Olive oil consumption, plasma oleic acid, and stroke incidence: The Three-City Study
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Olive oil consumption, plasma oleic acid, and stroke incidence

The Three-City Study

ABSTRACT

Objective: To determine whether high olive oil consumption, and high plasma oleic acid as an indirect biological marker of olive oil intake, are associated with lower incidence of stroke in older subjects.

Methods: Among participants from the Three-City Study with no history of stroke at baseline, we examined the association between olive oil consumption (main sample, n = 7,625) or plasma oleic acid (secondary sample, n = 1,245) and incidence of stroke (median follow-up 5.25 years), ascertained according to a diagnosis validated by an expert committee.

Results: In the main sample, 148 incident strokes occurred. After adjustment for sociodemographic and dietary variables, physical activity, body mass index, and risk factors for stroke, a lower incidence for stroke with higher olive oil use was observed (p for trend = 0.02). Compared to those who never used olive oil, those with intensive use had a 41% (95% confidence interval 6%–63%, p = 0.03) lower risk of stroke. In the secondary sample, 27 incident strokes occurred. After full adjustment, higher plasma oleic acid was associated with lower stroke incidence (p for trend = 0.03). Compared to those in the first tertile, participants in the third tertile of plasma oleic acid had a 73% (95% confidence interval 10%–92%, p = 0.03) reduction of stroke risk.

Conclusions: These results suggest a protective role for high olive oil consumption on the risk of stroke in older subjects. Neurology® 2011;77:1-1

GLOSSARY

3C Study = Three-City Study; BMI = body mass index; CI = confidence interval; HDL = high-density lipoprotein; HR = hazard ratio; MeDi = Mediterranean diet; MI = myocardial infarction; MUFA = monounsaturated fats.

Cerebrovascular events are responsible for a substantial clinical burden, with their incidence currently exceeding that of coronary heart disease, in particular in older age groups.1 Risk of stroke could be reduced by improvement of lifestyle factors, including diet,2 notably by increasing fruits and vegetables consumption3 and decreasing sodium intake, which is strongly correlated with hypertension.4 Adherence to the Mediterranean diet (MeDi)5 was related to a lower risk of mortality from cardiovascular diseases6 and to a reduction in major cardiovascular risk factors.7–9 High olive oil consumption is one of the most constant features of the MeDi, and may account for most of its cardioprotective properties.10 Olive oil contains 80% monounsaturated fats (MUFA) in the form of oleic acid, 20% polyunsaturated fats, and several antioxidant components, including phenolic compounds found in virgin olive oil.10 A higher...
consumption of olive oil has been associated with a decreased risk for myocardial infarction (MI),\textsuperscript{1,11,12} a lower risk of all-cause mortality after MI,\textsuperscript{1,12} and a lower carotid intima-media thickness.\textsuperscript{13} Olive oil was the only component of the MeDi specifically associated with lower blood pressure in a large European cohort.\textsuperscript{14}

To our knowledge, the assumption that high olive oil consumption may be associated with a reduced incidence of stroke independently of other dietary habits and stroke risk factors has never been explored. We investigated the relationship between olive oil consumption, plasma oleic acid as a biological marker of oleic acid intake, and 6-year stroke incidence in older participants in the Three-City (3C) Study.

**METHODS**

**Study population.** The 3C Study is an ongoing multicenter prospective cohort study of vascular risk factors for dementia which started in 1999–2000 and included 9,294 community dwellers in 3 French cities: Bordeaux (n = 2,104), Dijon (n = 4,931), and Montpellier (n = 2,259). Individuals living in one of these cities, aged 65 years and over and not institutionalized, were eligible for recruitment into the 3C Study.\textsuperscript{15} The baseline data collection included sociodemographic and lifestyle characteristics, symptoms and complaints, main chronic conditions, medication use, neuropsychological testing, clinical examination including blood pressure measurement, EKG, and blood sampling.

**Standard protocol approvals, registrations, and patient consents.** The protocol of the 3C Study has been approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris). All participants gave their written informed consent.

**Diagnosis of stroke.** History of stroke was assessed at baseline during a face-to-face interview conducted by trained psychologists and nurses, and stroke occurrence was recorded at each follow-up examination performed 2, 4, and 6 years after the baseline visit, either during a face-to-face interview or by self-questionnaire. At each follow-up, the participants were asked if they had had a stroke history or stroke symptoms, and if they had been hospitalized. In those who screened positive for stroke, further medical data were collected, including emergency medical service and hospitalization reports, neuroimaging reports, and interview with the patient’s physician or the family.\textsuperscript{16}

According to the diagnostic criterion of the World Health Organization,\textsuperscript{17} a stroke was defined as a new focal neurologic deficit of sudden or rapid onset, of presumed vascular origin, that lasted 24 hours or more, or leading to death. A specific validation committee composed of neurologists reviewed all available information and confirmed or not the diagnosis of stroke and its subtype (ischemic or hemorrhagic). Patients with TIAs were not included. Fatal events were classified according to the 10th revision of the International Classification of Diseases.\textsuperscript{18} More details on the procedure of stroke diagnosis are given in e-Methods on the Neurology® Web site at www.neurology.org.

**Dietary and biological nutritional data. Dietary data.** Frequency of consumption of broad categories of foods and preferred dietary fats used for dressing, cooking, or spreading were recorded in the whole 3C cohort at baseline, as described previously.\textsuperscript{19} As in a previous publication,\textsuperscript{20,21} 3 categories of olive oil consumption were defined: “no use,” “moderate use” (using olive oil for cooking or dressing alone), “intensive use” (using olive oil for both cooking and dressing). In subjects from the Bordeaux center, mean total energy intake per day was estimated from a 24-hour dietary recall performed during a face-to-face interview administered by trained dietitians. For subjects of the 2 other 3C centers (Dijon and Montpellier), total energy intake was imputed in sensitivity analyses.

**Plasma fatty acids.** Plasma fatty acids were determined at baseline from fasting blood samples in 1,364 subjects from the 3C Bordeaux center, according to a previously described methodology.\textsuperscript{22} The results for each fatty acid were expressed as percentage of total fatty acids, and the sum of all fatty acid species was equal to 100%. We used plasma oleic acid as an indirect biological marker of oleic acid intake provided by olive oil consumption.\textsuperscript{23}

**Other variables.** Sociodemographic variables included age, gender, and education. Usual alcohol consumption (number of glasses of alcoholic beverages per week) was recorded. Practice of physical activity was defined as regular when doing sport regularly or having at least 1 hour of leisure or household activity per day. All drugs consumed at least once a week during the last month were recorded and classified according to the World Health Organization’s Anatomic Therapeutic Chemical Classification.\textsuperscript{24} Body mass index (BMI) was computed as the weight/height\textsuperscript{2} (kg/m\textsuperscript{2}). Hypercholesterolemia was defined as a plasma total cholesterol $\geq$6.20 mmol/L or intake of cholesterol-lowering treatment. Stroke risk factors included in the present report were very close to those of the Framingham Stroke risk function, as previously defined in the 3C Study.\textsuperscript{25} They included smoking (number of pack-years), systolic blood pressure (average of 2 separate measures), antihypertensive therapy, diabetes (if fasting glycemia $\geq$7.0 mmol/L or antidiabetic treatment), cardiovascular disease (history of myocardial infarction or angina pectoris or coronary bypass or angioplasty or vascular surgery for lower limb arteritis), and atrial fibrillation (diagnosed by EKG or self-report).

**Statistical analyses. Main analysis.** The outcome of interest was occurrence of a first fatal or nonfatal ischemic or hemorrhagic stroke. The main analysis estimated the association between baseline olive oil consumption and risk of stroke over 6 years. Cox proportional hazard models were used to estimate hazard ratios (HR) for stroke and their confidence intervals (CI) in moderate and intensive users of olive oil compared to nonusers. Two multivariate models were successively performed: 1) adjustment for age, sex, education, and center and 2) further adjustment for regular consumption of other foods (regular consumption of fish $\geq$ once a week, meat $\geq$ twice a week, pulses $\geq$ once a week, raw vegetables $\geq$ twice a week, raw fruits $\geq$4 times a week), cooked fruits or vegetables $\geq$4 times a week, or cereals $\geq$once a day);\textsuperscript{26} more moderate or intensive use of omega-3 rich oils (colza, walnut, and soya oils), omega-6 rich oils (peanut, sunflower, grape, and corn oils), butter, or goose or duck fat; alcohol consumption; physical activity; other stroke risk factors (systolic blood pressure, antihypertensive therapy, diabetes, smoking, history of cardiovascular disease, atrial fibrillation); BMI; triglyceridemia; and hypercholesterolemia.

**Secondary analysis.** The association between baseline plasma oleic acid and 6-year risk of stroke was investigated on the Bordeaux 3C subsample. HRs for stroke were estimated...
in the second (T2) and third (T3) tertiles of plasma oleic acid compared to the first (T1) tertile. Since the relationship between oleic acid and stroke incidence was nonlinear, cutoffs were chosen a priori according to tertiles of the distribution to ensure enough power for comparisons, considering the relative few incident stroke cases. Three models were performed: 1) adjustment for age, sex, and education; 2) further adjustment for alcohol consumption, physical activity, stroke risk factors, BMI, triglyceridemia, and hypercholesterolemia; 3) in order to take into account collinearity between plasma fatty acids, a principal component analysis was performed on total saturated fatty acids, total omega-3 fatty acids, and total omega-6 fatty acids plasma proportions, and further adjustment for the first 2 principal components was performed.

Imputation of missing values. Physical activity data were missing in 10.6% of the main study sample. Alcohol use data were missing in 3.8% of the sample, and the probability to be missing was associated with stroke risk. Thus, missing values for physical activity and alcohol use were imputed by multiple imputation, as a function of all other covariates plus the number of medications used, recent weight loss >3 kg, and mobility restriction assessed by the Rosow scale. As total energy intake was available only in 1,048 subjects from the Bordeaux center, imputation of missing values and adjustment for total energy intake were performed in sensitivity analyses only, and the validity of imputations was closely checked (see e-Methods for more details).

RESULTS Among the 9,294 subjects from the baseline 3C sample, we excluded 415 subjects with stroke history at baseline (figure 1). Among the remaining 8,879 subjects, the main study sample consisted of the 8,709 participants with available data for olive oil consumption, and the secondary study sample of the 1,364 subjects from Bordeaux who had plasma fatty measurements at baseline. After exclusion of subjects with missing data for the main covariates and those without follow-up assessment, 7,625 subjects were included in the main study sample, and 1,245 in the secondary study sample.
Olive oil consumption and stroke incidence. Baseline characteristics of the main study sample as a function of olive oil use (22.8% of nonusers, 40.0% of moderate users, and 37.2% of intensive users) are presented in table 1 and table e-2. Intensive users of olive oil were more frequent in Montpellier (Mediterranean Sea, 41.9%) and Dijon (Middle East, 38.5%) than in Bordeaux (Atlantic Ocean, 28.1%).

Moderate and intensive olive oil users were younger and more educated than nonusers. They also had lower values or frequencies for several stroke risk factors, lower BMI (table 1), lower triglycerides, and lower total/high-density lipoprotein (HDL) cholesterol ratio (table e-2). They practiced more often regular physical activity, consumed more often fish and fruits and vegetables, and were more often users of omega-3 rich oils. Conversely, intensive olive oil users consumed less often omega-6 rich oils than non- or moderate olive oil users (table e-2).

In the main study sample, 148 incident strokes occurred over a 5.25-year median follow-up (mean...
4.9 years, range 1.5 months–6 years), including 115 of ischemic etiology, 28 hemorrhagic strokes, and 5 strokes of undetermined cause. A significant trend toward a lower incidence for stroke with higher olive oil use was observed (table 1). This trend was statistically significant for ischemic but not for hemorrhagic stroke (table e-2).

The multivariate association between olive oil use and 6-year incident stroke is shown in table 2. Compared to those who never used olive oil, those with intensive use had a 41% (95% CI 6%–63%) lower risk in the fully adjusted model (model 2). From model 1, further adjustment for regular use of omega-6 rich oils, but no other covariate, slightly increased the strength of the association between olive oil use and stroke risk (see intermediate model in table e-3). No other dietary variable was significantly associated with stroke incidence, considered either separately or together in model 2 (table e-4).

**Plasma oleic acid and stroke incidence.** Baseline characteristics of the secondary study sample from Bordeaux according to tertiles of plasma oleic acid proportion are presented in table 3 and table e-5. Higher plasma oleic acid proportion was significantly associated with higher olive oil consumption. Mean proportion of plasma oleic acid was 19.9% (SD 3.3) in nonusers of olive oil, 20.5% (SD 3.7) in moderate users, and 20.7% (SD 4.2) in intensive users ($p$ for trend = 0.002, $r^2 = 1\%$). The other significant dietary predictors of plasma oleic acid were added fats: omega-6 rich oils (negative association), butter, goose or duck fat (positive associations) (table e-6).

### Table 3 Characteristics of the 1,245 participants from the study sample from Bordeaux without stroke history at baseline and as function of baseline plasma oleic acid: The Three-City Study (1999–2005)

<table>
<thead>
<tr>
<th></th>
<th>All secondary study sample (n = 1,245)</th>
<th>Tertiles of plasma oleic acid proportion (% of total fatty acids)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 (3.5; 19.0) (n = 411)</td>
<td>T2 (19.1; 21.9) (n = 419)</td>
</tr>
<tr>
<td>Baseline olive oil, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No use</td>
<td>421 (33.8)</td>
<td>161 (39.2)</td>
<td>153 (36.5)</td>
</tr>
<tr>
<td>Moderate use</td>
<td>481 (38.6)</td>
<td>152 (37.0)</td>
<td>167 (39.9)</td>
</tr>
<tr>
<td>Intensive use</td>
<td>343 (27.6)</td>
<td>98 (23.8)</td>
<td>99 (23.8)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>477 (38.3)</td>
<td>149 (36.3)</td>
<td>150 (35.8)</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>74.3 (4.8)</td>
<td>73.8 (5.0)</td>
<td>74.6 (4.6)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or primary</td>
<td>436 (35.0)</td>
<td>152 (37.0)</td>
<td>144 (34.3)</td>
</tr>
<tr>
<td>Secondary</td>
<td>328 (26.3)</td>
<td>110 (26.8)</td>
<td>108 (25.8)</td>
</tr>
<tr>
<td>High school</td>
<td>272 (28.9)</td>
<td>84 (20.4)</td>
<td>95 (22.7)</td>
</tr>
<tr>
<td>University</td>
<td>209 (16.8)</td>
<td>65 (15.8)</td>
<td>72 (17.2)</td>
</tr>
<tr>
<td>SBP, mm Hg, mean (SD)</td>
<td>143.8 (21.4)</td>
<td>143.8 (22.4)</td>
<td>144.4 (21.5)</td>
</tr>
<tr>
<td>Antihypertensive therapy, n (%)</td>
<td>675 (54.2)</td>
<td>211 (51.3)</td>
<td>225 (53.7)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>113 (9.1)</td>
<td>29 (7.1)</td>
<td>31 (7.4)</td>
</tr>
<tr>
<td>Smoking, pack-years, mean (SD)</td>
<td>9.0 (18.5)</td>
<td>6.4 (14.1)</td>
<td>9.5 (19.9)</td>
</tr>
<tr>
<td>History of cardiovascular disease, n (%)</td>
<td>169 (13.6)</td>
<td>53 (12.9)</td>
<td>48 (11.5)</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>58 (4.7)</td>
<td>21 (5.1)</td>
<td>16 (3.8)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>26.4 (4.2)</td>
<td>26.0 (4.1)</td>
<td>26.5 (4.5)</td>
</tr>
<tr>
<td>6-y incident stroke, n (%) cases</td>
<td>27a (2.2)</td>
<td>13 (3.2)</td>
<td>10 (2.4)</td>
</tr>
<tr>
<td>Incidence/100 person-years (95% CI)</td>
<td>0.46 (0.29–0.63)</td>
<td>0.66 (0.30–1.02)</td>
<td>0.51 (0.19–0.83)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI – body mass index; CI – confidence interval; MUFA – monounsaturated fatty acids; SBP – systolic blood pressure; SFA – saturated fatty acids.

* Chi-square test for categorical variables, Cochran-Armitage test for binary variables ($p$ for trend), analysis of variance ($p$ for trend) for continuous variables, Cox proportional hazard model for incident stroke ($p$ for trend).

b Including 20 ischemic and 7 hemorrhagic strokes.
Contrarily to that observed with olive oil consumption, participants with higher plasma oleic acid proportion were slightly older and had a more severe burden of vascular risk factors (table 3 and table e-5).

In this secondary study sample, 27 incident strokes occurred over a 5.0-year median follow-up (range 6 months–6 years), including 20 ischemic and 7 hemorrhagic strokes. There was a significant trend toward a lower incidence for stroke with higher plasma oleic acid proportion (table 3). This trend was statistically significant for ischemic stroke. Compared to those in T1, participants with plasma oleic acid in T3 had a 73% (95% CI 10%–92%) to 75% (95% CI 14%–92%) reduction of stroke risk in, respectively, models 2 and 3 (table 4).

**Sensitivity analyses.** Further adjustment for imputed mean total daily energy intake in the main analysis did not substantially modify the results (e-Results), and the distributions of observed and imputed values conditional on energy intake were very close (figure e-1). Moreover, adjustment for the total/HDL cholesterol ratio instead of hypercholesterolemia, or exclusion of prevalent dementia cases at baseline (n = 96 in the main study sample and n = 23 in the Bordeaux sample), did not substantially modify the results (data not shown).

**DISCUSSION** In the present population-based study, intensive olive oil use was prospectively associated with a lower stroke risk after controlling for numerous confounding factors, including lifestyle and nutritional factors, main stroke risk factors, and blood lipids.

Several converging arguments suggest a protective role for high olive oil consumption on the risk of stroke in older subjects: 1) olive oil use was associated with lower stroke risk; 2) plasma oleic acid was associated with lower stroke risk; 3) plasma oleic acid was an indirect marker of olive oil consumption since higher plasma oleic acid was significantly associated with higher olive oil use; 4) no other dietary predictor of plasma oleic acid was significantly associated with stroke risk.

However, our results should be interpreted cautiously, since plasma oleic acid is not a specific marker of olive oil consumption. It can also derive from other food sources and from endogenous hepatic synthesis from saturated fats, so that plasma oleic acid may be a marker of olive oil consumption only in populations with moderate to high olive oil use. Accordingly, in our study, although higher plasma oleic acid was significantly associated with higher olive oil use, the intensity of the association was very low. Moreover, plasma oleic acid was also associated with higher consumption of butter and goose or duck fat, which are sources of oleic acid as well. This may explain the unfavorable pattern of risk factors associated with higher plasma oleic acid.

While epidemiologic studies published to date do not enable us to disentangle olive oil from other components of the MeDi, probably because they did not use olive oil but the MUFA-to-saturated fatty acids ratio as originally described, the current study supports the assumption that within the MeDi, olive oil may be a major protective component independently of other dietary components. It may also partly explain the protective association of olive oil and the MeDi with cognitive decline observed in the 3C Study. The demonstrated vascular beneficial effects of olive oil include blood pressure reduction, improvement of blood lipid profile, reduction of low-density lipoprotein susceptibility to oxidation,
and improvement of oxidative vascular damage and endothelial function,\(^1\) possibly through a modulation of key genes implied in vascular inflammation, foam cell formation, and thrombosis.\(^2\) These effects were primarily attributed to oleic acid\(^3\)–\(^5\) but also more recently to phenolic compounds found in virgin olive oil,\(^6\) which have a potent anti-inflammatory action.\(^7\) The protective association between olive oil use and stroke was significant in ischemic but not hemorrhagic stroke, for which the low number of cases limits study power.

Although vascular events outside the coronary arterial territory encompass acute coronary and peripheral vascular events, in particular in older subjects,\(^8\) a single large prospective cohort study found that higher adherence to the MeDi was related to lower stroke incidence.\(^9\) However, the associations between each component of the MeDi and stroke incidence were not presented separately. A study reported that higher plasma oleic acid was associated with higher 32-year incidence of stroke,\(^10\) a result that seems contradictory to our findings. However, this discrepancy may be explained by different dietary habits of the studied populations.

The main strengths of the present study include a longitudinal design with a large sample size, low attrition rate, and a standardized and centralized procedure for data recording and validation of clinical outcomes. Another major strength is the use of biological data to complete and validate associations observed with dietary data. This study also has some limitations. Since final stroke diagnosis depended on initial self-reporting, some stroke cases may have been missed by our process. Moreover, in spite of a deep investigation of every suspected incident case of stroke by an independent expert committee, we cannot rule out that some confirmed stroke cases may be false positives. Although we used plasma oleic acid as an indirect marker of olive oil intake to support our findings, the lack of quantitative data for olive oil use did not enable an accurate estimation of the association between plasma oleic acid and olive oil intake, which remained very low in our study. Thus, the validity of plasma oleic acid as an indirect marker of olive oil consumption in our population remains to be evaluated. While converging evidence suggests that due to its high content in polyphenols, virgin olive oil provides more cardiovascular benefits than refined olive oil,\(^11\) we were not able to distinguish the different types of olive oil consumed. However, olive oil consumers were expected to mostly consume virgin olive oil in our study, since 98% of the French market of olive oil is composed of extra virgin olive oil.\(^12\)

The high prevalence of stroke in older subjects emphasizes the need for primary and secondary prevention in this age group. Showing a strong association between intensive olive oil use and lower stroke incidence, our study suggests a novel approach of dietary recommendations to prevent stroke occurrence in elderly populations.

**AUTHOR CONTRIBUTIONS**

Design and conduct of the study: Dr. Barberger-Gateau, Dr. Berr, Dr. Tsourio. Collection: Dr. Barberger-Gateau, Dr. Berr, Dr. Tsourio. Management: Dr. Barberger-Gateau, Dr. Berr, Dr. Tsourio. Analysis and interpretation of data: Dr. Samieri, Dr. Proust-Lima, Dr. Féart. Preparation of the manuscript: Dr. Samieri, Dr. Barberger-Gateau. Review and approval of the manuscript: Dr. Samieri, Dr. Féart, Dr. Proust-Lima, Dr. Tsourio, Dr. Stapf, Dr. Berr, Dr. Barberger-Gateau. Statistical analysis was conducted by Dr. Samieri.

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**DISCLOSURE**

Dr. Samieri received research support from Institut Carnot LISA (Lipides pour l’Industrie et la Santé [Lipids for Industry, Safety and Health]). Dr. Féart reports no disclosures. Dr. Proust-Lima serves as a consultant for Danone and receives research support from INCA (Institut National du Cancer [National Institute for Cancer]). Dr. Peuchant reports no disclosures. Dr. Stapf serves as an Associate Editor for *Cerebrovascular Disease* and receives research support from the NIH/NINDS, Columbia University, and University Paris. Dr. Tsourio serves on scientific advisory boards for Merck Sharp & Dohme and Fondation Plan Alzheimer; serves on the editorial boards of *Neuropedimétrie and the Journal of Hypertension,* and receives research support from Agence Nationale de la Recherche and Fondation Plan Alzheimer. Dr. Berr has served on a scientific advisory board for Janssen; serves on the editorial advisory board for *Revue Épidémiologie et Santé Publique,* and receives research support from Agence Nationale de la Recherche and Fondation Plan Alzheimer. Dr. Samieri received research support from Institut Carnot LISA.

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