Title : Fish intake and MRI burden of cerebrovascular disease in older adults

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ABSTRACT

Background and Objectives: Fish intake may prevent cerebrovascular disease (CVD), yet the mechanisms are unclear, especially regarding its impact on subclinical damage. Assuming that fish may have pleiotropic effect on cerebrovascular health, we investigated the association of fish intake with global CVD burden based on brain MRI markers.

Methods: This cross-sectional analysis included participants from the Three-City Dijon population-based cohort (aged ≥65 years) without dementia, stroke, or history of hospitalized cardiovascular disease who underwent brain MRI with automated assessment of white matter hyperintensities, visual detection of covert infarcts, and grading of dilated perivascular spaces. Fish intake was assessed through a frequency questionnaire, and the primary outcome measure was defined as the first component of a factor analysis of mixed data applied to MRI markers. The association of fish intake with the CVD burden indicator was studied with linear regressions.

Results: In total, 1,623 participants (mean age 72.3 years, 63% women) were included. The first component of factor analysis (32.4% of explained variance) was associated with higher levels of all 3 MRI markers. Higher fish intake was associated with lower CVD burden. In a model adjusted for total intracranial volume, compared to participants consuming fish <1 time per week, those consuming fish 2 to 3 and ≥4 times per week had a $\beta = -0.19$ (95% confidence interval –0.37 to –0.01) and $\beta = -0.30$ (–0.57 to –0.03) lower indicator of CVD burden, respectively (*p* trend <0.001). We found evidence of effect modification by age such that the association of fish to CVD was stronger in younger participants (65-69 years) and not significant in participants ≥75 years of age. For comparison, in the younger age group, consuming fish 2 to 3 times a week was roughly equivalent (in the opposite direction) to the effect of hypertension.

Discussion: In this large population-based study, higher frequency of fish intake was associated with lower CVD burden, especially among participants <75 years of age,

suggesting a beneficial effect on brain vascular health before manifestation of overt brain disease.

Classification of Evidence: This study provides Class II evidence that in individuals without stroke or dementia, higher fish intake is associated with lower subclinical CVD at MRI.

Search Terms: [2] All Cerebrovascular disease/Stroke, [25] All Cognitive Disorders/Dementia, [54] Cohort studies, [59] Risk factors in epidemiology, [120] MRI

INTRODUCTION

Fish consumption, the primary source of long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA), may contribute to lower risk of age-related brain diseases. Higher fish intake or increased n-3 PUFA blood levels have been associated with lower cognitive decline[1] and with a reduced risk of stroke[2,3] and dementia.[4,5] However, little is known about their impact on subclinical brain damage before the onset of overt diseases. Identifying risk factors for early brain alterations that may accumulate with aging and eventually lead to stroke or dementia is critical for primary prevention.

Cerebrovascular disease (CVD) is a major contributor of cognitive aging. Clinical stroke and subclinical cerebrovascular damage, visualized and quantified on brain Magnetic Resonance Imaging (MRI) through various markers, including covert brain infarcts,[6] white matter hyperintensities (WMH),[7] and dilated perivascular spaces,[8] have been associated with increased dementia risk.[9,10] Most population-based studies on fish or n-3 PUFA and MRI markers of CVD focused on WMH and infarcts and reported beneficial associations,[11–16] although some studies also found no association.[13,17–20]

Fish intake may favor brain vascular health through various endophenotypes or mechanisms, including ischemic events and small vessel disease.[2,21,22] In the investigating factors with a pleiotropic effect such as fish, there is interest in capturing a global picture of the pathophysiologic burden by combining correlated biomarkers of underlying mechanisms into a continuous indicator that may provide more power than studying each phenotype individually.[23–25] We therefore investigated, in a large population-based cohort of older participants, the relationship between fish intake and an indicator of CVD burden (largely reflecting cerebral small vessel disease) combining WMH, covert infarcts, and dilated perivascular spaces.

METHODS

The primary research question was to identify whether fish intake is associated with lower subclinical CVD at MRI (Class II evidence).

Study population

The Three City (3C) Dijon study is a prospective cohort of 4,931 non-institutionalized community dwellers >65 years of age who were selected form the electoral rolls of Dijon (France) between March 1999 and March 2001.[26] At inclusion, face-to-face interviews were conducted to collect sociodemographic, medical, and lifestyle information, including a brief food frequency questionnaire; anthropometric and blood pressure measurements were performed; and neuropsychological testing was administered by psychologists.

Participants <80 years of age at enrollment were invited to participate to an ancillary MRI study between April 1999 and June 2001 (n = 2,763). Exclusion criteria for the MRI examination included having a cardiac pacemaker, a valvular prosthesis, or any other internal electrical/magnetic device; reporting a history of neurosurgery or aneurysm; experiencing claustrophobia; and reporting the presence of metal fragments in the eyes, brain, or spinal cord. Although 2,285 participants agreed to participate, only 1,924 scans were performed due to financial limitations.[26] We excluded participants with prevalent dementia (n = 8), with a history of stroke (n = 83), or reporting a history of hospitalization for cardiovascular disease (i.e., myocardial infarction, and cardiac, abdominal aortic, carotid, coronary, or leg artery surgery; n = 87). We also excluded participants with major acquisition artifacts on MRI scans (n = 9) or brain tumors (n = 7) and those with missing data for at least one of the studied MRI biomarkers (n = 107), leaving 1,623 individuals included in the analysis.

Standard protocol approvals, registrations, and patient consents

The protocol of the 3C study was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital (Paris, France); participants did not receive allowance and provided written informed consent.

Dietary assessment

At inclusion, a brief food frequency questionnaire was administrated to assess the frequency of consumption of 10 broad categories of foods (raw fruits, raw vegetables, cooked fruits and vegetables, legumes, cereals, fish, meat, eggs, dairy products, chocolate) recorded in 6 classes (never, <1 time per week, 1 time per week, 2–3 times per week, 4–6 times per week, and daily).[27] After extreme categories were grouped due to small numbers, fish intake was categorized in 4 classes: never or <1 time per week, 1 time per week, 2 to 3 times per week, and ≥4 times per week.

Neuroimaging

MRI acquisition (including T1, T2 and proton density [PD] sequences) was performed on 1.5-T Magnetom (Siemens, Erlangen, Germany) on average 0.3 years after baseline (range, 0–1.3 years). The neuroimaging protocol has been detailed in **eMethods**, doi.org/10.5061/dryad.hhmgqnkgz.

The T1- and T2-weighted images of each participant were analyzed using Statistical Parametric Mapping 99, and gray matter, white matter (WM) and cerebrospinal fluid (CSF) volumes were estimated with an optimized voxel-based morphometry protocol, as described elsewhere.[28] Total intracranial volume (TIV) was computed as the sum of CSF, gray matter and WM volumes, and bilateral hippocampal volume was automatically estimated.[29] MRI markers of CVD were estimated from T1-, T2-, and PD-weighted images, as describe below.

MRI markers of CVD burden

We studied 3 MRI markers of cerebrovascular health, all commonly found in the brain of older persons (with no history of transient ischemic attack or stroke) and strong predictors of the risk of stroke, cognitive decline, and dementia: WMH, covert brain infarcts and dilated perivascular spaces in the WM and basal ganglia.[6–8,30] WMH are focal hyperintense lesions in WM on T2-weighted or fluid-attenuated inversion recovery images; they may reflect axonal degeneration and demyelination as a consequence of chronic hypoperfusion or disruption of the blood-brain barrier associated with small vessel disease.[30] Covert infarcts are focal cavities with CSF-like signal intensity (hypointense on T1 and hyperintense on T2) that result from cell death following inadequate blood supply after vessel obstruction. Like infarcts, dilated perivascular spaces are CSF-like signal intensity lesions typically found in the basal ganglia and deep WM. They refer to enlargement of the perivascular compartments surrounding veins and perforating arteries due to accumulation of interstitial fluid, presumably as a consequence of small vessel disease.[31] Those 3 MRI markers can be differentiated by their localization in the brain and may have specific underlying mechanisms,[10] although (i) they all reflect, at least partly, cerebral small vessel disease as a common general pathophysiological process; (ii) they share risk factors (e.g., age, hypertension); and (iii) they are correlated with each other.[32] Thus, they may each represent observed measures of a single underlying CVD burden dimension.[23]

We estimated WMH load, that is, the percentage of WM occupied by WMH (continuous variable), with a validated fully automated processing which uses multispectral (T1, T2, PD) segmentation in a multistep procedure for the detection, quantification, localization, and statistical mapping of WMH on T2-weighted images, as detailed elsewhere.[33] The algorithm first removed areas of infarction and perivascular spaces from T2 images before proceeding to the detection of WMH.

153 Covert infarcts were visually rated on T1-, T2-, and PD-weighted images by a neurologist
154 using a standardized assessment grid. They were defined as lesions ≥3 mm in diameter, as
155 previously described.[8,34] Presence of covert infarcts was considered a binary indicator (yes, if
156 at least 1 infarct regardless of the localization; no otherwise).
157
158 Dilated perivascular spaces were defined as lesions with round, ovoid or linear shape <3 mm
159 in diameter with smooth delineated contours and located in areas supplied by perforating
160 arteries. Lesions fulfilling the same criteria but with a ≥3-mm diameter were differentiated
161 from infarcts with the use of multi-planar reformatting. Only lesions with a typical vascular
162 shape and following the orientation of perforating vessels (including cystic lesions with an
163 extension of vascular shape) were considered as dilated perivascular spaces.[32] For each
164 participant, each of the 124 axially-oriented slices was visually examined to evaluate the
165 global burden of dilated perivascular spaces and to identify the slice containing the largest
166 number in both basal ganglia and WM. One experienced reader, blinded to clinical data,
167 analyzed all images (the intra-rater agreement statistics indicated good reliability; k = 0.77 for
168 basal ganglia and k = 0.75 for WM on n = 100 MRIs).[32] The severity of dilated perivascular
169 spaces was first rated separately in basal ganglia and in WM with a 4-level severity score
170 defined by the number of dilated perivascular spaces. For basal ganglia, rating was based on
171 the slice containing the greatest number of dilated spaces. For rating in WM, we used the slice
172 containing the greatest number of dilated spaces or the entire set of 124 slices when very few
173 dilated spaces were observed. Details on the rating procedure have been published
174 previously.[8] Because there is no a priori hypothesis for a relation of fish intake with a specific
175 location of dilated perivascular spaces, we used a global indicator combining them in both
176 basal ganglia and WM (we did not consider dilated perivascular spaces in the hippocampus
177 because they were not associated with cognitive decline or dementia risk in the cohort and
178 they may partly reflect developmental mechanisms[35]). We defined 3 levels of severity:
179 ‘degree 1’, if low severity in both basal ganglia and WM (<5 dilated perivascular spaces for

basal ganglia and <10 in total WM); ‘degree 2’, if moderate severity in basal ganglia or WM (5 to >10 but still numerable for basal ganglia and/or 10 to 20 dilated spaces for WM); and ‘degree 3’, if high severity in basal ganglia or WM (innumerable dilated spaces [cribriform] for basal ganglia and/or >20 for WM).

Other variables

Sociodemographic and lifestyle variables included age, sex, tobacco consumption (pack-year; continuous), alcohol consumption (number of glasses of alcoholic beverages per week; continuous), and engagement in moderate to vigorous physical activity (recreational walking ≥1 hours per day or practicing sport ≥1 times per week; yes/no). Socioeconomic status was evaluated through both educational level (none/primary, secondary, high school, and university) and monthly income (<750 euros, 750–1500 euros, 1500–2250 euros, ≥2250 euros, and refused to answer). Intake frequency of foods potentially associated with brain health included fruits and vegetables, legumes, meats, and olive oil as the preferred source of added fat.[36] Vascular risk factors included history of non-hospitalized cardiovascular diseases (angina pectoris, cardiac rate disorder, heart failure, lower limbs arteritis; yes/no), hypertension (blood pressure ≥140/90 mmHg or treated; yes/no), hypercholesterolemia (blood cholesterol ≥6.2 mmol/L or treated; yes/no), diabetes (fasting blood glucose ≥7.0 mmol/L or treated; yes/no), body mass index (BMI; continuous), and use of antithrombotic treatment (yes/no). The presence of carotid plaques (yes/no) and common carotid artery intima-media thickness (continuous) were ascertained by carotid ultrasound.[26] *APOEε*4 allele carrier status (which may induce cerebrovascular dysfunction[37]) was considered dichotomously (carrying at least 1 versus no ε4 allele).

Statistical analyses

Characterization of a global indicator of CVD burden

To define a global indicator of CVD burden, we investigated the common dimension underlying the 3 MRI-based phenotypes (WMH load [%, continuous], covert brain infarcts [yes/no, categorical nominal] and dilated perivascular spaces [3 severity levels, categorical ordinal]). We used Factor Analysis of Mixed Data (FAMD), a dimension-reduction approach close to principal component analysis (PCA). The general goal is to reduce the dimension of a dataset into a few summary variables, defined as linear combination of predictors, that explain as much as possible of the variance of data. While PCA is adapted to continuous variables, FAMD allows a mixture of continuous and categorical variables. FAMD combines PCA with multiple component analysis used for categorical variables. In FAMD, continuous variables are scaled to a unit variance (as in PCA), while categorical variables are transformed into a disjunctive table (i.e., 1 column per modality) and scaled to account for the number of individuals presenting each modality. Principal components were identified by orthogonal rotation, which allows direct interpretation of the component loadings in terms of correlation between variables and dimensions (see **Figure 1** legend and **eMethods**, doi.org/10.5061/dryad.hhmgqnkgz).

We retained the first FAMD component to represent CVD burden. It explained 32.4% of the variance and was characterized by higher coefficients with increasing WMH load, presence of infarcts, and degree 3 dilated perivascular spaces (**Figure 1**). We focused on the first component because (i) the aim of the FAMD was to identify a primary outcome measure for use as a dependent variable in subsequent analyses, (ii) the first component was the only component with an explained variance (32.4%) above the average (25.0%) (**eFigure 1**, doi.org/10.5061/dryad.hhmgqnkgz), and (iii) it had a meaningful clinical interpretation, summarizing CVD burden through a balanced contribution of all 3 endophenotypes (39.4%, 31.2%, and 29.4% for WMH, covert infarcts and dilated perivascular spaces, respectively).

The individual score associated with the first FAMD component was then calculated as the linear combination of the value (for WMH load) or the prevalence of the modalities (for infarcts and dilated perivascular spaces), weighted by its specific coefficient (**Figure 1** legend and **eMethods**, doi.org/10.5061/dryad.hhmgqnkgz). This score quantified the extent of CVD in a continuous scale. It was further normalized using a latent process model before being entered as an outcome in the models.[38]

Association of fish intake with CVD burden

The association of fish consumption (4 intake categories) with the indicator of CVD burden (continuous, normalized) was estimated from linear regression models, first adjusted for TIV only and then further adjusted for other potential confounders cited above. The linear trend across fish intake categories was examined using a continuous variable in which participants in a given category were assigned the approximated median number of weekly fish intake occasions (i.e., 0.5, 1, 2.5, and 5 for <1, 1, 2–3, and ≥4 times per week, respectively). Effect modification by age was investigated.

Supplementary analyses

We performed a series of secondary analyses. First, to address the possibility of reverse causality, we run sensitivity analyses excluding participants with cognitive impairment (Mini Mental State Examination <26). Second, we examined the robustness of our results to any selection bias using propensity score-weighted analysis that accounts for the probability of noninclusion in the analytic sample (**eMethods**, doi.org/10.5061/dryad.hhmgqnkgz). Third, we investigated the association of fish intake with each of the 3 CVD biomarkers individually. Last, we evaluated the associations of fish consumption with cerebral brain volumes (hippocampus, total gray matter and total WM).

Missing data for covariates were imputed by multiple imputations (using chained equations with fully conditional specification method; M = 5 imputations). Statistical analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria; PCAmixdata package). Two-sided P-values were used with $\alpha = 0.05$ threshold for statistical significance.

Data availability statement

Anonymized data will be shared by request to the 3C scientific committee. Supplementary data (eMethods, eTables 1 and 2, eFigures 1, 2 and 3) are available from Dryad (doi.org/10.5061/dryad.hhmgqnkgz).

RESULTS

Among the 1,623 participants included, the mean age was 72.3 (SD, 4.1) years with 63% women (**Table 1**). Compared to participants excluded, participants included in the analysis were more often women, slightly younger, and in better vascular and general health (**eTable 1**, doi.org/10.5061/dryad.hhmgqnkgz). Their dietary intakes did not differ, except for fruits/vegetables.

Participants consumed fish 1.9 (SD, 1.1) times per week, on average, with prevalence of intake frequencies ranging from 10.7% for those with fish consumption less than once a week, 37.2% for once a week, 46.6% for 2 to 3 times per week, and 5.6% for ≥4 times per week. Participants with more frequent fish consumption had a higher educational level, were less likely to have low income, engaged more often in moderate to vigorous physical activity, had lower BMI, and had slightly better cognitive performances (**Table 1**). In contrast, no difference was observed across fish intake categories for sex, age, tobacco and alcohol consumption, diabetes, hypertension, hypercholesterolemia, or *APOE*ε4 status. Regarding

dietary consumption, participants with higher frequency of fish intake tended to more often consume olive oil, legumes, fruits and vegetables but less often consume meat.

Overall, WMH represented 2.2% of WM volume on average; 8.1 % of participants had covert infarcts, and 6.2% had severe dilated perivascular spaces (**Table 2**). By construction, increasing CVD burden indicator was associated with higher WMH load and increasing prevalence of infarcts and severe dilated perivascular spaces (**Figure 1, Table 2**). WMH load contributed linearly to the score, while the presence of infarcts and severe dilated perivascular spaces was limited to the fourth quartile of CVD indicator. In the highest quartile of CVD score, the mean WMH load was 4.3% (SD, 3.0%), 32.5% of participants had covert infarcts, and 24.6% had severe dilated perivascular spaces. In contrast, in the lowest quartile of score, WMH load was 1.1% (SD, 0.6%) on average, no infarct was detected, and degree 1 dilated perivascular spaces were the most prevalent (58.1%). Participants with higher global CVD burden indicator were more often men, were older, had higher BMI, had more hypertension, and tended to perform worse at cognitive tests (**eTable 2**, doi.org/10.5061/dryad.hhmgqnkgz).

In the TIV-adjusted model, higher frequency of fish consumption was associated with lower CVD burden (*p* for trend <0.001) (**Figure 2A**). Participants consuming fish 2 to 3 and ≥4 times per week had a $\beta = -0.19$ unit (95% confidence interval –0.37 to –0.01) and $\beta = -0.30$ unit (–0.57 to –0.03) lower global indicator of CVD burden, respectively, compared to those consuming fish less than once per week.

Moreover, we found effect modification by age (*p* for fish-by-age interaction = 0.04). Aged-stratified analyses indicated stronger associations for younger participants (*p* for trend = 0.003 in participants 65–69 years of age, 0.006 in those 70–74 years of age, and 0.40 among those ≥75 years of age; **Figure 2B**). Among individuals 65 to 69 years of age (**Figure 2B, green**), compared to eating fish less than once per week, consuming fish 2 to 3 and ≥4 times per week

was significantly associated with lower CVD burden (β = –0.30 [–0.58 to –0.02] and –0.60 [–1.02 to –0.18], respectively, in TIV-adjusted model; **Figure 2B, dotted lines**). Among participants 70 to 74 years of age (**Figure 2B, purple**), the linear trend was significant, but the associations of each fish intake categories with CVD burden indicator were not significant (β = –0.20 [–0.49 to 0.09] and –0.41 [–0.87 to 0.05] for 2–3 and ≥4 times per week in TIV-adjusted model). Among individuals ≥75 years of age, fish intake was not significantly associated with CVD burden (β = –0.09 [–0.43 to 0.25] and 0.22 [–0.30 to 0.74] for 2–3 and ≥4 times per week).

To help interpret these differences, we compared the effect estimates for fish intake categories to those of hypertension (that was associated with increasing CVD score) in the age-stratified, TIV-adjusted model. Hypertension was associated with a β = 0.27 (0.09 to 0.45) higher CVD score in participants 65 to 69 years of age, β = 0.31 (0.10 to 0.52) in those 70 to 74 years of age, and β = 0.26 (–0.01 to 0.52) in those ≥75 years of age (*p* for hypertension-by-age interaction = 0.91). Thus, in the younger age group (65–69 years), in whom fish was most strongly associated with CVD burden, consuming fish 2 to 3 times a week was roughly equivalent (in the opposite direction) to the effect of hypertension, and consuming fish ≥4 times had double that effect.

Multivariable adjustment did not meaningfully modify the results in any age group (**Figure 2B, plain lines**). Likewise, findings were virtually unchanged by additional adjustment for subclinical atherosclerosis or antithrombotic medication use (data available upon request).

Furthermore, none of the other food groups was associated to the indicator of CVD burden, and there was no evidence of interaction with age (**eFigure 2**, doi.org/10.5061/dryad.hhmgqnkgz).

Supplementary analyses

Excluding participants with cognitive impairment at baseline (n = 189) slightly attenuated the association between fish and CVD burden (β = –0.21 [–0.40 to –0.02] and –0.28 [–0.57 to 0.01] for consuming fish 2 to 3 and ≥4 times per week compared to <1 time a week) in TIV-adjusted model, but the trend remained significant (*p* for trend = 0.008). Accounting for selection bias in analysis did not meaningfully change the results (**eFigure 3**, doi.org/10.5061/dryad.hhmgqnkgz).

When we investigated each MRI phenotype individually, we found no significant interaction of fish intake with age. Fish intake appeared linearly associated with lower WMH load (*p* for trend <0.001) and with lower odds of covert brain infarcts (*p* for trend = 0.03) but not with dilated perivascular spaces (*p* for trend ≥0.18 for higher degrees vs degree 1) in fully adjusted models. The association of fish with dilated perivascular spaces appeared nonlinear, with fish intake ≥1 time a week significantly associated with lower odds of degree 3 (versus degree 1) dilated spaces (odds ratio = 0.66 [0.49 to 0.88], 0.43 [0.32 to 0.57], and 0.69 [0.62 to 0.77] for 1, 2–3 and ≥4 times per week, respectively).

Last, fish intake was not significantly associated with gray matter, WM and hippocampal volumes (results available upon request).

DISCUSSION

In this large cohort of older adults, we found a strong association of fish intake frequency with lower global CVD burden evaluated through a combined measure of 3 MRI-based phenotypes, including WMH, covert brain infarcts and dilated perivascular spaces. There was an effect modification by age such that association of fish to CVD burden was stronger in younger participants (<75 years of age). In the younger age group (65–69 years), the effect

estimate of consuming fish at least twice a week on CVD burden was roughly equivalent in magnitude (in the opposite direction) to the effect estimate of hypertension. When fish was consumed ≥4 times per week, the effect was double the effect found with hypertension.

Our results confirm previous epidemiological studies reporting an association between higher fish intake and lower risk of stroke,[2,3] although few studies investigated the association of fish consumption with subclinical MRI markers of cerebrovascular health and findings have been mixed overall. In the large Cardiovascular Health Study (n = 3,660; age ≥65 years), compared to participants consuming fish less than once per month, those with at least 3 servings of fish per week had lower prevalence of WM lesions and subclinical infarcts (although fish was not associated with the incidence of novel infarcts).[16] In contrast, both the Northern Manhattan Study on strokes (n = 966, mean age 72 years) and the Washington Heights–Inwood Community Aging Project (WHICAP, n = 707, mean age 80 years) did not find any association of fish intake with WMH[17] or infarcts.[20]

Most previous studied on fish and WMH or infarcts did not report any effect modification by age.[16] A notable exception is WHICAP, in which, in accordance with our findings, age-stratification indicated stronger association of the Mediterranean diet with infarcts for younger participants.[20]

The effect of fish on brain vasculature may be largely attributed to long-chain n-3 PUFA, but again, associations between higher blood n-3 PUFA and lower WMH load[11–15] or fewer infarcts[11] have been conflicting in the literature.[11,13,18,19] For example, in 1,575 participants from the Framingham Offspring cohort (mean age 67 years), higher red blood cell docosahexaenoic acid level was associated with lower WMH volume, although the relation did not remain significant after adjustment for vascular risk factors.[13] In the Oregon Brain Aging Study cohort (n = 42, mean age 87 years), a nutrient biomarker pattern characterized

by higher plasma n-3 PUFA was associated with lower WMH volume, but only among participants without depression.[12] Our findings are consistent with the single study that, to the best of our knowledge, combined, among relatively young older adults (mean age 64 years, n = 220), several MRI phenotypes (i.e., microbleeds, lacunar infarcts, high-grade WM changes, and perivascular spaces) to relate blood n-3 PUFA to overall CVD burden. However, the study was among acute ischemic stroke patients, limiting comparability with our findings.[22]

As with observational studies, the few clinical trials investigating the effect of fish oil or long-chain n-3 PUFA supplementation on (mostly clinical) brain aging outcomes reported inconsistent results.[39] Despite large sample sizes, trials on n-3 PUFA supplementation (alone or in combination with other domains) such as Multidomain Alzheimer Preventive Trial (MAPT) or DO-HEALTH (Vitamin D3, Omega3, Home Exercice, Healthy Aging, and Longevity Trial) failed to evidence effect on cognitive decline.[40,41] To date, the only trial focused on vascular MRI markers, the PUFA trial (n = 102, age ≥75 years) reported no effect of n-3 PUFA supplementation on 3-year WMH accumulation.[42] Of note, many trials included relatively old population (e.g., mean age at recruitment 75 years in both MAPT and DO-HEALTH), at an age range when the protective association of fish to CVD burden was no longer apparent in our study.

The general beneficial role of n-3 PUFA for the vasculature has been long documented.[43] It includes antiatheroma and anti-arrhythmic properties and favorable effects on blood pressure[21] and on endothelial membrane fluidity.[43] There may be also specific pathways linking n-3 PUFA but also other nutrients provided by fish such as vitamin D and selenium to cerebral small vessel disease.[10] Long-chain n-3 PUFA may preserve WM myelin sheath integrity,[44] β-amyloid clearance[45] and protect against disruption of the blood-brain barrier.[46] Vitamin D is implicated in vasoprotective mechanisms and blood pressure regulation,[47] and

selenium is involved in atherogenesis and low-density lipoprotein cholesterol oxidation.[48] All 3 nutrients have well-documented anti-inflammatory properties.[47–49]

A strength of this study is its high-resolution 3-dimensional MRI images and evaluation of 3 complementary markers of CVD, including use of a cutting-edge automatic algorithm for detection and quantification of WMH on T2-weighted images, which has provided robust estimates of WMH volumes with very little operator intervention and a good reproducibility.[33] The use of a high resolution 3D MRI with small voxel size ($1.0\times0.98\times0.98$ mm^3) and multiplanar reformatting technique provides high-sensitivity sequences for the detection of dilated perivascular spaces of small size (2 mm) and diverse orientation with a high reliability.[8] Moreover, we were able to control for a large number of potential confounders. We used FAMD to derive a continuous indicator of CVD burden because previous studies have shown increased sensitivity of continuous quantification of small vessel disease compared to simple rating scores.[23–25] Different statistical approaches have been proposed, and a methodological study should be conducted to formally compare methods.

The main limitation of our study is the cross-sectional design, which precludes us from establishing that exposure (fish intake) precedes outcome (CVD burden). We assumed that fish intake evaluated at 1 time point at baseline reflects longer-term dietary habits. In another 3C center (Bordeaux), fish intakes evaluated by repeated food frequency questionnaires were relatively stable over a 5-year period,[50] and correlated well to baseline plasma levels of long-chain omega-3.[5] Although we excluded prevalent cases of dementia and stroke and participants with history of hospitalized cardiovascular diseases, who are susceptible to have modified their dietary habits after a clinical event, it remains possible that individuals with infraclinical CVD (as studied here) may have spontaneously changed their diet due to subtle cognitive changes. In addition, individuals included in the study were healthier than the overall cohort population; however, taking into account the probability of inclusion using

propensity-score-weighting did not modify the association results. Moreover, although the observed association between increasing fish intake and lower CVD burden was robust to adjustment for a large number of potential confounders, as in any observational study, residual confounding may still persist. Last, despite an overall large sample size, age-stratified analyses were based on relatively small sample size in some subgroups, and findings should be replicated.

In this large population-based cohort of older adults free of dementia or history of stroke and cardiovascular diseases, fish intake was associated with lower global cerebrovascular burden at MRI, especially among participants <75 years of age. Our study illustrates the interest of summarizing CVD burden through coevaluation of WMH load, covert infarcts, and dilated perivascular spaces, that largely reflect cerebral small vessel disease, into a single continuous measure. Such an approach led to powerful predictors of cognitive decline in previous studies.[23,24] Our results suggest a stronger beneficial effect of fish intake on brain vasculature for younger participants, which should be taken into account in the design of future studies. To date, few protective factors against the development of subclinical cerebrovascular damage that lead to stroke or dementia have been identified. If confirmed in prospective studies or clinical trials, the beneficial role of fish intake for the preservation of cerebrovascular health in very early brain aging stages may lead to relatively simple and inexpensive preventive strategies.

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DISCLOSURES

A. Thomas, F. Crivello, B. Mazoyer, S. Debette, C. Tzourio and C. Samieri report no disclosures relevant to the manuscript.

APPENDIX 1: AUTHORS

Name	Location	Contribution
Aline Thomas, MSc	Bordeaux, France	Designed and conceptualized study; analyzed the data; performed statistical analysis; drafted the manuscript
Fabrice Crivello, PhD	Bordeaux, France	Major role in acquisition of data; provided significant advice; revised the manuscript
Bernard Mazoyer, MD, PhD	Bordeaux, France	Major role in experiment design and acquisition of data; provided significant advice; revised the manuscript
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Cécilia Samieri	Bordeaux, France	Supervised the research project; designed and conceptualized study; drafted the manuscript

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Table 1. Participants' characteristics according to increasing fish intake categories, the 3C Dijon study, 1999-2000 (n = 1,623)

	Total population (N = 1,623)	Fish intake (times /week)			
		<1 (n = 173)	1 (n = 603)	2-3 (n = 756)	≥4 (n = 91)
Age (years), mean (SD)	72.3 (4.1)	72.3 (3.8)	72.1 (4.0)	72.5 (4.1)	72.1 (3.9)
Female, n (%)	1,020 (62.8)	119 (68.8)	341 (56.6)	499 (66.0)	61 (67.0)
Educational level, n (%)					
None or primary	263 (16.2)	41 (23.7)	102 (16.9)	107 (14.2)	13 (14.3)
Secondary	723 (44.6)	79 (45.7)	277 (46.0)	329 (43.6)	38 (41.8)
High school	305 (18.8)	34 (19.7)	107 (17.8)	152 (20.1)	12 (13.2)
University	330 (20.4)	19 (11.0)	116 (19.3)	167 (22.1)	28 (30.8)
Monthly income, n (%)					
<750 euros	48 (3.1)	10 (5.9)	13 (2.2)	22 (3.0)	3 (3.5)
750–1500 euros	448 (28.7)	72 (42.6)	163 (28.0)	185 (25.6)	28 (32.9)
1500–2250 euros	449 (28.8)	43 (25.4)	179 (30.8)	208 (28.8)	19 (22.4)
≥2250 euros	584 (37.5)	44 (26.0)	218 (37.5)	290 (40.1)	32 (37.6)
Refused to answer	30 (1.9)	0 (0.0)	9 (1.5)	18 (2.5)	3 (3.5)
Moderate to vigorous physical activity, n (%)	1,219 (79.7)	112 (69.6)	436 (76.2)	596 (84.1)	75 (85.2)
Smoking (packs/year), mean (SD)	7.4 (15.9)	6.4 (12.9)	8.3 (17.9)	7.0 (15.1)	6.4 (13.4)
Alcohol (drinks/week), mean (SD)	8.7 (10.1)	6.7 (11.5)	9.9 (10.5)	8.4 (9.4)	6.7 (9.0)
BMI (kg/m²), mean (SD)	25.3 (3.8)	26.1 (4.2)	25.4 (3.7)	25.1 (3.6)	25.2 (3.7)
Diabetes, n (%)	125 (7.8)	16 (9.4)	46 (7.7)	55 (7.3)	8 (8.8)
Hypertension, n (%)	1,244 (76.6)	130 (75.1)	471 (78.1)	571 (75.5)	72 (79.1)
Hypercholesterolemia, n (%)	901 (55.9)	99 (58.2)	312 (52.0)	435 (57.8)	55 (60.4)
History of cardiovascular disease[a], n (%)	327 (21.3)	35 (21.7)	115 (20.1)	154 (21.5)	23 (26.7)
Antithrombotic treatment, n (%)	186 (11.5)	13 (7.5)	72 (11.9)	90 (11.9)	11 (12.1)
Carotid plaque at ultrasound, n (%)	725 (46.1)	82 (48.8)	270 (46.3)	339 (46.2)	34 (38.6)
CCA-IMT (mm), mean (SD)	0.68 (0.11)	0.68 (0.10)	0.69 (0.11)	0.68 (0.11)	0.67 (0.10)
*APOE*ε4 status, n (%)	354 (21.8)	40 (23.4)	117 (19.6)	184 (24.5)	13 (14.3)
MMSE score (range, 0-30), mean (SD)	27.7 (1.8)	27.6 (2.0)	27.7 (1.7)	27.7 (1.8)	27.7 (1.8)
BVRT score (range, 0-15), mean (SD)	11.8 (1.9)	11.4 (2.1)	11.7 (1.8)	11.8 (1.9)	12.1 (1.7)
TMT-A score, mean (SD)	30.5 (10.0)	30.1 (10.1)	30.2 (10.0)	30.7 (10.1)	32.1 (9.8)
TMT-B score, mean (SD)	14.3 (7.1)	13.5 (7.2)	14.3 (7.0)	14.2 (7.2)	16.1 (6.9)
IST score, mean (SD)	34.2 (6.7)	34.0 (7.1)	34.3 (6.5)	34.0 (6.8)	35.7 (7.1)
Meat (times/week), mean (SD)	4.8 (1.9)	5.2 (2.2)	5.3 (1.7)	4.4 (1.8)	3.2 (1.8)
Fruits and vegetables (times/week), mean (SD)	17.4 (3.9)	16.5 (4.2)	17.1 (3.8)	17.7 (3.9)	17.9 (3.9)
Legumes (times/week), mean (SD)	0.62 (0.56)	0.57 (0.42)	0.61 (0.56)	0.64 (0.51)	0.68 (0.98)
Olive Oil (preferred source of added fat), n (%)	619 (42.1)	47 (31.1)	193 (35.5)	328 (47.5)	51 (60.7)

Abbreviations: 3C = Three-City; *APOE*ε4 = ε4 allele of the apolipoprotein E gene; BMI = body mass index; BVRT = Benton Visual Retention Test; CCA-IMT = common carotid artery intima-media thickness; IST = Isaacs Set Test; MMSE = Mini-Mental State Examination; TMT-A/B = Trail Making Test part A/B.

Means and percentages are of non-missing values. Missing values (by decreasing percent): 9.4% for olive oil consumption; 7.1% for alcohol consumption; 5.7% for physical activity; 5.1% for non-hospitalized cardiovascular disease; 4.4% for CCA-IMT; 4.3% for TMT-B; 3.9% for monthly income; 3.1% for carotid plaque; 1.5% for TMT-A; 1.4% for tobacco consumption; 1.0% for diabetes; 0.8% for *APOE*ε4 status and BVRT score; 0.6% for hypercholesterolemia; 0.5% for IST score; 0.1% for educational level, BMI, MMSE, and fruits and vegetables intake.

[a] Non-hospitalized angina pectoris, cardiac rate disorder (including arterial fibrillation), heart failure or lower limbs arteritis (history of hospitalized cardiovascular diseases were excluded from study sample).

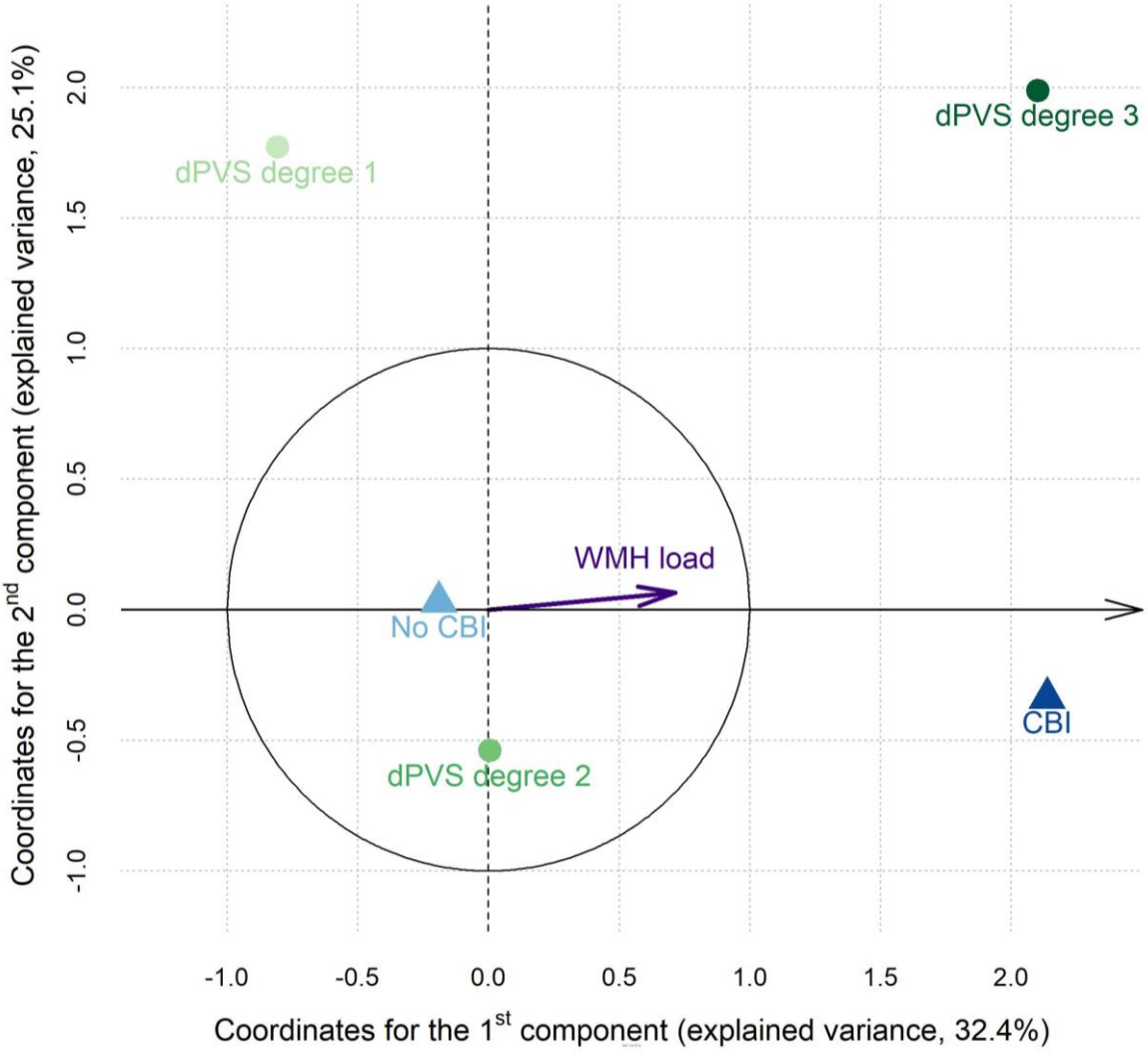
Table 2. Contribution of each MRI phenotype to the global CVD burden indicator,[a] the 3C Dijon study, 1999-2000 (n = 1,623)

	Total population	Quartiles of global CVD burden indicator			
		Q1	Q2	Q3	Q4
WMH load (%), mean (SD)	2.2 (2.0)	1.1 (0.6)	1.3 (0.4)	2.2 (0.5)	4.3 (3.0)
Covert brain infarcts, n (%)	132 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	132 (32.5)
Dilated perivascular space severity, n (%)					
Degree 1	269 (16.6)	236 (58.1)	9 (2.2)	7 (1.7)	17 (4.2)
Degree 2	1,254 (77.3)	170 (41.9)	397 (97.8)	398 (98.3)	289 (71.2)
Degree 3	100 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)	100 (24.6)

Abbreviations: 3C = Three-City; CVD = cerebrovascular disease; WMH = white matter hyperintensities

[a] A global indicator of CVD burden was defined as the first component of a Factor Analysis of Mixed Data applied to 3 brain MRI markers (WMH load, covert brain infarcts and dilated perivascular spaces; 32.4% of data variance explained by the first component).

Figure 1. First factorial plane of a Factor Analysis of Mixed Data applied to the 3 MRI phenotypes of CVD (emphasizing the first principal component [x axis] used as a global CVD burden indicator), the 3C Dijon study, 1999-2000 (n = 1,623)



Abbreviations: 3C = Three-City; CBI = covert brain infarct; CVD = cerebrovascular disease; dPVS = dilated perivascular spaces; MRI = magnetic resonance imaging; WMH = white matter hyperintensities

The objective of this graphical representation is to interpret the global indicator of cerebrovascular burden calculated from the first component of the Factor Analysis of Mixed Data (FAMD). In dimension-reduction approaches, *principal components* are linear combinations of variables weighted by geometrically defined *eigenvectors* in a *variable space* (defined by p variables). Here, the 3 MRI markers are represented by p = 6 variables/modalities (continuous WMH load; 2 CBI and 3 dPVS modalities).

A new space, the *factorial space* (maximum of p dimensions), is constructed by sequentially identifying the orthogonal eigenvectors that best summarize the data while keeping most of its information. The *first factorial plane* of this new space is defined by the first (x axis) and second (y axis) components, which explain most of the variance in the data. This is the best 2-

dimensional summary of the data that is emphasized in the figure. The figure displays the projection of variables/modalities onto this first factorial plane. The *factorial coordinates* (defined in each factorial plane) inform about the contribution of each variable on each component. Hence, the first component, defined by elevated coordinates for WMH load, CBI and dPVS degree 3, represents individuals with higher values for the 3 markers.

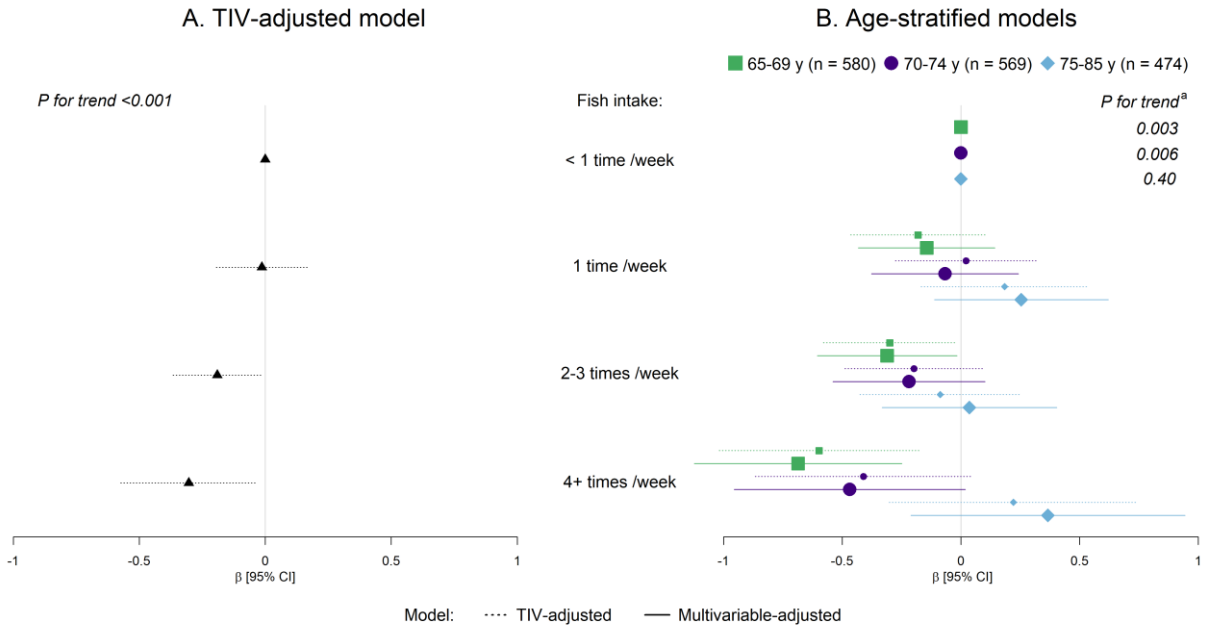
In FAMD, coordinates of continuous and categorical variables are not directly comparable because they are computed from different methodologies: linear correlation between the variable and the component for continuous variables, and barycenter of projected observations which possess the modality (normalized by the inverse of the modality frequency) for categorical variables.

There is a direct link between factorial coordinates of each variable in the factorial space as plotted here, eigenvectors from the variable space, and the coefficients of the linear combinations of variables for definition of principal components. Eigenvectors are defined by the $p = 6$ factorial coordinates divided by the squared root of the variance explained by the component (i.e., the *eigenvalue*). In FAMD, *coefficients* are computed as follows: eigenvector element divided by the empirical standard deviation of the variable for continuous variables, and eigenvector element for each modality of categorical variables.

Here, the first FAMD component score (i.e., the "CVD burden indicator" labeling the first component) was defined as the linear combination of each variable/modality weighted by its corresponding coefficient as follows: CVD indicator score = 0.312 WMH load – 0.709 dPVS d1 + 0.005 dPVS d2 + 1.847 dPVS d3 – 0.166 no CBI + 1.878 CBI; where d1, d2, and d3 are degrees 1, 2, and 3.

The figure superimposes a correlation circle for WMH load (continuous) and factorial map for dPVS and CBI (categorical). The first component that represents the global CVD burden indicator in our analyses was emphasized with a solid line (x axis line) and with a light-to-dark color scaling set proportional to variables/modalities factorial coordinates indicating their contribution to that component.

Figure 2. Association of fish intake with the global cerebrovascular disease burden indicator, the 3C Dijon study, 1999-2000 (n = 1,623)



β coefficient and 95% confident intervals (CIs) were estimated by linear regression models with fish intake as a main explanatory variable (upper intake categories versus <1 time per week as a reference). Panel A: model adjusted for total intracranial volume (TIV). Panel B: age-stratified models, (i) adjusted for TIV (dotted lines) and (ii) further adjusted for sex; educational level; monthly income; body mass index; tobacco consumption; alcohol consumption; intakes of fruits and vegetables, legumes, meat and olive oil; engagement in moderate to vigorous physical activity; diabetes; history of non-hospitalized cardiovascular diseases; hypertension; hypercholesterolemia; and carrying ε4 allele of the apolipoprotein E gene (solid lines).

The *p*-value for linear trend across categories was obtained using a continuous variable in which participants in a given category were assigned the median number of times of fish intake per week.

[a] The *p*-values for linear trends across fish intake categories estimated in age-stratified TIV-adjusted model (*p* for trend in multivariable-adjusted models were as follows: <0.001 in 65-69 years, 0.02 in 70-75 years, and 0.91 in ≥75 years).