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Daneshmand R

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Associations of estimated delta-5-desaturase and delta-6-desaturase activities with stroke risk factors and risk of stroke: the Kuopio Ischaemic Heart Disease Risk Factor Study

Roya Daneshmand, Sudhir Kurl, Tomi-Pekka Tuomainen, and Jyrki K Virtanen

All from the University of Eastern Finland, Kuopio Campus, Institute of Public Health and Clinical Nutrition, Kuopio, Finland.

Correspondence to Jyrki K. Virtanen, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland., PO Box 1627, 70211 Kuopio, Finland. E-mail jyrki.virtanen@uef.fi, Tel +358-40-3552957, Fax +358-17-162936.

Running title: Desaturase activities and risk of stroke

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Keywords: desaturases, polyunsaturated fatty acids, stroke, risk factors, prospective study

Abstract

Stroke is the leading cause of morbidity and mortality. The role of polyunsaturated fatty acids (PUFA) in reducing the risk of stroke is uncertain. The concentrations of PUFAs in human body are determined both by dietary intake and by activities of desaturase enzymes. Desaturase enzymes have been associated with chronic diseases, but little is known about their association with stroke risk. We investigated the associations of the delta-6-desaturase (D6D) and delta-5-desaturase (D5D) activities with stroke risk factors and risk of stroke among 1842 men from the prospective, population-based Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), aged 42-60 years and free of CVD at baseline in 1984-1989. ANCOVA and Cox regression models were used for the analyses. Whole serum desaturase activities were estimated as product-to-precursor ratios: gamma-linolenic acid/linoleic acid for D6D and arachidonic acid/dihomo-gamma-linolenic acid for D5D. Higher D6D activity was associated with higher systolic and diastolic blood pressure, body mass index, serum insulin and triglyceride concentrations, and worse Homeostatic Model Assessment (HOMA) indices. In contrast, higher D5D activity was associated with lower systolic and diastolic blood pressure, body mass index, serum insulin, LDL-cholesterol, triglyceride and C-reactive protein concentrations, higher HDL cholesterol concentration, and better HOMA indices. During the mean follow-up of 21.2 years, 202 stroke cases occurred. Neither D6D activity (multivariable-adjusted extreme-quartile HR=1.18, 95% CI 0.80-1.74) nor D5D activity (HR=1.06, 95% CI 0.70-1.60) were associated with stroke risk. In conclusion, higher D5D activity was favorably associated and higher D6D activity unfavorably associated with several stroke risk factors, but not with risk of incident stroke.

Introduction

Polyunsaturated fatty acids (PUFA) have an important role in the prevention of cardiovascular diseases[1,2]. The levels of the essential fatty acids linoleic acid and alpha-linolenic acid in body are determined by diet, but the levels of other PUFAs are influenced not only by diet, but also by desaturase enzymes[3]. Delta-5-desaturase (D5D) and delta-6-desaturase (D6D) catalyse the endogenous synthesis of the long-chain PUFA from the essential fatty acids and are considered as key enzymes for PUFA conversion[4,5]. Especially D6D is a rate-limiting enzyme in the whole PUFA pathway, because it is the enzyme that converts the n-6 PUFA linoleic acid and the n-3 PUFA alpha-linolenic acid to longer-chain n-6 and n-3 PUFAs. D5D and D6D are encoded respectively by the fatty acid desaturase genes FADS1 and FADS2, which have been shown to be the strongest genetic predictors of circulating PUFA concentrations[6].

D5D and D6D activities and FADS1 and FADS2 polymorphisms have been associated with risk of chronic diseases, such as type 2 diabetes and cardiovascular diseases[3]. However, little is known about the association with stroke risk[7], although D5D and D6D activities have been rather consistently associated with several stroke risk factors, such as high blood pressure, high body mass index and obesity, worse serum lipid profile, inflammation, and insulin resistance. In general, D6D activity has been associated with a higher risk of these risk factors[8-14] and D5D activity with a lower risk[8-13,15-17]. However, many of the studies have been small. Therefore, in order to elucidate the role of the D5D and D6D activities in the development of stroke, we investigated the associations of the estimated D5D and D6D activities with risk of incident stroke and cross-sectionally with stroke risk factors in 1842 middle-aged and older men from Finland.

Methods

Study design and population

KIHD was designed to explore the associations between risk factors and risk of cardiovascular disease, atherosclerosis, stroke and other chronic diseases[18]. The baseline examinations were performed in 1984–1989. All men who were 42, 48, 54 or 60 years old and living in the city of Kuopio or the surrounding areas were invited and 2682 (82.9 % of those eligible) participated in the baseline examinations. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Research Ethics Committee of the University of Kuopio. Written informed consent was obtained from all participants.

Men with history of cardiovascular disease or stroke (n=709) or with missing data on serum PUFA (n=131) were excluded, leaving 1842 men.

Serum fatty acid measurements

Serum esterified and non-esterified fatty acids were specified in one gas chromatographic run without preseparation as described[19]. Serum fatty acids were extracted with chloroform-methanol. Chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards purchased from NU-Check Prep Inc. (MA, USA). Each analyte had individual reference standard, and an internal standard was eicosane. Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, PA, since 1999 Agilent Technologies Inc.) with a flame ionization detector. Results were obtained in micromoles per liter and presented as proportion of total serum fatty acids. The coefficient of variation (CV%) for repeated measurements of fatty acids was 9.6% for linoleic acid (LA, 18:2n-6), 11.7% for gamma-linolenic acid (18:3n-6), 8.3% for dihomo-gamma-linolenic acid (20:3n-6), 9.2% for arachidonic acid (20:4n-6). Desaturase enzyme activities were estimated as the ratio of product to precursor and were calculated as the ratio of arachidonic acid to dihomo-γ-linolenic acid for the D5D activity and as the ratio of gamma-linolenic acid to linoleic acid for the D6D activity[20].

Other measurements

The subjects gave fasting blood samples between 8 and 10AM at the baseline examinations in 1984-1989. They were instructed to abstain from ingesting alcohol for three days and from smoking and eating for 12 hours prior to giving sample. Detailed descriptions of the determination of serum lipids and lipoproteins, assessment of medical history and medications, family history of diseases, smoking, and alcohol consumption, have been published[21]. Plasma glucose was measured using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. Serum insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk, Bagsvaerd, Denmark). Diabetes was defined as self-reported diabetes mellitus or fasting blood glucose of 6.7 mmol/L or more. Insulin resistance and sensitivity and β-cell function were estimated by HOMA computer algorithm[22,23]. Education was assessed in years by using self-administrated questionnaire. Physical activity was assessed using the KIHD 12-Month Leisure-Time Physical Activity Questionnaire[24]. Serum C-reactive protein (CRP) was measured with an immunometric assay (Immulite High

Sensitivity CRP Assay, DPC, Los Angeles, CA, USA). Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in meters. Dietary intake of foods and nutrients was assessed at the time of blood sampling using 4-day food recording[25].

Ascertainment of follow-up events

Incident strokes between years 1984-1992 were observed through FINMONICA stroke register[26]. Information regarding the stroke incident between years 1993 and 2012 was collected through computerized linkage to the national hospital discharge registry. The diagnosis of stroke was based on sudden onset of clinical signs or focal or global disturbance of cerebral function lasting 24 hours (except in the case of sudden death or if interrupted by surgical intervention) with no apparent cause other than a vascular origin. Each suspected stroke (International Classification of Diseases [ICD]-9 codes 430-439 and ICD-10 codes I60-I68 and G45-G46) was classified into 1) a definite stroke, 2) no stroke, or 3) an unclassifiable event. The FINMONICA stroke register data were annually rechecked with the data obtained from the computerized national hospital discharge and death registers. Definite strokes and unclassifiable events were included in the group of any stroke. Each definite stroke was classified into 1) an ischemic stroke (ICD-9 codes 433 434; ICD-10 code I63) or 2) a haemorrhagic stroke (ICD-9 codes 430 431; ICD-10 codes I60–I61). If the subject had multiple nonfatal strokes during follow-up, the first stroke was considered as the end point. CT was performed in 90% of the patients by 1993, and CT, MRI, and autopsy reached 100% by 1997[27]. Every resident of Finland has a unique personal identifier that is used in registers. There were no losses to follow-up.

Statistical analysis

Subjects were divided into quartiles according to estimated D6D and D5D activities. The univariate relationships between estimated D6D and D5D activities and baseline characteristics were assessed by means and linear regression (for continuous variables) or χ^2 tests (for categorical variables). The mean values of risk factors in the estimated D6D and D5D activity quartiles were analysed using analysis of covariance (ANCOVA). Associations between estimated D6D and D5D activities and risk of incident stroke were analysed using Cox regression models. Two different models were used to adjust for potential confounders. First model was adjusted for age and examination year. The second model further included body mass index, smoking, physical activity, and alcohol intake. All quantitative variables were entered as continuous variables. The covariates in the models were chosen to comply with the recent analyses regarding the n-3 and n-6 PUFAs and risk of stroke in this study population[28].

Further adjustment for potential confounders education and waist circumference did not appreciably change the associations (change in estimates <5%). Cohort mean was used to replace missing values in covariates (<0.5%). Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. All *P* values were two-tailed (α=0.05). Data were analysed using SPSS 23.0 for Windows (IBM Corp., Armonk, NY).

Results

At baseline, higher estimated D5D activity was mainly associated with lower BMI and higher alcohol intake, whereas higher estimated D6D activity was associated with lower age, lower education and higher BMI and higher alcohol intake (Table 1). They were also more likely to live in a rural area and have type 2 diabetes. Those with higher estimated D5D activity had higher serum concentrations of total n-3 PUFA, total n-6 PUFA, linoleic acid and arachidonic acid but lower gamma-linolenic acid and dihomo-γ-linolenic acid concentrations, whereas those with higher estimated D6D activity had higher concentrations of eicosapentaenoic acid, docosapentaenoic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid and arachidonic acid but lower concentrations of total n-6 PUFA, total n-3 PUFA, linoleic acid and docosahexaenoic acid (Table 1).

The Table 2 shows the associations of the estimated desaturase activities with stroke risk factors. After multivariable adjustments, higher estimated D6D activity was associated with higher diastolic blood pressure, body mass index, serum insulin concentration, serum triglyceride concentration, HOMA insulin resistance, HOMA β-cell function, and lower HOMA insulin sensitivity. Although the *P* for trend across the quartiles was statistically significant also for the association with systolic blood pressure, visual inspection did not support a linear association (Table 2). In contrast, higher estimated D5D activity was associated with lower systolic and diastolic blood pressure, body mass index, serum insulin concentration, serum LDL-cholesterol concentration, serum triglyceride concentration, C-reactive protein concentration, HOMA insulin resistance, HOMA β-cell function, and higher HDL cholesterol concentration and HOMA insulin sensitivity. No statistically significant associations were found with blood glucose.

During the average follow-up of 21.2 years (min-max 0.3-28.8 y), 202 men (11.0%) experienced a stroke. Of all strokes, 153 were ischemic and 51 were haemorrhagic strokes. Despite the statistically significant associations with several stroke risk factors (Table 2), we did not find statistically significant associations between the estimated D5D or D6D activities and risk of incident stroke (Table 3). Entering the estimated desaturase activities as tertiles or continuously (per 1 SD) instead of quartiles

into the models did not reveal any statistically significant associations, either. For example, the HR (95% CI) for any stroke, ischaemic stroke and haemorrhagic stroke for 1 SD change in the estimated D5D activity were 0.99 (0.86-1.15), 0.98 (0.83-1.16) and 0.95 (0.72-1.27), respectively. The respective HRs (95% CI) for the estimated D6D activity were 1.06 (0.93-1.22), 1.09 (0.93-1.27) and 0.98 (0.75-1.29) (other data not shown).

Because a long follow-up could potentially attenuate the associations between exposures that are measured only at baseline, we also investigated the associations with a shorter, 12.8 y mean follow-up. However, we did not find statistically significant associations with this shorter follow-up, either [multivariable-adjusted extreme-quartile HR for total stroke (104 events)=1.34, 95% CI 0.77-2.34, P-trend=0.30 for the estimated D6D activity and HR=0.90, 95% CI 0.50-1.63, P-trend=0.64 for the estimated D5D activity]. Because diabetes can affect the desaturase activities and many risk factors shown in the Table 2, we also investigated the associations after excluding those with type 2 diabetes (n=83). However, the associations with risk factors and with risk of incident stroke remained similar (data not shown). Similarly, excluding participants with lipid-lowering or anti-inflammatory medication (n=89) had no appreciably impact on the associations (data not shown).

Discussion

In this prospective cohort study among men from eastern Finland, higher estimated D5D activity had a favorable association and higher estimated D6D activity an unfavorable association with several stroke risk factors. However, neither were associated with the risk of incident stroke.

Overall, the impact of PUFA metabolism on stroke risk is not well established. Although estimated desaturase activities have been associated with several stroke risk factors, the impact on stroke incidence is so far very little investigated. Our findings of the associations between estimated D5D or D6D activities and stroke risk factors are consistent with the results from most previous studies, where high estimated D6D activity has generally had adverse associations with stroke risk factors, while high estimated D5D activity has been favorably associated. For example, higher estimated D6D activity has been associated with higher serum triglycerides[9,12,15,17], higher LDL cholesterol[9,15], lower HDL cholesterol[12,15], higher CRP[12,14], higher blood pressure[17], higher BMI and obesity[10-12,15,17], and higher HOMA insulin resistance[17], whereas higher estimated D5D activity has been associated with lower serum triglycerides[8,9,12,15,17], lower LDL cholesterol[9], higher HDL cholesterol[12], lower CRP[12,14], lower blood pressure[8,19], lower BMI[10-12,16], and lower HOMA insulin resistance[17]. Although these studies have been cross-sectional, also a longitudinal study found similar differences

between the estimated D5D and D6D activities[13]. Similarly, FADS1/FADS2 polymorphisms have been shown to associate, for example, with serum lipid levels and glucose metabolism[3,29], adding more evidence for the impact of D5D and D6D activities on these risk factors. A recent study also found that two FADS1/FADS2 SNPs were associated with higher risk of ischaemic stroke in a Chinese population, possibly by influencing serum lipid levels[30].

The reasons for the opposite associations of the D6D and D5D activities are not completely known. D6D activity is downregulated and D5D activity upregulated in the presence of a high-PUFA diet[31], so the desaturase activities may reflect the beneficial effects of the essential fatty acids, especially linoleic acid, on the risk factors[32]. On the other hand, D5D is the key enzyme for the production of the n-6 PUFA arachidonic acid and the n-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid, which have important roles as precursors in the production of eicosanoids and other bioactive compounds[33].

Previous studies have indicated an association between estimated D6D and D5D activities and risk of cardiovascular disease[3,34]. However, despite the observed associations with the stroke risk factors, estimated D5D or D6D activities were not associated with the risk of stroke in our study. This finding is consistent with the result of the only previous prospective study, which did not find statistically significant associations between estimated D5D or D6D activities and risk of stroke[7]. A possible explanation for why we found an association between stroke risk factors but not with stroke incidence maybe that the impact of the desaturase enzymes on the risk factors is too small and weak to have a significant impact on the risk of stroke. Especially the association with one of the strongest risk factors for stroke, high blood pressure, was quite modest and may not be clinically significant. The lack of association with the desaturase activities in the current study is also supported by the similar lack of association of the serum n-3 and n-6 PUFAs with the risk of stroke in this study population[28].

The strengths of the present study include the prospective and population-based design, and the extensive database of potential confounders and mediators. Several limitations should also be acknowledged. First, a potential weakness is the use of fatty acid product-to-precursor ratio to estimate hepatic desaturase activities indirectly, instead of directly measuring the desaturase activity. Direct measurement of enzyme activity is very impractical in large studies, but the use of a product-to-precursor ratio as a substitute measure to estimate desaturase activity is well established[3,20,31]. However, desaturase activities are commonly estimated from phospholipid or cholesterol ester fatty acids, not from the whole serum fatty acids like in our study. This makes the direct comparison of the estimated desaturase activities challenging. For example, because the proportion of gamma-linolenic acid in phospholipids is usually very low, the estimated D6D activity when using phospholipid fatty

acids is calculated as the ratio of dihomo-gamma-linolenic acid to linoleic acid, which also describes the activity of the elongase that converts gamma-linolenic acid to dihomo-gamma-linolenic acid. However, D5D and D6D activities estimated from whole serum have been shown to strongly associate with a known intron variant of the FADS1 gene, which provides indirect validation for the use of also whole serum fatty acids to estimate the desaturase activities[35]. Second, we had information on the estimated desaturase activities only from the baseline, which may not be representative for the entire long follow-up. This could potentially create exposure misclassification and thus random error, which would attenuate the associations towards the null in the analyses with incident stroke events. Because this kind of error would not affect the cross-sectional analyses with the risk factors, the discrepancy between the findings with the risk factors and with the risk of incident events may at least partly be explained the lack of repeated measurement of desaturase activities. On the other hand, the cross-sectional analyses are prone to reverse causation. Third, because of the low number of especially haemorrhagic stroke events, the findings regarding the associations between the desaturase activities and stroke risk should be interpreted cautiously. However, we did not find any associations with stroke risk if we analysed the associations in tertiles or as continuously instead of using quartiles, which indicates that the lack of association with incident events may not be due to low number of events. Finally, the study included only middle-aged and older Caucasian men, so the results may not be generalizable to other populations or to women.

In summary, estimated D5D and D6D activities were both associated with several risk factors for stroke, higher estimated D5D activity showing generally favorable associations and higher estimated D6D activity unfavorable associations. In spite of these associations with the risk factors, the desaturase activities were not associated with risk of incident stroke. Further studies in larger study populations are needed to elucidate the impact of the D5D and D6D activities on the risk of stroke. In addition, there is a need for more studies to clarify that what factors determine the activities of these enzymes and what biological mechanisms could explain the opposite associations of these desaturases with several risk factors for chronic diseases.

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Conflict of interests

None.

Authorship

The authors' contributions were as follows: R.D., S.K., T-P.T. and J.K.V. contributed to the conception and design of the research; S.K., and T-P.T. acquired data; R.D. and J.K.V. analysed data and interpreted the results; R.D. drafted the manuscript; all authors critically revised the paper and approved the final version.

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Table 1. Baseline characteristics according to quartiles of estimated serum delta-5-desaturase and delta-6-desaturase activities

	Delta-5-desaturase activity		*P* for trend	Delta-6-desaturase activity		*P* for trend
	Q1 (<2.99)	Q4 (>4.28)		Q1	Q4	
Number of subjects	460	461		460	460	
Age (y)	52.5 (5.1)*	52.3 (5.6)	0.72	52.8 (5.4)	51.7 (5.4)	0.003
Education (y)	8.9 (3.4)	9.1 (3.7)	0.83	9.1 (3.6)	8.5 (3.0)	0.01
Leisure-time physical activity (kcal/d)	129 (175)	149 (156)	0.25	148 (164)	130 (172)	0.30
Body mass index (kg/m^2)	27.3 (3.6)	26.2 (3.1)	<0.001	26.0 (3.1)	27.4 (3.6)	<0.001
Marital status, married (%)	87	86	0.89	88	86	0.66
Living in rural area (%)	32	27	0.12	24	35	<0.001
Current smoker (%)	29	28	0.59	31	27	0.27
Diabetes (%)	5	4	0.55	4	6	0.04
Family history of stroke (%)	19	19	0.96	19	17	0.50
Hypertension medication before stroke (%)	72	69	0.52	68	71	0.14
Alcohol intake (g/wk)	59 (105)	98 (139)	<0.001	59 (94)	96 (158)	<0.001
Total serum n-3 PUFA (%)	4.6 (1.0)	6.5 (2.0)	<0.001	5.4 (1.6)	5.2 (1.3)	0.01
Alpha-linolenic acid (%)	0.8 (0.3)	0.7 (0.2)	<0.001	0.8 (0.2)	0.7 (0.2)	<0.001
Eicosapentaenoic acid (%)	1.2 (0.5)	2.2 (1.3)	<0.001	1.6 (0.9)	1.7 (0.7)	0.08
Docosapentaenoic acid (%)	0.5 (1.0)	0.6 (0.1)	<0.001	0.5 (0.1)	0.6 (0.1)	0.001
Docosahexaenoic acid (%)	2.0 (0.5)	3.0 (0.8)	<0.001	2.6 (0.7)	2.3 (0.7)	<0.001

Total serum n-6 PUFA (%)	29.7 (4.5)	33.2 (4.4)	<0.001	34.3 (4.2)	28.6 (4.3)	<0.001
Gamma-linolenic acid (%)	0.3 (0.1	0.2 (0.1)	<0.001	0.2 (0.04)	0.4 (0.1)	<0.001
Dihomo-γ-linolenic acid (%)	1.5 (0.2)	1.1 (0.2)	<0.001	1.2 (0.2)	1.5 (0.2)	<0.001
Linoleic acid (%)	25.4 (4.3)	27.3 (4.5)	<0.001	29.5 (4.0)	23.3 (3.8)	<0.001
Arachidonic acid (%)	3.9 (0.7)	5.7 (1.0)	<0.001	4.6 (1.0)	2.9 (1.0)	<0.001

PUFA, polyunsaturated fatty acids. Delta-5-desaturase activity was calculated as the ratio of arachidonic acid to dihomo-γ-linolenic acidy and delta-6-desaturase activity as the ratio of gamma-linolenic acid to linoleic acid.

*Values are means (SD) or percentages.

Table 2. Associations of the estimated serum delta-5-desaturase and delta-6-desaturase activities with risk factors for stroke in 1842 men from the Kuopio Ischaemic Heart Disease Risk Factor Study

	Quartile of serum desaturase activity				
	1	2	3	4	*P* for trend
Systolic blood pressure, mmHg					
Delta-5-desaturase	<2.99	2.99-3.51	3.52-4.28	>4.28	
Model 1[†]	136 (0.8)[*]	134 (0.8)	134 (0.8)	132 (0.8)	0.002
Model 2[‡]	135 (0.7)	134 (0.7)	134 (0.7)	133 (0.7)	0.04
Delta-6-desaturase	<0.007	0.007-0.010	0.011-0.014	>0.014	
Model 1	132 (0.8)	134 (0.8)	134 (0.8)	137 (0.8)	<0.001
Model 2	133 (0.7)	135 (0.7)	134 (0.7)	136 (0.7)	0.02
Diastolic blood pressure, mmHg					
Delta-5-desaturase					
Model 1	90 (0.5)	89 (0.5)	89 (0.5)	88 (0.5)	<0.001
Model 2	90 (0.5)	89 (0.5)	89 (0.4)	88 (0.5)	0.005
Delta-6-desaturase					
Model 1	87 (0.5)	89 (0.5)	89 (0.5)	91 (0.5)	<0.001
Model 2	88 (0.5)	89 (0.5)	89 (0.4)	90 (0.5)	0.02
Body mass index, kg/m^2					
Delta-5-desaturase					
Model 1	27.3 (0.2)	26.8 (0.2)	26.5 (0.2)	26.2 (0.2)	<0.001
Model 2	27.4 (0.2)	26.9 (0.2)	26.5 (0.2)	26.1 (0.2)	<0.001
Delta-6-desaturase					
Model 1	26.0 (0.2)	26.5 (0.2)	26.9 (0.2)	27.5 (0.2)	<0.001
Model 2	26.0 (0.2)	26.5 (0.2)	26.9 (0.2)	27.4 (0.2)	<0.001

LDL Cholesterol, mmol/L					
Delta-5-desaturase					
Model 1	3.9 (0.04)	4.0 (0.1)	4.0 (0.04)	4.0 (0.04)	0.11
Model 2	3.9 (0.04)	4.0 (0.04)	4.1 (0.04)	4.0 (0.1)	0.09
Delta-6-desaturase					
Model 1	3.9 (0.04)	4.0 (0.04)	4.1 (0.04)	4.0 (0.04)	0.17
Model 2	3.9 (0.1)	4.0 (0.04)	4.1 (0.04)	4.0 (0.1)	0.15
HDL Cholesterol, mmol/L					
Delta-5-desaturase					
Model 1	1.2 (0.01)	1.3 (0.01)	1.3 (0.01)	1.4 (0.01)	<0.001
Model 2	1.2 (0.01)	1.3 (0.01)	1.3 (0.01)	1.4 (0.01)	<0.001
Delta-6-desaturase					
Model 1	1.3 (0.01)	1.3 (0.01)	1.3 (0.01)	1.3 (0.01)	0.21
Model 2	1.3 (0.01)	1.3 (0.01)	1.3 (0.01)	1.3 (0.01)	0.66
Triglycerides, mmol/L					
Delta-5-desaturase					
Model 1	1.6 (0.03)	1.4 (0.03)	1.1 (0.03)	1.0 (0.03)	<0.001
Model 2	1.6 (0.03)	1.4 (0.03)	1.1 (0.03)	1.0 (0.03)	<0.001
Delta-6-desaturase					
Model 1	1.1 (0.03)	1.2 (0.03)	1.3 (0.03)	1.5 (0.03)	<0.001
Model 2	1.1 (0.03)	1.2 (0.03)	1.3 (0.03)	1.5 (0.03)	<0.001
Blood glucose, mmol/L					
Delta-5-desaturase					
Model 1	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	0.20

Model 2	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	0.68
Delta-6-desaturase					
Model 1	4.6 (0.04)	4.7 (0.04)	4.7 (0.04)	4.8 (0.04)	0.01
Model 2	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	4.7 (0.04)	0.30
Serum insulin, mU/L					
Delta-5-desaturase					
Model 1	13.1 (0.3)	11.3 (0.3)	10.4 (0.3)	9.6 (0.3)	<0.001
Model 2	12.4 (0.3)	11.2 (0.3)	10.6 (0.3)	10.2 (0.3)	<0.001
Delta-6-desaturase					
Model 1	9.9 (0.3)	10.6 (0.3)	11.6 (0.3)	12.4 (0.3)	<0.001
Model 2	10.6 (0.3)	10.8 (0.3)	11.4 (0.3)	11.6 (0.3)	0.01
C-reactive protein, mg/L					
Delta-5-desaturase					
Model 1	2.8 (0.2)	2.1 (0.2)	2.1 (0.2)	2.0 (0.2)	0.01
Model 2	2.7 (0.2)	2.1 (0.2)	2.2 (0.2)	1.9 (0.2)	0.01
Delta-6-desaturase					
Model 1	2.0 (0.2)	2.2 (0.2)	2.4 (0.2)	2.4 (0.2)	0.16
Model 2	2.1 (0.2)	2.2 (0.2)	2.4 (0.2)	2.3 (0.2)	0.44
HOMA Insulin resistance					
Delta-5-desaturase					
Model 1	1.6 (0.04)	1.5 (0.04)	1.3 (0.04)	1.3 (0.04)	<0.001
Model 2	1.6 (0.03)	1.5 (0.03)	1.4 (0.03)	1.3 (0.03)	<0.001
Delta-6-desaturase					
Model 1	1.3 (0.04)	1.4 (0.04)	1.5 (0.04)	1.6 (0.04)	<0.001

Model 2	1.4 (0.03)	1.4 (0.03)	1.5 (0.03)	1.5 (0.03)	<0.001
HOMA β-cell function					
Delta-5-desaturase					
Model 1	118.6 (1.7)	112.8 (1.8)	106.7 (1.7)	103.2 (1.7)	<0.001
Model 2	117.1 (1.6)	114.1 (1.6)	109.7 (1.6)	107.5 (1.6)	<0.001
Delta-6-desaturase					
Model 1	105.9 (1.8)	107.2 (1.8)	113.2 (1.8)	114.9 (1.8)	<0.001
Model 2	108.3 (1.7)	107.9 (1.7)	112.3 (1.7)	112.7 (1.7)	0.03
HOMA insulin sensitivity					
Delta-5-desaturase					
Model 1	75.2 (1.7)	81.3 (1.7)	91.0 (1.7)	95.2 (1.7)	<0.001
Model 2	78.4 (1.5)	82.0 (1.5)	90.0 (1.5)	92.4 (1.5)	<0.001
Delta-6-desaturase					
Model 1	95.2 (1.7)	89.7 (1.7)	81.9 (1.7)	76.3 (1.7)	<0.001
Model 2	91.3 (1.5)	88.5 (1.5)	83.1 (1.5)	80.1 (1.6)	<0.001

HOMA, Homeostasis Model Assessment. Delta-5-desaturase activity was calculated as the ratio of arachidonic acid to dihomo-γ-linolenic acidy and delta-6-desaturase activity as the ratio of gamma-linolenic acid to linoleic acid.

*Values are mean (SEM).

†Model 1: adjusted for age and examination year.

‡Model 2: adjusted for Model 1 plus body mass index, smoking, physical activity, and alcohol intake.

Table 3. Risk of incident total stroke, ischaemic stroke and haemorrhagic stroke in quartiles of estimated serum delta-5-desaturase and delta-6-desaturase indices

	Quartile of serum desaturase activities				
	1	2	3	4	*P* for trend
All stroke cases, (n=202)					
Delta-5-desaturase	<2.99	2.99-3.51	3.51-4.28	>4.28	
	(n=460)	(n=461)	(n=461)	(n=460)	
Events, n	47	61	47	47	
Model 1[†]	1(referent)	1.29 (0.88-1.88)[*]	0.95 (0.63-1.42)	0.98 (0.65-1.46)	0.54
Model 2[‡]	1(referent)	1.35 (0.92-1.98)	1.01 (0.67-1.51)	1.06 (0.70-1.60)	0.81
Delta-6-desaturase	<0.007	0.007-0.010	0.011-0.014	>0.014	
	(n=460)	(n=456)	(n=465)	(n=461)	
Events, n	50	44	51	57	
Model 1	1(referent)	0.89 (0.59-1.33)	1.07 (0.73-1.59)	1.27 (0.87-1.85)	0.12
Model 2	1(referent)	0.87 (0.58-1.31)	1.02 (0.69-1.51)	1.18 (0.80-1.74)	0.25
Ischaemic stroke cases, (n=153)					
Delta-5-desaturase					
Events, n	37	50	32	34	
Model 1	1(referent)	1.33 (0.87-2.03)	0.81 (0.51-1.30)	0.89 (0.56-1.42)	0.27

Model 2	1(referent)	1.41 (0.92-2.16)	0.88 (0.55-1.41)	1.00 (0.62-1.60)	0.54	365
						366
Delta-6-desaturase						367
Events, n	38	36	34	45		368
Model 1	1(referent)	0.96 (0.61-1.51)	0.95 (0.60-1.51)	1.31 (0.85-2.03)	0.17	369
Model 2	1(referent)	0.94 (0.59-1.48)	0.90 (0.57-1.44)	1.24 (0.80-1.93)	0.27	370
Haemorrhagic stroke cases (n=51)						
Delta-5-desaturase						
Events, n	11	13	16	11		
Model 1	1(referent)	1.20 (0.54-2.67)	1.39 (0.65-3.00)	1.00 (0.43-2.31)	0.99	
Model 2	1(referent)	1.24 (0.55-2.76)	1.48 (0.68-3.20)	1.03 (0.44-2.43)	0.94	
Delta-6-desaturase						
Events, n	12	8	17	14		
Model 1	1(referent)	0.68 (0.28-1.68)	1.49 (0.71-3.11)	1.24 (0.57-2.69)	0.32	
Model 2	1(referent)	0.66 (0.27-1.63)	1.39 (0.66-2.92)	1.06 (0.48-2.33)	0.58	

Delta-5-desaturase activity was calculated as the ratio of arachidonic acid to dihomo-γ-linolenic acidy and delta-6-desaturase activity as the ratio of gamma-linolenic acid to linoleic acid.

*Values are hazard ratios (95% confidence interval).

†Model 1: adjusted for age and examination year.

‡Model 2: adjusted for Model 1 plus body mass index, smoking, physical activity, and alcohol intake.