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

67 plasma concentrations of acylcarnitines and amino acids, is either dysregulated from

68 a younger age in South-Asians than in Europeans or increases more rapidly with

69 age. A previous study by Tillin *et al.* found that serum concentrations of the amino

70 acids isoleucine, phenylalanine, tyrosine and alanine were higher in South-Asian

71 men than among European men [10]. In addition, a small study found differences in

72 amino acids and acylcarnitines between middle aged men and women of South-

73 Asian and European descent [11]. In our cross-sectional study, we addressed the

74 following questions; (1) How do acylcarnitines and amino acids differ by age in 18-70

75 year old Dutch and South-Asian Surinamese men and women living in Amsterdam

76 the Netherlands? (2) Do age-trends in acylcarnitines and amino acids differ between

77 these ethnic groups?

78 **Methods**

79 *Population*

80 We used baseline data of the Healthy Life in an Urban Setting (HELIUS) study,

81 collected between 2011 and 2015. HELIUS is a multi-ethnic cohort study among six

82 ethnic groups living in Amsterdam. A detailed description of the design was

83 previously published [12, 13]. In brief, participants were randomly sampled from the

84 municipal register, stratified by ethnicity. Questionnaires, physical examinations, and

85 biological samples were obtained. Full data were collected among 22,165

86 participants, from whom we selected those of Dutch and South-Asian Surinamese

87 ethnicity (n=7,607). We then excluded participants who did not provide permission for

88 data linkage or storage of biological material (n=671), and those who had less than

89 two vials of EDTA-plasma available in the biobank (n=186). In addition, participants

90 with T2D based on self-report, increased fasting glucose (≥7.0 mmol/L), increased

91 HbA1c (≥ 48 mmol/mol) or use of glucose lowering medication were excluded

92 (n=773), because the current study is part of a HELIUS sub-study aimed at studying

93 causes of incident T2D. From the 5,977 participants (3,972 of Dutch origin and 2,005

94 of South-Asian Surinamese origin) who remained in the study, we took a random

95 sample of 350 participants per ethnic group in whom metabolites were determined

96 using the sample function in the R statistical software package. HELIUS was

97 approved by the Institutional Review Board of the Amsterdam Medical Center (MREC

98 10/100# 17.10.1729). All participants provided written informed consent.

99 *Measurements*

100 Ethnicity was defined by the individual's country of birth combined with the parental

101 countries of birth. Dutch ethnicity was assigned to participants born in the

102 Netherlands, with both parents born in the Netherlands. South-Asian Surinamese

103 ethnicity was assigned to participants born in Suriname with at least one parent born

104 in Suriname (1st generation) or born in the Netherlands with both parents born in

105 Suriname (2nd generation) combined with self-reported South-Asian ethnic origin.

106 The total reported fat intake and total energy intake were derived from an ethnic

107 specific food frequency questionnaire which was taken among a subsample of the

108 HELIUS cohort, as described in detail elsewhere [14]. The FFQ data were available

109 for 259 participants of our study sample, of whom 58 participants were Dutch men,

110 47 South-Asian men, 67 Dutch women and 87 South-Asian women.

111 *Laboratory methods*

112 Blood was collected after a fasting period of at least 10 hours. Acylcarnitines and free

113 carnitine were determined in plasma by tandem-MS as described previously [15].

114 Amino acids were determined in plasma by tandem-MS as previously described [16].

115 *Statistical analyses*

116 We first examined the distributions of the metabolite data. Metabolites with more than

117 5% of the data below the detection limit were excluded from further analyses as

118 imputation may lead to inaccuracies (acylcarnitines C5:1, C5OH, C4DC, C53M3OH,

119 C8DC, C14OH, C16:1OH, C16OH, C18:2OH, C18:1OH and C18OH) [17]. We

120 described the ethnic differences in these excluded metabolites by a description of the

121 percentage of data below the detection limit per ethnic group and an overview of the

122 median concentration of the values measured above the detection limit. For the

123 included metabolites, we imputed half the detection limit for any measurements

124 below the detection limit. Finally, outliers for glycine (n=1), serine (n=1) and

125 asparagine (n=1) were regarded as missing. Then, we inspected the normal

126 distribution of variables by plotting histograms and checking skewness and kurtosis.

127 As many metabolites were not-normally distributed, the acylcarnitines and amino

128 acids were 10log transformed before further analysis.

129 Second, we made a summary score of the metabolites conducting a principal

130 component analysis (PCA). PCA was used as the included metabolites are highly

131 correlated, and PCA is then able to reduce the dimensionality of the dataset. We first

132 checked the sampling adequacy by the Kaiser-Meyer-Olkin measure. Further, we

133 checked whether the correlation between metabolites was sufficiently large with the

134 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

158 scatterplots in the total population and stratified by sex. We analysed the association

159 of age with metabolites by linear regression, in which the metabolite concentration

160 was extrapolated to age zero years and was set to 100%. In addition, the interaction

161 of age with sex was checked due to indications of sexual dimorphism in the

162 metabolome [20]. This was done by adding an interaction term between age and sex.

163 As we found an interaction between age and sex, we stratified for sex in all analyses.

164 We then adjusted for BMI in our models as Yu *et al.* indicated that BMI was

165 significantly correlated with both age and metabolite concentrations [19]. Because

166 BMI may reflect different levels of intra-abdominal fat storage in European than in

167 South-Asian populations we checked whether results for PC1 and PC2 were robust

168 when additionally adjusted for waist-to-hip ratio (WHR). Subsequently, we checked

169 whether the association between metabolites and age differed by ethnic group by

170 adding an interaction term between age and ethnicity in our models.

171 Finally, metabolite levels may be influenced by the amount of substrate available.

172 Therefore, we checked whether the results were consistent when adjusted for fat and

173 energy intake. This was done in a subgroup for whom a food frequency questionnaire

174 (n=259) was available. All analyses were conducted in R studio version 0.99.903

175 [21].

176 **Results**

177 *Baseline characteristics*

178 The mean age among Dutch men (46.6; SD 13.4) was higher than among South-

179 Asian Surinamese men (43.1; SD 12.7), while women of both ethnic groups were of

180 similar age (Supplemental Material 1). Mean BMI was lower among Dutch women

181 than among South-Asian Surinamese women, but did not differ among men. Fat and

182 energy intake, available for a subset of the population, were lower among South-

183 Asian Surinamese than among Dutch in both men and women.

184 *PCA*

185 The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.90, with all scores

186 above 0.50 (range 0.53-0.98). Moreover, the Bartlett's Test of Sphericity showed that

187 the correlation between metabolites was sufficiently large to perform a PCA χ^2 (946)

188 = 24084, p<0.001. The scree plot (Supplemental Material 2) showed an inflexion at

189 the third principal component (PC), therefore the first two components were retained.

190 These could explain 40.1% of the variance in the data. Table 1 shows the factor

191 loadings after rotation. The items that cluster on the same components suggest that

192 PC1 represents the metabolites reflective of the acylcarnitine metabolism, while PC2

193 represents the metabolites reflective of the amino acid metabolism.

194 *Baseline metabolite concentrations*

195 Some ethnic differences in metabolite concentrations were observed. PC1

196 (acylcarnitine) indicated that metabolite concentrations were higher among South-

197 Asian Surinamese men than among Dutch men (Table 2), while PC2 (amino acids)

198 indicated higher metabolite concentrations in both South-Asian Surinamese men and

199 women than Dutch men and women. Similar results were found for the age-adjusted

200 analyses (data not shown), and the age- and BMI-adjusted analyses (Supplemental

201 Material 3).

202 Similarly, ethnic differences in individual metabolite concentrations were observed

203 (Table 2), although the direction of the differences varied. Free carnitine

204 concentrations were higher among South-Asian Surinamese participants than Dutch

205 participants. Moreover, some medium- and long-chain acylcarnitine concentrations,

206 in particular C10:1, C14:2 and C18:2, were higher among South-Asian Surinamese

207 than Dutch men and women, others (e.g. C16:1, C18:1 and C18 in men) were

208 significantly lower in South-Asian Surinamese than in Dutch participants. This is also

209 illustrated by the significantly elevated C10:1/C10:0 ratio in South-Asian Surinamese

210 participants.

211 For individual amino acids, larger ethnic differences were observed among men than

212 among women (Table 3), but the directionality of the results was similar in both

213 sexes. Most amino acid concentrations were higher among South-Asian Surinamese

214 than among Dutch, but glycine and glutamine (in men) concentrations were lower.

215 Ethnic differences in metabolite concentrations remained similar after age-adjustment

216 (data not shown) or age- and BMI-adjustment (Supplemental Material 4), although

217 there were some shifts in significance levels. Finally, there were no major ethnic

218 differences in baseline concentrations of the excluded metabolites (Supplemental

219 Material 5).

220 *Metabolite patterns by age*

221 No differences in age trends for metabolites were observed by ethnicity in either men

222 or women (Tables 3 and 4). PC1, reflective of the acylcarnitine metabolism,

223 decreased by age in all groups (Fig 1). Because of the negative factor loadings of

224 this principal component, this implies an increase in acylcarnitine levels with age.

225 This was also reflected in the individual acylcarnitines, particularly in women. In

226 women, age trends in most acylcarnitines were observed, especially in those

227 reflected in PC1. For instance, C16:1-carnitine increased with age (113.3% (95% CI:

228 107.7 ; 119.1) per 10 years increment in age in Dutch women). Similar results were

229 observed for South-Asian women (111.0% (95% CI: 106.2 ; 116.1)).

230 Age patterns for PC2 were less clear (Fig 1). No age trend was observed in men,

231 while in women PC2 increased (statistically significant in South-Asian Surinamese

232 women). Similarly, only citrulline and glutamine increased with age in both men, while

233 citrulline, glutamine, phenylalanine, tyrosine, glycine, ornithine and arginine

234 concentrations increased in women. Asparagine, with a relatively low loading on

235 PC2, on the other hand, decreased with age in women.

236 *Sensitivity analyses*

237 All analyses were repeated while adjusted for fat intake and adjusted for energy

238 intake in a subgroup of participants with a food frequency questionnaire available

239 (data not shown). The ethnic differences in PC1, PC2, and the acylcarnitine levels

240 remained largely similar to the main results, as did the age trends. However, many

241 acylcarnitine levels were no longer statistically significantly different between ethnic

242 groups. Moreover, the magnitude of the changes in metabolite concentrations by age

243 decreased. For instance, the C18:0-carnitine concentration was 112.9% (95%-CI:

244 108.5; 117.4) for a ten years increase in age compared to the baseline value in Dutch

245 women in the unadjusted analysis, while in the for fat adjusted analysis it was

246 101.1% (100.5; 101.7). Nevertheless, the directionality of the results was similar to

247 the main results. Additional adjustment of PC1 and PC2 for WHR did not alter the

248 results (data not shown).

249 **Discussion**

250 Our study suggests that concentrations of some acylcarnitines and amino acids,

251 reflective of a dysfunctional LCFA metabolism, are higher among South-Asian

252 Surinamese men than Dutch men. In women, amino acid concentrations were higher

253 among South-Asian than Dutch participants. Most metabolite concentrations increase

254 with age, especially in women, and trends are similar in South-Asian Surinamese and

255 Dutch participants. Together, this suggests that metabolic profiles differ between both

256 ethnic groups at any age in adulthood, but the progression of dysregulation of the

257 LCFA metabolism with aging is similar in both ethnic groups.

258 *Ethnic differences*

259 We found that mean amino acid levels were higher among South-Asian Surinamese

260 than Dutch populations, this confirms previous results by Tillin *et al.* that showed

261 aromatic amino acid concentrations to be higher among South-Asian men than

262 European men living in the UK [10], and it expands the evidence to women. Similar

263 ethnic differences in amino acid concentrations were also reported in a small study

264 by van Valkengoed *et al.* [11]. Like in the two previous studies [10, 11], the branched-

265 chain amino acid levels (leucine, isoleucine and valine) were higher in South-Asian

266 Surinamese than Dutch participants. Additionally, it is worth noting that the pattern of

267 the elevated amino acids in South-Asian Surinamese (elevations of methionine,

268 alanine, phenylalanine, tyrosine and lysine) corresponds to that of what is seen in

269   persons with reduced liver function [22], possibly reflecting altered liver amino acid

270   metabolism. No data were available to adjust for liver function. It can also not be

271   excluded that the dietary composition could cause the observed ethnic differences.

272   In addition, the study by van Valkengoed *et al.* reported higher mono- and

273   polyunsaturated acylcarnitine concentrations in South-Asian Surinamese than in

274   Dutch living in the Netherlands [11]. Furthermore, lower concentrations in saturated

275   acylcarnitine concentrations among South-Asian Surinamese than among Dutch

276   were reported. Our study showed similar trends, for instance C18:0-carnitine

277   concentrations were lower among South-Asian Surinamese than Dutch participants

278   while C18:2-carnitine concentrations were higher. Also, the C10:1/C10:0 ratio, which

279   represents the relative abundance of unsatured vs saturated fatty acids, is clearly

280   higher in South-Asian Surinamese participants. This is likely partially reflective of

281   ethnic differences in dietary intake, and is consistent with the observation that South-

282   Asian Surinamese consume more (poly)unsaturated fatty acids than the Dutch [23].

283   Our analyses were adjusted for BMI as the study by Yu et al. indicated metabolites to

284   be correlated with both age and BMI [19]. The correlation between BMI and

285   metabolites may be due to storage of fat in the body. However, BMI does not reflect

286   storage of intra-abdominal fat content similarly in Europeans and South-Asians [24].

287   To account for ethnic differences in distribution of bodyfat storage we adjusted for

288   WHR in sensitivity analyses, however, this did not alter our results. We, therefore, do

289   not expect differences in intra-abdominal fat storage to have affected our results, but

290   future studies may measure fat content directly or use biomarkers such as

291   adiponectin for confirmation.

Age-related differences

293 Acylcarnitines concentrations originating from fatty acid degradation increased with

294 age, especially in women. Up to now, not many studies that investigated age-trends

295 in acylcarnitines in adult populations are available. Consistent with our study, Yu *et*

296 *al.* showed a clear linear increase in most acylcarnitine concentrations with age in

297 populations from Germany and the UK [19]. Yu *et al.* also reported on amino acids

298 and in accordance with that study, we also found large sex differences and age-

299 trends in amino acids which were most prominent among women. Our study

300 suggested a (non-significant) reduction in tryptophan by age, as did the study by Yu

301 *et al.* Additionally, the study by Yu et al. suggested a reduction in histidine, which was

302 not measured in our study.

303 Our results for amino acids were partly consistent with a study by Kouchiwa *et al.*

304 who showed both increases and decreases in amino acid concentrations with age in

305 a Japanese population [25]. Most of our results were consistent with this study,

306 although not all findings were statistically significantly replicated [25]. Some amino

307 acids, e.g. serine, did not show clear consistent age trends. The small differences in

308 metabolite concentrations between both studies may be related to dietary intake as

309 intake differs between ethnic groups and by age [26, 27]. To study the effect of

310 dietary intake on differences in metabolite concentrations, analyses were additionally

311 adjusted for dietary intake in sensitivity analyses in a subset of the population. This

312 did not affect our results and we, thus, assume that dietary intake has limited effect

313 on our findings. The study of Kouchiwa *et al.* did, however, not adjust for dietary

314 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

338 in metabolite concentrations may also be used to identify those at high risk to

339 develop type 2 diabetes and cardiovascular disease. Currently, there is a lack of

340 quality biomarkers that can distinguish those at higher risk for type 2 diabetes and

341 cardiovascular disease. But if markers are identified this may help to target high risk

342 populations. Furthermore, this will possible help to develop preventive strategies

343 aimed to delay or prevent metabolic disturbances.

344 *Limitations*

345 Our study is not exempt of limitations. First, the results may be affected by selective

346 participation or inclusion of participants. Although participants within HELIUS were

347 randomly selected from the general population, recruitment rates into the study were

348 low (28%). However, comparisons of responders and non-responders suggest that

349 participants represent the general population [13]. We further compared HELIUS

350 participants who consented to data linkage and stored physical material to those who

351 did not, no clear differences were observed (data not shown). We therefore assume

352 that our included population represents the general population. However, we limited

353 our analyses to participants who were not diagnosed with T2D at baseline. This may

354 potentially have excluded participants with most dysregulated LCFA metabolism,

355 reflected in serum metabolite levels and may also explain differences in metabolite

356 concentrations with previous studies. Because the prevalence of T2D is higher

357 among South-Asian Surinamese participants than among Dutch participants [29],

358 differences in metabolite profiles between both ethnic groups may be larger in the

359 overall population.

360 Second, we used a cross-sectional study design, therefore our results may reflect

361 cohort-effects, characteristics of older participants may be different from younger

362 ones. Although this effect is not likely because of the observed linear relationship

363 between age and metabolites, longitudinal studies are required to document age

364 related effects.

365 Finally, the measured metabolite concentrations may have been insufficient to

366 adequately characterize differences between the ethnic groups. Metabolite levels

367 may fluctuate. Our analysis could therefore have benefitted from multiple

368 measurements. Additionally, although plasma metabolite concentrations measured in

369 this study reflect the dysregulation of the LCFA metabolism, the measured levels

370 may not necessarily reflect the role of the processes in all individual organ

371 compartments and its interplay in the body [30]. Metabolite concentrations measured

372 in specific target organs may, therefore, yield different results.

373 **Concluding remarks**

374 We studied the differences in plasma metabolites related to the LCFA metabolism

375 between South-Asians and Europeans and their development with age. We found

376 that many metabolite concentrations reflective of a dysregulated LFCA metabolism

377 are higher among South-Asian Surinamese than among Dutch participants. With

378 aging, metabolite concentrations increase at a similar rate in both ethnic groups,

379 indicating that differences in LCFA metabolism between South-Asian Surinamese

380 and Dutch exist already at the start of adulthood. It remains to be established

381 whether the observed differences in LCFA metabolism potentially marks or explains

382 the earlier onset of type 2 diabetes and cardiovascular disease among South-Asian

383 Surinamese.

Competing interests

385 The authors have no competing interests to declare.

Author contributions

387 MM and IGMV designed the study. RP established the HELIUS study cohort. MM

388 conducted the analyses and wrote the manuscript. IGMV and FV contributed to the

389 writing. CCM, RP, FV and IGMV reviewed the manuscript. All authors read and

390 approved the final manuscript.

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