The Association between Plasma Stearoyl Co-A Desaturase-1 (SCD-1) Activity and Risk of Incident Type 2 Diabetes from the Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Objective: Stearoyl Co-A desaturase (SCD-1), the rate limiting enzyme in monounsaturated fatty acid synthesis, is critical for lipid synthesis, lipid oxidation and possibly type 2 diabetes. This study characterized SCD-1 activity through fatty acids ratios in baseline plasma measurements and established their association with incident diabetes in the Atherosclerosis Risk in Communities (ARIC) study.

Research Design & Methods: In 2782 Caucasian adults (age 45-64, 47% men) free of diabetes, baseline SCD-1 activity was approximated by plasma fatty acid ratios in cholesterol esters (SCD16c-16:1/16, SCD18c-18:1/18:0) and plasma phospholipids (SCD16p-16:1/16:0, SCD18p-18:1/18:0). Incident diabetes was ascertained during three follow up visits. The relationship between SCD-1 activity and incident diabetes was evaluated by Cox proportional hazards regression.

Results: During follow-up, 208 (7.5%) participants developed diabetes (mean 8 years ± SD 2.1 years). The various SCD-1 ratios correlated with each other ($p<0.001$). After adjusting for age and gender, higher levels of SCD16c and SCD16p were positively associated with incident diabetes. Higher levels of SCD18p were inversely associated with incident diabetes. After additional adjustment for parental history of diabetes, smoking, blood pressure, activity, carbohydrate intake, fiber intake, saturated fat intake, Waist-hip ratio, education, alcohol, and body-mass-index (BMI), only SCD16c remained positively associated with incident diabetes (RR=1.1: 95%CI 1.01-1.30 ; p=0.03).

This association disappeared with additional adjustment for insulin resistance.

Conclusions: Approximation of SCD-1 activity by SCD16c was positively associated with incident diabetes but this association was weak, especially when considering common phenotypic measures (i.e. BMI and Waist-hip ratio). Measurements of SCD-1 activity should be further refined.

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Introduction

Given the epidemic of Type 2 diabetes, there is much interest in biomarkers, particularly serum fatty acids, in predicting incident diabetes. Previously, epidemiologic data from the Atherosclerosis Risk in Communities (ARIC) cohort demonstrated a positive association between incident diabetes and fasting plasma free fatty acid levels (1) as well as the proportion of saturated fatty acid in plasma (2).

The composition of plasma fatty acids depends on many factors, including fatty acid intake (3), age, gender, exercise, endogenous synthesis and genetic predisposition (4). In particular, the role of Stearoyl Co-A desaturase (SCD-1), the rate limiting enzyme in monounsaturated fatty acid synthesis and a critical player in lipid synthesis and oxidation, needs to be considered. SCD-1, a delta9 desaturase, introduces a double bond at the c-9 location, with preferred substrates being palmitoyl (16:0) and stearoyl CoA (18:0) (5).

This enzyme is found in many lipogenic tissues, specifically the liver (6), adipose tissue (6) and muscle (7). SCD-1 deficiency improves insulin sensitivity likely due to increased lipid oxidation (8), improved skeletal muscle insulin signaling (9) and enhanced hepatic AMP-Kinase activity (10). Inhibition of SCD-1 activity may be a potential mechanism for treatment of obesity and diabetes (11). Recent studies inhibiting SCD-1 expression *in vivo* by antisense oligonucleotide injection prevented diet induced obesity in mice (12) and specifically improved hepatic insulin resistance in rats and mice (13).

Many approximations of SCD-1 desaturase activity, using the product/precursor ratio in plasma, exist (14-18). One of the earliest studies, using a mouse model with a targeted SCD-1 knockout and a mouse model with naturally occurring SCD-1 deficiency, showed the palmitoleate/palmitate (16:1/16:0) and oleate/stearate (18:1/18:0) ratio in plasma was proportional to SCD-1 gene dosage (14). Further evidence recommended against approximating SCD-1 activity in whole plasma due to heterogeneity in plasma lipid subcomponents: free fatty acids, cholesterol esters, phospholipids, and triglycerides (4; 19). As fasting cholesterol esters, triglycerides, and phospholipids are primarily derived from the liver, the approximation of SCD-1 activity by the product/substrate ratio in plasma cholesterol esters and plasma phospholipids has been attributed to hepatic SCD-1 activity (15).

Although epidemiological studies have demonstrated that higher levels of SCD-1 activity was associated with the metabolic syndrome (16), insulin resistance (17) (18) and diabetes (20), a prospective cohort study describing the association between baseline SCD-1 activity and incident diabetes has not yet been published. The goal of this study was to use a large, prospective cohort, the Atherosclerosis Risk in Communities Study (ARIC), to evaluate SCD-1 activity through the precursor-product ratio (16:1/16:0, 18:1/18:0) in cholesterol esters and plasma phospholipids as a biomarker for incident diabetes. The hypothesis was that higher levels of SCD-1 activity would be associated with higher risk of incident diabetes.

Material and Methods

Subjects:

The Atherosclerosis Risk in Communities Study (ARIC) is a population based cohort study of 15792 participants (ages 45-64 years) enrolled from 4 communities (Forsyth County NC, Jackson MS, Minneapolis MN, Washington County MD) during 1987-1989 with follow-up exams approximately 3 years apart (1990-1992, 1993-1995, 1996-1998) (21). This study was restricted to participants (n=4009) from the Minneapolis field center, which is the only center with measurement of plasma fatty acid composition from phospholipid and cholesterol esters. Plasma was collected and stored at baseline and subsequently analyzed for fatty acid composition 2 years after the initial collection. The ARIC study was approved by the Institutional Review Board (IRB) of each participating center. The current study was specifically approved by the IRB at the University of Minnesota.

Of the participants in the Minneapolis cohort, 37 subjects were nonwhite and excluded from the analysis (due to small numbers). Of the remaining 3972 subjects, an additional 1190 participants were excluded because they had one or more of the following at baseline: prevalent or unknown diabetes status (n=432), missing fatty acid measurements (n=37) and use of medications (lipid lowering agents and bile acid sequestrants) which may affect the lipid profile (n=906 total subjects, n=721 subjects not previously excluded). After the applying the exclusion criteria, 2782 participants were available for analysis.

Plasma Fatty Acid Measurement:

Plasma collection and fatty acid composition measurements have been previously described (2; 3). Briefly, fasting blood was collected in EDTA, centrifuged and frozen at -70 degrees Celsius until analysis approximately 2 years later. The lipid extract was obtained by exposing plasma to methanol and chloroform under a nitrogen atmosphere. The cholesterol ester and phospholipid fractions were separated using a silica gel plate (Silica Gel H: Analtech, Newark DE) and two stage mobile phase development with 80:20:1 (by vol) and 40:60:1 (by vol) solvents of petroleum ether, diethyl ether, and glacial acetic acid. Dichlorofluorescein was used to visualize the phospholipid, cholesterol ester, triacylglycerol, and free fatty acid bands under UV light. The phospholipid and cholesterol esters were scraped into separate test tubes and converted into methyl esters of fatty acids by boron trifluoride catalysis (22). The methyl esters were separated and measured using gas chromatography (model 5890 Hewlett Packard, Avondale, PA) equipped with a glass capillary column (J&W Scientific, Folsom CA) and a flame ionization detector. Each fatty acid peak was identified by comparison to synthetic standards of known fatty acid composition. The relative amount was quantified by integrating the area under the specific peak and dividing the results by the total area for all fatty acids. Short term (reliability coefficient: 0.5 to 0.93 for plasma cholesterol esters, 0.31 to 0.89 for plasma phospholipids) and long term repeatability (reliability coefficient: 0.35 to 0.83 for plasma cholesterol esters, 0.30 to 0.81 for plasma phospholipids) of this technique in quantifying fatty acid composition has been previously reported (23).

Given the precedent set by previous epidemiological studies (16-18), plasma fatty acid composition from phospholipid and cholesterol esters was used as a surrogate measure of SCD-1 activity. As these samples were obtained in a fasting state, the assumption is that the measured SCD-1 activity primarily represents hepatic SCD-1 activity (15). Given that SCD-1 converts 16:0 to 16:1 and 18:0 to 18:1, SCD-1 activity was approximated by 4 different measures: 16:1/16:0 in plasma cholesterol esters (SCD16c), 16:1/16:0 in plasma phospholipids (SCD16p), 18:1/18:0 in plasma cholesterol esters (SCD18c) and 18:1/18:0 in plasma phospholipids (SCD18p).

Primary Outcomes:

Incident diabetes was defined by one or more of the following: self-reported physician diagnosis, fasting (≥ 8 hours) serum glucose greater than or equal to 7.0 mmol/l (126 mg/dL), non-fasting serum glucose greater than or equal to 11.1 mmol/l (200 mg/dL), or use of anti-diabetes medication within the past two weeks, at any of the three follow up visits for the ARIC study. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the previous one, as previously described (24).

Other Measurements:

The covariates in the analysis were measured at baseline and included : age, gender, smoking history (cigarette-years), education level, alcohol use, parental history of diabetes, body mass index (BMI), dietary fiber intake, dietary carbohydrate intake, dietary saturated fat intake, and blood pressure (25). These covariates were selected as possible confounders due to their association with

altering the plasma lipid profile (cigarette smoking (26), dietary fiber intake (4), dietary carbohydrate intake (6), saturated fat intake (4), alcohol use (27)) and association with incident diabetes (parental history of diabetes, physical activity, education, waist-hip ratio, BMI, HOMA-IR, hypertension) (28). Trained interviewers collected information on alcohol intake and dietary intake using an adaptation of Willett's food frequency questionnaire (29). Insulin and glucose were measured as previously reported (30). Insulin resistance was represented by the homeostatic model assessment (HOMA-IR) (31). Physical activity information at baseline was assessed by the Baecke questionnaire (32) and summarized quantitatively as total activity (work, leisure, and sport activity: range 3-15) and play activity (leisure and sport activity: range 2-10). Adjustment of cigarette smoking including consideration of both smoking history and smoking years, but as this did not change the results in a sensitivity analysis, smoking years was used in the final model.

Statistical analysis:

All analysis was conducted using SAS (version 9.2 - SAS institute, Inc, Cary, North Carolina). Baseline characteristics were summarized for those with and without incident diabetes and compared using two-sample t-tests. The chi-squared test was used for categorical outcomes. Pearson's correlation coefficients were computed between the different SCD-1 ratios.

After confirming the Cox proportional hazards assumption, the proportional hazards model was used to evaluate the association of the SCD-1 ratio with incident diabetes, adjusting for covariates of interest. Model 1 adjusted

for age and gender. Model 2 additionally adjusted model 1 for other risk factors, specifically parental history of diabetes (absent/present), cigarette smoking (cigarette years), mean arterial pressure (mmHg), physical activity (total of work, leisure and sport activity), dietary fiber intake (grams/day), carbohydrate intake (% of total kcal), saturated fat intake (% of total kcal), alcohol use (grams/day), education (<high school, high school, > high school), waist-hip ratio, and BMI (kg/m^2). The third model additionally adjusted model 2 for insulin resistance (HOMA-IR). Differences in risk factors between groups (ie participants with/without incident diabetes, extreme quintiles of SCD-1) were measured using a two way unpaired T-test. Statistical significance was set at $p<0.05$ for all analyses.

Results

Demographics:

Among the 2782 subjects (47% men, n=1317), 208 (7.5%) participants developed diabetes over a mean of 8 years (± SD 2.1 years) of follow-up. Compared to subjects without diabetes, subjects who developed incident diabetes had the following variables significantly higher at baseline: BMI, Waist-hip ratio, fasting glucose, HOMA-IR, mean arterial pressure, dietary fiber intake, and SCD16c ratio. Subjects who developed incident diabetes were more likely to be men and have a parental history of diabetes, higher smoking exposure, and lower educational level **(Table 1).** Compared to subjects without diabetes, subjects who developed incident diabetes had lower levels of play activity (as measured by a combination of sport and leisure) and lower SCD18p ratio at baseline. Baseline saturated fat intake, alcohol intake, carbohydrate intake, total activity, age, SCD16p and SCD18c ratios were not different.

Correlation between SCD1 ratios:

The different SCD-1 ratios were positively and significantly correlated with each other (all $p<0.001$). Correlations with SCD16c were 0.78 (SCD16p), 0.66 (SCD18c) and 0.55 (SCD18p). Correlations with SCD16p were 0.48 (SCD18c) and 0.58 (SCD18p). Correlation of SCD18c with SCD18p was 0.64.

Correlation between SCD1 ratios and Baseline Risk Factors for Diabetes

The different approximations of SCD-1 (SCD16c, SCD16p, SCD18c, SCD18p) correlated with known risk factors for diabetes, especially insulin resistance (HOMA-IR), BMI, and Waist-hip ratio. HOMA-IR was positively

correlated with SCD16c (0.1) and SCD16p (0.06) and negatively correlated with SCD18c (-0.17) and SCD18p (-0.24) (all $p<0.01$). BMI was positively correlated with SCD16c (0.12) and SCD16p (0.06) and negatively correlated with SCD18c (-0.16) and SCD18p (-0.22) (all $p<0.01$). Waist-hip ratio positively correlated with SCD16c (0.08) and negatively correlated with SCD18c (-0.14) and SCD18p (-0.11) (all $p<0.01$). The Waist-hip ratio was not correlated with SCD16p (-0.02 : $p = 0.22$),

Baseline risk factors by quintiles of SCD16C ratio:

Given the association between quintiles of SCD16 ratio and incident diabetes, differences in baseline risk factors between the lowest and highest quintiles of SCD16c were examined. For SCD16c **(Table 2),** the highest quintile of SCD16c was associated with higher age, alcohol intake, BMI, Waist-hip ratio, Mean Arterial Pressure, fasting glucose, and HOMA-IR. The participants with the highest quintile of SCD16c had a lower percentage of men, lower education level, and higher rates of smoking. The participants in the highest quintile of SCD16c had lower dietary fiber intake, carbohydrate intake, and activity. Family history of diabetes and dietary saturated fat intake were not different between the extreme quintiles of SCD16c. Similar findings were observed between SCD16p (data not shown) and SCD16c with the exception of the following: carbohydrate intake (no difference between extreme quintiles of SCD16p), saturated fat intake (no difference between extreme quintiles of SCD16p), dietary fiber intake (no difference between extreme quintiles of SCD16p), fasting glucose (no difference

between extreme quintiles of SCD16p), and Waist-hip ratio (no difference between extreme quintiles of SCD16p).

The differences in baseline risk factors between the lowest and highest quintiles of SCD18p were also examined. For SCD18p **(Table 3),** the highest quintile of SCD18p was associated with higher smoking history, higher saturated fat intake and higher alcohol intake. The highest quintile of SCD18p was associated with lower percentage of men, education, carbohydrate intake, fiber intake, BMI, Waist-hip ratio, glucose and HOMA. There was no difference in age, parental history of diabetes, activity level, or mean arterial pressure between the extreme quintiles.

Association between SCD1 ratios and Incident Diabetes

The associations of SCD-1 quintiles with incident diabetes were examined for each SCD-1 ratio separately. In Model 1 (age and gender adjusted), there was a significant positive association between SCD-1 quintiles and incident diabetes for both SCD16c and SCD16p. In contrast, there was a significant negative association between SCD18p quintiles and incident diabetes (**Figure 1**). The associations of SCD16c quintiles and incident diabetes persisted when adjusting for additional risk factors (Model 2), but disappeared when additionally adjusting for HOMA-IR (Model 3). The association between SCD16p quintiles and SCD18p quintiles with incident diabetes disappeared when other risk factors (Model 2, Model 3) were considered.

Discussion

To our knowledge, this is the first study examining SCD-1 activity, as represented by various plasma fatty acid ratios (SCD16c, SCD16p, SCD18c, SCD18p) and the associations with incident diabetes in a prospective cohort of men and women. This study complemented previous epidemiologic data from the Atherosclerosis Risk in Communities (ARIC) cohort which showed a positive association between incident diabetes and fasting plasma free fatty acid levels (1) as well as the proportion saturated fatty acid composition in plasma (2). The approximations of SCD-1 activity (SCD16c, SCD16p, SCD18c, SCD18p) were moderately to highly correlated with each other. However, SCD16c and SCD16p were positively associated with typical risk factors for incident diabetes (BMI, HOMA-IR, Waist-hip ratio) whereas SCD18c and SCD18p were inversely associated with these risk factors. The approximation of SCD-1 activity by SCD16c (16:1/16:0 ratio in plasma cholesterol esters) had the strongest association with incident diabetes. However, the association between SCD16c and incident diabetes was attenuated when considering further risk factors for type 2 diabetes (i.e. BMI and Waist-hip ratio) and was absent when considering insulin resistance (HOMA-IR).

Although novel in its measure of incident diabetes in a prospective cohort of women and men, the ARIC study generally agrees with previous literature. SCD-1 activity, as approximated by SCD16c, has been associated with reduced insulin sensitivity (17) and the metabolic syndrome (16). These previous studies (16; 17), however, were limited to men and examined the association between baseline levels of SCD16c and insulin sensitivity (as measured by the

hyperinsulinemic, euglycemic clamp) or metabolic syndrome (16) twenty years later. Despite the independent association between SCD16c and insulin sensitivity when adjusting for additional risk factors (i.e. BMI, glucose, diastolic blood pressure, triglycerides, HDL, physical activity, smoking, socioeconomic status, insulin, saturated fat intake), the strongest predictor of insulin sensitivity remained BMI (17). Likewise, the association between SCD16c and development of the metabolic syndrome disappeared when adjusting for BMI or physical activity (16).

When examining SCD-1 activity as approximated by the different plasma phospholipids (SCD16p, SCD18p) and the predictive value of incident diabetes, the results for SCD16p, but not SCD18p, were consistent with Patel et al. (20). Using a smaller (cases:n=199, control:n=184)) case-control study with narrower criteria (no laboratory assessment) for the diagnosis of diabetes, Patel et al, reported a positive association between SCD1 activity (as measured by SCD16p or SCD18p) and incident diabetes (20). The difference in SCD18p and incident diabetes in the Patel versus the current ARIC study may be explained by several factors. The current ARIC study is a larger prospective study (n=2782) with broader inclusion criteria (including laboratory assessment) for diabetes (24). Although the duration of follow-up (~8 years) was comparable, the current ARIC study enrolled a younger population (mean age 53) compared with the Patel et.al study (mean age 64) (20). The current ARIC study reported an association between dietary saturated fat intake and SCD18p quintiles but no association between dietary saturated fat intake and quintiles of SCD16c or SCD16p. In the

ARIC cohort, the contribution of oleate (18:1 comprises 12% of total energy) to the diet was higher than palmitate (16:0 comprises 6.9% of total energy) (3), which may potentially alter plasma fatty acid levels of 18:1 (4) as indicated by the low reliability coefficient (0.39) for 18:1 observed in plasma phospholipids (23).

Although our study showed that measures of SCD-1 activity may be independently associated with incident diabetes and risk factors for type 2, this association was weak, especially when compared to common phenotypic measures (i.e. BMI and Waist-hip ratio) and were attenuated after further adjustment for risk factors. SCD-1 activity may be associated with incident diabetes in several ways. SCD-1 deficient mice have reduced body fat, increased insulin sensitivity and are protected from the metabolic effects of a high fat diet (8). This effect appears to be due to increased lipid oxidation (8), reduced lipid synthesis (8), and enhanced insulin signaling in the muscle, white adipose tissue, liver, and heart (33). Alternatively, one of the products of SCD-1 activity, palmitoleic acid (16:1) may play a role in incident diabetes, although the details remain unclear. Diabetes has been associated with high levels of palmitoleic acid (16:1) in serum free fatty acids (34), plasma/serum cholesterol esters (2) and plasma phospholipids (35).

The resources of the ARIC study were uniquely suited to examine the association between SCD-1 activity and incident diabetes. The correlation between dietary fat composition, as measured by the food frequency questionnaire and plasma fatty acid composition has been previously published (3). Baseline measures of diet, activity, plasma free fatty acid, and plasma fatty

acid composition were available for a proportion of study participants, with previously described associations between fasting plasma free fatty acid (1) and proportion of plasma saturated fatty acid (2) with incident diabetes. The use of an older cohort (age group 45-64 at baseline) with 8 years of follow up ensured a reasonable event rate of incident diabetes. Finally, the diagnosis of diabetes was confirmed through glucose measures rather than strictly by self-report. However, as previously published, the timing of the onset of diabetes was uncertain and ascertained by interpolation between visits (1; 24).

Our study was novel in its assessment of multiple approximations of SCD-1 activity (SCD16c, SCD16p, SCD18c, SCD18p ratios) and evaluation of these ratios relative to incident diabetes in the setting of long term, prospective follow-up. The values for SCD16c obtained in this study were comparable to those previously reported in studies using the 16:1/16:0 ratio in cholesterol esters (0.33±SD 0.09) (16; 17). The values of SCD16p and SCD18p were also comparable to previous literature using the 16:1/16:0 ratio or 18:1/18:0 ratio in plasma phospholipids to approximate SCD-1 activity (20).

A limitation of the study, but not unique to the current study, was the use of the plasma product-precursor ratio for SCD-1 in approximating SCD-1 activity. The ratio of 16:1/16:0 measured in serum has been shown to correlate with the 16:1/16:0 ratio in hepatic tissue (36). The 16:1/16:0 ratio in hepatic tissue has been shown to reflect hepatic SCD-1 mRNA (37). In the setting of a stable diet, plasma fatty acid variation was highest for 16:1 in cholesterol esters, suggesting that alterations in 16:1 levels in plasma may be due to desaturase activity (38).

However, dietary intake, alcohol intake, smoking, physical activity, and medication may influence fatty acid composition (4), with varying effects depending on the specific lipid (3). Analytical variation may affect reproducibility, obscuring changes in SCD16p due to low levels of 16:1 in plasma phospholipids (approximate 1% molar %) compared with plasma cholesterol esters (5% molar %) (4). Another limitation of the study is the one-time baseline measurement of plasma fatty acid composition, which does not incorporate time-dependent variation in SCD-1 activity/plasma fatty acid composition. Lastly, due to increased lipolysis, free fatty acid levels are approximately twice as high in humans with insulin resistance (39), and type 2 diabetes (40) compared with normal humans. Whether the increased free fatty acid levels affect plasma phospholipid composition and plasma cholesterol ester composition remains unknown; nevertheless, the association between increased free fatty acids and reduced insulin sensitivity may confound the reported findings.

Using a prospective cohort to examine the relationship between SCD-1 and incident diabetes, this study found that the approximation of SCD-1 activity by the 16:1/16:0 ratio in plasma cholesterol esters had the strongest association with incident diabetes. However, this association remained weak, especially when compared to more easily accessible phenotypic measures such as BMI and Waist-hip ratio and was substantially attenuated when adjusting for additional risk factors (parental history of diabetes, cigarette smoking, mean arterial pressure, physical activity, dietary fiber, saturated fat intake, alcohol use,

education, Waist-hip ratio, BMI, HOMA-IR). Further refinement of measurements for SCD-1 activity is recommended.

Tables & Figures

Table 1: Baseline characteristics of participants with and without incident diabetes

	No diabetes (n=2574)	Incident Diabetes (n=208)	P value
Age (years)	53.3 (0.1)	53.6 (0.4)	0.4
Gender (male)	45.9%	65.4%	<0.01
Education (<high school)	5.0%	9.2%	0.04
Positive Parental History of diabetes (%)	18.8%	29.8%	<0.01
Positive hx of smoking (%)	60.9%	72.9%	<0.01
Cigarette Years Smoked	303.7 (7.5)	416.7 (29)	<0.01
Diet-fiber intake (grams)	16.1 (0.1)	17.1 (0.6)	0.04
Diet Saturated fat intake (%kcal)	12.6 (0.1)	12.6 (0.2)	0.9
Diet-Carbohydrate intake (%kcal)	46.4 (0.2)	45.6 (0.6)	0.2
Alcohol intake (g/day)	8.2 (0.3)	9.3 (1.1)	0.3
Total Activity [3 (low) to 15 (high)]	7.4 (0.03)	7.3 (0.1)	0.4
Sport+Leisure Activity [2 (low) to 10 (high)]	5.2 (0.02)	5.0 (0.08)	<0.01
BMI (kg/m2)	26.1(0.1)	30.4 (0.3)	<0.01
Waist hip ratio	0.9 (0.002)	1.0 (0.005)	<0.01
Mean Arterial Pressure (mmHg)	87.1 (0.2)	92.7 (0.7)	<0.01
Fasting glucose (mmol/l)	5.4 (0.01)	6.0 (003)	<0.01
HOMA-IR	2.1 (0.03)	4.1 (0.2)	<0.01
SCD16c	0.2 (0.002)	0.3 (0.007)	<0.01
SCD16p	0.02 (0.0001)	0.03 (0.0004)	0.1
SCD18c	18.5 (0.1)	17.8 (0.2)	0.2
SCD18p	0.7(0.002)	0.6 (0.008)	<0.01

Results reported as mean (Standard Error)

SCD16c represents 16:1/16:0 in plasma cholesterol esters.

SCD16p represents 16:1/16:0 in plasma phospholipids.

SCD18c represents 18:1/18:0 in plasma cholesterol esters.

SCD18p represents 18:1/18:0 in plasma phospholipids.

Activity derived from Baecke Survey (32).

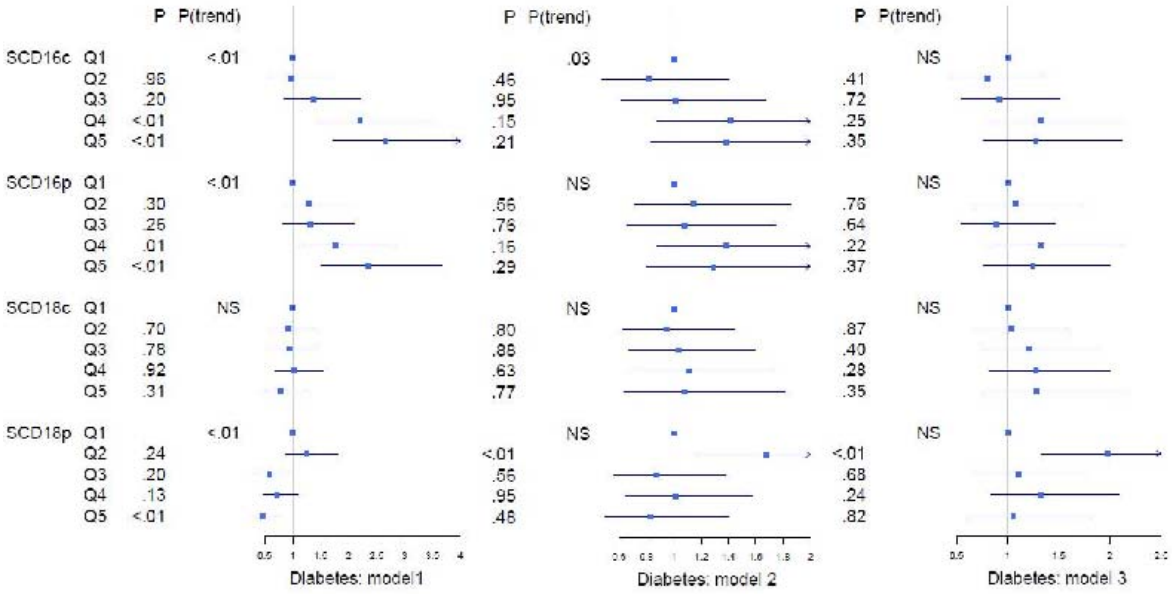

Figure 1: Hazard Ratios for association of SCD-1 quintiles with Incident Diabetes. Model 1: Adjusted for age and gender. Model 2: Additionally adjusted for other risk factors: parental history of diabetes, cigarette smoking, mean arterial pressure, physical activity, dietary fiber intake, carbohydrate intake, saturated fat intake, alcohol use, education, waist-hip ratio and BMI.. Model 3: Model 2 additionally adjusted for HOMA-IR. Quintile 1 (lowest level of SCD-1 ratio) is the common reference group. P trend measured the association between the linear relationship across quintile levels and incident diabetes. Statistical significance was set at $p<0.05$ for all analyses.

Table 2: Mean Baseline Risk Factors by Extreme Quintiles of SCD16c

	Lowest quintile (n=556)	Highest quintile (n=556)	p
SCD16c (range)	0.08-0.17	0.3 -1.2	
Age (years)	52.9 (0.2)	53.8 (0.2)	0.01
Gender (male)	60.4%	36.2%	p<0.01
Education (<high school)	4.9%	5.9%	p<0.01
Positive parental history of diabetes (%)	19.6%	23.2%	0.37
Positive history of smoking	54.7%	72.9%	p<0.01
Cigarette years smoked	248.6 (15.2)	436.1 (18.0)	p<0.01
Diet-fiber intake (grams)	17.4 (0.3)	15.2 (0.3)	p<0.01
Diet-Carbohydrate (%kcal)	47.4 (0.3)	44.5 (0.4)	p<0.01
Diet-Saturated fat (%kcal)	12.4 (0.1)	12.3 (0.1)	0.96
Alcohol intake (g/day)	3.8 (0.3)	16.9 (0.9)	p<0.01
Total Activity [3 (low) to 15 (high)]	7.7 (0.1)	7.1 (0.1)	p<0.01
Sport+Leisure Activity [2 (low) to 10 (high)]	5.4 (0.05)	4.9 (0.05)	p<0.01
BMI (kg/m2)	25.4 (0.1)	27.3 (0.2)	p<0.01
Waist hip Ratio	0.89 (0.004)	0.92 (0.003)	p<0.01
Mean Arterial Pressure	85.6 (0.4)	90.2 (0.5)	p<0.01
Fasting glucose (mmol/l)	5.4 (0.02)	5.6 (0.02)	p<0.01
HOMA-IR	2.0 (0.1)	2.6 (0.1)	p<0.01

- Results reported as mean (Standard Error)

SCD16c represents 16:1/16:0 in plasma cholesterol esters.

Activity derived from Baecke Survey (32).

Table 3: Mean Baseline Risk Factors by Extreme Quintiles of SCD18p

	Lowest quintile (n=556)	Highest quintile (n=556)	p
SCD18p (range)	0.37-0.56	0.74-1.4	
Age (years)	53.7 (0.2)	53.2 (0.2)	0.09
Gender (male)	51.6%	45.1%	0.03
Education (<high school)	6.0%	6.3%	0.03
Positive parental history of diabetes (%)	22%	16%	0.07
Positive history of smoking	57%	70%	p<0.01
Cigarette years smoked	268.6 (15.0)	406.8 (17.5)	p<0.01
Diet-fiber intake (grams)	16.8 (0.3)	15 (0.3)	p<0.01
Diet-Saturated fat (%kcal)	12.3 (0.1)	12.7 (0.1)	0.01
Diet-carbohydrate (%kcal)	47.1 (0.4)	44.7 (0.4)	p<0.01
Alcohol intake (g/day)	4.9 (0.3)	14.6 (0.9)	p<0.01
Total Activity [3 (low) to 15 (high)]	7.4 (0.1)	7.4 (0.1)	0.56
Sport+Leisure Activity [2 (low) to 10 (high)]	5.2 (0.1)	5.1 (0.1)	0.34
BMI (kg/m2)	27.7 (0.2)	25.1 (0.2)	p<0.01
Waist hip Ratio	0.93 (0.003)	0.90 (0.004)	p<0.01
Mean Arterial Pressure (mm Hg)	87.8 (0.4)	87.6 (0.5)	0.77
Fasting glucose (mg/dL)	5.6 (0.02)	5.4 (0.02)	p<0.01
HOMA-IR	2.9 (0.1)	1.8 (0.1)	p<0.01

- Results reported as mean (Standard Error)

SCD18p represents 18:1/18:0 in plasma phospholipids.

Activity derived from Baecke Survey (32).

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