Linoleic Acid, Other Fatty Acids, and the Risk of Stroke

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Background and Purpose—The role of serum fatty acids as a risk factor for stroke and stroke subtypes is largely unknown.

Methods—A prospective nested case-control study of Japanese 40 to 85 years of age was conducted through the use of frozen serum samples from 7450 participants in cardiovascular risk surveys collected from 1984 to 1989 for 1 community and 1989 to 1992 for the other 2 communities. By the end of 1998, we identified 197 incident strokes whose subtypes were confirmed by imaging studies. Three controls per case were selected by matching for sex, age, community, year of serum storage, and fasting status.

Results—Compared with controls, total (n=197), hemorrhagic (n=75), and ischemic (n=122) strokes had similar proportions of n3 polyunsaturated fatty acids, lower proportions of linoleic and arachidonic acids, and higher proportions of saturated and monosaturated acids, determined by gas chromatography. The multivariate odds ratios associated with a 1-SD increase in linoleic acid (5%) after adjustment for hypertension, diabetes, serum total cholesterol, and other cardiovascular risk factors were 0.72 [95% confidence interval (CI), 0.59 to 0.89] for total stroke, 0.66 (95% CI, 0.49 to 0.88) for ischemic stroke, 0.63 (95% CI, 0.46 to 0.88) for lacunar infarction, and 0.81 (95% CI, 0.59 to 1.12) for hemorrhagic stroke. The respective odds ratios for saturated fatty acids (4%) were 1.13 (95% CI, 1.05 to 1.65), 1.35 (95% CI, 1.01 to 1.79), 1.44 (95% CI, 1.03 to 2.01), and 1.21 (95% CI, 0.82 to 1.80). Further adjustment for other fatty acids attenuated these relations, but the relation between linoleic acid and risk of ischemic stroke remained statistically significant.

Conclusions—A higher intake of linoleic acid may protect against ischemic stroke, possibly through potential mechanisms of decreased blood pressure, reduced platelet aggregation, and enhanced deformability of erythrocyte cells. **(*Stroke*. 2002;33:2086-2093.)**

Key Words: fatty acids ■ fatty acids, unsaturated ■ follow-up studies ■ linoleic acid ■ stroke

Fatty acid composition influences various physiological and biochemical processes, including blood pressure regulation,[1–3] glucose metabolism,[4,5] lipid metabolism,[6] platelet aggregation,[7,8] and erythrocyte deformability.[9,10] However, the effects of serum fatty acid composition on the risk of stroke have not been examined extensively.

Several case-control studies have shown that a lower proportion of linoleic acid in blood, platelets, erythrocytes, or adipose tissue is associated with increased risk of total stroke or ischemic stroke.[11–14] In a prospective study, serum α-linolenic acid (an n3 polyunsaturated fatty acid) but not linoleic acid was associated with reduced stroke risk among men at high risk of cardiovascular disease.[15] In that study, the classification of stroke subtypes was not conducted, although the effects of serum fatty acids are likely to differ among stroke subtypes. No prospective study has reported a significant association between other fatty acids and the risk of stroke and stroke subtypes.

Japanese have a higher mortality from both ischemic and hemorrhagic strokes[16] and exhibit lower proportional levels of serum linoleic acid and higher levels of serum saturated and n3 polyunsaturated fatty acids[17] compared with white Americans and Japanese Americans. These cross-cultural differences suggest that high serum saturated fatty acids and low serum linoleic acid are associated with an increased risk of ischemic stroke and that a high proportion of n3 polyunsaturated fatty acids is associated with an increased risk of hemorrhagic stroke. To examine this hypothesis, we conducted a prospective nested case-control study of men and women in 3 Japanese communities using stored serum samples.

Materials and Methods

Surveyed Populations

The surveyed populations comprised 9174 men and women 40 to 85 years of age who participated in cardiovascular risk surveys between

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1984 and 1993 in a central rural community (Kyowa; census population for 40 to 85 years of age, 6874) and between 1989 and 1992 in a northeast rural community (Ikawa, n= 2822) and a southwest rural community (Noichi, n=5199). The participation rate in cardiovascular risk surveys among men and women 40 to 84 years of age was 62% in Kyowa, 76% in Ikawa, 53% in Noichi, and 62% for the total population. The serum sample of 1.0 to 2.0 mL for each participant was stored at −80°C for 1 to 9 years (average, 4.5 years).

Surveillance of Stroke and Classification of Stroke Subtypes

The participants were followed up to determine incident strokes occurring by the end of 1998. The follow-up was done by annual cardiovascular risk surveys to obtain histories of incident strokes; for nonparticipants, the ascertainment of stroke was done by mailing questionnaires and by the use of death certificates. From death certificates, cases with stroke as an underlying cause of death (International Classification of Disease 9 classification, 430 to 438) were selected. We also used national insurance claims, ambulance records, reports by local physicians, and reports by public health nurses and health volunteers for stroke ascertainment.[18]

To confirm the diagnosis, all living patients were visited or invited to take part in risk factor surveys to obtain medical history; if cases were still alive, neurological examinations were done by study physicians and medical records were reviewed. For deceased patients, histories were obtained from families and medical records were reviewed.

Stroke was defined as a focal neurological disorder with rapid onset that persisted at least 24 hours or until death and was confirmed by CT and/or MRI.[19] A diagnosis of embolic infarction was made when evidence of an embolic source was present in the medical records and if imaging studies and neurology consult supported the diagnosis. Classification of other stroke subtypes, ie, large-artery occlusive infarction, lacunar infarction, subarachnoid hemorrhage, and intraparenchymal hemorrhage, was conducted from imaging studies. Strokes with negative findings in imaging studies and unclassified strokes were not included in the present study. For each incident stroke, 3 controls were selected by matching for sex, age (±2 years), community, year of serum storage, and fasting status at serum collection (<8 and ≥8 hours).

Determination of Serum Total Fatty Acid Composition

The composition of serum total fatty acids was examined in incident stroke cases and in 3 controls. The distribution of the number of years serum had been stored was 2% for <1 year, 9% for 2 years, 14% for 3 years, 22% for 4 years, 20% for 5 years, 11% for 6 years, 8% for 7 years, 12% for 8 years, and 2% for 9 to 10 years. Sera were obtained from nonfasting samples, and the distribution of time since last meal was 30% for ≤1 hour, 40% for 2 hours, 19% for 3 hours, 8% for 4 to 7 hours, and 3% for ≥8 hours.

Lipids were extracted from the stored serum with chloroform and methanol and were saponified with potassium hydroxide and ethanol.[17] Fatty acids were transesterified with BF3-methanol, and the methyl esters were analyzed in a Hitachi 263-80 gas chromatograph

TABLE 1. Risk Characteristics Among Cases and Controls by Stroke Subtype

	No.	Age, y	Men, %	Systolic BP, mm Hg	Diastolic BP, mm Hg	Hypertension, %	BMI, kg/m^2	Ethanol Intake, g/d	Current Smokers, %	Serum Cholesterol, mmol/L	Triglycerides, mmol/L	Impaired Glucose Tolerance, %	Diabetes, %
Total stroke													
Cases	197	65.4	53	143‡	83‡	58‡	24.0†	14.4	36*	5.09	1.42*	19	10*
Controls	591	65.4	53	135	79	42	23.1	12.5	28	5.02	1.28	17	5
P													
Ischemic stroke													
Cases	122	66.8	62	141†	81	60†	23.8*	16.3	39	5.07	1.39*	20	13†
Controls	366	66.8	62	135	78	43	23.0	14.4	32	4.94	1.23	19	4
Lacunar infarction													
Cases	95	66.9	61	140†	81	63‡	23.8*	16.3	39	5.07	1.37	18	12*
Controls	285	66.8	61	135	78	41	23.0	13.1	29	4.94	1.21	17	4
Large-artery occlusive infarction													
Cases	19	66.6	68	148	81	58	24.3	16.1	47	5.18	1.53	16	21*
Controls	57	66.6	68	138	80	51	22.9	16.1	44	5.03	1.38	31	4
Embolic infarction													
Cases	8	67.1	63	134	78	25	22.8	17.3	13	4.79	1.27	50	0
Controls	24	67.6	63	136	77	50	23.3	26.0	38	4.75	1.10	17	0
Hemorrhagic stroke													
Cases	75	63.0	39	146‡	86‡	55*	24.3	11.2	32*	5.12	1.47	19	4
Controls	225	63.0	39	135	79	40	23.4	9.5	20	5.15	1.36	13	6
Intraparenchymal hemorrhage													
Cases	45	64.6	42	147†	87‡	58	24.7	12.0	33*	4.94	1.34	23	5
Controls	135	64.7	42	135	80	46	23.4	8.8	18	5.19	1.34	17	6
Subarachnoid hemorrhage													
Cases	30	60.6	33	145*	86*	50	23.7	9.9	30	5.39	1.68*	13	3
Controls	90	60.5	33	134	79	31	23.3	10.4	23	5.09	1.38	9	7

BP indicates blood pressure; BMI, body mass index. Triglycerides were expressed as geometric mean.
*P<0.05, †P<0.01, ‡P<0.001 vs controls.

TABLE 2. Case-Control Differences in Mean Fatty Acid Composition of Total Serum Lipids by Stroke Subtype

	Saturated Fat			Monounsaturated Fat		n6-Polyunsaturated Fat				n3-Polyunsaturated Fat			
	Miristic 14:0	Palmitic 16:0	Stearic 18:0	Palmitoleic 16:1	Oleic 18:1	Linoleic 18:2	γ-Linolenic 18:3n6	Dihomo-r-Linolenic 20:3	Ara-chidonic 20:4	α-Linolenic 18:3n3	Eicosa-pentaenoic 20:5	Docosa-pentanenoic 22:5	Docosa-hexaenoic 22:6
Total stroke													
Cases	1.2†	23.8†	7.9	3.9‡	21.4†	26.5‡	0.4	0.9	4.6†	1.0	3.6	0.5	4.3
Controls	1.1	23.0	7.9	3.6	20.9	28.2	0.2	0.8	4.9	1.1	3.5	0.5	4.3
Ischemic stroke													
Cases	1.2†	24.0†	8.0	4.0‡	21.4*	26.1‡	0.2	0.9	4.7	1.0	3.6	0.6	4.5
Controls	1.1	23.0	8.1	3.5	20.8	27.9	0.2	0.8	4.9	1.0	3.7	0.5	4.4
Lacunar infarction													
Cases	1.2†	24.2†	8.1	4.0‡	21.4*	25.9‡	0.2	0.9	4.7	1.0	3.5	0.5	4.4
Controls	1.1	23.0	8.2	3.5	20.7	28.1	0.2	0.8	4.9	1.0	3.7	0.5	4.4
Large-artery occlusive infarction													
Cases	1.2	23.5	7.8	3.8	21.4	25.9	0.2	0.9	4.5	1.0	4.5	0.6	4.8
Controls	1.1	23.4	8.0	3.7	21.7	27.0	0.2	0.8	4.6	1.1	3.5	0.5	4.3
Embolic infarction													
Cases	1.2	22.8	7.4	3.7	21.1	28.0	0.2	0.8	5.2	1.0	3.4	0.6	4.7
Controls	1.1	22.7	7.7	3.3	20.2	28.3	0.2	0.9	5.3	1.0	4.2	0.5	4.6
Hemorrhagic stroke													
Cases	1.2	23.4	7.8	3.8	21.5	27.1*	0.6	0.9	4.5†	1.1	3.4	0.5	4.1
Controls	1.1	23.1	7.6	3.6	20.9	28.6	0.2	0.8	4.9	1.1	3.4	0.5	4.2
Intraparenchymal hemorrhage													
Cases	1.1	23.2	7.9	3.8	21.8*	27.5	0.2	0.8	4.5†	1.1	3.5	0.5	4.2
Controls	1.1	23.1	7.5	3.6	20.7	28.7	0.2	0.8	5.0	1.1	3.5	0.5	4.1
Subarachnoid hemorrhage													
Cases	1.2	23.8	7.7	3.9	21.1	26.6	1.2	0.9	4.5	1.1	3.2	0.6	4.2
Controls	1.1	23.0	7.7	3.7	21.2	28.6	0.2	0.9	4.7	1.1	3.1	0.5	4.2

*$P<0.05$, †$P<0.01$, ‡$P<0.001$ vs controls.

(Hitachi Corp) with a 3-m glass column with 3-mm internal diameter (Unisole 3000, Gas-Chro Corp). An injection temperature of 250°C, a column temperature of 220°C, and a column flow of 40 m/min of nitrogen were used. Peaks were determined by a flame ionization detector and were quantified with an electronic integrator (Hitachi Corp). Compositions of individual serum fatty acids were expressed as percentages of the total area of 13 major fatty acid peaks from 14:0 to 22:6.

We used duplicated serum aliquots for 31 samples frozen at −80°C in 1990 and measured serum fatty acids in 1990 for the nested case-control study and again 8 years later. There was an increase in the compositions of saturated fat (29.2% versus 30.3%, $P<0.001$) and 20:3 (0.85% versus 0.98%, $P=0.003$), and a decline in the compositions of monounsaturated fatty acids (22.9% versus 22.4%, $P=0.004$), 18:3n6 (0.31% versus 0.24%, $P=0.01$), and 20:4 (5.6% versus 5.5%, $P=0.01$), but no changes were seen for other fatty acids, including linoleic acid (28.4% versus 28.3%, $P=0.39$) and n3 polyunsaturated fatty acids (12.8% versus 12.3%, $P=0.14$). Another 31 pairs of samples collected from nonfasting subjects for the nested-control study in 1993 and fasting subjects in 1994 exhibited no difference in the composition of each fatty acid, including linoleic acid (29.3% versus 29.9%, $P=0.49$) and n3 polyunsaturated fatty acids (10.6% versus 10.9%, $P=0.60$) according to fasting status.

Determination of Confounding Variables

An interview was conducted to ascertain histories of cigarette smoking (never, ex, and current), ethanol intake (never, ex, and current daily intake), and medication use for high blood pressure and high serum glucose levels. Height in stocking feet and weight in light clothing were measured. Body mass index was calculated as weight (kilograms) divided by height (meters) squared.

Systolic and diastolic blood pressures were measured by trained observers using a standard mercury sphygmomanometer on the right arm of seated participants after a 5-minute rest. Hypertension was defined as systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥95 mm Hg, and/or the use of antihypertensive medication; normotension was defined as systolic blood pressure <140 mm Hg, diastolic blood pressure <90 mm Hg, and no antihypertensive medication. All others were classified as having borderline hypertension.

Serum total cholesterol, triglycerides, and glucose were measured by enzymatic methods (SMAC, Technicon Instrument Corp). The measurements of serum lipids were successfully standardized by the Lipid Standardization Program (Center for Disease Control, Atlanta, Ga).[20] Impaired glucose tolerance was defined as a fasting glucose of 6.1 to 6.9 mmol/L and/or a nonfasting glucose of 7.8 mmol/L without use of medication for diabetes. Diabetes was defined as a fasting glucose of ≥7.0 mmol/L, a nonfasting glucose of ≥11.1 mmol/L, and/or the use of medication for diabetes.

Statistical Analysis

The paired Student's *t* test was used to compare the mean values of baseline cardiovascular risk factors and serum fatty acid compositions between incident cases and controls. The χ^2 test was used to compare proportions between cases and controls. Univariate odds ratios of total stroke and stroke subtypes associated with a 1-SD increase in each fatty acid composition were calculated by use of the conditional logistic regression model. The multivariate odds ratio was estimated according to quartiles of selected serum fatty acids and with a change of 1-SD of these fatty acids in the controls with

conditional logistic regression models adjusted for body mass index (kg/m^2), cigarette smoking status (never, ex, and current), current ethanol intake (g/d), hypertension status (normal, borderline, and hypertension), serum total cholesterol levels (mmol/L), and log-transformed triglyceride levels (mmol/L) and serum glucose category (normal, impaired glucose tolerance, and diabetes). Further adjustments for selected serum fatty acids of statistical significance with the risk of stroke and stroke subtypes were also conducted. All probability values for statistical significance were 2 tailed, and all confidence intervals (CIs) were estimated at the 95% level.

Results

During follow-up, we identified 197 incident strokes: 75 hemorrhagic strokes (45 intraparenchymal hemorrhages, 30 subarachnoid hemorrhages), 114 ischemic strokes (95 lacunar infarction, 19 large-artery occlusive infarctions, and 8 embolic infarctions). Table 1 shows risk characteristics of total stroke and each stroke subtype compared with controls. The average age was 65 years for total stroke, varying from 61 years for subarachnoid hemorrhage to 67 years for lacunar infarction. The proportion of men was 53% for total stroke, varying from 33% for subarachnoid hemorrhage to 68% for large-artery occlusive infarction. Systolic and diastolic blood pressure levels and the prevalence of hypertension were higher for total stroke than in controls; this trend was most evident for lacunar infarction and hemorrhagic stroke but was not observed for embolic infarction. Mean body mass index and the prevalence of diabetes were higher in cases than in controls for total stroke, ischemic stroke, and lacunar infarction. The prevalence of impaired glucose tolerance did not differ between cases and controls for total stroke or stroke subtypes. The prevalence of current smoking was higher in cases than controls for total stroke and hemorrhagic stroke. Mean serum cholesterol levels tended to be higher in ischemic strokes than controls, whereas cholesterol levels tended to be lower in intraparenchymal hemorrhages than controls. Mean serum triglyceride levels were higher in cases than controls for total stroke, ischemic stroke, and subarachnoid hemorrhage. Mean ethanol intake tended to be higher in cases than controls for total, ischemic, and hemorrhagic strokes.

The serum compositions of total fatty acids are displayed in Table 2. Saturated (14:0 and 16:0) and monounsaturated (16:1 and 18:1) fatty acids were higher and n6 polyunsaturated fatty acids (18:2 and 20:4) were lower in participants who had had stroke than in controls. These case-control differences in serum fatty acid compositions were more evident for ischemic stroke, specifically lacunar infarction, than for other stroke subtypes. There was no difference in n3 polyunsaturated fatty acids between the total stroke group and controls or between any stroke subtype and controls.

Table 3 shows univariate odds ratios and 95% confidence intervals (CIs) for total stroke and stroke subtypes associated with a 1-SD increase in each fatty acid. Risk of total stroke was positively associated with saturated fatty aids (14:0 and 16:0) and monounsaturated fatty acids (16:1 and 18:1) and inversely associated with n6 polyunsaturated fatty acids, more specifically linoleic and arachidonic acids (18:2 and 20:4). The association was similarly observed for ischemic stroke, more specifically lacunar infarction and hemorrhagic stroke, more specifically intraparenchymal hemorrhage. After adjustment for cardiovascular risk factors, these associations remained significant except for the relation between arachidonic acid and risk of total and hemorrhagic stroke and the relation between monosaturated fatty acids and risk of ischemic and hemorrhagic strokes (not shown in Table 3).

Because serum saturated and linoleic acids were consistently associated with the risk of stroke, we examined the relationships of these fatty acids with other major cardiovascular risk factors, ie, blood pressure levels, diabetes, and serum total cholesterol levels among participants who remained free of strokes (n= 591). No association was observed between saturated fatty acids and blood pressure levels. A 5.0% higher linoleic acid level was associated with 0.4 mm Hg (95% CI, 0. 1 to 1.9; P=0.56) lower systolic and 1.3 mm Hg (95% CI, 0.3 to 2.2; P=0.008) lower diastolic blood pressure levels after adjustment for age, sex, body mass index, and ethanol intake. The multivariate odds ratio for diabetes associated with 4.0% higher saturated fatty acids after controlling for age, sex, body mass index, and ethanol intake was 0.7 (95% CI, 0.4 to 1.1, P=0.09), whereas the odds ratio with 5.0% higher linoleic acid was 1.7 (95% CI, 1.1 to 2.7; P=0.01). A 4.0% higher saturated fatty acid level was associated with 0.07 mmol/L (95% CI, 0.003 to 0.14; P=0.04) lower serum total cholesterol, whereas a 5.0% higher linoleic acid was associated with 0.01 mmol/L (95% CI, −0.06 to 0.08; P=0.73) lower serum total cholesterol after adjustment for age, sex, and body mass index.

Table 4 provides multivariate odds ratios for total strokes and stroke subtypes according to quartiles of saturated and linoleic acids and odds ratios associated with a 1-SD increase in these fatty acids (4% and 5%, respectively). Subarachnoid and intraparenchymal hemorrhages were combined as hemorrhagic stroke because univariate trends were similar and the respective numbers of incident cases were small. The data on large-artery occlusive infarction and embolic infarction were not shown because of the small number of cases.

Serum saturated fatty acids were positively associated with the risk of total stroke, ischemic stroke, and lacunar infarction in a dose-response fashion. After adjustment for hypertension, diabetes, serum total cholesterol levels, and other cardiovascular risk factors, these positive relationships remained significant. The positive associations disappeared after further adjustment for serum linoleic acid and monounsaturated fatty acids that were correlated with saturated fatty acids (Pearson's correlation coefficient among controls, −0.67 and 0.07, respectively).

Because of a high correlation between saturated fatty acids and linoleic acid, we further examined the relation between saturated fatty acids and the risk of ischemic stroke stratified by below and above the median of linoleic acid; the multivariate odds ratio of ischemic stroke associated with a 1-SD increase in saturated fatty acids (4%) was 1.45 (95% CI, 0.88 to 2.37) for persons who had lower linoleic acid and 0.90 (95% CI, 0.38 to 2.14) for those who had higher linoleic acid.

Serum linoleic acid was inversely associated with the risk of total stroke, ischemic stroke, lacunar infarction, and hemorrhagic stroke in a dose-response fashion. After adjustment for cardiovascular risk factors, these inverse relationships remained significant for total stroke, ischemic stroke, and lacunar infarction but not for hemorrhagic stroke. The

TABLE 3. Univariate Odd Ratios of Stroke and Stroke Subtypes According to 1-SD Increase in Each Fatty Acid

	Saturated Fat			Monounsaturated Fat	
	Miristic 14:0	Palmitic 16:0	Stearic 18:0	Palmitoleic 16:1	Oleic 18:1
Total stroke	1.34‡ (1.14–1.58)	1.35‡ (1.14–1.59)	1.03	1.39‡ (1.17–1.65)	1.32† (1.11–1.56)
Ischemic stroke	1.47‡ (1.19–1.82)	1.47‡ (1.19–1.82)	0.80	1.51‡ (1.21–1.89)	1.23* (1.01–1.51)
Lacunar infarction	1.50‡ (1.19–1.90)	1.63‡ (1.27–2.08)	0.83	1.58‡ (1.23–2.03)	1.29* (1.03–1.61)
Large-artery occlusive infarction	1.63	1.09	0.63	1.15	0.83
Embolic infarction	1.16	1.03	0.66	1.57	1.57
Hemorrhagic stroke	1.16	1.17	1.81* (1.04–3.16)	1.23	1.51† (1.12–2.02)
Intraparenchymal hemorrhage	1.09	1.04	2.12* (1.03–4.39)	1.25	1.63* (1.10–2.40)
Subarachnoid hemorrhage	1.26	1.36	1.36	1.21	1.34

95% CIs are shown when odds ratios are statistically significant.
*$P<0.05$, †$P<0.01$, ‡$P<0.001$ vs controls.

reduced association between serum linoleic acid and the risk of hemorrhagic stroke occurred after controlling for the hypertension category only (not shown in Table 4). Further adjustment for saturated and monounsaturated fatty acids, which were correlated with serum linoleic acid (Pearson's correlation coefficients among controls between monounsaturated fatty acids and linoleic acid, −0.47), did not alter the relations with total stroke and ischemic stroke. Associations between linoleic acid and the risk of ischemic stroke were similarly observed for persons who had levels below and above the median level of saturated fatty acids; the multivariate odds ratio of ischemic stroke associated with a 1-SD increase in linoleic acid (5%) was 0.77 (95% CI, 0.59 to 1.19) for persons who had lower saturated fatty acids and 0.67 (95% CI, 0.45 to 1.00) for those who had higher saturated fatty acids.

The n3 polyunsaturated fatty acids were not associated with the risk of total stroke or any stroke subtypes (not shown in Table 4).

Discussion

The major finding of the present study was that serum linoleic acid was inversely associated with the risk of total stroke, ischemic stroke, and more specifically lacunar infarction. These associations remained significant even after adjustment for known cardiovascular risk factors and the matching variables of age, sex, years of serum storage, and community. Further adjustment for other fatty acids such as saturated and monounsaturated fatty acids did not materially alter the relation with risk of total stroke and ischemic stroke. Serum saturated fatty acids were positively associated with the risk of total stroke, ischemic stroke, and lacunar infarction, but these associations disappeared when adjusted further for linoleic and monounsaturated fatty acids. The adjustment for these fatty acids may be unreliable because saturated fatty acids and linoleic acid were strongly correlated. However, the analysis is likely to be correct statistically because we verified that the relationship of saturated fatty acids and stroke was strongly attenuated within persons who had below the median level of linoleic acid and within those who had above the median. The small effect of adjustment for other fatty acids on the inverse relation between serum linoleic acid and the risk of ischemic stroke suggests that linoleic acid per se reduces the risk of ischemic stroke.

The exact mechanisms of the apparent protective effect of serum linoleic acid on the risk of ischemic stroke are not clear at present, although a few possibilities can be proposed. First, dietary intake of linoleic acid may lower blood pressure; therefore, high serum linoleic acid may reduce the risk of ischemic stroke. Previous clinical trials,[3,4] but not all,[21] have supported a blood pressure–lowering effect. The present study showed lower diastolic blood pressure levels in association with higher serum linoleic acid. However, our finding of an inverse relationship between serum linoleic acid and risk of ischemic stroke was not entirely explained by a lowering effect of blood pressure levels because adjustment for blood pressure did not eliminate the association. Second, a previous study of whites showed that dietary intake of linoleic acid may improve glucose tolerance.[5] In the present study, however, the prevalence of diabetes was higher with higher serum linoleic acid, so it is unlikely that the reduced risk of ischemic stroke associated with higher serum linoleic acid was mediated by improved glucose tolerance. Third, it is well known that dietary linoleic acid has a cholesterol-lowering effect.[6] In the present study, serum linoleic acid was not significantly associated with serum total cholesterol levels. Even if a modest cholesterol-lowering effect exists, it is unlikely to influence the risk of ischemic stroke because only very high levels (eg, ≥7.2 to 8.0 mmol/L) of serum total cholesterol are associated with increased risk of ischemic stroke[22,23] and few subjects in our study had such high cholesterol levels. Finally, linoleic acid also reduces platelet aggregation[8] and enhances erythrocyte deformation,[10] both of which may contribute to improved circulation in small blood vessels and a reduced risk of lacunar infarction.

Serum linoleic acid and n3 polyunsaturated fatty acids reflect diet, whereas serum saturated fatty acid and monounsaturated fatty acid do not because of metabolic conversion of other fatty acids that obscures the relationship of these fatty acids in the serum and diet.[4,24] Our previous study of 507 middle-aged Japanese men showed that the Pearson coefficient between dietary intake of fatty acids (percent of total

TABLE 3. Continued

n6-Polyunsaturated Fat				n3-Polyunsaturated Fat			
Linoleic 18:2	γ-Linolenic 18:3n6	Dihomo-r-Linolenic 20:3	Arachidonic 20:4	α-Linolenic 18:3n3	Eicosapentaenoic 20:5	Docosapentanenoic 22:5	Docasahexaenoic 22:6
0.65‡ (0.54–0.79)	1.04	1.11	0.78† (0.65–0.93)	0.86	1.01	1.17	1.03
0.60‡ (0.46–0.77)	1.12	1.14	0.86	0.83	0.99	1.21	1.08
0.57‡ (0.43–0.76)	1.12	1.10	0.86	0.82	0.89	1.17	1.02
0.66	1.40	1.34	0.84	0.82	2.28* (1.14–4.57)	1.35	1.53
0.92	0.68	0.96	0.89	0.89	0.63	1.59	1.06
0.73* (0.55–0.95)	0.94	1.05	0.65† (0.47–0.89)	0.93	1.04	1.13	0.93
0.79	0.71	1.20	0.52	0.83	1.01	1.09	0.87
0.62* (0.38–1.00)							
	1.20	0.85	0.87	1.10	1.12	1.15	1.10

energy), assessed by a 24-hour dietary recall, and serum fatty acid compositions was 0.34 ($P<0.001$) for linoleic acid, −0.005 ($P=0.90$) for saturated fatty acids, −0.10 ($P=0.03$) for monounsaturated fatty acids, and 0.26 ($P<0.001$) for n3 polyunsaturated fatty acids.[25] Reanalysis of that study indicated that dietary intake of linoleic acid was 3.7% (9.5 g/d) in the lowest quintile, 4.4% (10.9 g/d) in the second quintile, 4.9% (12.2 g/d) in the third quintile, and 5.4% (13.3 g/d) in the highest quintile of serum linoleic acid when the same cut points as those in the present study were used; no such gradient was seen in parallel analysis of dietary intake and serum content of saturated fatty acid.

We observed an inverse association between serum linoleic acid and risk of hemorrhagic stroke in the univariate analysis, but this was no longer significant after adjustment for hypertension status. Because there was a significant inverse association between linoleic acid and blood pressure levels, a protective effect of serum linoleic acid on the risk of hemorrhagic stroke may be mediated in part by a blood pressure–lowering effect.

There was no association of n3 polyunsaturated fatty acids with the risk of hemorrhagic or ischemic strokes. This finding is not surprising because serum n3 polyunsaturated fatty acid levels (range, 2.7% to 22.0%) were still lower than that of Greenland Eskimos[26] in whom an excess mortality from hemorrhagic stroke was reported.[27] Elongation of bleeding time appears only when the proportion of serum n3 fatty acids exceeds ≈20%,[28] corresponding to only 2% of the subjects in the present study. One possible reason for the lack of association between n3 polyunsaturated fatty acids and ischemic stroke was that most of the subjects in the present study had quite high levels of n3 polyunsaturated fatty acids, which are likely to protect against ischemic stroke. Previous prospective studies have demonstrated that a small amount of fish and n3 polyunsaturated fatty acid intake or several weekly servings of fish were associated with a significant risk reduction for ischemic stroke.[29,30]

The strength of the present study is the large number of strokes confirmed by imaging studies, which allowed us to investigate the relationship between serum fatty acids and total stroke, as well as stroke subtypes. A previous study of whites showed that α-linolenic acid, an n3 polyunsaturated fatty acid primarily in vegetables, was inversely associated with risk of total stroke. However, the number of total strokes included in that study was not large (n=96),[15] and no stroke subtype classification was conducted. In our study, no association was evident between α-linolenic acid and risk of total, hemorrhagic, or ischemic strokes.

The present study has certain limitations. First, dehydration of polyunsaturated fatty acids during preservation at −80°C may be a potential problem. Subsample analysis, however, demonstrated that there were no material changes in serum n3 or n6 polyunsaturated fatty acid composition between the 1990 and 1998 values. Furthermore, mean values of fatty acids were similar to those reported in our previous studies in which the serum samples were preserved at −80°C for only 3 months until analysis.[17,25] Second, the serum samples were nonfasting, and the time of blood collection may affect fatty acid composition. However, subsample analysis revealed no significant difference in serum fatty acid composition between fasting and nonfasting samples. Third, the generalizability of the present data to Western countries is unknown. The proportion of stroke subtypes in the present study was 38% for hemorrhagic strokes, 48% for lacunar infarctions, 10% for large-artery occlusive infarctions, and 4% for embolic infarctions, which were similar to previous reports among Japanese, whereas those in Western countries were ≈15% to 20%, 15% to 25%, 50% to 60%, and 10%, respectively.[31] Because ischemic stroke was mostly lacunar infarction among Japanese and large-artery occlusive infarction among whites, the present study implies the importance of linoleic acid in the prevention of lacunar stroke. A potential effect on large-artery occlusive infarction was uncertain because of the limited number of cases.

In conclusion, our observational study suggests that serum linoleic acid may protect against ischemic stroke and lacunar infarction, possibly because of lowering of blood pressure levels and improvement in small-vessel circulation via reduced platelet aggregation and enhanced erythrocyte deformability. This finding implies the potential importance of dietary intake of linoleic acid for the prevention of ischemic stroke. A clinical trial is necessary to confirm the causality between linoleic acid intake and risk of ischemic stroke.

TABLE 4. Univariate and Multivariate Odds Ratios of Stroke and Stroke Subtypes According to Serum Saturated and Linoleic Acids

	Quartiles of Serum Fatty Acids				OR Associated With a 1-SD Increase in Serum Fatty Acid	*P*
	1 (Low)	2	3	4 (High)		
Total saturated acids, %						
Median	27.5	30.5	32.9	36.7		
Range	23.8–29.0	29.1–31.5	31.6–34.4	34.5–52.4		
Total stroke						
Cases, n	37	48	49	63		
Controls, n	147	148	148	148		
Multivariate OR*	1.0	1.28 (0.75–2.16)	1.28 (0.73–2.22)	1.84 (1.00–3.40)	1.31 (1.05–1.65)	0.02
Multivariate OR†	1.0	0.96 (0.54–1.72)	0.82 (0.43–1.57)	1.07 (0.50–2.32)	1.12 (0.83–1.51)	0.45
Ischemic stroke						
Cases, n	20	33	27	42		
Controls, n	87	97	85	97		
Multivariate OR*	1.0	1.58 (0.80–3.11)	1.49 (0.70–3.15)	2.51 (1.09–5.74)	1.35 (1.01–1.79)	0.04
Multivariate OR†	1.0	1.17 (0.54–2.53)	0.92 (0.38–2.21)	1.33 (0.48–3.71)	1.09 (0.76–1.58)	0.64
Lacunar infarction						
Cases, n	13	26	23	33		
Controls, n	66	82	64	73		
Multivariate OR*	1.0	1.57 (0.70–3.54)	1.68 (0.69–4.10)	3.17 (1.21–8.31)	1.44 (1.03–2.01)	0.03
Multivariate OR†	1.0	1.17 (0.46–3.00)	1.07 (0.36–3.17)	1.90 (0.55–6.63)	1.24 (0.81–1.91)	0.32
Hemorrhagic stroke						
Cases, n	17	15	22	21		
Controls, n	60	51	63	51		
Multivariate OR*	1.0	0.95 (0.39–2.30)	1.03 (0.43–2.48)	1.14 (0.43–2.99)	1.21 (0.82–1.80)	0.34
Multivariate OR†	1.0	0.70 (0.27–1.83)	0.66 (0.23–1.94)	0.77 (0.22–2.69)	1.11 (0.64–1.93)	0.72
Linoleic acid, %						
Median	22.4	26.4	29.9	33.7		
Range	12.4–24.8	24.9–28.3	28.4–31.6	31.7–43.6		
Total stroke						
Cases, n	70	58	41	28		
Controls, n	146	148	149	148		
Multivariate OR*	1.0	0.97 (0.61–1.54)	0.56 (0.33–0.84)	0.41 (0.23–0.74)	0.72 (0.59–0.89)	0.002
Multivariate OR†	1.0	0.97 (0.59–1.59)	0.58 (0.32–1.08)	0.43 (0.20–0.93)	0.76 (0.58–1.00)	0.05
Ischemic stroke						
Cases, n	46	37	24	15		
Controls, n	97	92	92	85		
Multivariate OR*	1.0	0.95 (0.53–1.73)	0.47 (0.23–0.96)	0.33 (0.14–0.74)	0.66 (0.49–0.88)	0.005
Multivariate OR†	1.0	0.95 (0.51–1.77)	0.48 (0.21–1.08)	0.33 (0.12–0.93)	0.68 (0.47–0.98)	0.04
Lacunar infarction						
Cases, n	36	29	18	12		
Controls, n	73	71	69	72		
Multivariate OR*	1.0	1.00 (0.50–1.98)	0.48 (0.21–1.12)	0.34 (0.14–0.83)	0.63 (0.46–0.88)	0.006
Multivariate OR†	1.0	1.11 (0.54–2.29)	0.60 (0.23–1.55)	0.43 (0.13–1.42)	0.70 (0.46–1.08)	0.11
Hemorrhagic stroke						
Cases, n	24	21	17	13		
Controls, n	49	56	57	63		
Multivariate OR*	1.0	1.06 (0.48–2.35)	0.71 (0.31–1.63)	0.50 (0.20–1.26)	0.81 (0.59–1.12)	0.20
Multivariate OR†	1.0	1.13 (0.46–2.78)	0.96 (0.33–2.85)	0.62 (0.17–2.29)	0.98 (0.60–1.58)	0.92

*Adjusted for body mass index (kg/m^2), cigarette smoking status (never, ex, and current), current ethanol intake (g/d), hypertension status (normal, borderline, and hypertension), serum total cholesterol levels (mmol/L), log-transformed triglyceride levels (mmol/L), and serum glucose category (normal, glucose intolerance, and diabetes), as well as matching for sex, age, community, year of serum stored, and fasting status.

†Further adjusted for serum monounsaturated fatty acids and either saturated or linoleic acids (quartiles).

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Linoleic Acid, Other Fatty Acids, and the Risk of Stroke

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