# Associations of dietary polychlorinated biphenyls and long-chain omega-3 fatty acids with stroke risk

Maria Kippler [a], Susanna C. Larsson [a], Marika Berglund [a], Anders Glynn [b], Alicja Wolk [a], Agneta Åkesson [a,*]

[a] Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE 171 77, Stockholm, Sweden
[b] Department of Risk and Benefit Assessment, National Food Agency, Box 622, SE 751 26, Uppsala, Sweden

## ARTICLE INFO

## ABSTRACT

Background: Little is known about joint exposure to polychlorinated biphenyls (PCBs) and long-chain omega-3 fatty acids [eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)], through fish consumption, on cerebrovascular disease risk.
Objective: To explore associations of dietary PCB exposure and EPA-DHA intake with risk of different stroke subtypes.
Methods: This was assessed in the prospective population-based Cohort of Swedish Men including 39,948, middle-aged and elderly men, who were free of cardiovascular disease and cancer at baseline in 1997. Validated estimates of dietary PCBs and EPA-DHA were obtained via a food frequency questionnaire.
Results: During 12 years of follow-up, 2286 and 474 incident cases of ischemic stroke and hemorrhagic stroke, respectively, were ascertained through register linkage. Dietary PCB exposure and EPA-DHA intake were associated with hemorrhagic stroke but not ischemic stroke. Men in the highest quartile of dietary PCB exposure (median 412 ng/day) had a multivariable- and EPA-DHA-adjusted RR of hemorrhagic stroke of 2.77 [95% confidence interval (CI), 1.48–5.19] compared with men in the lowest quartile (median 128 ng/day; p for trend <0.01). The corresponding RRs in men with and without hypertension were 5.45 (95% CI, 1.34–22.1) and 2.37 (95% CI 1.17–4.79), respectively. The multivariable- and PCB-adjusted RR of hemorrhagic stroke for the highest quartile of EPA-DHA intake (median 0.73 g/day) versus the lowest quartile (median 0.18 g/day) was 0.42 (95% CI, 0.22–0.79).
Conclusion: Dietary PCB exposure was associated with an increased risk of hemorrhagic stroke, whereas a protective association was observed for dietary EPA-DHA intake.

## 1. Introduction

Exposure to polychlorinated biphenyls (PCBs) has been associated with increased risk of stroke in two prospective studies (Bergkvist et al., 2014; Lee et al., 2012) and in some ecological studies (Sergeev and Carpenter, 2010, 2011; Shcherbatykh et al., 2005), and with hypertension, hypercholesterolemia, atherosclerosis and diabetes (Donat-Vargas et al., 2015; Kreiss et al., 1981; Lee et al., 2010, 2011; Lind et al., 2012). Although the production of PCBs has been banned for >30 years, they are still widespread due to their high persistence in the environment and accumulation in adipose tissue (half-lives up to decades) (IARC, 2015). Dietary exposure – the main

exposure route – occurs predominantly via consumption of fat of animal origin, especially fatty fish (Bergkvist et al., 2012; Malisch and Kotz, 2014).

Consumption of fatty fish, rich in long-chain omega-3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], is encouraged in cardiovascular guidelines (Gidding et al., 2009). These long-chain omega-3 fatty acids may lower blood pressure and blood triglyceride concentrations, decrease inflammation, and improve vascular function (Mozaffarian and Wu, 2011). In meta-analyses, fish consumption was modestly inversely associated with ischemic stroke risk but not significantly associated with hemorrhagic stroke risk (Chowdhury et al., 2012; Larsson and Orsini, 2011; Xun et al., 2012). On the contrary, a higher incidence of hemorrhagic stroke has been observed in Greenland Eskimos, consuming a marine diet rich in long-chain omega-3 fatty acids, than in Danes (Ostergaard Kristensen, 1983), suggested to be caused by a higher proportion of long-chain omega-3 fatty acids in their platelets, reducing platelet aggregation and prolonging bleeding time (Dyerberg and Bang, 1979). Noteworthy, the marine-based Eskimo

* Corresponding author at: Division of Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Box 210, 171 77 Stockholm, Sweden.
E-mail addresses: maria.kippler@ki.se (M. Kippler), susanna.larsson@ki.se (S.C. Larsson), marika.berglund@ki.se (M. Berglund), anders.glynn@slv.se (A. Glynn), alicja.wolk@ki.se (A. Wolk), agneta.akesson@ki.se (A. Åkesson).

diet is also high in PCBs (Bjerregaard et al., 2001); thus, confounding by PCB exposure cannot be excluded.

We aimed to assess the associations of validated estimates of dietary PCB exposure and dietary long-chain omega-3 fatty acids intake, with the risk of stroke subtypes in a large population-based prospective cohort of men.

## 2. Materials and methods

### 2.1. Study population

The Cohort of Swedish Men, a population-based prospective cohort, was established in 1997 to 1998 when all men, aged 45 to 79 years, residing in two counties in Central Sweden received an invitation to participate in the study. Participants completed a self-administrated questionnaire, including almost 350 questions concerning diet and other lifestyle factors. In total, 48,850 men, representative of Swedish males aged 45–79 years, in terms of age, level of education, and prevalence of overweight (Norman et al., 2002), returned a completed questionnaire (response rate 49%). The study was approved by the Regional Ethical Review Board in Stockholm, Sweden, and return of the completed questionnaire was considered to imply informed consent.

For the present analysis, we excluded men with incorrect personal identification number and those who died before baseline (January 1, 1998; n = 352). We also excluded those who were diagnosed with cancer [not including non-melanoma skin cancer (identified through the National Cancer Register)] (n = 2592), had prevalent cardiovascular disease [myocardial infarction, angina, and stroke (identified through the Swedish Patient Register)] (n = 5476), duplicate diagnoses on the same date (n = 34), or reported implausible energy intake [±3 standard deviation (SD) of mean log transformed energy] (n = 448). Thus, the analytical cohort for the present analysis consisted of 39,948 men.

### 2.2. Dietary PCB exposure, long-chain omega-3 fatty acids and other covariates

Questions on diet consisted of a 96-item food frequency questionnaire (FFQ), constructed to reflect the men's average consumption of different foods and beverages during the last year. The consumption of frequently consumed foods (e.g., dairy products and bread) was assessed using open-ended questions, whereas consumption of other foods was assessed by pre-specified serving sizes and eight predefined frequency categories, ranging from never to ≥3 times per day.

The dietary exposure to PCBs was estimated through recipe-based databases created for the FFQ. The PCB-database, described in detail elsewhere (Bergkvist et al., 2012), was based on PCB congener 153 at the time of baseline, mainly because it is the most abundant PCB in food on the Swedish market. PCB153 is also a very good indicator of total PCBs, dioxinlike PCBs, and the related polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) in food as well as in human serum (Bergkvist et al., 2012; Covaci et al., 2002). The concentrations of PCBs, EPA-DHA, and methylmercury (MeHg) in foods were obtained from the Swedish National Food Agency and the Swedish Environmental Protection Agency. Each participant's total intake was estimated by summing the multiplied average concentration of each of the compounds with the consumption frequency and portion-size for each food. The total intake was adjusted for total energy intake (average: 2600 kcal/day) using the residual-regression method (Willett and Stampfer, 1986). The FFQ-based dietary PCB estimates have been validated against six PCB congeners in serum (correlation coefficient, r = 0.30 to 0.58; *p* values <0.05) in 201 women, aged 56–85 years from the same region (Bergkvist et al., 2012). FFQ-based dietary intake of EPA-DHA has been validated against 14 24-h dietary recall interviews (r = 0.6 for both EPA and DHA) in 248 men, aged 40–74 years, from the study area (Messerer et al., 2004) and against

adipose tissue concentrations of EPA and DHA [r = 0.32 and 0.48, respectively for concurrent, and r = 0.21 and 0.33, respectively for past exposure assessment (6 years prior to the adipose tissue sampling)] in women (Wallin et al., 2014). The dominant source of dietary MeHg is fish consumption, which in turn, correlates well with hair mercury concentrations in Swedish populations (r ≥ 0.7) (Strom et al., 2011).

From the questionnaire we also obtained information on education level, family history of myocardial infarction <60 years, history of high cholesterol and hypertension (yes/no), use of aspirin, body weight and height, smoking habits, use of alcohol and dietary supplements, and physical activity. Total daily physical activity was assessed with a validated questionnaire and expressed as metabolic equivalents (METs) (Norman et al., 2001).

### 2.3. Case ascertainment of stroke

Dates of first incident cases of stroke that occurred between January 1, 1998 and December 31, 2010 were ascertained through computerized linkage via the study participants' personal identification number to the Swedish Patient Register and the Swedish Cause of Death Register. Strokes were classified as ischemic stroke (International Classification of Disease, 10th Revision, code I63) and hemorrhagic stroke, including intracerebral hemorrhage (I61) and subarachnoid hemorrhage (I60).

### 2.4. Statistical analyses

We followed the men from January 1, 1998 until the date of the first stroke, death or end of follow-up (December 31, 2010), whichever was the initial event. Cox proportional hazards regression models, with attained age (in years) as the underlying time scale, were used to estimate hazard ratios (herein referred to as relative risks, RR) and 95% confidence intervals (95% CI) of different stroke subtypes [ischemic stroke, intracerebral hemorrhage, and hemorrhagic stroke (intracerebral and subarachnoid hemorrhage combined)]. Men were categorized into quartiles of dietary PCB exposure and dietary EPA-DHA intake at baseline. The multivariable-adjusted models were controlled for education level (less than high school, high school, or university), family history of myocardial infarction before the age 60 years (yes/no), high cholesterol (yes/no), history of hypertension (yes/no), atrial fibrillation before baseline 1998 (yes/no), use of aspirin (yes/no), body mass index (<20, 20–24.9, 25–29.9, ≥30 kg/m$^2$), smoking status (never, past or current), alcohol consumption (0, >0–4.9, 5.0 – <15, ≥15.0 g/day), total physical activity (quartiles, MET-hours/day), use of fish oil supplements (yes/no), energy intake (continuous, kcal/day), consumption of fruit and vegetables (quartiles, servings/week), red and processed meat (quartiles, servings/week) and dairy products (quartiles, servings/week), dietary intake of saturated fatty acids (quartiles, g/day) and dietary MeHg exposure (quartiles, μg/day). In an additional model, we further adjusted for either intake of EPA-DHA (quartiles, g/day) or dietary PCB exposure (quartiles, ng/day). The Schoenfeld's residual test did not indicate any violation of the proportional hazard assumption. To explore the potential effect of the strong correlation between PCBs and EPA-DHA (r = 0.95), we performed analyses stratified by both dietary intake of EPA-DHA and dietary PCB exposure (using split by the medians). We stratified our analyses by i) history of hypertension - as high blood pressure may be an intermediate risk factor in the causal pathway between dietary PCB exposure and stroke, and by ii) exposure to PCBs in utero [categorized as being born before (≤1930) or after (>1930) the industrial use of PCBs started]. Potential interactions were assessed using the likelihood ratio test, comparing models with and without an interaction term. Linear trend across quartiles was tested using the median concentration within categories as a continuous variable. All statistical analyses were performed in STATA, software version 12 (Intercooled STATA; StataCorp LP, Collage Station, TX, USA).