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Associations of phospholipid fatty acids with incidence of type 2 diabetes: a CARRS nested case-control study

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MBBS, Nishtar Medical University, 2017

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An abstract of

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Abstract

Associations of phospholipid fatty acids with incidence of type 2 diabetes: a CARRS nested case-control study

By Muhammad Zia ul Haq

Aims: Higher burden of type 2 diabetes mellitus (T2DM) in South Asians could be explained using biomarkers such as fatty acids (FAs). We aimed to investigate whether certain FA combinations are associated with incidence T2DM in the CARRS study cohorts.

Methods: We employed a nested case-control approach to select participants from CARRS-1 ($N_{diabetes\text{-}incident\text{-}cases}$=200, $N_{controls}$=200 from N=4,017) and CARRS-2 ($N_{diabetes\text{-}incident\text{-}cases}$ =200, $N_{controls}$=200 from N=4,802). Participants in both the cohorts were followed up for up to 5-years and incident diabetes cases were ascertained based upon self-report and no-medication use, fasting glucose ≥126mg/dl and HbA1c≥6.5%. Controls were age- and sex-matched individuals who were diabetes-free at baseline and follow-up. In cases and controls, serum phospholipid FAs including polyunsaturated fatty acids (PUFA), monounsaturated fatty acids, total fatty acids, omega-6 FAs, omega-3 FAs, saturated fatty acids (SFA), unsaturated FAs, linoleic acid and docosahexaenoic acid were measured using nuclear magnetic resonance imaging. Principal component (PC) analysis was performed to decompose data from many correlated FA variables into composite variables. First PC with eigen value greater than 1 was chosen to represent and called as FA pattern score which was loaded with PUFA, omega-6 FA, and SFA. The association of FA pattern score was then investigated with incidence of T2DM while adjusting for potential confounders.

Results: We observed a weak protective effect of FA pattern-score in association with incidence of T2DM (OR 0.94, 95% CI 0.88-1.02, *p-value:* 0.14) after adjusting for potential confounders. This association was strengthened by further adjusting for BMI (OR 0.91, 95% CI 0.83-1.00, *p-value*: 0.04) and HDL-C and triglycerides (OR 0.88, 95% CI 0.80-0.97, *p-value*: 0.01). In obesity

stratified analysis (BMI < 18.5 kg/m^2, BMI 18.5 – 24.9 kg/m^2, BMI ≥25 kg/m^2), the protective effect of FA pattern-score in association with incident T2DM was only observed among normal weight participants (OR 0.83, 95% CI 0.72 – 0.97, *p-value:* 0.016).

Conclusions: Our study demonstrated that an FA pattern-score characterized by a combination of PUFA, omega-6 FAs, and SFA was associated with a lower risk of T2DM in South Asians and this association was more pronounced in normal weight individuals.

Keywords

Fatty acids combination; Type 2 Diabetes; South Asians; fatty acids

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To the dreams that keep us awake.

Introduction

Diabetes is a major and rising health problem in South Asia with greater susceptibility and comparably earlier disease onset than in other race/ethnic groups. Diabetes has met the definition of an epidemic in South Asia[1] with an estimated 6 out of 10 people having either diabetes or prediabetes[2], and with the expectations that this will grow due to increasing trends of obesity in Asia[1, 3]. Uniquely, people of South Asian origin develop rapid resistance to insulin with 4-6 times higher incidence of T2DM, at lesser grades of obesity and younger age, as compared to Europeans[1, 4]. Theories explaining this difference attribute it to poor adaptation to the Western diet, genetic susceptibility to the disease, and reduced oxidative capacity, as well as lower capability of FAs utilization at the whole-body level. Additionally, suggested mechanisms include insulin resistant phenotype, impaired insulin secretion, and impaired insulin action owing to low lean-muscle weight in South Asians[1, 4, 5].

A variation in T2DM expression and intensity across populations can be explained using metabolomics[2, 6]. Metabolomics, which serves as a 'real world endpoint' to understand and prevent metabolic disorders globally, is defined as the study of the metabolome: the small molecule complements of a biological system (including drug- or microbiome-related metabolites)[7, 8]. The common methodologies in practice to quantify metabolomics include chromatography, two-dimensional electrophoresis, mass spectrometry, functional magnetic resonance, positron emission tomography, and protein/gene sequencing[9, 10]. Mass spectrometry (MS) and NMR spectroscopy are the two common methodologies to quantify metabolomics, including FAs[8, 11, 12]. MS has superior sensitivity but a higher price and sub-optimal quality control for large cohorts. NMR has faster sample preparation,

shorter analysis time, and lower costs. Though less sensitive than MS, it is robust, reproducible, and provides an absolute measurement of many metabolites particularly amino acids, FAs, and other metabolomics including lipoproteins[12]. NMR spectroscopy uses the magnetic properties of protons to gain a structural picture of a molecule, and hence its identity.

Some of the recognized diagnostic metabolites including glycated hemoglobin (HbA1c), advanced glycation end-products (AGEs), amino acids, FAs, and glycoproteins, have been implicated in T2DM incidence as emphasized by many studies[9, 13-15].

Several studies have implied a role of individual FAs in the association, prevention, progression, and potential treatment of T2DM through improvement in insulin resistance, complications, and glycemic control [8, 16-21]. In experimental diabetic animals, increasing concentrations of indole propionic acid, acetic acid, DHA, and decreasing concentrations of short chain FA butyrate have been observed in association with obesity leading to insulin resistance[16, 17]. Similarly, studies have found a positive association between dietary intake of 6-PUFA, SFA (stearic and palmitic acids), trans FAs (elaidic acid), and blood concentrations of medium- and long-chain saturated and unsaturated FAs with glycemia, incidence of prediabetes, and T2DM[20-25]. On the other hand, increased concentrations of total fatty acids (TOTFA) levels, long- chain omega-3 FAs, and short-chain FAs are associated with decreased incidence of T2DM[13, 18-21]. Furthermore, eicosapentaenoic acid, docosahexaenoic acid (DHA), and monounsaturated fatty acids (MUFA) including oleic and palmitoleic acids are correlated with improvement in metabolic alterations[21].

Most studies exploring the association of FAs with T2DM have focused on individual FAs and their role in T2DM incidence [8, 16-21, 26] and no such studies have been conducted in South Asian populations. Imamura et al, using Europe-wide EPIC study concluded that a combination of individual FAs, characterized by high concentrations of LA, stearic acid, odd-chain FAs, and very long-chain saturated FAs, was associated with lower incidence of T2DM[27]. To our knowledge, no study has explored longitudinal associations of FA combinations with T2DM in South Asians despite metabolic differences, and relatively higher prevalence of T2DM in South Asian populations[1, 4, 5].

Therefore, we aimed to investigate whether certain FA combinations are associated with incidence of T2DM in South Asians. We first identified a combination of FAs in pooled samples from the CARRS1 and CARRS2 cohorts, that explained maximum variation in FAs to derive an FA pattern score. Then we tested the association of the established FA pattern score with incidence of T2DM. Subsequently, we evaluated, if the established FA-pattern score and T2DM incidence association is mediated by obesity and metabolic biomarkers. This helped ascertain the potential mechanism of the role of phospholipids in T2DM and potential pharmacological interventions among South Asians.

Materials and Methods

Source Population

CARRS is a prospective cohort study in three major urban cities of South Asia: Delhi, Chennai, and Karachi. For the current analysis, we used a nested case-control design within the Karachi arm of the CARRS, details of which have been reported previously[2, 28]. The recruitment was completed in two waves, first, for CARRS 1, and then for CARRS 2;

with follow up assessments. Karachi is a metropolitan urban setting with a large, growing (due to continued births and migration from various parts of the country), and diverse population of ~13 million[28]. Moreover, the demographic heterogeneity of this urban capital continues to symbolize rapid social, economic, epidemiologic, and nutrition/lifestyle transition in South Asian region[28].

Sampling

The CARRS study recruited a representative sample of 4,017 participants in 2010/2011 (CARRS1) and 4,802 participants in 2015 (CARRS2) through multi-stage cluster random sampling in Karachi. Two participants, one male and one non-pregnant female, aged ≥ 20 years old, were selected from each household and baseline questionnaires, anthropometric measurements, and biological samples were collected.

The coordinating site was Agha Khan University Hospital, Karachi, Pakistan. At participant households and neighborhood camps, field teams collected a fasting blood sample from study participants, at baseline and follow up, in both cohorts. The samples for the study came from aliquots of plasma and serum that were stored at the coordinating site in cryo-vials at -80 °C.

Study Population

For our current prospective nested case-control study, we identified a sub-cohort of 200 participants in CARRS1 who developed T2DM in the 5 year follow up period (cases) and a random sample of 200 individuals matched for age and sex who remained diabetes-free for the whole 5 year follow up period (controls). Furthermore, 200 cases and 200 controls were selected from CARRS2 by the same method.

Definition of cases and controls

Incident T2DM (cases) came from follow up examinations among Karachi CARRS1 and CARRS2 participants (i.e., fasting plasma glucose (FPG) ≥ 126 mg/dl, or HbA1c ≥ 6.5%, or self-reported physician-diagnosed diabetes, or reported use of diabetes medications) who were diabetes-free at baseline (i.e. FPG<126mg/dl and HbA1c<6.5% and no self-reported diagnosis or diabetes medication use).

Controls were defined as CARRS1 and CARRS2-Karachi participants having all the following at both baseline and throughout the 5-year follow-up period: FPG < 126 mg/dl, and HbA1c < 5.7%, and no self-reported physician-diagnosed diabetes, and no reported diabetes medication use.

Assessment of FAs and other variables

Quantitative NMR metabolite profiling was performed to quantify a total of 225 metabolites including 9 FA metabolites concentrations in the serum samples. We selected following metabolites: PUFA, MUFA, TOTFA, omega- 6 FAs, omega-3 FAs, SFA, unsaturated FAs, LA and DHA. We computed BMI using height (m^2) and weight (kg); estimated serum triglyceride by enzymatic method, which uses the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase to determine glycerol [29].

Furthermore, HDL-C was measured directly from serum, low density lipoprotein (LDL) cholesterol was computed by Friedewald's formula[30], and HbA1c by high performance liquid chromatography method (HPLC)[28]. Finally, fasting serum concentration of glucose was determined using glucose oxidase method[28].

Demographic variables including age, sex, socioeconomic status (estimated by household income and years of education)[31], smoking history (categorized as tobacco smoked ever or not), past medical history of hypertension, depression, heart disease, and stroke were self-reported in during questionnaire administration[28].

Statistical Analysis

Statistical analyses were performed using Stata 17.0. Two-sided *p-values* <0.05 were used for all analyses.

Collinearity, interaction, and confounding analysis were performed, and history of stroke was dropped due to significant collinearity with history of heart disease.

Descriptive statistics were generated for participants' age, sex, level of education, income, smoking history, history of heart disease, anthropometric measurements, and metabolic parameters in CARRS1 and CARRS2 separately. Statistical comparisons were performed between cases and controls in each of the cohorts **(Table 1 a & b)**. The data from both cohorts was appended for further analyses.

Derivation of FA pattern score

To help understand the variation in FAs in the sample, PC analysis was performed by incorporating the measures of TOTFA, MUFA, PUFA, SFA, omega-6 FAs, omega-3 FAs, LA, DHA, and unsaturated FAs. Eigenvalues divided by 5 were assessed representing the percent of variance explained by the variables. Sampling adequacy was confirmed using a Kaiser-Meyer-Olkin measure that indicates the proportion of variance in variables that might be caused by underlying factors[32]. High values (close to 1.0) generally indicate that a factor analysis may be useful with the data [33].

Among the PCs with eigenvalues greater than 1, the PC explaining most variation among FAs concentrations was chosen to represent FA pattern scores. Loading values corresponding to individual FAs were constructed for FA pattern scores.

Associations with T2DM

The exposure variable, FA-pattern score, was incorporated in the model as a continuous variable. First, chi-square tests were performed for preliminary inspection of potential associations between other factors and T2DM. Finally, binary logistic regression analyses were performed in order to assess the associations of FA pattern scores with incident T2DM. Results were reported as ORs with 95% CI. Sex, age, smoking history, educational background, and a history of heart disease were included in models as potential confounders (Model I). FA pattern score-T2DM association analyses were additionally adjusted for BMI (Model II), and HDL-C and triglycerides (Model III). Predicted probabilities were calculated for each model and goodness of fit was evaluated through percent correctly predicted values. As a comparison, binary logistic regression analyses were performed using individual FA metabolites in association with incident T2DM. Finally, obesity stratified analyses were performed as sensitivity analyses based on the BMI level (Underweight: BMI <18.5 kg/m^2; Normal weight: BMI 18.5 – 24.9 kg/m^2; Overweight: BMI ≥ 25 kg/m^2) categorized per CDC definition[34].

Analysis of metabolic factors

Further, we performed correlation analysis of the FA-pattern score with each of the selected metabolic risk factors (BMI, triglycerides, HDL-C, and HbA1c) by treating all

metabolic risk factor variables as continuous variables to understand the mechanism of association of FA pattern score with risk of T2DM.

Results

Clinical characteristics

The study population included 392 participants from the CARRS 1 cohort with 47% males and 394 participants from the CARRS 2 cohort with 38% males (**Table 1**). We did not observe any statistically significant differences regarding demographic variables including education, income, smoking history, and history of depression between cases and controls in both the CARRS1 and CARRS2 cohorts. However, the metabolic parameters, including measures of BMI, HbA1c, LDL-C, HDL-C, and triglycerides were statistically different among cases and controls across both CARRS 1 and CARRS 2 cohorts (*p-value* <0.05).

Principle component analysis

PC analysis produced seven PCs with eigenvalues of first two PCs greater than 1. The first PC (PC1) explained 70% of variation in FA concentrations (**supplementary figure 2**) and had highest loading values for PUFA (0.41), LA (0.4), omega-6 FA (0.4), SFA (0.4) and lowest for unsaturated FAs (-0.04) (**Supplementary Table 1**). PC1 was subsequently referred to as the FA pattern score.

Association of the FA pattern score with incidence of T2DM

We observed that the FA pattern score had a marginal protective role in association with incident T2DM (OR 0.94, 95% CI 0.88-1.02, *p-value*: 0.14) while adjusting for age, sex,

education level, smoking history, and history of heart disease in Model I (**Table 2a).** The protective association was strengthened by further adjusting for BMI (OR 0.91, 95% CI 0.83-1.00, *p-value*: 0.04) in Model II and HDL-C and triglycerides (OR 0.88, 95% CI 0.80-0.97, *p-value*: 0.013) in Model III. In comparison, the total FAs also exhibited a weak protective trend against T2DM after adjusting for potential confounders (OR 0.76 95% CI 0.76-1.11). Among the individual FAs, strongest protective effects against T2DM were noted for PUFA, LA, and omega-6 FAs (**Table 2a**).

Considering adjustment for obesity strengthened the associations between FA pattern score and incident T2DM, we performed obesity stratified analysis. We observed a similar protective role of FA pattern score against T2DM in the normal weight individuals (OR 0.83 95% CI 0.72 – 0.97, *p-value*: 0.02). There was no protective role in the overweight and obese population (OR 0.94 95% CI 0.83-1.06, *p-value*: 0.31) and underweight population (OR 1.37 95% CI 0.49-3.86, *p-value*: 0.54).

Correlation of the FA pattern score with metabolic factors

We observed that FA pattern score demonstrated a positive correlation with triglycerides (r: 0.18, *p-value*: <0.001), HDL-C (r: 0.10, *p-value*: 0.04), and HbA1c (r: 0.10, *p-value*: 0.03) (**Table 3**). We did not observe statistically significant correlations with BMI.

Discussion

Our study demonstrated that a combination of FAs, with higher composition of PUFA, omega-6 FAs, SFA, and omega-3 FAs was associated with a lower future risk of T2DM among South Asians. Furthermore, the association of FA pattern score in relation to T2DM was significantly protective in normal weight individuals, but there was no association in overweight or underweight individuals potentially due to small sample size. We also observed moderate positive correlations between FA pattern score in relation to metabolic biomarkers.

The findings of our study concurred with the protective role of FAs on T2DM incidence as noted with individual FAs and combinations of FAs previously. First, our study confirmed the effect reflected in a Mendelian randomization study that concluded an inverse association between TOTFA and omega-3 FAs with the incidence of T2DM[18]. Secondly, we observed a weakly positive association of FA pattern score consisting of high PUFA composition with HbA1c **(Table 3)**, unlike beneficial glycemic control suggested in a systematic review of human randomized controlled intervention studies[35]. Finally, our study demonstrated a similar protective association of FA pattern score, with high concentration of omega-6 FA in relation with T2DM incidence in South Asian populations as observed by Imamura et al. in European populations[27]. In agreement with previous studies, we found no significant association between omega-3 FA and risk of T2DM[36-38] and no harmful effect of higher omega-6 and lower omega-3 FA concentrations on T2DM[39]. Furthermore, a protective effect of LA in association with T2DM was found in concurrence with previous research[40, 41]. However, our findings disagreed with studies that concluded protective effects of omega-3 on T2DM. [19, 42, 43].

FAs have been proven to produce transcription factors that affect glucose homeostasis and changes in phospholipid FA concentrations in plasma membranes have been found to modulate insulin resistance[44-46]. These transcription factors including peroxisome proliferator-activated receptors (PPARs) which are affected by FAs and have a protective role against inflammatory conditions that could give rise to T2DM[47]. One of the PPARs enzymes, PPAR alpha has been implicated in controlling the rate limiting step in de novo lipogenesis (DNL) in the liver[44]. Activation of PPAR alpha suppresses DNL which has been known to produce intermediates of pro inflammatory pathways, some of which have been implicated in development of T2DM[47-50]. According to metanalyses of randomized controlled trials, supplementation of fish oils containing certain FAs may decrease adiposity, increase adiponectin, lower circulating triglycerides, and inflammatory markers[51, 52]. In a similar manner, FAs have been demonstrated to improve glycolipid metabolism, leading to reductions in obesity and insulin resistance[16]. Free FAs (FFA) have been shown to improve glucose-mediated insulin secretion[53]. In terms of inflammation, FAs are precursors to eicosanoids, which play an important role in regulation of inflammation[42]. These mechanisms provide plausible explanations of our findings.

Our study has some important pharmacological and pathophysiological implications on understanding incidence and management of T2DM in South Asians. First, combinations of FA groups may have a greater role in reduction of incidence of T2DM in South Asians than individual FAs. Secondly, drugs targeting FA receptors, metabolism and PPAR alpha maybe more effective in the South Asians for T2DM prevention and management as suggested by a previous review[54]. Finally, a combination of FAs with higher PUFA,

omega-6 FA, SFA, omega-3 FA concentrations and lower unsaturated FA concentrations could have a stronger protective effect in the normal weight South Asian individuals against T2DM incidence as compared to overweight or obese individuals.

To our knowledge, this is the first study evaluating the role of combinations of FAs with the incidence of T2DM in South Asians. Our study is strengthened by prospective data collection in CARRS which provided clarity of the temporal sequence of exposure and outcome. The nested case-control design with cumulative sampling is another strength of this study due to cost effectiveness. Additional strengths include use of NMR for measuring samples and addressing potential confounders. Finally, obesity-stratified analysis to emphasize the role of obesity in association of FA pattern score with T2DM makes our study unique.

Nevertheless, our study had several limitations including small sample size, due to missing data of FA variables in both cohorts. To address that, we pooled data from the CARRS1 and CARRS2 cohorts for analysis. Other limitations included lack of generalizability (study only included South Asian population), reliance on self-reported physician diagnosis, lack of data on biomarkers of inflammation and potential residual confounding. Finally, we had very limited data regarding individual FAs and our findings are restricted to FA groups and should be interpreted carefully.

In conclusion, our findings, taken in the context of those from previous studies, suggest that FA combinations characterized by PUFA, omega-6 FAs, and SFA may reduce future risk of T2DM in non-obese south Asian populations. This calls for investigating this relationship with larger sample sizes of South Asian populations, studying combinations

of individual FAs instead of groups, and studying efficacy of antidiabetic drugs that modulate PPARs for the management of T2DM.

Ethics approval

CARRS-surveillance study has been approved by the Institutional Review Boards (IRBs) of Public Health Foundation of India, New Delhi, All India Institute of Medical Sciences, New Delhi, Madras Diabetes Research Foundation, Chennai, India, Aga Khan University, Karachi, Pakistan, and Emory University, Atlanta, USA. Furthermore, the study has received regulatory approval from the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), USA and the Health Ministry Screening Committee of India, New Delhi.

Tables and Figures

Table 1. Baseline characteristics of cases and controls

	CARRS1 (n=392)			CARRS2 (n=394)		
Baseline characteristics	**Cases**	**Controls**	*p-value*	**Cases**	**Controls**	*p-value*
N	197	195		194	200	
Age (years), mean (SD)	44 (11)	44 (11)	0.97	47 (12)	46 (12)	0.66
Male %	47%	46%	0.99	38%	39%	0.83
Education %			0.079			0.80
Professional degree or postgraduate	2%	5%		2%	4%	
Graduate	11%	14%		8%	11%	
Secondary School	30%	28%		21%	21%	
High school	27%	33%		27%	27%	
Primary School	2%	2%		6%	7%	
Literate, no formal education	5%	2%		3%	4%	
Illiterate	23%	16%		33%	28%	
Income (RS.), (%)			0.44			0.20
<10,000 (US$ 200)	35%	27%		40%	42%	
10,000–20,000	46%	47%		49%	40%	
>20,000 (US$ 400)	20%	26%		3%	18%	
Unknown	0%	1%		1%	4%	
Smoking history, (%)	23%	22%	0.76	31%	33%	0.74
History of Depression			0.52			0.86
No	91%	93%		70%	70%	
Moderate	8%	7%		29%	29%	
Severe	1%	0%		1%	1%	
Examinations, mean (SD)						
Waist circumference (cm)	95.94 (11.84)	88.89 (12.52)	<0.001			
Weight (kg)	72.48 (14.35)	66.05 (13.58)	<0.001			

BMI (kg/m2)	29.91 (6.04)	27.15 (5.60)	<0.001	27.97 (6.33)	25.63 (6.21)	<0.001
Metabolic parameters, mean (SD)						
HbA1c (%)	5.81 (0.38)	5.44 (0.43)	<0.001	5.68 (0.45)	5.27 (0.29)	<0.001
LDL-c (mg/dl)	112.55 (27.13)	107.78 (28.75)	0.093			
HDL-c (mg/dl)	41.66 (13.24)	43.87 (12.78)	0.094	41.23 (10.04)	43.62 (11.75)	0.031
Triglycerides (mg/dl), median (IQR)	139 (108 196)	114 (83 157)	<0.001	126.5 (92 183)	107 (74 144)	<0.001
Total cholesterol (mg/dl)	0.95 (0.57 1.49)	0.91 (0.58 1.33)	0.14	-0.18 (-0.54 - 0.16)	-0.08 (-0.55 - 0.57)	0.060
Creatinine (mg/dl)	0.06 (0.01)	0.06 (0.01)	0.062	0.08 (0.83)	-0.07 (1.16)	0.15

Table 2 a. Association of the FA pattern score with incidence of type 2 diabetes: CARRS sub-cohort (n=470)

Model	**Model I*** **OR (95% CI) (n=462)**	**Model II †** **OR (95% CI) (n=441)**	**Model III ‡** **OR (95% CI) (n= 440)**
FA pattern score	0.94 (0.88 - 1.02)	0.91 (0.83 – 1.00)	0.88 (0.80 - 0.97)
P value	0.14	0.04	0.01
Polyunsaturated Fatty acids	0.81 (0.67 - 0.98)	0.77 (0.62 – 0.95)	0.73 (0.58 - 0.92)
Docosahexaenoic acid	0.84 (0.67 – 1.01)	0.78 (0.63 – 0.97)	0.76 (0.61 – 0.95)
Linoleic acid	0.81 (0.67 – 0.98)	0.77 (0.62 – 0.95)	0.72 (0.57 – 0.91)
Monounsaturated Fatty Acids	1.09 (0.90 - 1.32)	1.00 (0.81 – 1.23)	0.93 (0.74 - 1.16)
Omega-3 Fatty acids	0.92 (0.76 - 1.11)	0.85 (0.70 – 1.05)	0.83 (0.67 - 1.02)
Omega-6 Fatty Acids	0.80 (0.66 - 0.97)	0.76 (0.62 – 0.94)	0.73 (0.58 - 0.91)
Saturated Fatty acids	0.95 (0.79 – 1.15)	0.88 (0.72 – 1.07)	0.80 (0.64 - 1.00)
Unsaturated Fatty Acids	0.96 (0.80 – 1.16)	0.98 (0.80 – 1.20)	1.08 (0.87 - 1.35)
Total fatty acids	0.92 (0.76 - 1.11)	0.85 (0.70 – 1.05)	0.79 (0.63 - 0.99)

*****Adjusting for potential confounders age, sex, education, smoking, and history of heart disease

†Adjusting for BMI in addition to all potential confounders in model I

‡ Adjusting for triglycerides and HDL-C in addition to all potential confounders in model II

Table 2 b. Association of the FA pattern score with incidence of type 2 diabetes: CARRS sub-cohort – Stratified analysis

Model	Odds ratio (95% CI) (n=264) Overweight and Obese (BMI >24.9)	Odds ratio (95% CI) (n=155) Normal weight (18.5 ≤ BMI ≤ 24.9)	Odds ratio (95% CI) (n=22) Underweight (BMI < 18.5)
FA pattern score	0.94 (0.83 – 1.06)	0.83 (0.72 – 0.97)	1.37 (0.49 – 3.86)
***P* trend**	0.31	0.016	0.54
Total fatty acids	0.89 (0.67 – 1.18)	0.72 (0.52 – 1.01)	1.25 (0.17 – 9.25)

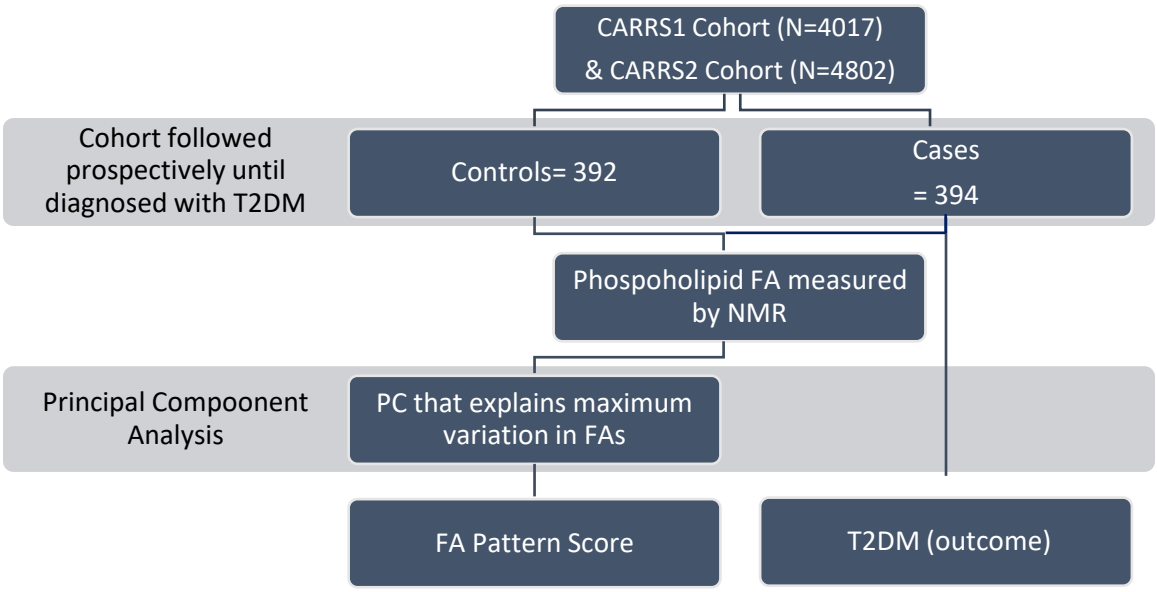
*Adjusting for potential confounders age, sex, education, smoking, and history of heart disease

Table 3. Correlation of the FA pattern score with metabolic factors CARRS sub-cohort (n=470) and with metabolic factors in the larger CARRS cohort

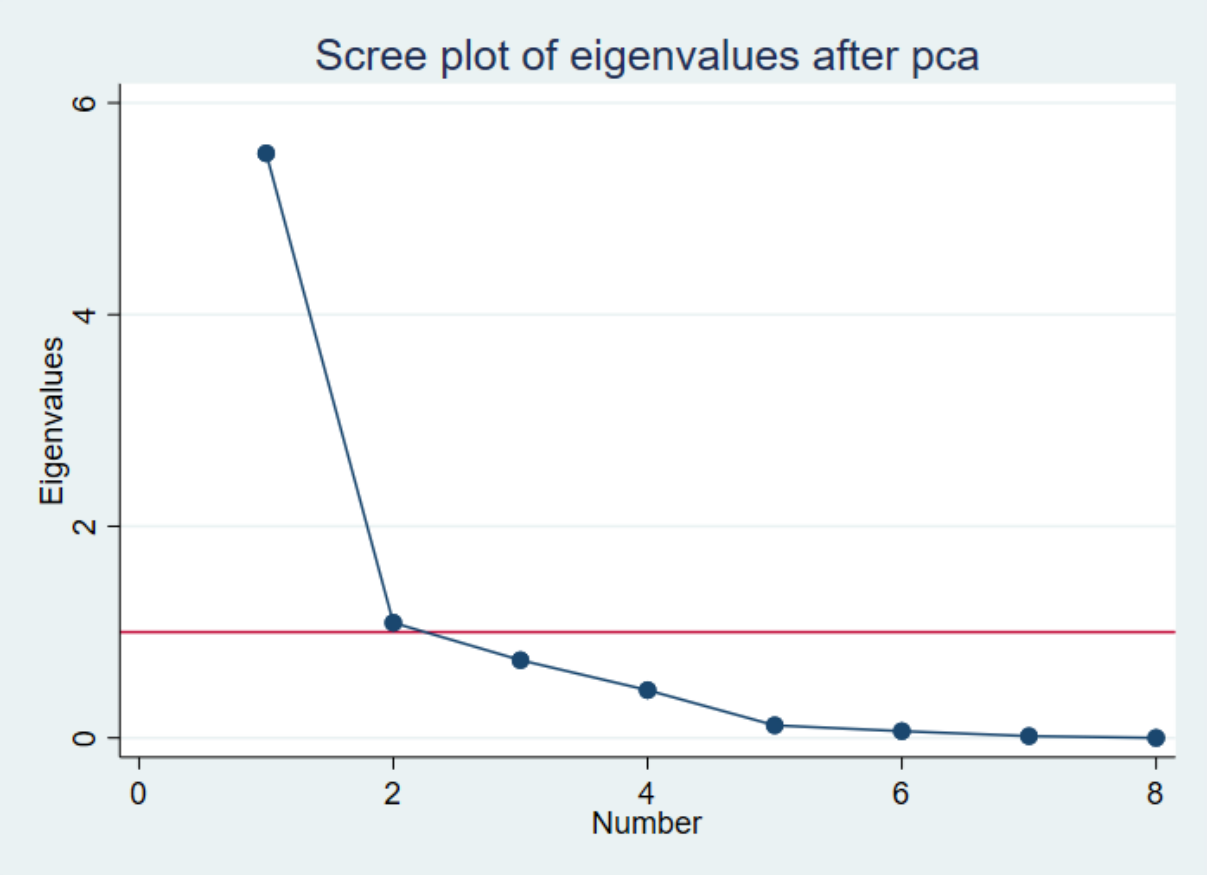
Metabolic factor	**Correlation with FA pattern score: r (*p-value*)**
BMI	0.04 (0.44)
Triglycerides	0.18 (<0.001)
HDL-c	0.10 (0.04)
HbA1c	0.10 (0.03)

Supplementary Table 1. Relative concentrations of plasma FAs and their correlations with the identified FA pattern score: CARRS sub-cohort (n= 470)

Fatty Acids	Loading values for first Principal component (FA pattern score)
Polyunsaturated Fatty acids	0.4103
Linoleic acid	0.4045
Omega-6 Fatty Acids	0.4011
Saturated Fatty acids	0.3976
Omega-3 Fatty acids	0.3587
Monounsaturated Fatty Acids	0.3315
Docosahexaenoic acid	0.3297
Unsaturated Fatty Acids	-0.0426



Supplementary Figure 1. Nested case-control design of study



Supplementary Figure 2. Principal component score and eigenvalues

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