Plasma cholesteryl ester fatty acids do not mediate the association of ethnicity with type 2 diabetes: results from the HELIUS study

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List of abbreviations:

95% CI – 95% confidence interval; CE – cholesteryl ester; CEFA - cholesteryl ester fatty acid; DAG – directed acyclic graph; FA - fatty acid; FBG – fasting blood glucose; FFQ – food frequency questionnaire; HELIUS - Healthy Life in an Urban Setting; MET - metabolic equivalent; OR – odds ratio; SFA – saturated fatty acid; SQUASH - Short Questionnaire to Assess Health; T2D - type 2 diabetes.

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Abstract

Scope: Ethnic minority groups have a higher risk of type 2 diabetes (T2D) than the host population. Our aim was to identify whether plasma cholesteryl ester fatty acids (CEFA) mediate the ethnic differences in type 2 diabetes.

Methods and results: We included 202 Dutch, 206 South-Asian Surinamese, 205 African Surinamese, 215 Turkish and 213 Moroccan origin participants of the HELIUS study (Amsterdam, the Netherlands). Logistic regression was used to determine the associations between plasma CEFA and T2D. Mediation analysis was used to identify whether CEFA contributed to the association between ethnicity and T2D. We adjusted for ethnicity, age, sex, smoking, physical activity and BMI. Associations between plasma CEFA and T2D were similar across all ethnic groups. Although differences in plasma CEFA across ethnic groups were observed, CEFA did not mediate the differences in T2D prevalence between ethnic groups.

Conclusion: Although ethnic differences in plasma CEFA were found and CEFA were associated with T2D, CEFA did not contribute to the difference in T2D prevalence between ethnic groups. If confirmed, this implies that maintenance of the more beneficial CEFA profiles in the non-Dutch ethnic groups may be encouraged to prevent an even higher prevalence of T2D in these groups.

Introduction

The burden of type 2 diabetes (T2D) differs greatly across ethnic groups living in the same geographical location [1-4]. Disparities were for instance observed between groups of Dutch, Ghanaian, Turkish, Moroccan, South-Asian Surinamese and African Surinamese ethnicity living in the Netherlands [5-7]. Results from the

HELIUS study conducted in Amsterdam, for instance, showed that ethnic minority populations are at increased risk for T2D compared to the Dutch host population [8]. The prevalence of T2D among non-Dutch ethnic groups was, adjusted for all relevant covariates, three to five times higher than among the Dutch. The causes of these differences in prevalence of T2D have not yet been fully elucidated.

Previous work has shown that differences in the amount and type of lipids in the diet may be associated with insulin resistance and T2D [9, 10]. Proposed mechanisms for this include the differences in liquidity of various fatty acids (FAs), which influences metabolic regulation [11]. Cell membranes with a higher amount of unsaturated FAs are more fluid than membranes with a lower amount of unsaturated FAs, which influences the responsiveness to insulin [11]. Moreover, individual FAs may activate or reduce inflammatory immune cells or reduce the storage capacity of β-cells [12-15].

There are several biomarkers of dietary lipid intake. In general, biomarkers better reflect FA that can only be derived exogenously (e.g essential fatty acids) than those that are also produced endogenously [16]. In the current study, we used cholesteryl ester fatty acids (CEFA) in plasma as the biomarker. CEFA reflect dietary fat intake during the past weeks with a low variance [16]. Differences in lipid intake between ethnic groups exist due to differences in dietary habits of various ethnic groups, this may also be reflected in biomarkers. In general, traditional diets of non-European ethnic groups are healthier than European diets. A dietary shift towards less healthy diets is observed after migration to European countries [17]. However, previous studies have shown that several ethnic minority groups, including South-Asians and Turks, living in European countries had more beneficial lipid intake and lipid profiles

than the host population [18, 19]. These observations may be similar for ethnic minority groups living in the Netherlands, but are not yet described.

There are indications that a less healthy diet is more detrimental for the health of specific ethnic groups. A 5-day experimental high-fat, high-calorie diet showed for instance reduced insulin sensitivity, increased fasting glucose and insulin concentrations in South Asians but not in Caucasians [20]. Moreover, previous work among Caucasians, East Asians and South Asians living in Canada suggested that associations between FAs and markers of insulin resistance differ across ethnic groups [21]. Our study set out to further investigate the ethnic differences in plasma fatty acids and the association with T2D among the largest ethnic groups living in Amsterdam, the Netherlands, and to study whether these may explain ethnic disparities in prevalence of T2D. First, we described the differences in plasma CEFA percentages between people of Dutch, Turkish, Moroccan, South-Asian Surinamese and African Surinamese living in the Netherlands. Second, we investigated the association of plasma CEFA with T2D prevalence across ethnic groups. Finally, we determined whether differences in T2D prevalence between ethnic groups were mediated by differences in plasma CEFA.

Methods

Population

Baseline data, collected between 2011 and 2015, from the HELIUS study were used. HELIUS is a multi-ethnic cohort among six ethnic groups living in Amsterdam; a detailed description of the design is available elsewhere [22]. In brief, participants were randomly sampled from the municipality registry, stratified by ethnicity. Data

were collected among nearly 25.000 participants; questionnaires, physical examinations and biological samples were obtained. Within HELIUS, a dietary patterns substudy was conducted; participants that had agreed to be approached for additional research were invited to fill in a Food Frequency Questionnaire (n=5358 participants completed this questionnaire, [23]). CEFA were measured for random subsamples of participants that had filled in the FFQ within three month of having had their blood collected. The aim was to include around 200 participants (50% men) of each ethnic group. All had data on T2D available. In total, 202 participants of Dutch, 206 of South-Asian Surinamese, 205 of African Surinamese, 215 of Turkish and 213 of Moroccan ethnicity were included. HELIUS was approved by the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729) and all participants provided informed written consent.

Measurements

Ethnicity was defined by the individual's country of birth combined with the parental countries of birth. For the Dutch sample, we invited people who were born in the Netherlands and of whom both parents were born in the Netherlands. Non-Dutch ethnic origin was assigned to participants born abroad with at least one parent born abroad (1st generation) or born in the Netherlands with both parents born abroad (2nd generation). The Surinamese group was further classified according to self-reported ethnic origin into "African", "South-Asian", "Javanese" or "other".

Information on pack years of smoking and physical activity score were determined from the questionnaire. The number of packyears was calculated by multiplying the number of packs (containing 20 cigarettes) smoked a day by the number of years. Smoking cigars and pipe tobacco were also included by calculating the equivalent

rates of tobacco. The physical activity score was derived by the Short Questionnaire to Assess Health enhancing physical activity (SQUASH), which includes questions on activities at work and school, leisure time, household activities, commuting activities and other daily activities and the intensity at which the activity was executed [24]. Results of the SQUASH were converted to minutes per week and multiplied by the metabolic equivalent (MET) intensity score. Body Mass Index (BMI) was determined by dividing measured body weight (kg) by height squared (m^2). The total reported fatty acid intake and total energy intake were derived from a ethnicity specific food frequency questionnaire (FFQ, [25]).

T2D was defined by self-reported physician diagnosis and/or measured fasting blood glucose (FBG) levels of ≥ 7.0 $mmol.l^{-1}$ and/or measured HbA_{1c} levels of ≥ 48 mmol/mol and/or anti-diabetic medication use. Data on self-reported physician diagnosis was obtained from the questionnaire. Anti-diabetic medication use was recorded during the physical examination, to which participants had been asked to bring their prescribed medications. FBG and HbA_{1c} concentrations were determined from fasting blood samples. HbA_{1c} concentrations were determined by HPLC (TOSOH, Japan) in whole blood. Glucose concentrations were determined by spectrophotometry in plasma (hexokinase primary enzyme; Roche Diagnostics, Japan).

FA levels were determined in cholesteryl esters (CE) in plasma that had been stored at -20˚C until measurement by gas-liquid chromatography (Division of Human Nutrition and Epidemiology, Wageningen University). Isolated CE were incubated with acidified methanol. Peak retention times and area percentages of total CEFA were identified by using known CE standards (FAME components from Sigma (MO)

and Nuchek (MN)) and analyzed with the Agilent Technologies ChemStation software (Agilent, Amstelveen, The Netherlands). CEFA levels were expressed as percentage of the total CEFA levels. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were calculated by summing all the CEFA with 12-24 carbon atoms (n=9, 7 and 21). Individual CEFA with more than 5% of the measurements below the detection limit were excluded from the analyses, because numbers near the detection limit are considered less precise and mean values may no longer be accurate when imputation techniques are applied [26]. These CEFA were however included in the estimated total SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA levels. Due to skewed distributions of CEFA levels, fractions of CEFA levels were logit-transformed before analysis.

Statistical analyses

Baseline characteristics of the participants, as well as CEFA were presented by means and standard deviations (SD) for continuous normally distributed variables, by medians and interquartile ranges for continuous not-normally distributed variables and by numbers of observations and percentages for categorical variables. Ethnic differences in covariates and plasma CEFA levels were tested with a one-way-ANOVA combined with a Tukey-HSD post-hoc test or, where applicable, a Kruskal-Wallis and a post-hoc dunn-test. Differences between ethnic groups in plasma CEFA levels were investigated using multiple linear regression, these analyses were age, sex, smoking, physical activity and BMI adjusted.

The association of plasma CEFA levels with T2D in the overall population and per ethnic group was investigated using logistic regression and presented as odds ratios

with their corresponding 95% confidence interval (OR [95% CI]). We investigated whether the association between CEFA and T2D differed by sex by adding an interaction term between CEFA*sex in our models, due to indications that the association between FAs and T2D may be sex-specific [21]. No evidence for interaction by sex was found (data not shown). Therefore, we did not stratify for sex. We also examined the interaction between CEFA*ethnicity. We examined the consistency of the analysis with the definition for T2D not including HbA_{1c}, but the associations were consistent independent of the definition used (data not shown).

Finally, we analyzed whether differences in T2D prevalence between ethnic groups were mediated by CEFA. We used the mediation package developed for R by Imai et al. [27] to estimate which part of the total effect of ethnicity could be contributed to an indirect effect of CEFA. A quasi-Bayesian approximation with 10.000 Monte Carlo draws was used to determine 95% confidence intervals (95% CI). Mediation analysis with categorical determinants are still only available for dichotomous comparisons, therefore the Dutch group was used as reference group and other ethnic groups were compared to the reference group in succession. Our main interest were ethnic differences compared to the Dutch ethnic group and we limited our discussion to these results. The Dutch ethnic group is the host population and the group with the lowest prevalence of T2D of the included ethnic groups. We however additionally showed ethnic differences between non-Dutch ethnic groups.

Analysis of contribution by assessment of a greater than 10% change in odds for T2D by logistic regression yielded similar results to the mediation analysis (data not shown).

All analyses were conducted in R studio version 0.99.903 [28], with the exception of logistic regressions that were conducted in IBM SPSS Statistics 23 (2014, Chicago). We used step-wise adjustment for confounders in our models, and adjusted for ethnicity, age, sex, smoking status, physical activity score and BMI. This was in accordance with our conceptual model developed with software from Daggity. The directed acyclic graph (DAG) is shown at http://dagitty.net/dags.html?id=603aCm and includes all possible covariates. P-values <0.05 were considered as statistically significant.

Results

Baseline characteristics

Mean age ranged from 41.4 (95% CI 39.8; 43.0) in the Moroccan group to 48.9 (95% CI 47.4; 50.3) years in the African Surinamese group (Table 1). Median energy intake and median total fatty acids intake were the highest among the Turkish, and the lowest among the Moroccan for energy intake and the South-Asian Surinamese participants for fatty acids intake. Dutch participants scored the highest on the physical activity score, while Turkish participants scored the lowest. Mean BMI ranged from 25.0 (95% CI 24.4; 25.5) among Dutch to 28.3 kg/m^2 (95% CI 27.6; 29.0) among Turkish. Prevalence of diabetes was the highest among South-Asian Surinamese (23.3%), while it was the lowest among Dutch participants (4.5%).

Plasma CEFA in ethnic groups

Baseline plasma CEFA levels varied significantly across ethnic groups, as shown in Table 2. C16:0, C18:1n9 and C18:2n6 were the FAs with the highest percentage among all ethnic groups. Median SFA percentages ranged from 11.9% to 12.8%,

mean plasma MUFA from 19.2% to 24.1% and mean PUFA from 61.4% to 67.3%. Significant differences between ethnic groups were observed for the logit-transformed percentages of total PUFA, MUFA and SFA as well as for individual CEFA (Appendix 1).

Associations FAs and T2D

We found that higher levels of SFA were significantly associated with a higher odds for T2D in the overall population, whereas lower odds of T2D were observed for higher levels of PUFA and n6-PUFA (Table 3). The direction of the associations between SFA, MUFA, PUFA and n6-PUFA and T2D were similar across ethnic groups. We found no evidence of interaction between CEFA and ethnicity (Appendix 2), but we had limited power and confidence intervals were wide. No associations were observed for n3-PUFA. The associations of a few individual SFA, MUFA and PUFA with T2D in the overall population contrasted with the observed overall association (Appendix 3). For instance, although total PUFA were associated with a lower odds for T2D, y-linoleic acid (C18:3n6) and arachidonic acid (C20:4n6) were associated with a higher odds for T2D.

Mediation by CEFA in the association of ethnicity with T2D prevalence

Mediation analyses showed a significant total effect of ethnicity and CEFA on T2D for SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA (Table 4 with Dutch as a reference group; Appendix 4 for comparisons between ethnic minority groups). However, none of the indirect effects were significant, which indicated that the difference in prevalence of T2D across ethnic groups was not mediated by CEFA. For instance, a total effect on T2D of South-Asian Surinamese ethnicity compared to the Dutch

reference group of c = 0.20 (p-value <0.01) was found, while there was no observed indirect effect of SFA. Similar results were observed for the other ethnic groups and grouped CEFA. Moreover, no statistically significant indirect effects were observed for any of the individual CEFA (data not shown).

Discussion

Our study confirmed differences in plasma CEFA between ethnic groups. Proportions of PUFA were lower in participants of Dutch ethnicity compared to the other ethnic groups, while MUFA were higher. The Dutch had higher proportions of SFA compared to participants of South-Asian Surinamese ethnicity but lower compared to participants of Turkish and Moroccan ethnicity. The associations of CEFA with T2D showed a similar direction across ethnic groups. Plasma CEFA and T2D were statistically significant positively associated with total SFA and negatively with total PUFA. In line with the observed more favorable CEFA profile in ethnic groups at high risk for T2D and similar associations of CEFA with T2D, our study suggests that differences in T2D prevalence between ethnic groups are not mediated by differences in plasma CEFA.

Our findings suggest that plasma FA profiles are more favorable among ethnic minority groups than among the Dutch. This is in line with previous studies describing lipid intake or lipid profiles among various ethnic groups living in other European countries [18, 19, 29]. A systematic review by Gilbert and Khokhar described that native diets are generally more healthy than European diets [17]. However, after migration native dietary components are often replaced by less healthy alternatives ubiquitously available in European countries [17]. Lipid intake in the past week (combined with the endogenous metabolism) is reflected in plasma

CEFAs [16], and due to our results we can therefore assume that lipid intake of ethnic minority groups living in the Netherlands is, for now, more healthy than that of the Dutch.

The associations of CEFA with T2D are consistent with those previously described for the general European population in a prospective case-cohort study by Forouhi et al. [30, 31]. Similar to that study, we observed that associations of individual FAs with T2D differ to what is observed when FAs are grouped based on saturation [30]. Forouhi et al. suggested that odd-chained SFA may be negatively associated with T2D, our results indicate this as well [31]. To our knowledge, only one previous study investigated the association between individual FAs and T2D or metabolic markers of T2D in a multi-ethnic population and this study was conducted in Canada [21]. Ralston et al. only reported positive associations of FAs with markers of insulin resistance, while our study observed positive as well as negative associations of FAs with T2D. This may be attributable to the differences in methodology, as we measured FAs as proportions in plasma, while Ralston et al. calculated absolute values of FAs [32]. In contrast to the study by Ralston et al., our study did not identify evidence for different associations between ethnic groups [21]. This may be attributable to the assessment of such ethnic differences in the studies. Ralston et al. based their conclusion on a comparison of significance levels of the associations between FAs and markers of insulin resistance between the various ethnic groups, while in our study the interaction between FAs and T2D was studied. Nevertheless, the lack of interaction by ethnicity in our study may also be due to limited power.

Our study was the first to investigate whether FA mediate the observed T2D disparities between ethnic groups. The observed lack of mediation is due to the

direction of the associations combined with the observed CEFA percentages in the respective ethnic groups. The ethnic groups with the highest prevalence of T2D had higher levels of CEFA negatively associated with T2D, while the ethnic group in which T2D was less prevalent had higher levels of CEFA positively associated with T2D. For instance, the South-Asian Surinamese group had a high prevalence of T2D, but we observed this group to have the lowest levels of SFA (positively associated with T2D) and the highest levels of PUFA (negatively associated with T2D).

This does not mean that our results are irrelevant. The Dutch ethnic group has the lowest prevalence of T2D, although their plasma CEFA profiles are most unfavorable. As the association of CEFA with T2D is similar across ethnic groups, we may expect that if CEFA levels of non-Dutch ethnic groups were similar to those of the Dutch, the prevalence of T2D among these groups would be even higher. Under the assumption that the association between CEFA and T2D is a causal relationship and if our observations are further confirmed in future studies, maintenance of the more favorable CEFA levels in non-Dutch ethnic groups should be encouraged to avoid higher prevalences of T2D within these groups. This is a positive finding, because we know from a previous study within HELIUS that the dietary intake is rather robust across generations. Therefore, a shift in CEFA levels seems unlikely [33]. In the current sample, for instance, CEFA levels not clearly differentiated across generations (data not shown).

The current study did not further clarify the causes of ethnic differences in prevalence of T2D. Future studies need to identify these in order to eventually decrease ethnic disparities. The review by Sattar et al. indicated that differences in

adiposity and skeletal muscle might be involved in the higher prevalence of T2D among South Asians compared to White Europeans [34]. Chatterjee et al. identified novel risk factors for T2D among African Americans with a higher prevalence for T2D than the host population, such as a low birth weight, vitamin D and sleep duration [35]. Future studies on ethnic differences in the prevalence of T2D that include different populations may still need to consider CEFA as a possible explanation. We showed that the association between CEFA and T2D is consistent across ethnic groups. Therefore, in ethnic groups with a higher intake of SFA or lower intake of PUFA than the host population, CEFA may explain some of the disparities between ethnic groups.

Strengths and limitations

Our study has several strengths and limitations. First we used biomarker data to determine plasma FAs. A strength of the use of biomarker data compared to self-report is that self-reported dietary intake is often prone to misreporting [36]; especially lipid intake, as the type and quantity of lipid intake is poorly recognized by individuals [16]. However, the disadvantage of CEFA as a biomarker for dietary lipid intake in the past weeks is that it also reflects endogenous FA metabolism [16]. There are indications that the lipid metabolism between ethnic groups differs. This might also play a role in observed differences [37, 38]. Another disadvantage is that the expression of FAs as proportions may cause different results than FAs expressed as absolute amounts [32]. Future studies are needed to confirm our results with absolute values of FAs. Second, some CEFA measurements were below the detection limit. The handling of non-detectable values might have affected our results, possible multiple-imputation techniques would have led to different results

[32]. However, imputation is considered less accurate in case over 5% of the measurements are below the detection limit [26]. Third, cross-sectional data was used. Therefore, we cannot draw conclusions on the direction of our associations. However, previous prospective studies showed that FAs are related to the risk for T2D and we therefore expect that T2D is a consequence of the lipid profile rather than a cause [39, 40]. And last, our sample size was limited. Future studies may include more participants to increase power to further study the possible interaction between ethnicity and sex.

Concluding remarks

This study aimed to clarify whether the disparities in T2D prevalence between ethnic groups could be explained by CEFA. However, our findings suggest that CEFA do not mediate the ethnic differences in the prevalence of T2D across ethnic groups. We confirmed in a multi-ethnic population that plasma CEFA are similarly associated with T2D across ethnic groups. This confirms that FAs are potential important parameters to prevent T2D across multiethnic groups. Fortunate, we observed that ethnic minority groups in the Netherlands at high risk for T2D had relatively more favorable CEFA profiles than the Dutch ethnic group. Maintenance of these more favorable profiles should be encouraged.

Competing interests

The authors have no competing interests to declare.

Authors contributions

MM, CC, MN, JG and IGMV designed the study. MBS established the HELIUS study cohort and managed the data. MM conducted the analyses and wrote the manuscript. IGMV contributed to the writing. CC, MN, MBS, JG and IGMV reviewed the manuscript. All authors read and approved the final manuscript.

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Graphic Abstract

Ethnic minority groups in the Netherlands have a higher risk for type 2 diabetes than the host population. Differences in the amount and type of lipids in the diet and their metabolism may mediate ethnic differences in type 2 diabetes prevalence. We found that plasma cholesteryl ester fatty acids are similarly associated with type 2 diabetes across ethnic groups. Therefore, cholesteryl ester fatty acids are important parameters to prevent type 2 diabetes across multiethnic groups. However, ethnic differences were not mediated by plasma cholesteryl ester fatty acids as these profiles were more beneficial in ethnic minority groups than in the Dutch ethnic group.

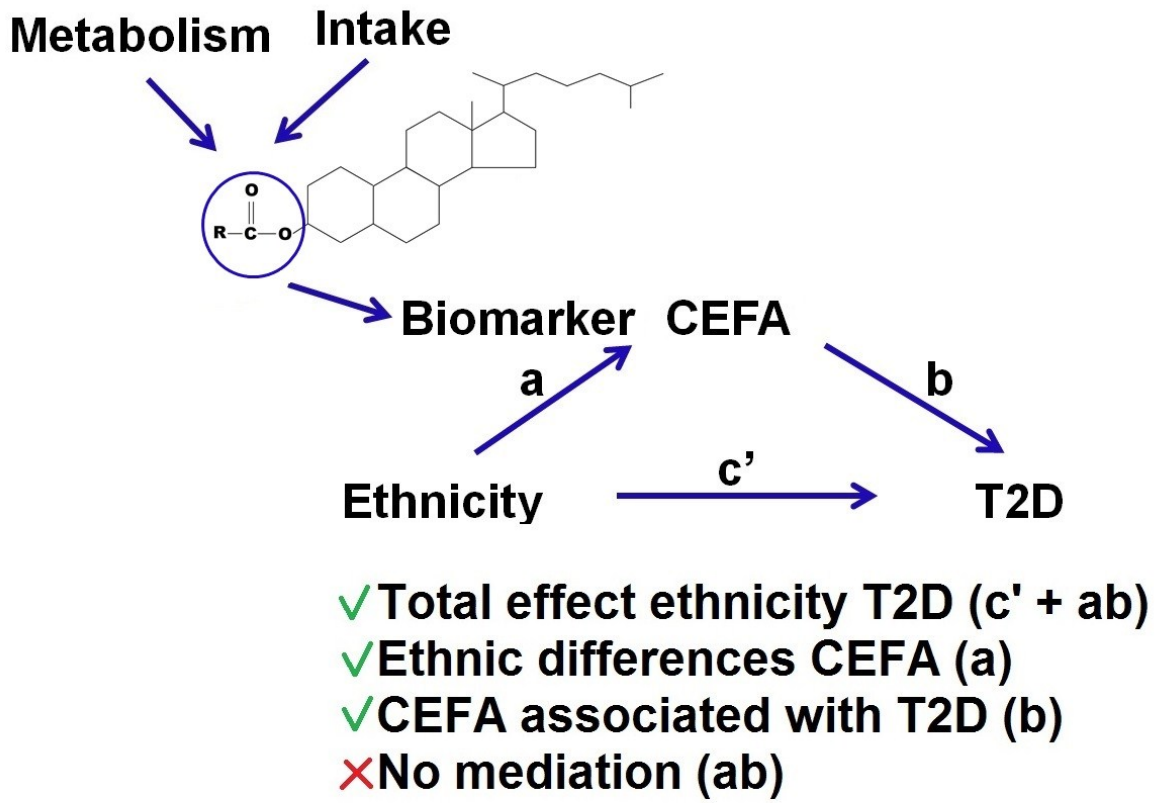

Table 1: Baseline characteristics per ethnic group

	Dutch	South-Asian Surinamese	African Surinamese	Turkish	Moroccan
	n=202	n=206	n=205	n=215	n=213
Age (years)	45.6 (14.1)[d,e]	46.3 (12.2)[d,e]	48.9 (10.7)[d,e]	42.1 (10.7)[a,b,c]	41.4 (11.6)[a,b,c]
Sex (% men)	101 (50.0)	103 (50.0)	103 (50.2)	107 (49.8)	105 (49.3)
Pack years smoking	0.9 (0.0-12.3)[b,c,d,e]	0.1 (0.0-5.6)[a,d]	0.0 (0.0-3.7)[a,e]	0.0 (0.0-3.8)[a,b,e]	0.0 (0.0-3.0)[a,c,d]
Energy intake (kcal / day)	2066 (1741-2477)	2075 (1679-2483)[d]	2156 (1594-2755)	2266 (1641-2910)[e]	1997 (1525-2714)
Total fatty acids (g)	74.5 (58.0-92.8)[a,b,c]	66.3 (53.0-84.2)[d,e]	67.6 (50.1-90.5)[a,d]	82.1 (55.5-113.2)[a,b,c,e]	68.2 (49.7-102.9)[b,d]
Physical activity score (min / week)	7334 (5085-9450)[b,d,e]	6428 (3945-9708)[a,c,d]	7268 (4200 - 11112)[b,d,e]	5640 (3126-8950)[a,b,c]	6120 (3770-9060)[a,c]
BMI (kg/m^2)	25.0 (3.9)[c,d,e]	26.0 (4.4)[c,d]	28.0 (5.0)[a,b]	28.3 (5.3)[a,b]	27.1 (4.5)[a]
Generation (% first)		169 (82)	179 (87)	167 (78)	161 (76)
Years in the Netherlands		33.1 (8.2)[c,d,e]	29.7 (10.3)[b]	29.1 (8.4)[b]	27.7 (8.6)[b]
Diabetes (%)	9 (4.5)	48 (23.3)	26 (12.7)	23 (10.7)	32 (15.0)

Data are mean (SD), median (IQR) or n (%).

BMI = Body Mass Index; WC = Waist Circumference; WHR = Waist Hip Ratio.

[a] Significant different ($p<0.05$) from the Dutch.

[b] Significant different ($p<0.05$) from the South-Asian Surinamese.

[c] Significant different ($p<0.05$) from the African Surinamese.

[d] Significant different ($p<0.05$) from the Turkish.

[e] Significant different ($p<0.05$) from the Moroccan.

Table 2: Mean and median baseline percentages of cholesteryl ester fatty acids per ethnic group

CEFA (% of total)		Dutch	South-Asian Surinamese	African Surinamese	Turkish	Moroccan
Median total SFA		12.5 (11.8 ;13.1)[b,d]	11.9[**] (11.2 ; 12.9)[a,c,d,e]	12.4 (11.8 ; 13.2)[b,d]	12.8[*] (11.7 ; 14.6)[a,b,c]	12.5 (11.7 ; 13.7)[b]
Median myristic acid	C14:0	0.64 (0.53 ; 0.79)[b,c,e]	0.51[**] (0.42 ; 0.62)[a,d,e]	0.53[**] (0.42 ; 0.65)[b,d]	0.62 (0.50 ; 0.83)[b,c,e]	0.58[**] (0.44 ; 0.71)[a,b,c,d]
Mean pentacyclic acid	C15:0	0.17 (0.04)[b,c,d,e]	0.14[**] (0.07)[a,d,e]	0.14[**] (0.05)[a,d,e]	0.23[**] (0.07)[a,b,c]	0.24[**] (0.09)[a,b,c]
Median palmitic acid	C16:0	10.8 (10.2 ; 11.2)[b,d]	10.4[*] (9.9 ; 11.3)[a,c,d,e]	10.8 (10.3 ; 11.5)[b]	10.9[*] (10.0 ; 12.4)[a,b,e]	10.6 (10.0 ; 11.6)[a,b,d]
Median stearic acid	C18:0	0.76 (0.65 ; 0.89)[b,d,e]	0.70[*] (0.64 ; 0.80)[a,c,d,e]	0.77 (0.70 ; 0.87)[b,d,e]	0.87[**] (0.74 ; 1.15)[a,b,c]	0.84[**] (0.74 ; 1.05)[a,b,c]
Mean total MUFA		24.1 (3.3)[b,c,d,e]	19.2[**] (3.7)[a,c,d,e]	20.9[**] (3.2)[a,b,d,e]	23.1[*] (4.3)[a,,b,c]	23.0[*] (3.9)[a,b,c]
Median cis-7 hexadecenoic acid	C16:1 n-9	0.45 (0.40 ; 0.50)[d]	0.43 (0.39 ; 0.51)[e]	0.44 (0.40 ; 0.50)	0.43[*] (0.39 ; 0.50)[a,e]	0.47 (0.41 ; 0.52)[b,d]
Median palmitoleic acid	C16:1 n-7	2.5 (1.8 ; 3.2)[b,c,d,e]	1.6[**] (1.3 ; 2.1)[aa,d,e]	1.7[**] (1.3 ; 2.2)[a,d,e]	1.8[**] (1.4 ; 2.4)[a,b,c,e]	1.5[**] (1.1 ; 1.9)[a,b,c,d]
Mean oleic acid	C18:1 n-9	19.7 (2.4)[b,c]	15.8[**] (3.0)[a,c,d,e]	17.3[**] (2.65)[a,b,d,e]	19.2 (3.8)[b,c]	19.6 (3.6)[b,c]
Median cis-vaccenic acid	C18:1 n-7	1.2 (1.0 ; 1.4)[b]	1.0[**] (0.9 ; 1.2)[a]	1.2 (1.0 ; 1.4)[b]	1.2 (1.1 ; 1.4)[b]	1.2 (1.1 ; 1.4)[b]
Mean total PUFA		61.5 (59.0 ; 63.9)[b,c]	67.3[**] (64.2 ; 69.9)[a,c,d,e]	65.4[**] (63.2 ; 67.9)[a,b,d,e]	63.6 (58.5 ; 66.3)[b,c]	63.6 (59.0 ; 66.2)[b,c]

Median total n6		58.9 (56.2 ; 61.7)[b,c,d,e]	65.1[**] (61.6 ; 68.0)[a,c,d,e]	63.0[**] (60.3 ; 65.6)[a,b,d,e]	61.5[**] (56.8 ; 64.4)[a,b,c]	61.3[**] (57.0 ; 64.4)[a,b,c]
Mean linoleic acid	C18:2 n-6	50.0 (5.0)[b,c]	54.1[**] (5.8)[a,c,d,e]	51.7[*] (5.1)[a,b]	51.1 (6.9)[b]	51.5 (5.8)[b]
Median γ-linoleic acid	C18:3 n-6	0.95 (0.72 ; 1.20)[b,c,e]	1.1[**] (0.8 ; 1.5)[a,c,d,e]	1.0[*] (0.8 ; 1.[a,b,d,e]3)	0.88 (0.69 ; 1.14)[b,c]	0.86[*] (0.66 ; 1.14)[b,c]
Mean dihomo-γ-linolenic acid	C20:3 n-6	0.75 (0.19)[d]	0.76 (0.17)	0.80 (0.18)	0.89[*] (0.21)[a]	0.80 (0.20)
Mean arachidonic acid	C20:4 n-6	6.8 (1.7)[b,c]	8.2[**] (2.11)[a,c,d,e]	8.8[**] (1.93)[a,b,d,e]	6.8 (1.9)[b,c]	6.9 (1.9)[b,c]
Median total n3		2.3 (1.8 ; 2.7)[b,c,d,e]	1.9[**] (1.5 ; 2.4)[a,d,e]	1.9[**] (1.6 ; 2.5)[a,d,e]	1.3[**] (1.0 ; 1.7)[a,b,c,e]	1.7[**] (1.3 ; 2.0)[a,b,c,d]
Median eicosapentaenoic acid	C20:5 n-3	0.98 (0.70 ; 1.31)[b,c,d,e]	0.79[**] (0.55 ; 1.15)[a,d,e]	0.78[*] (0.56-1.15)[a,d,e]	0.44[**] (0.30 ; 0.66)[a,b,c,e]	0.57[**] (0.38 ; 0.84)[a,b,c,d]
Mean docosahexaenoic acid	C22:6-n3	0.57 (0.21)[b,c,d]	0.64[*] (0.23)[a,d,e]	0.68[**] (0.22)[a,d,e]	0.46[**] (0.16)[a,b,c,e]	0.58 (0.17)[b,c,d]

Percentages of cholesteryl ester fatty acids were compared to the Dutch group. [*] p-value <0.05. [**] p-value <0.001. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[a] Significant different (p<0.05) from the Dutch.

[b] Significant different (p<0.05) from the South-Asian Surinamese.

[c] Significant different (p<0.05) from the African Surinamese.

[d] Significant different (p<0.05) from the Turkish.

[e] Significant different (p<0.05) from the Moroccan.

Table 3: Association of cholesteryl ester fatty acids with type 2 diabetes in the total population and stratified by ethnicity

CEFA (SD)	Total population		Dutch		SA Surinamese		African Surinamese		Turkish		Moroccan	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Total SFA	1.27 (1.06 ; 1.53)	0.01	2.15 (0.86 ; 5.38)	0.07	1.09 (0.68 ; 1.75)	0.71	1.57 (0.75 ; 3.30)	0.23	1.30 (0.99 ; 1.70)	0.06	1.21 (0.82 ; 1.79)	0.34
Total MUFA	1.23 (0.99 ; 1.53)	0.06	1.70 (0.61 ; 4.79)	0.31	1.26 (0.87 ; 1.82)	0.22	1.10 (0.62 ; 1.96)	0.74	1.25 (0.79 ; 1.96)	0.35	1.20 (0.73 ; 1.98)	0.47
Total PUFA	0.77 (0.63 ; 0.94)	0.01	0.46 (0.17 ; 1.20)	0.11	0.77 (0.51 ; 1.17)	0.22	0.85 (0.46 ; 1.57)	0.32	0.74 (0.53 ; 1.01)	0.06	0.85 (0.55 ; 1.32)	0.48
Total n6 PUFA	0.77 (0.63 ; 0.94)	0.01	0.44 (0.17 ; 1.1	0.10	0.75 (0.51 ; 1.12)	0.16	0.90 (0.50 ; 1.63)	0.73	0.75 (0.54 ; 1.04)	0.09	0.85 (0.55 ; 1.33)	0.48

			5)									
Total n3 PUFA	1.05 (0.85 ; 1.31)	0.65	1.35 (0.60 ; 3.02)	0.47	1.34 (0.93 ; 1.93)	0.12	0.92 (0.53 ; 1.58)	0.75	0.48 (0.27 ; 0.85)	0.01	1.24 (0.66 ; 2.35)	0.51

OR= odds ratio per standard deviation increase in the logit-transformed cholesteryl ester fatty acid fraction, in the model adjusted for ethnicity (total population only), age, sex, pack years of smoking, physical activity score and body mass index; 95% CI = 95% confidence interval; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; Type 2 diabetes was defined by self-reported physician diagnosis and/or measured fasting glucose levels of ≥7.0 mmol/L and/or measured HbA_{1c} levels of ≥48 mmol/mol and/or anti-diabetic medication.

Table 4: Ethnic differences in type 2 diabetes (compared to the Dutch) and mediation by cholesteryl ester fatty acids

		South-Asian Surinamese		African Surinamese		Turkish		Moroccan	
CEFA		Effect (95% CI)	p-value	Effect (95% CI)	p-value	Effect (95% CI)	p-value	Effect (95% CI)	p-value
Total SFA	Total effect	0.20 (0.13 ; 0.26)	<0.01	0.07 (0.014 ; 0.13)	0.02	0.10 (0.03 ; 0.17)	0.01	0.15 (0.07 ; 0.22)	<0.01
	Indirect effect	-0.005 (-0.02 ; 0.01)	0.26	-0.001 (-0.01 ; 0.01)	0.66	0.01 (< -0.001 ; 0.02)	0.07	0.004 (-0.001 ; 0.01)	0.18
Total MUFA	Total effect	0.20 (0.13 ; 0.26)	<0.01	0.073 (0.014 ; 0.13)	0.02	0.10 (0.03 ; 0.17)	0.01	0.15 (0.07 ; 0.22)	<0.01
	Indire	-0.03 (-	0.17	-0.02 (-	0.12	-0.01	0.22	-0.005	0.34

	ct effect	0.08)		0.05 ; 0.01)		(-0.02 ; 0.004)		(-0.02 ; 0.01)	
Total PUFA	Total effect	0.20 (0.14 ; 0.27)	<0.01	0.07 (0.02 ; 0.13)	0.02	0.09 (0.02 ; 0.17)	0.01	0.15 (0.07 ; 0.22)	<0.01
	Indirect effect	-0.03 (-0.07 ; 0.01)	0.12	-0.02 (-0.05 ; 0.003)	0.09	-0.002 (-0.01 ; 0.003)	0.41	-0.003 (-0.01 ; 0.003)	0.27
Total n6 PUFA	Total effect	0.15 (0.07 ; 0.22)	<0.01	0.07 (0.02 ; 0.13)	0.02	0.10 (0.02 ; 0.17)	0.01	0.15 (0.07 ; 0.22)	<0.01
	Indirect effect	-0.01 (-0.03 ; 0.01)	0.38	0.02 (-0.05 ; 0.004)	0.11	-0.005 (-0.01 ; 0.001)	0.10	-0.01 (-0.02; 0.003)	0.24
Total n3 PUFA	Total effect	0.20 (0.14 ; 0.27)	<0.01	0.07 (0.01 ; 0.13)	0.02	0.09 (0.02 ; 0.16)	0.01	0.15 (0.07 ; 0.22)	<0.01
	Indirect effect	-0.009 (-0.02 ; 0.001)	0.06	<0.001 (-0.01 ; 0.01)	0.93	0.03 (-0.01 ; 0.06)	0.10	-0.01 (-0.02 ; 0.003)	0.24

Effect is the estimate per standard deviation increase in the logit-transformed cholesteryl ester fatty acid fraction, in the model adjusted for age, sex, pack years of smoking, physical activity score and body mass index. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.