102 ± 36.7	168 ± 80.6
3M.C4.3OH	F	0.30	21.5 ± 7.82[a]	28.3 ± 10.9[b]	28.5 ± 9.63[a]	25.7 ± 6.89	28.5 ± 6.81
	M	0.05	29.4 ± 11.3[a]	35.7 ± 14.5[b]	28.5 ± 7.37[a]	28.5 ± 6.82*	34.3 ± 10.8
C16.2	F	0.30	2.55 ± 1.75[a]	2.04 ± 0.99[a]	4.01 ± 1.66[b]	2.20 ± 0.83	3.23 ± 1.23
	M	0.24	2.89 ± 2.21[a]	2.36 ± 1.42[a]	4.12 ± 2.50[b]	2.08 ± 0.90	3.62 ± 1.79

(Continues)

TABLE 2 (Continued)

Metabolite (μM)	Sex	Corr w. Age (R^2)	Normal waist circumference			Elevated waist circumference	
			Young adults ($n=86$)	Middle agers ($n=106$)	Older people ($n=69$)	Middle agers ($n=99$)	Older people ($n=91$)
C7	F	0.29	0.86 ± 0.62[a]	1.00 ± 0.73[a,b]	1.33 ± 0.68[b]	1.09 ± 0.71	1.35 ± 0.64
	M	0.28	0.81 ± 0.44[a]	0.98 ± 0.58[a,b]	1.28 ± 0.74[b]	1.03 ± 0.82	1.35 ± 0.83
C4.OH.b	F	0.29	28.9 ± 16.6[a]	26.0 ± 30.2[a]	52.1 ± 28.2[b]	27.7 ± 18.2	48.6 ± 35.1
	M	0.20	32.4± 27.0	35.6 ± 38.5	46.6 ± 31.7	28.0 ± 15.1	47.6 ± 27.5
iso.C13	F	0.28	7.90 ± 5.72[a]	15.9 ± 37.7[a,b]	24.2 ± 24.5[b]	10.9 ± 6.26	24.1 ± 20.3
	M	0.40	10.3 ± 5.67[a]	14.5 ± 11.3[a,b]	21.3 ± 15.1[a]	16.2 ± 20.4	24.2 ± 17.3
C11	F	0.27	2.20 ± 1.33[a]	2.21 ± 1.11[a]	3.24 ± 1.51[b]	2.33 ± 1.16	2.82 ± 1.20
	M	0.23	2.29 ± 1.02[a]	2.09 ± 1.11[a]	3.1 ± 1.25[b]	1.72 ± 0.59	2.86 ± 1.11
C9	F	0.26	3.0 ± 1.85	4.17 ± 4.15	4.68 ± 2.73	3.51 ± 1.98	5.25 ± 3.99
	M	0.27	3.09 ± 1.55[a]	3.35 ± 2.40[a,b]	4.95 ± 2.62[b]	2.90 ± 1.76	5.33 ± 5.02
C4	F	0.26	68.7 ± 41.8	62.7 ± 26.6	96.2 ± 68.7	115 ± 148*	111 ± 73.3
	M	0.23	71.1 ± 34.0	86.3 ± 49.3	96.8 ± 59.8	103 ± 45.9	113 ± 72.7
C12	F	0.26	75.9 ± 36.1[a]	64.9 ± 31.5[a]	100 ± 35.6[b]	73.3 ± 34.9	91.8 ± 30.3
	M	0.24	84.2 ± 39.0[a]	71.8 ± 37.7[a]	107 ± 41.1[b]	62.4 ± 21.9	103 ± 40.3
C12.1	F	0.24	104 ± 44.1[a]	83.9 ± 32.3[a]	134 ± 43.9[b]	104 ± 41.7	125 ± 47.5
	M	0.12	119 ± 49.7[a,b]	95.6 ± 46.9[a]	130 ± 51.1[b]	88.2 ± 29.5	134 ± 55.5
X2M.C4.1	F	0.24	8.15 ± 3.50[a]	10.7 ± 4.16[b]	10.4 ± 3.75[c]	9.22 ± 3.15*	10.4 ± 3.42
	M	0.22	10.0 ± 4.57	12.1 ± 5.86	12.9 ± 5.52	8.78 ± 3.30	12.9 ± 5.34
C8.1	F	0.24	126 ± 65.9	111 ± 70.3	169 ± 101	154 ± 61.1*	175 ± 98.8
	M	0.21	134 ± 60.0[a,b]	133 ± 60.0[a]	160 ± 80.3[b]	189 ± 103.8*	180 ± 106
C4.1	F	0.23	0.37 ± 0.21	0.38 ± 0.60	0.61 ± 0.35	0.40 ± 0.23	0.68 ± 0.70
	M	0.29	0.40 ± 0.26[a]	0.37 ± 0.30[a]	0.62 ± 0.39[b]	0.37 ± 0.18	0.70 ± 0.48
C5.M.DC	F	0.22	23.1 ± 17.9	21.3 ± 14.1	31.4 ± 23.1	22.2 ± 11.0	36.4 ± 26.6
	M	0.42	11.4 ± 9.41[a]	17.0 ± 8.54[a]	27.4 ± 21.8[b]	15.2 ± 5.68	28.5 ± 18.2
X5M.C6	F	0.21	1.18 ± 1.06	1.23 ± 1.33	1.52 ± 1.09	1.36 ± 1.00	1.81 ± 1.26
	M	−0.03	2.13 ± 2.33	1.49 ± 0.94	1.93 ± 1.61	1.57 ± 0.71	2.10 ± 1.21
C8	F	0.17	153 ± 86.3[a,b]	139 ± 64.0[a]	186 ± 69.5[b]	162 ± 83.3	176 ± 73.5
	M	0.22	147 ± 64.9[a,b]	137 ± 72.0[a]	190 ± 83.3[b]	134 ± 57.3	188 ± 90.7
C10.1	F	0.16	94.4 ± 41.3[a,b]	84.1 ± 32.0[a]	107 ± 34.4[b]	96.4 ± 38.2	106 ± 39.7
	M	0.08	115 ± 45.6[a]	88.7 ± 35.7[b]	122 ± 52.2[a]	81.2 ± 30.0	118 ± 46.8
C10	F	0.11	283 ± 116	251 ± 115	300 ± 113	284 ± 138	300 ± 115
	M	0.17	276 ± 146	243 ± 119	319 ± 127	224 ± 84.9	319 ± 125
X2M.C3.1	F	0.09	1.87 ± 1.60	2.83 ± 3.28	5.06 ± 6.55	2.88 ± 2.46	5.14 ± 6.61
	M	−0.05	3.39 ± 3.72	3.08 ± 3.05	4.08 ± 3.15	3.03 ± 2.34	4.51± 3.46
C5.DC	F	0.09	46.0 ± 14.5	41.0 ± 9.22	48.2 ± 16.7	41.5 ± 12.4	48.8 ± 13.4
	M	0.01	57.7 ± 15.9	50.5 ± 15.1	56.1 ± 16.2	50.5 ± 15.1	56.1 ± 19.2
C14.1.trans	F	0.08	0.50 ± 0.66	0.82 ± 1.55	0.63 ± 0.75	0.45 ± 0.53	0.83 ± 2.09
	M	−0.09	4.14 ± 11.0	0.19 ± 0.16	0.92 ± 1.02	0.19 ± 0.32	1.92 ± 7.06
X2M.C3	F	0.08	61.1 ± 32.2	72.3 ± 33.0	59.9 ± 34.5	61.8 ± 30.1	78.1 ± 51.3
	M	0.18	68.9 ± 28.8	80.3 ± 34.4	74.5 ± 33.6	65.3 ± 27.7	94.1 ± 47.1
C6.1	F	0.08	17.8 ± 17.1	15.9 ± 21.4	19.8 ± 8.39	19.8 ± 15.9	21.9 ± 15.1
	M	−0.07	23.7 ± 16.7	16.9 ± 12.8	21.2 ± 14.2	18.1 ± 8.54	21.7 ± 16.0

TABLE 2 (Continued)

Metabolite (μM)	Sex	Corr w. Age (R^2)	Normal waist circumference			Elevated waist circumference	
			Young adults ($n = 86$)	Middle agers ($n = 106$)	Older people ($n = 69$)	Middle agers ($n = 99$)	Older people ($n = 91$)
C14.2	F	0.07	29.6 ± 18.2[a]	21.2 ± 8.70[b]	33.9 ± 10.1[a]	24.5 ± 8.23	29.2 ± 11.1
	M	−0.04	38.2 ± 22.2[a]	26.0 ± 14.3[b]	35.1 ± 16.3[a]	22.8 ± 7.32	33.6 ± 13.5
C10.OH	F	0.03	31.4 ± 15.7[a]	24.3 ± 9.84[b]	34.6 ± 15.2[a]	25.3 ± 10.3	29.4 ± 11.0
	M	−0.02	40.2 ± 18.2[a]	17.0 ± 15.2[b]	39.5 ± 16.7[a]	25.2 ± 9.85	36.0 ± 14.0
X3M.C4	F	−0.12	43.9 ± 27.9[a,b]	55.5 ± 25.8[a]	36.5 ± 12.3[b]	51.6 ± 20.7	43.8 ± 19.0
	M	−0.11	56.6 ± 25.8[a]	79.6 ± 31.7[b]	47.8 ± 24.7[a]	86.0 ± 33.0	61.4 ± 21.6
iso.C11.a	F		0.29 ± 0.14[a]	0.39 ± 0.22[a]	0.53 ± 0.29[b]	0.35 ± 0.16	0.46 ± 0.23
	M		0.34 ± 0.17[a]	0.38 ± 0.19[a,b]	0.49 ± 0.25[b]	0.31 ± 0.22	0.47 ± 0.24

Note: Acylcarnitine species are sorted based on the strength of the correlation with age. Data are presented as mean ± standard deviation. p values of < .05 were regarded as statistically significant. Mean differences were assessed between young adults, middle agers, and older people with normal waist circumference based on a multi-group comparison with ANOVA and post-hoc Tukey test; Middle agers, respectively, older people with normal and elevated waist circumference were compared according to distribution by using t test or Wilcoxon-signed ranked test. Labeled means in a row with a common superscript letter do not differ, $p < .05$. *, different from normal waist circumference, $p < .05$.

TABLE 3 Amino acids with age-related *decreases* in plasma in females (F) and males (M).

Metabolite (μM)	Sex	Corr w. Age (R^2)	Normal waist circumference			Elevated waist circumference	
			Young adults ($n = 86$)	Middle agers ($n = 106$)	Older people ($n = 69$)	Middle agers ($n = 99$)	Older people ($n = 91$)
His	F	−0.48	79.6 ± 10.4[a]	71.4 ± 7.7[b]	67.7 ± 7.3[b]	71.9 ± 6.9	68.2 ± 8.3
	M	−0.37	78.5 ± 8.9[a]	75.5 ± 8.9[a,c]	69.8 ± 7.0[b]	73.0 ± 10.1	71.1 ± 9.2
Met	F	−0.24	21.3 ± 3.0	20.0 ± 2.6	20.1 ± 2.6	20.0 ± 2.8	19.4 ± 2.0
	M	−0.34	24.7 ± 2.5[a]	22.4 ± 3.2[b]	21.9 ± 3.5[b]	23.3 ± 3.5	21.9 ± 3.3
Leu	F	−0.27	109 ± 13.1	100 ± 15.2	98.8 ± 12.9	106 ± 23.1	103 ± 12.3
	M	−0.33	133 ± 22.0[a]	130 ± 23.8[a]	113 ± 15.9[b]	130 ± 18.5	119 ± 17.9
Asn	F	−0.35	43.3 ± 7.4[a]	40.7 ± 6.8[a,b]	38.6 ± 5.5[b]	36.4 ± 4.6*	35.6 ± 5.4
	M	−0.31	44.4 ± 5.4[a]	40.3 ± 5.9[b]	40.9 ± 6.5[a,b]	36.7 ± 5.0*	38.5 ± 5.6
Ser	F	−0.13	111 ± 24.5	108 ± 18.8	108 ± 22.1	104 ± 21.9	102 ± 22.5
	M	−0.28	111 ± 19.8[a]	96.8 ± 17.8[b]	98.6 ± 15.5[b]	95.4 ± 20.4	95.2 ± 19.4
Ile	F	−0.29	57.1 ± 8.6[a]	47.9 ± 7.8[b]	48.8 ± 7.1[b]	52.2 ± 7.8*	53.2 ± 7.0
	M	−0.28	68.7 ± 12.9[a]	64.5 ± 13.9[a,b]	57.0 ± 8.8[b]	66.2 ± 10.2	62.1 ± 12.2
Thr	F	−0.47	127 ± 28.5[a]	105 ± 20.2[b]	101 ± 20.2[b]	103 ± 20.0	95.8 ± 17.3
	M	−0.23	118 ± 21.8[a]	105 ± 19.8[b]	110 ± 22.6[a,b]	104 ± 20.3	97.5 ± 17.3
Val	F	−0.18	164 ± 20.3[a]	157 ± 26.5[a]	149 ± 17.1[b]	172 ± 19.5	165 ± 18.1
	M	−0.23	194 ± 27.5[a]	194 ± 29.1[a,b]	173 ± 26.6[b]	195 ± 24.1*	181 ± 24.5*
Trp	F	−0.23	46.4 ± 7.1	45.7 ± 6.9	41.3 ± 6.9	44.3 ± 6.4	42.5 ± 6.6
	M	−0.16	51.7 ± 7.8[a]	48.2 ± 9.3[a]	48.5 ± 10.1[b]	46.6 ± 7.4	47.5 ± 9.2

Note: Data are presented as mean ± standard deviation. p values of < .05 were regarded as statistically significant. Mean differences were assessed between young adults, middle agers, and older people with normal waist circumference based on a multi-group comparison with ANOVA and post-hoc Tukey test; Middle agers, respectively, older people with normal and elevated waist circumference were compared according to distribution by using t test or Wilcoxon-signed ranked test. Labeled means in a row with a common superscript letter do not differ, $p < .05$. *, different from normal waist circumference, $p < .05$.

Skeletal muscle mass has the highest impact on the concentrations of medium-chain dicarboxylic acylcarnitines including adipoylcarnitine (C6-DC), pimeloylcarnitine (C7-DC), suberoylcarnitine (C8-DC), and sebacylcarnitine (C10-DC). Last, CRP levels seem to be related to plasma concentrations of crotonylcarnitine (C4:1).

TABLE 4 Amino acids with age-related *increases* in plasma in females (F) and males (M).

			Normal waist circumference			Elevated waist circumference	
Metabolite (μM)	**Sex**	**Corr w. Age (R^2)**	**Young adults ($n=86$)**	**Middle agers ($n=106$)**	**Older people ($n=69$)**	**Middle agers ($n=99$**	**Older people ($n=91$)**
Orn	F	0.67	36.8 ± 11.9[a]	51.7 ± 13.3[b]	64.0 ± 14.7[c]	46.6 ± 9.5	64.6 ± 12.2
	M	0.45	49.9 ± 11.9[a]	53.1 ± 11.4[a]	65.1 ± 15.9[b]	52.7 ± 10.4	64.3 ± 16.5
Cit	F	0.65	22.4 ± 4.8[a]	28.8 ± 6.1[b]	33.6 ± 5.4[c]	25.9 ± 6.2	32.7 ± 7.2
	M	0.46	25.8 ± 5.6[a]	28.5 ± 4.5[a]	33.3 ± 5.9[b]	26.3 ± 5.1	31.8 ± 7.3
Glu	F	0.53	23.8 ± 11.0[a]	27.7 ± 10.1[a]	37.1 ± 11.7[b]	37.9 ± 16.1	45.9 ± 17.9
	M	0.34	30.3 ± 9.2	40.1 ± 17.3	40.1 ± 15.1	62.5 ± 19.9	52.4 ± 22.7
tau.M.His	F	0.45	3.3 ± 0.9[a]	3.5 ± 0.9[a]	4.5 ± 1.7[b]	3.7 ± 1.0	5.1 ± 1.7
	M	0.34	4.1 ± 0.9	4.4 ± 1.2	4.8 ± 1.1	4.5 ± 1.1	5.8 ± 1.8
Tyr	F	0.43	49.4 ± 10.8[a]	56.8 ± 8.6[b]	59.1 ± 10.9[b]	61.4 ± 9.1	60.1 ± 7.3
	M	0.17	57.5 ± 8.1	59.7 ± 8	58.6 ± 10.6	69.8 ± 12.3	64.5 ± 11.6
Kynurenine	F	0.40	1.4 ± 0.5[a]	1.5 ± 0.2[a]	1.8 ± 0.6[b]	1.6 ± 0.4	2.0 ± 0.3
	M	0.36	1.6 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	1.9 ± 0.3	2.2 ± 0.6
Betaine	F	0.40	17.9 ± 7.6[a]	25.6 ± 9.2[b]	28.7 ± 8.5[b]	21.9 ± 6.3	24.7 ± 7.6
	M	0.12	29.3 ± 7.8	29.7 ± 7.5	30.3 ± 7.0	26.9 ± 4.7	31.5 ± 8.5
AADP	F	0.37	0.9 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	1.3 ± 0.3
	M	0.29	1.2 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.7 ± 0.4	1.7 ± 0.6
Butyrobetaine	F	0.36	0.5 ± 0.1[a]	0.6 ± 0.1[b]	0.6 ± 0.2[b]	0.6 ± 0.1	0.6 ± 0.2
	M	0.00	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2
Sarcosine	F	0.35	2.5 ± 0.4[a]	2.4 ± 0.6[a]	3.0 ± 0.5[b]	2.5 ± 0.8	2.9 ± 0.5
	M	0.18	3.0 ± 0.6	2.9 ± 0.8	3.2 ± 0.6	2.9 ± 1.1	3.2 ± 0.6
Taurine	F	0.34	47.4 ± 12.9[a]	69.1 ± 33.1[b]	67.1 ± 12.0[b]	63.2 ± 19.3	65.4 ± 15.5
	M	0.36	50.4 ± 10.9[a]	58.8 ± 12.2[a,b]	59.1 ± 11.3[b]	58.2 ± 12.9	62.2 ± 12.7
GAA	F	0.33	1.6 ± 0.6[a]	2.1 ± 0.6[b]	2.1 ± 0.4[b]	1.9 ± 0.4	1.9 ± 0.4
	M	0.13	2.0 ± 0.5	2.1 ± 0.5	2.2 ± 0.4	2.3 ± 0.5	2.1 ± 0.5
GABA	F	0.30	0.13 ± 0.04[a]	0.13 ± 0.03[a]	0.17 ± 0.04[b]	0.13 ± 0.03	0.15 ± 0.03
	M	0.13	0.12 ± 0.02	0.13 ± 0.03	0.17 ± 0.26	0.12 ± 0.03	0.15 ± 0.03
Gly	F	0.30	187 ± 54.6[a]	234 ± 56.6[b]	226 ± 53.5[b]	214 ± 68.1	226 ± 67.8
	M	−0.14	213 ± 27.3	190 ± 37.9	208 ± 43.6	169 ± 29.4	186 ± 41.4
TMAO	F	0.29	2.4 ± 1.3[a]	3.1 ± 2.2[a]	5.8 ± 7.7[b]	2.7 ± 1.1	5.3 ± 5.6
	M	0.23	3.2 ± 2.1	3.8 ± 2.8	4.8 ± 4.4	3.4 ± 1.9	6.0 ± 6.9
Creatine	F	0.25	15.1 ± 9.8[a]	22.1 ± 10.7[b]	19.4 ± 9.2[a,b]	24.7 ± 9.9	25.2 ± 11.7
	M	0.11	11.2 ± 6.9	14.4 ± 7.5	11.3 ± 5.6	21.3 ± 9.4	15.7 ± 7.7
BAIB	F	0.21	1.6 ± 0.6	1.6 ± 0.6	1.8 ± 0.9	1.4 ± 0.6	2.1 ± 1.1
	M	0.42	1.4 ± 0.8[a]	1.8 ± 0.7[a]	2.3 ± 1.1[b]	1.4 ± 0.7	2.3 ± 1.2
DMG	F	0.21	2.1 ± 0.8	2.0 ± 0.8	2.5 ± 0.7	2.0 ± 0.7	2.5 ± 0.8
	M	0.16	2.6 ± 0.8	2.5 ± 0.7	2.9 ± 0.9	2.3 ± 0.6	3.0 ± 1.3
Trimethyllysine	F	0.19	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.2
	M	0.10	0.6 ± 0.3	0.5 ± 0.1	0.6 ± 0.5	0.5 ± 0.1	0.6 ± 0.4
Asp	F	0.18	5.0 ± 1.1	5.3 ± 1.6	5.3 ± 1.4	5.1 ± 1.2	5.4 ± 1.1
	M	0.00	5.6 ± 1.0	5.4 ± 1.0	5.3 ± 0.9	5.9 ± 0.8	5.7 ± 1.3
Kynurenic.acid	F	0.17	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
	M	0.22	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.04	0.05 ± 0.03	0.06 ± 0.03
Choline	F	0.17	389 ± 122.7	395.3 ± 127.4	453.2 ± 177.5	376 ± 94.2	445 ± 173
	M	0.07	379 ± 127.8	421.3 ± 126.8	395.7 ± 100.6	435 ± 151	407 ± 129

TABLE 4 (Continued)

Metabolite (μM)	Sex	Corr w. Age (R^2)	Normal waist circumference			Elevated waist circumference	
			Young adults ($n = 86$)	Middle agers ($n = 106$)	Older people ($n = 69$)	Middle agers ($n = 99$	Older people ($n = 91$)
Lys	F	0.15	153 ± 33.5	161 ± 26.1	160 ± 25.3	167 ± 22.9	165 ± 23.3
	M	0.09	158 ± 24.1[a]	174 ± 28.8[b]	161 ± 30.0[a,b]	174 ± 25.8	169 ± 30.5
IAA	F	0.15	1.5 ± 0.7	1.6 ± 0.8	1.7 ± 0.8	1.4 ± 0.7	1.7 ± 0.8
	M	0.22	1.6 ± 0.6	1.9 ± 1.0	1.8 ± 0.7	1.5 ± 0.7	2.3 ± 1.6

Note: Data are presented as mean ± standard deviation. p values of <.05 were regarded as statistically significant. Mean differences were assessed between young adults, middle agers, and older people with normal waist circumference based on a multi-group comparison with ANOVA and post-hoc Tukey test; Middle agers, respectively, older people with normal and elevated waist circumference were compared according to distribution by using t test or Wilcoxon-signed ranked test. Labeled means in a row with a common superscript letter do not differ, $p < .05$. *, different from normal waist circumference, $p < .05$.

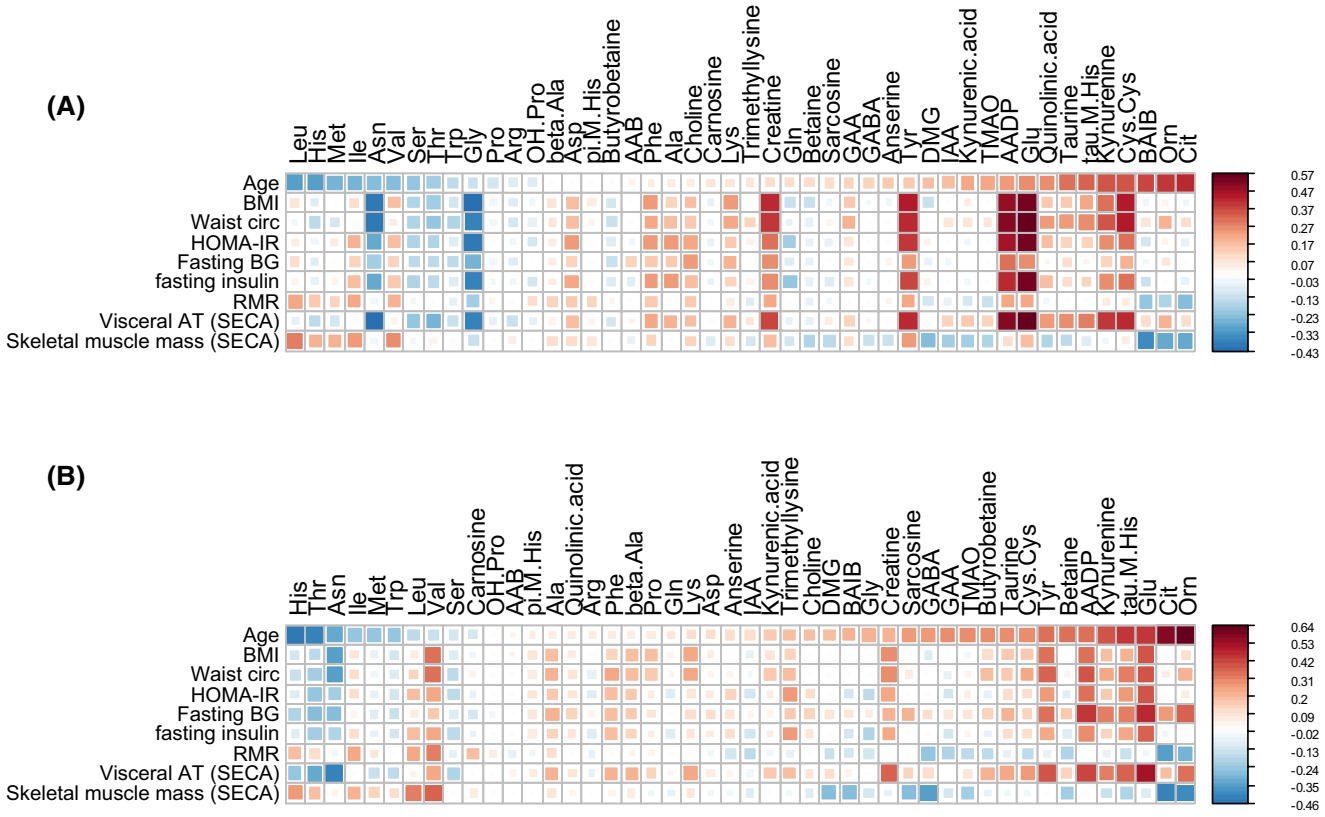

FIGURE 3 Correlations between fasting plasma metabolite concentrations and clinical and anthropometric parameters in males across all age groups ($n = 227$) (A) and females across all age groups ($n = 230$) (B). Blue color indicates negative, and red color indicates positive correlations. Metabolites are sorted according to their correlation with age.

4 | DISCUSSION

In this study, we determined plasma metabolite concentrations in extensively phenotyped cohorts of healthy young, middle-aged, and older adults and searched for age-related patterns. Also, we were interested to investigate the influence of an elevated waist circumference and other adiposity markers on plasma metabolite profiles and, therefore, compared age- and adiposity-related plasma metabolite patterns.

4.1 | Age-related amino acid alterations are indicative of changes in protein turnover and nitrogen balance

Overall, higher age was characterized by decreasing circulating essential amino acids and increasing intermediates of protein and amino acid metabolism. The finding of reduced concentrations of essential amino acids at an advanced age was already observed in previous studies. As

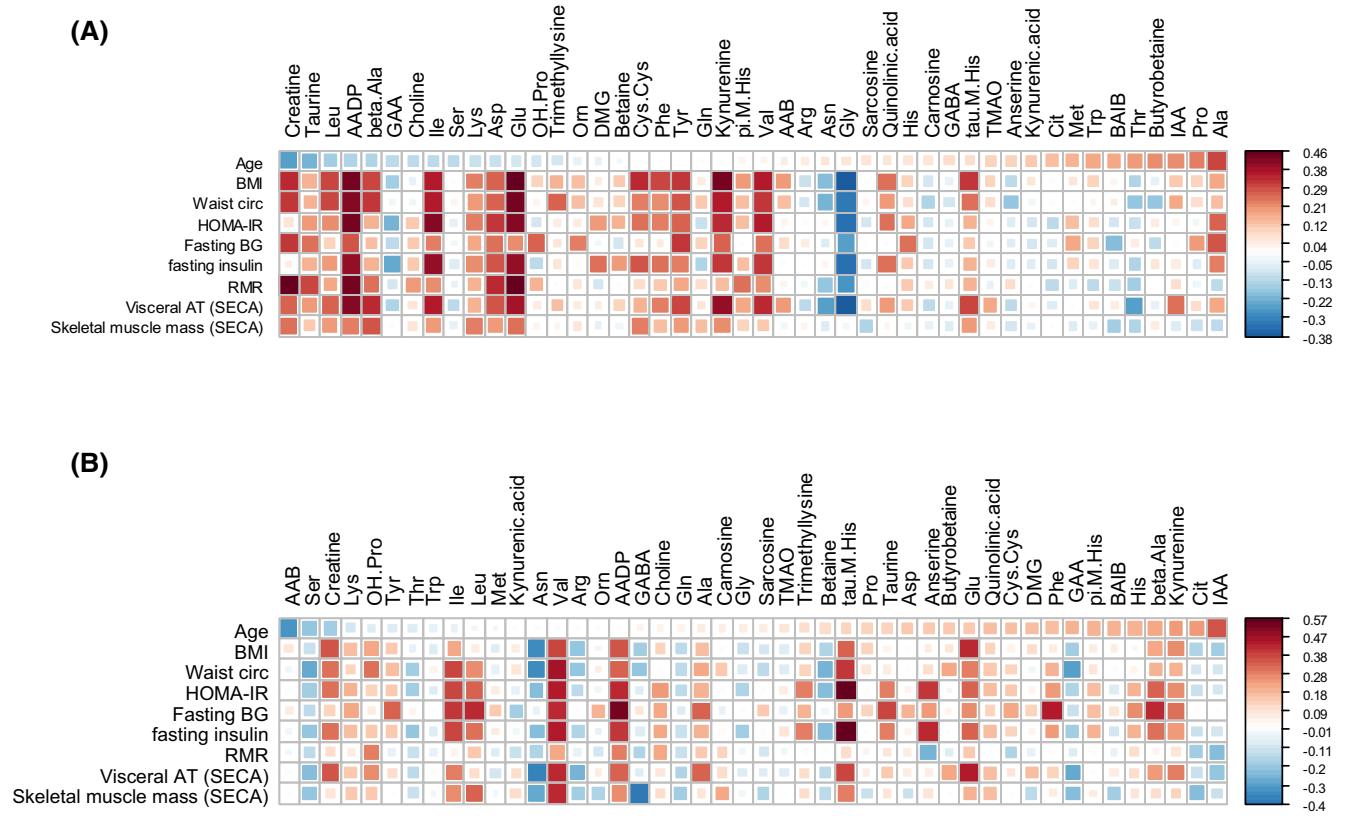

FIGURE 4 Correlations between fasting plasma metabolite concentrations and clinical and anthropometric parameters in older males ($n = 81$) (A) and older females ($n = 79$) (B). Blue color indicates negative, and red color indicates positive correlations. Metabolites are sorted according to their correlation with age.

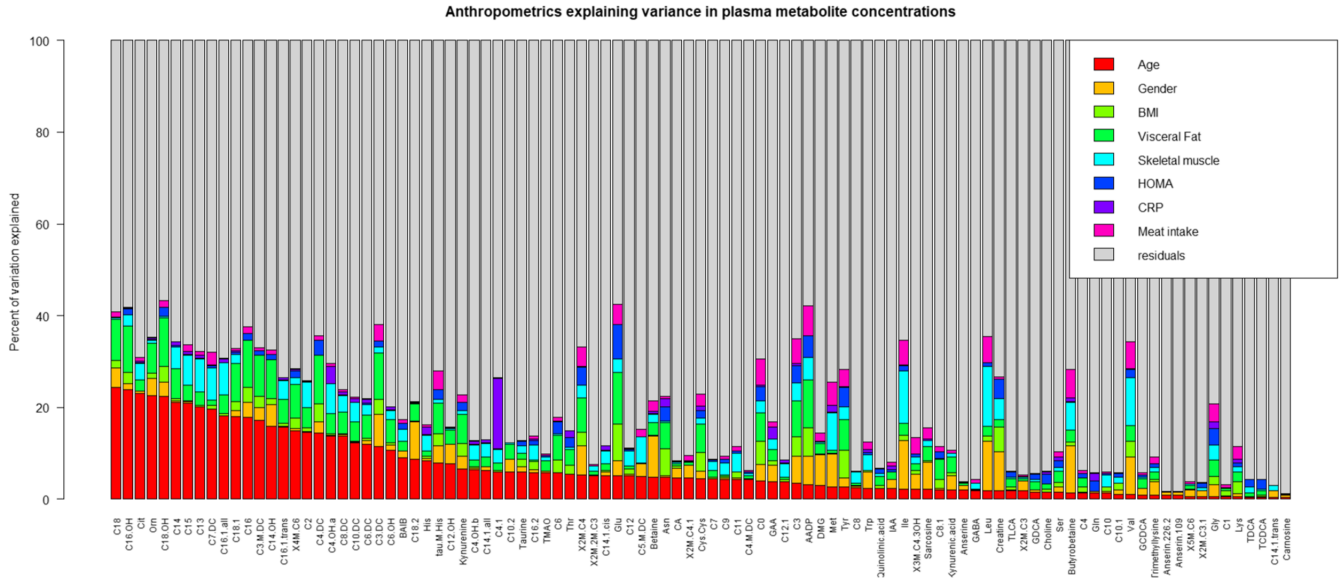

FIGURE 5 Anthropometrics explaining variance in plasma metabolite concentrations. Multiple regression results display the percentage of explained variation of fasting plasma concentrations by suggested major clinical, anthropometric, and nutritional contributors.

one reason, Pitkänen et al.[17] reported that the decrease in serum amino acid concentrations was associated with decreased energy and protein intake during aging. Similar to Kouchiwa et al.,[18] we observed a specific reduction in Gly concentrations in men. We also found increases for a cluster of metabolites specifically related to nitrogen balance,

protein turnover, and amino acid metabolism. This cluster includes the urea cycle intermediates Cit and Orn, the proteolysis marker τ-M-His, intermediates of Trp and Lys breakdown (AADP, Kynurenine, Kynurenic acid), and end-products of nitrogen disposal (creatine and its precursor GAA).

Notably, the increase of these metabolites was especially prominent in older female adults. The strong increase in τ-M-His concentrations in this group suggests that these women might be particularly susceptible to muscle loss. Moreover, the increase of trimethyllysine and butyrobetaine observed here is plausible, as these metabolites are derived from posttranslational modification of Lys and are released into the bloodstream upon protein breakdown. A dependency of trimethyllysine concentrations on the rate of protein turnover as well as a correlation with τ-M-His concentrations was shown previously.[19] Overall, these changes are indicative of a state of increased proteolysis, which is a characteristic of older people and is especially prominent in older females. Our findings are in line with the observation of "anabolic resistance" in older people, which means that the anabolic response to glucose-induced hyperinsulinemia as well as to a high amino acid load in muscle is blunted.[20] Anabolic resistance also includes a high proteolysis rate at fasting conditions.[21] Our data suggest that this process may likely be affected by the degree of insulin resistance and the individual's ability to compensate by increasing insulin output. Whereas males display a compensatory increase in insulin secretion with increasing age, this is not seen in females (see Table 1).

A further tendency for age-related diseases such as cancer or neurodegeneration can be hypothesized from alterations in Trp and the kynurenine pathway, which are relevant for immune regulation and neuronal function.[22] Elevated levels of metabolites in this pathway were also associated with cardiovascular disease[23] and diabetes.[24]

Although the process of protein breakdown is considered to increase the plasma level of essential amino acids, a reduced muscle mass is associated with decreased fasting levels of essential amino acids as shown previously.[25] However, it was proposed from experimental data that muscle mass in older citizens can be maintained by a higher intake of protein.[26]

4.2 | The adiposity-related metabolite profile includes typical markers for insulin resistance and diabetes

Metabolomics studies have identified a range of metabolite changes that are associated with obesity, insulin resistance, and/or diabetes. These include increases in branched-chain and aromatic amino acids[27] and their derived acylcarnitines.[7] But also alpha-amino adipic acid,[28] kynurenine, an increased Glu/Gln ratio,[7] as well as decreased concentrations of Gly,[27] Asn, and beta-aminoisobutyric acid (bAib)[29] was shown. In principle, our data confirm the findings in individuals with normal and increased waist circumference but extends these observations to well-defined age groups for both sexes. However, our data indicate that there seems to be no specific metabolite pattern characterizing an increased metabolic risk as many classes and pathways are affected. Other authors suggest certain phosphatidylcholines as discriminative for an increased cardiometabolic risk, however, this study compared a group of metabolically unhealthy obese with a group considered as metabolically healthy obese.[30] A very recent systematic review including 20 studies identified BCAA, aromatic amino acids, certain lipids (e.g., palmitic acid), and propionylcarnitine to be associated with an unhealthy phenotype.[31] Due to the lower number of volunteers with increased waist circumference in our study, we would not be able to pick up these differences between these groups. We, nevertheless, could confirm a higher level of acylcarnitine in the high-risk group compared with our lean controls.

4.3 | Age- and adiposity-related metabolite profiles differ for essential amino acids, asparagine, and urea cycle intermediates

It is well-accepted that elevated waist circumference or increased body weight and higher age are associated with impaired insulin sensitivity. Especially in older people but also with overweight, a decreased skeletal muscle mass and further impairments of metabolic health, primarily the tolerance to macronutrients and their disposal into tissues can be found. Therefore, it might be expected that plasma metabolite profiles alter in parallel between overweight and higher age. Indeed, a number of metabolites change in the same direction, including increases in AADP, Glu, Tyr, and kynurenine (Table 4). However, a large number of metabolites change in opposite directions. Whereas age was associated with decreases in essential amino acids and increases in urea cycle intermediates, adiposity was related to increases in the concentrations of these metabolites. This is a unique finding suggesting that the aging process and elevated waist circumference/adiposity have different/opposite effects on specific pathways. Therefore, these data may have implications for the search for biomarkers for the diagnosis and prediction of insulin resistance and metabolic syndrome. However, confirmation by other studies is needed.

4.4 | Aging and adiposity relate to accumulations of fatty acid- and amino acid-derived acylcarnitines, corresponding to mitochondrial dysfunction

Aging and obesity can both be characterized as a dysfunctional mitochondrial state (Natarajan et al. 2020; Prasun 2020). These observations could be underlined with our results as the clearest finding reported here is increased levels of acylcarnitine species in older individuals. Increases were found for medium- and long-chain acylcarnitines derived from mitochondrial fatty acid metabolism and include hydroxy and dicarboxy derivates as well as odd-numbered species from peroxisomal oxidation pathways. These findings extend the recent findings of Jarell et al.,[32] who reported on increased levels of C20-carbon length acylcarnitine species in healthy older individuals. However, acylcarnitine species derived from branched-chain amino acids and Trp and Lys were not increased in relation to age.

The obvious explanation for these increases seems to be an age-related decline in mitochondrial function. A reduced mitochondrial activity would result in an accumulation of intermediates from oxidation pathways and the impaired transfer of electrons to oxygen in the electron transfer chain leads to the generation of reactive oxygen species. It is suggested that the shuttling of acyl chains from the cells into the blood circulation in the form of acylcarnitines serves to regenerate free inner-mitochondrial CoA to keep upright mitochondrial metabolism.[33] Age-related accumulations of acylcarnitines in the circulation, thus, likely reflect inner-mitochondrial accumulation of intermediate oxidation products.

Interestingly, whereas age relates to an elevation of fatty acid-derived acylcarnitines, adiposity is related to acylcarnitines derived from specific amino acids, including Val, Ile, Thr, and Met. The origins of these differences might be found in the different oxidation end-products that transfer their electrons to coenzyme Q in the electron transfer chain. An age-related reduction in coenzyme Q10 levels was reported previously.[34] Coenzyme Q is an important converging point of amino acid and fatty acid oxidation pathways. It accepts electrons from NADH (via complex I) and from FADH2 (via electron transfer flavoproteins [ETFs]). It furthermore accepts electrons derived from the oxidation of succinate in complex II. Importantly, succinate is the end-point of amino acid oxidation pathways, including those of Val, Ile, Thr, and Met. If the availability of coenzyme Q is limited, the different electron-donating complexes are in competition, causing an upstream accumulation of intermediates, in this case, acyl-CoA species being converted to acylcarnitines.

In summary, we found marked alterations of amino acids and acylcarnitines across the age groups as well as specific changes in association with elevated waist circumference, body composition, and insulin sensitivity markers.

AUTHOR CONTRIBUTIONS

HH, TS, and DV conceived and designed the research; BB and TS performed the research and acquired the data; PG was responsible for metabolomics, he also analyzed and interpreted the data. All authors were involved in drafting and revising the manuscript.

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DISCLOSURES

The authors have declared that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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