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The association of acylcarnitines and amino acids with age in Dutch and South-Asian Surinamese living in Amsterdam

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Abstract

Background: Type 2 diabetes and cardiovascular disease occur more frequently, and at a younger age in South-Asians than Europeans. This may be related to differences in regulation of the fatty acid metabolism during aging. We compared age-related acylcarnitine and amino acid concentrations.

Methods: We measured types of acylcarnitine and amino acid concentrations in plasma (by tandem-MS) in a random subsample of 350 Dutch and 350 South-Asian Surinamese origin participants of the HELIUS study (Amsterdam, The Netherlands). We derived principal components (PCs) from the metabolites. Linear regression was used to assess differences in PCs and individual metabolite concentrations, and their age-trends between the groups by sex. We adjusted for BMI and intake of fat and total energy.

Results: Mean age was 44.8 (SD 13.3) years. Many metabolite concentrations were higher among South-Asian Surinamese participants compared to Dutch participants; amino acids in women, and both acylcarnitines and amino acids in men. Metabolite levels increased similarly with age in both ethnic groups. Results remained similar after adjustment.

Conclusion: Ethnic differences in metabolite concentrations suggest that fatty acid and amino acid metabolism are more dysregulated among South-Asian Surinamese compared to Dutch from a young age. During adulthood metabolites increase similarly in both ethnic groups.
Introduction

Type 2 diabetes and cardiovascular disease occur more frequently in populations of South-Asian than European origin. The incidence of these diseases increases with age [1, 2], but often with a younger age of onset in those of South-Asian origin [3, 4]. The causes of these differences are not fully understood.

Recently, increased plasma levels of long-chain fatty acid (LCFA) and amino acid metabolism, including acylcarnitines and branched-chain amino acids (BCAA), have been associated with type 2 diabetes and cardiovascular disease [5-7]. During aging LCFA homeostasis is dysregulated, ultimately leading to ectopic fat accumulation, increased use of non-oxidative pathways such as ceramide biosynthesis, and lipoapoptosis [8]. Impaired BCAA catabolism is reflected by higher levels of BCAAs (leucine, isoleucine and valine) and the accumulation of acylcarnitines derived from the intermediates of BCAA metabolism [9]. Different long-chain acylcarnitines, intermediates of LCFA catabolism, have been shown to accumulate in type 2 diabetes and cardiovascular diseases [6, 7] indicating impaired fatty acid oxidation. It has been suggested recently that diminished branched-chain amino acid (BCAA) catabolism impairs the use of fatty acid oxidation products (acetyl-CoA) by causing “anaplerotic stress” due to reduced levels of BCAA-derived tricarboxylic cycle (TCA) intermediates [9].

To study the differences in etiology of diseases related to the LCFA and amino acid metabolism, differences in related metabolite levels between South-Asian Surinamese and the Dutch were studied as well as changes in metabolite levels over age. We hypothesized that the LCFA and amino acid metabolism, as reflected in
plasma concentrations of acylcarnitines and amino acids, is either dysregulated from
da younger age in South-Asians than in Europeans or increases more rapidly with
age. A previous study by Tillin et al. found that serum concentrations of the amino
acids isoleucine, phenylalanine, tyrosine and alanine were higher in South-Asian
men than among European men [10]. In addition, a small study found differences in
amino acids and acylcarnitines between middle aged men and women of South-
Asian and European descent [11]. In our cross-sectional study, we addressed the
following questions; (1) How do acylcarnitines and amino acids differ by age in 18-70
year old Dutch and South-Asian Surinamese men and women living in Amsterdam
the Netherlands? (2) Do age-trends in acylcarnitines and amino acids differ between
these ethnic groups?

Methods

Population

We used baseline data of the Healthy Life in an Urban Setting (HELIUS) study,
collected between 2011 and 2015. HELIUS is a multi-ethnic cohort study among six
ethnic groups living in Amsterdam. A detailed description of the design was
previously published [12, 13]. In brief, participants were randomly sampled from the
municipal register, stratified by ethnicity. Questionnaires, physical examinations, and
biological samples were obtained. Full data were collected among 22,165
participants, from whom we selected those of Dutch and South-Asian Surinamese
ethnicity (n=7,607). We then excluded participants who did not provide permission for
data linkage or storage of biological material (n=671), and those who had less than
two vials of EDTA-plasma available in the biobank (n=186). In addition, participants
with T2D based on self-report, increased fasting glucose (≥7.0 mmol/L), increased HbA1c (≥ 48 mmol/mol) or use of glucose lowering medication were excluded (n=773), because the current study is part of a HELIUS sub-study aimed at studying causes of incident T2D. From the 5,977 participants (3,972 of Dutch origin and 2,005 of South-Asian Surinamese origin) who remained in the study, we took a random sample of 350 participants per ethnic group in whom metabolites were determined using the sample function in the R statistical software package. HELIUS was approved by the Institutional Review Board of the Amsterdam Medical Center (MREC 10/100# 17.10.1729). All participants provided written informed consent.

Measurements

Ethnicity was defined by the individual’s country of birth combined with the parental countries of birth. Dutch ethnicity was assigned to participants born in the Netherlands, with both parents born in the Netherlands. South-Asian Surinamese ethnicity was assigned to participants born in Suriname with at least one parent born in Suriname (1st generation) or born in the Netherlands with both parents born in Suriname (2nd generation) combined with self-reported South-Asian ethnic origin.

The total reported fat intake and total energy intake were derived from an ethnic specific food frequency questionnaire which was taken among a subsample of the HELIUS cohort, as described in detail elsewhere [14]. The FFQ data were available for 259 participants of our study sample, of whom 58 participants were Dutch men, 47 South-Asian men, 67 Dutch women and 87 South-Asian women.

Laboratory methods
Blood was collected after a fasting period of at least 10 hours. Acylcarnitines and free carnitine were determined in plasma by tandem-MS as described previously [15]. Amino acids were determined in plasma by tandem-MS as previously described [16].

Statistical analyses

We first examined the distributions of the metabolite data. Metabolites with more than 5% of the data below the detection limit were excluded from further analyses as imputation may lead to inaccuracies (acylcarnitines C5:1, C5OH, C4DC, C53M3OH, C8DC, C14OH, C16:1OH, C16OH, C18:2OH, C18:1OH and C18OH) [17]. We described the ethnic differences in these excluded metabolites by a description of the percentage of data below the detection limit per ethnic group and an overview of the median concentration of the values measured above the detection limit. For the included metabolites, we imputed half the detection limit for any measurements below the detection limit. Finally, outliers for glycine (n=1), serine (n=1) and asparagine (n=1) were regarded as missing. Then, we inspected the normal distribution of variables by plotting histograms and checking skewness and kurtosis. As many metabolites were not normally distributed, the acylcarnitines and amino acids were 10log transformed before further analysis.

Second, we made a summary score of the metabolites conducting a principal component analysis (PCA). PCA was used as the included metabolites are highly correlated, and PCA is then able to reduce the dimensionality of the dataset. We first checked the sampling adequacy by the Kaiser-Meyer-Olkin measure. Further, we checked whether the correlation between metabolites was sufficiently large with the Bartlett’s Test of Sphericity. The PCA was conducted on the log-transformed
metabolites with orthogonal rotation. Data were zero centered and scaled before analysis. The extracted principal components were characterized as the main outcome while individual metabolites were evaluated in secondary analyses. Therefore, we only corrected the analyses of individual metabolites for multiple testing using the Holm method [18]. Glutamate and glutamine and asparagine and aspartate can be converted into each other in the samples. Therefore, we additionally examined these metabolites combined. It has been suggested that levels of unsaturated acylcarnitines may be higher in South-Asian Surinamese than in the Dutch [11]. Therefore, we additionally explored the C10:1/C10 carnitine ratio as this ratio adequately reflects the relative presence of unsaturated and saturated fatty acids.

Third, we examined baseline characteristics and metabolite concentrations among men and women in each ethnic group. We calculated means and standard deviations (SD) for continuous normally distributed variables, medians and interquartile ranges for continuous non-normally distributed variables and numbers of observations and percentages for categorical variables. Ethnic differences in the means of normally distributed variables were analysed by a t-test, while ethnic differences in the medians of non-normally distributed variables were analysed by Kolmogorov-Smirnov tests. The chi-square test was used to check for ethnic differences in categorical variables. Additionally, ethnic differences in metabolite concentrations adjusted for age and for age and BMI were studied by linear regression.

Then, we analysed the association of metabolites with age. Yu et al. reported the association to be linear [19], we verified this visually in our population by plotting
scatterplots in the total population and stratified by sex. We analysed the association of age with metabolites by linear regression, in which the metabolite concentration was extrapolated to age zero years and was set to 100%. In addition, the interaction of age with sex was checked due to indications of sexual dimorphism in the metabolome [20]. This was done by adding an interaction term between age and sex. As we found an interaction between age and sex, we stratified for sex in all analyses. We then adjusted for BMI in our models as Yu et al. indicated that BMI was significantly correlated with both age and metabolite concentrations [19]. Because BMI may reflect different levels of intra-abdominal fat storage in European than in South-Asian populations we checked whether results for PC1 and PC2 were robust when additionally adjusted for waist-to-hip ratio (WHR). Subsequently, we checked whether the association between metabolites and age differed by ethnic group by adding an interaction term between age and ethnicity in our models. Finally, metabolite levels may be influenced by the amount of substrate available. Therefore, we checked whether the results were consistent when adjusted for fat and energy intake. This was done in a subgroup for whom a food frequency questionnaire (n=259) was available. All analyses were conducted in R studio version 0.99.903 [21].

Results

Baseline characteristics

The mean age among Dutch men (46.6; SD 13.4) was higher than among South-Asian Surinamese men (43.1; SD 12.7), while women of both ethnic groups were of
similar age (Supplemental Material 1). Mean BMI was lower among Dutch women
than among South-Asian Surinamese women, but did not differ among men. Fat and
energy intake, available for a subset of the population, were lower among South-
Asian Surinamese than among Dutch in both men and women.

**PCA**

The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.90, with all scores
above 0.50 (range 0.53-0.98). Moreover, the Bartlett’s Test of Sphericity showed that
the correlation between metabolites was sufficiently large to perform a PCA $\chi^2 (946)$
= 24084, p<0.001. The scree plot (Supplemental Material 2) showed an inflexion at
the third principal component (PC), therefore the first two components were retained.
These could explain 40.1% of the variance in the data. Table 1 shows the factor
loadings after rotation. The items that cluster on the same components suggest that
PC1 represents the metabolites reflective of the acylcarnitine metabolism, while PC2
represents the metabolites reflective of the amino acid metabolism.

**Baseline metabolite concentrations**

Some ethnic differences in metabolite concentrations were observed. PC1
(acylcarnitine) indicated that metabolite concentrations were higher among South-
Asian Surinamese men than among Dutch men (Table 2), while PC2 (amino acids)
indicated higher metabolite concentrations in both South-Asian Surinamese men and
women than Dutch men and women. Similar results were found for the age-adjusted
analyses (data not shown), and the age- and BMI-adjusted analyses (Supplemental
Material 3).
Similarly, ethnic differences in individual metabolite concentrations were observed (Table 2), although the direction of the differences varied. Free carnitine concentrations were higher among South-Asian Surinamese participants than Dutch participants. Moreover, some medium- and long-chain acylcarnitine concentrations, in particular C10:1, C14:2 and C18:2, were higher among South-Asian Surinamese than Dutch men and women, others (e.g. C16:1, C18:1 and C18 in men) were significantly lower in South-Asian Surinamese than in Dutch participants. This is also illustrated by the significantly elevated C10:1/C10:0 ratio in South-Asian Surinamese participants.

For individual amino acids, larger ethnic differences were observed among men than among women (Table 3), but the directionality of the results was similar in both sexes. Most amino acid concentrations were higher among South-Asian Surinamese than among Dutch, but glycine and glutamine (in men) concentrations were lower.

Ethnic differences in metabolite concentrations remained similar after age-adjustment (data not shown) or age- and BMI-adjustment (Supplemental Material 4), although there were some shifts in significance levels. Finally, there were no major ethnic differences in baseline concentrations of the excluded metabolites (Supplemental Material 5).

Metabolite patterns by age

No differences in age trends for metabolites were observed by ethnicity in either men or women (Tables 3 and 4). PC1, reflective of the acylcarnitine metabolism, decreased by age in all groups (Fig 1). Because of the negative factor loadings of
this principal component, this implies an increase in acylcarnitine levels with age. This was also reflected in the individual acylcarnitines, particularly in women. In women, age trends in most acylcarnitines were observed, especially in those reflected in PC1. For instance, C16:1-carnitine increased with age (113.3% (95% CI: 107.7 ; 119.1) per 10 years increment in age in Dutch women). Similar results were observed for South-Asian women (111.0% (95% CI: 106.2 ; 116.1)).

Age patterns for PC2 were less clear (Fig 1). No age trend was observed in men, while in women PC2 increased (statistically significant in South-Asian Surinamese women). Similarly, only citrulline and glutamine increased with age in both men, while citrulline, glutamine, phenylalanine, tyrosine, glycine, ornithine and arginine concentrations increased in women. Asparagine, with a relatively low loading on PC2, on the other hand, decreased with age in women.

Sensitivity analyses

All analyses were repeated while adjusted for fat intake and adjusted for energy intake in a subgroup of participants with a food frequency questionnaire available (data not shown). The ethnic differences in PC1, PC2, and the acylcarnitine levels remained largely similar to the main results, as did the age trends. However, many acylcarnitine levels were no longer statistically significantly different between ethnic groups. Moreover, the magnitude of the changes in metabolite concentrations by age decreased. For instance, the C18:0-carnitine concentration was 112.9% (95%-CI: 108.5; 117.4) for a ten years increase in age compared to the baseline value in Dutch women in the unadjusted analysis, while in the for fat adjusted analysis it was 101.1% (100.5; 101.7). Nevertheless, the directionality of the results was similar to
the main results. Additional adjustment of PC1 and PC2 for WHR did not alter the results (data not shown).

**Discussion**

Our study suggests that concentrations of some acylcarnitines and amino acids, reflective of a dysfunctional LCFA metabolism, are higher among South-Asian Surinamese men than Dutch men. In women, amino acid concentrations were higher among South-Asian than Dutch participants. Most metabolite concentrations increase with age, especially in women, and trends are similar in South-Asian Surinamese and Dutch participants. Together, this suggests that metabolic profiles differ between both ethnic groups at any age in adulthood, but the progression of dysregulation of the LCFA metabolism with aging is similar in both ethnic groups.

**Ethnic differences**

We found that mean amino acid levels were higher among South-Asian Surinamese than Dutch populations, this confirms previous results by Tillin et al. that showed aromatic amino acid concentrations to be higher among South-Asian men than European men living in the UK [10], and it expands the evidence to women. Similar ethnic differences in amino acid concentrations were also reported in a small study by van Valkengoed et al. [11]. Like in the two previous studies [10, 11], the branched-chain amino acid levels (leucine, isoleucine and valine) were higher in South-Asian Surinamese than Dutch participants. Additionally, it is worth noting that the pattern of the elevated amino acids in South-Asian Surinamese (elevations of methionine, alanine, phenylalanine, tyrosine and lysine) corresponds to that of what is seen in
persons with reduced liver function [22], possibly reflecting altered liver amino acid metabolism. No data were available to adjust for liver function. It can also not be excluded that the dietary composition could cause the observed ethnic differences.

In addition, the study by van Valkengoed et al. reported higher mono- and polyunsaturated acylcarnitine concentrations in South-Asian Surinamese than in Dutch living in the Netherlands [11]. Furthermore, lower concentrations in saturated acylcarnitine concentrations among South-Asian Surinamese than among Dutch were reported. Our study showed similar trends, for instance C18:0-carnitine concentrations were lower among South-Asian Surinamese than Dutch participants while C18:2-carnitine concentrations were higher. Also, the C10:1/C10:0 ratio, which represents the relative abundance of unsaturated vs saturated fatty acids, is clearly higher in South-Asian Surinamese participants. This is likely partially reflective of ethnic differences in dietary intake, and is consistent with the observation that South-Asian Surinamese consume more (poly)unsaturated fatty acids than the Dutch [23].

Our analyses were adjusted for BMI as the study by Yu et al. indicated metabolites to be correlated with both age and BMI [19]. The correlation between BMI and metabolites may be due to storage of fat in the body. However, BMI does not reflect storage of intra-abdominal fat content similarly in Europeans and South-Asians [24]. To account for ethnic differences in distribution of bodyfat storage we adjusted for WHR in sensitivity analyses, however, this did not alter our results. We, therefore, do not expect differences in intra-abdominal fat storage to have affected our results, but future studies may measure fat content directly or use biomarkers such as adiponectin for confirmation.
**Age-related differences**

Acylcarnitines concentrations originating from fatty acid degradation increased with age, especially in women. Up to now, not many studies that investigated age-trends in acylcarnitines in adult populations are available. Consistent with our study, Yu et al. showed a clear linear increase in most acylcarnitine concentrations with age in populations from Germany and the UK [19]. Yu et al. also reported on amino acids and in accordance with that study, we also found large sex differences and age-trends in amino acids which were most prominent among women. Our study suggested a (non-significant) reduction in tryptophan by age, as did the study by Yu et al. Additionally, the study by Yu et al. suggested a reduction in histidine, which was not measured in our study.

Our results for amino acids were partly consistent with a study by Kouchiwa et al. who showed both increases and decreases in amino acid concentrations with age in a Japanese population [25]. Most of our results were consistent with this study, although not all findings were statistically significantly replicated [25]. Some amino acids, e.g. serine, did not show clear consistent age trends. The small differences in metabolite concentrations between both studies may be related to dietary intake as intake differs between ethnic groups and by age [26, 27]. To study the effect of dietary intake on differences in metabolite concentrations, analyses were additionally adjusted for dietary intake in sensitivity analyses in a subset of the population. This did not affect our results and we, thus, assume that dietary intake has limited effect on our findings. The study of Kouchiwa et al. did, however, not adjust for dietary intake and may therefore not be completely comparable to ours. The study was
conducted in Japan where dietary changes by age may be different from those in the Netherlands, and may, therefore, have affected metabolite trends by age differently.

The increase of LCFA with age may indicate a progressively dysregulated metabolism. This may reflect decreasing renal function. However additional adjustment for renal function did not alter our results (data not shown).

The dysregulation of the LCFA metabolism may be associated with the increase in incidence of chronic diseases such as T2D and CVD with age, as previous studies indicated associations between both acylcarnitines and amino acids with T2D and CVD [5-7, 10]. We cannot, however, exclude reversed causality, pre-diabetes and CVD may cause disturbances in the LCFA metabolism as well. Furthermore, risk factors such as dietary intake may influence both metabolite levels as the risk for T2D and CVD. This study is the first to show that the observed age-trends in acylcarnitines and amino acids are similar in South-Asian Surinamese and Dutch populations. However, mean metabolite concentrations are higher in South-Asians than Dutch already at the start of adulthood. As these higher metabolite levels are associated with T2D and CVD, our findings are consistent with the observation that South-Asians develop T2D and CVD at a younger age than the Dutch [3, 4]. As the age of participants in the study population was limited to 18-70 year adults, our results cannot be extrapolated to younger age groups.

Future studies are needed to investigate whether ethnic differences in metabolite concentrations exist from birth or develop during childhood [28]. If elucidated how and when during the life course differences in metabolite concentrations arise, this may help to identify how to prevent disturbance of the LCFA metabolism. Differences
in metabolite concentrations may also be used to identify those at high risk to
develop type 2 diabetes and cardiovascular disease. Currently, there is a lack of
quality biomarkers that can distinguish those at higher risk for type 2 diabetes and
cardiovascular disease. But if markers are identified this may help to target high risk
populations. Furthermore, this will possible help to develop preventive strategies
aimed to delay or prevent metabolic disturbances.

Limitations

Our study is not exempt of limitations. First, the results may be affected by selective
participation or inclusion of participants. Although participants within HELIUS were
randomly selected from the general population, recruitment rates into the study were
low (28%). However, comparisons of responders and non-responders suggest that
participants represent the general population [13]. We further compared HELIUS
participants who consented to data linkage and stored physical material to those who
did not, no clear differences were observed (data not shown). We therefore assume
that our included population represents the general population. However, we limited
our analyses to participants who were not diagnosed with T2D at baseline. This may
potentially have excluded participants with most dysregulated LCFA metabolism,
reflected in serum metabolite levels and may also explain differences in metabolite
concentrations with previous studies. Because the prevalence of T2D is higher
among South-Asian Surinamese participants than among Dutch participants [29],
differences in metabolite profiles between both ethnic groups may be larger in the
overall population.
Second, we used a cross-sectional study design, therefore our results may reflect cohort-effects, characteristics of older participants may be different from younger ones. Although this effect is not likely because of the observed linear relationship between age and metabolites, longitudinal studies are required to document age related effects.

Finally, the measured metabolite concentrations may have been insufficient to adequately characterize differences between the ethnic groups. Metabolite levels may fluctuate. Our analysis could therefore have benefitted from multiple measurements. Additionally, although plasma metabolite concentrations measured in this study reflect the dysregulation of the LCFA metabolism, the measured levels may not necessarily reflect the role of the processes in all individual organ compartments and its interplay in the body [30]. Metabolite concentrations measured in specific target organs may, therefore, yield different results.

**Concluding remarks**

We studied the differences in plasma metabolites related to the LCFA metabolism between South-Asians and Europeans and their development with age. We found that many metabolite concentrations reflective of a dysregulated LFCA metabolism are higher among South-Asian Surinamese than among Dutch participants. With aging, metabolite concentrations increase at a similar rate in both ethnic groups, indicating that differences in LCFA metabolism between South-Asian Surinamese and Dutch exist already at the start of adulthood. It remains to be established whether the observed differences in LCFA metabolism potentially marks or explains
the earlier onset of type 2 diabetes and cardiovascular disease among South-Asian Surinamese.

**Competing interests**

The authors have no competing interests to declare.

**Author contributions**

MM and IGMV designed the study. RP established the HELIUS study cohort. MM conducted the analyses and wrote the manuscript. IGMV and FV contributed to the writing. CCM, RP, FV and IGMV reviewed the manuscript. All authors read and approved the final manuscript.

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