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Effect of fatty fish or nut consumption on concentrations of persistent organic pollutants in overweight or obese men and women: A randomized controlled clinical trial

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Abstract *Background and aims:* While excess energy intake and physical inactivity constitute the obvious causes of body fat accumulation, persistent organic pollutants (POPs) are novel factors that have been linked to cardiometabolic disorders. Major sources of POPs are animal fats including fatty fish. Given the putative protective effects of fish on cardiovascular disease, we explored whether high consumption of fatty fish increased serum concentrations of POPs.
Methods and results: Men and women aged 35–70 years with body mass index between 25 and 38 kg/m^2 and at least 1 cardiometabolic component were randomized to high intakes of fatty fish (mostly farmed salmon, ~630 g/week; n = 45), high intakes of nuts (~200 g/week; n = 42) or a control group following their usual diet but restricting fatty fish and nuts for 6 months (n = 44). Concentrations of 15 POPs (5 organochlorinated compounds, 2 dioxin-like polychlorinated biphenyls and 8 non-dioxin-like polychlorinated biphenyls) and cardiometabolic risk factors were measured at baseline and end of the study. Results showed that changes in concentrations of individual and classes of POPs did not differ between the dietary groups and controls ($p > 0.05$). Among cardiometabolic risk factors HDL-cholesterol increased in the fatty fish group compared to controls (+0.10 mmol/L, CI (0.05–0.20); $p = 0.005$) while no changes were observed in the group consuming nuts.
Conclusion: Fatty fish consumption for 6 months did not increase the serum concentrations of POPs in individuals with overweight or obesity and metabolic risk. While this finding appears reassuring regarding short-term intakes of farmed salmon, long term variations in POPs in adipose stores require further study.

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Background

Excess body fat and its adverse health consequences appear to be rapidly increasing in prevalence in most areas of the world. In the wake of the obesity epidemic, cardiometabolic disorders including metabolic syndrome and type 2 diabetes have resulted in major public health challenges [1]. In Norway, more than one-fifth of the adult population is obese, and the prevalence of abdominal obesity has increased disproportionately in women [2].

Major causes of obesity include excess energy intake and physical inactivity, but emerging evidence has linked environmental toxins, such as persistent organic pollutants (POPs) with the development of obesity, metabolic syndrome and type 2 diabetes [3]. POPs are lipophilic chemicals that bioaccumulate in adipose tissue of living organisms for decades. They are also measurable in serum and usually vary according to age and gender [4]. Associations between POPs stored in adipose tissue and circulating blood concentrations may differ according to the compound and previous exposure [5].

POPs appear to disrupt metabolic regulation, possibly leading to weight gain [6] and increased risk of metabolic syndrome [7]. Furthermore, a body of evidence links of POPs to type 2 diabetes. A meta-analysis that included 72 observational studies found sufficient evidence for a positive association of some POPs and type 2 diabetes, despite studies' heterogeneity [8]. Furthermore, POPs may contribute to cardiovascular disease (CVD). The PREDIMED-CANARIAS cohort study found that people at high risk for CVD showed a higher level of contamination by POPs [9]. Others found associations between several POPs and carotid atherosclerosis [10].

Populations are mainly exposed to POPs through the consumption of fatty animal food, especially of marine origin. A study found that the most consistent association between foods and concentrations of POPs was fish followed by dairy and meat, while vegetables, fruit and cereals were rarely related to POPs [11]. These results seem to indicate that modifying dietary patterns may be useful to decrease the burden of POPs and potentially the risk of CVD [9].

Among types of fish, evidence to date points to farmed Atlantic salmon as a dietary source of some POPs, because of contaminants in fish feed [12]. Burdens of POPs appear to be higher in farmed compared to wild salmon; furthermore farmed salmon from Europe has previously been shown to be more contaminated than farmed salmon from South and North America [13]. In recent years aquaculture feed has been increasingly based on plant oils, instead of feed of animal origin [14]. On the other hand, a recent study found lower levels of POPs in farmed compared to wild salmon [15]. Cooking methods also affect the concentrations of POPs. For example, cooking of raw salmon leads to significant loss of lipids and lower content of POPs per unit of weight [16].

The potential harms of POPs in farmed fish complicate the relations between eating fish and CVD. Including fish in the diet has been generally associated with good health outcomes. Recently a reduced risk of CVD was found among Mediterranean populations who reported consuming fish, particularly fatty fish $\geq$4 times weekly versus $<$2 times weekly [17]. However, a meta-analysis of 14 prospective studies found regional differences in the relation between fish consumption and all-cause and CVD mortality; studies conducted in Western countries showed a U-shaped curve, indicating that large intakes may be detrimental [18].

Another food often recommended to prevent CVD is nuts. Studies strongly support a protective association between nut consumption and CVD [19]. A review of meta-analyses that appeared in 2018 suggested that nuts may be associated with lower all-cause mortality and CVD and coronary heart disease mortality, while there was no association between nut consumption and type 2 diabetes [20]. The PREDIMED dietary trial provided experimental evidence to support the protective effect of nuts on CVD [21]. Nuts could be a food alternative to people that either do not tolerate or dislike fatty fish and would not be expected to increase POP concentrations, though of course nut allergy also affects subsets of the population.

Given this background, the primary aim of this study was to clarify the effects of eating fatty fish (predominantly salmon and mackerell) or nuts (a mixture of walnuts, hazelnuts and almonds) on concentrations of POPs that are typically found in fatty fish compared to avoidance of fatty fish and nuts. We studied overweight and obese men and women with high risk of developing cardiometabolic disturbances that may be caused by high intakes of POPs.

Methods

Men and women aged 35–70 years with body mass index (BMI) 25–38 kg/m^2 were recruited through advertisement in newspapers, through the Facebook page of Oslo.

University Hospital as well as from patient referrals to the Section of Preventive Cardiology, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway. The study was approved by the local Regional Ethics Committee (Approval number 2015/1930) and conducted according to the Declaration of Helsinki. All participants provided written informed consent before any procedures were performed. The study, with protocol description was registered at www.clinicaltrials.gov (NCT02589756). The first participant was included in January 2016 and the last 6-month follow-up was in October 2017.

Inclusion criteria included 1 component of metabolic syndrome in addition to waist circumference ($\geq$102 cm for men or $\geq$88 cm for women), i.e. blood pressure $\geq$130/85 mmHg or use of antihypertensive medication, fasting glucose $\geq$5.6 mmol/L, HDL cholesterol $\leq$1.3 mmol/L for women or $\leq$1.0 mmol/L for men, or triglycerides $\geq$1.7 mmol/L. Exclusion criteria were cigarette smoking, diabetes, allergy to or dislike of fish or nuts, chronic disease including cancer, gastrointestinal disease or CVD,

morbid obesity (BMI of ≥38 kg/m^2 with obesity-related health conditions or ≥40 kg/m^2 alone) and self-reported weight fluctuations (+/− 5 kg in the last 6 months), eating disorder, history of bariatric surgery, use of anti-obesity drugs or other drugs affecting body weight, such as anty-psychotics or glucocorticoids.

Study design

At the screening visit, medical history and demographic information were recorded, including educational level and alcohol consumption and a medical examination was performed. Participants were also asked to fill out a food frequency questionnaire (FFQ) as described further below. Inclusion and exclusion criteria were applied after blood test results were available (usually on the same day). Thereafter, participants started with a run-in period of 2 weeks where they were asked not to consume salmon, mackerel or other fatty fish or nuts to provide a dietary baseline. Follow-up visits were scheduled at biweekly intervals up to 3 months, and thereafter every 6 weeks up to 6 months, for a maximum of 10 visits in total. Blood samples, weight, waist and hip circumferences, and blood pressure were taken at screening, randomization and 6 months. At every visit study food was distributed to participants in the fish or nuts groups. Participants were examined by the medical physician (SD) and were offered dietary instruction given by the nutritionists. The dietary instructions were mainly about how to incorporate the intervention foods in the diet. All participants received a booklet with recipes according to their study group.

Randomization

Randomization was performed by a stratified sampling procedure with gender and BMI (grouped as 25 to <30 kg/m^2 and 30−38 kg/m^2) as the strata. A statistician prepared a computer-generated random number list. **The clinic study assistant** opened numbered and sealed envelopes consecutively with no exceptions.

Dietary interventions

Participants randomized to the fatty fish group were asked to consume 4 portions (fillets) of farmed salmon provided in frozen portions (a total of 500−560 g weekly) and 1 tin of mackerel in tomato sauce (110 g) weekly. The energy content of the total amount of fish was ∼1400 kcal/week. This group was asked to avoid eating nuts. Fish was provided free of charge for the 6 months.

Participants randomized to the nuts group were asked to consume ∼100 g walnuts, ∼50 g hazelnuts and ∼50 g almonds weekly, also providing ∼1400 kcal/week. This recommendation is similar in composition and amount to the nut-mix used in the PREDIMED intervention study [21]. Nuts were provided free of charge for the 6 months. This group was asked to avoid eating fatty fish. However, lean fish was allowed.

The control group consumed their usual diet, but was asked to avoid fatty fish and nuts. The control group was also allowed to eat lean fish. At the end of the project this group received a gift card of 1200 Norwegian kroner (approximately 125 euros) as compensation for not receiving food during the project.

Data collection of dietary intake

Collection of dietary intake was done using the FFQ developed in conjunction with the Norwegian MoBa study [22]. Data were collected at screening and at 6 months. The FFQ at screening reflected the habitual diet the past year prior to the start of the study, while the FFQ at 6 months reflected the participants' diet during the study period of the last 6 months. The FFQ consisted of 255 different food and drink items. The food items were divided into categories like "bread, crisp bread and crackers" or "hot meals", with following suitable subcategories to specify details on each particular food. Drinks, desserts and snacks were mapped in the same manner as other food items in the attempt to include all sources of energy. The frequency of the reported food items was given in either number per day, number per week or number per month, with the possibility to mark only 1 of the 3 frequency intervals. Portion sizes were given for units of bread (slices), liquids (cups/glasses) and fruit. In cases where they were not given, consumption frequencies were converted into food amounts (g/day) by considering standard Norwegian portion sizes for men and for women.

The participants were given instructions on how to fill out the form and to consider seasonal food such as for Christmas or summer, as a mean throughout the year. Food items consumed less than 6 or 12 times a year (in the screening or end of study FFQ, respectively) were not queried.

The FFQs were examined by trained nutritionists in order to ensure accuracy as far as possible. Corrections were made in collaboration with each individual participant. Data on food items and nutrient consumption were calculated using FoodCalc [23] and the Norwegian food composition table from 2003 [24]. The calculations were performed in the programs originally developed for this FFQ at Norwegian Institute of Public Health.

Checks of compliance with assigned foods were done at each visit by the dietitian/responsible physician by direct query as to whether all of the assigned food had been consumed. Participants in the group assigned to fatty fish reported full compliance with the assigned weekly amounts with the exception of 2 participants. One of these participants consumed 1 portion less weekly than the 4 weekly assigned portions due to prestudy established nausea and esophageal reflux while the other participant reported a pause of 2 weeks in fish intake during a

vacation after the 3-month visit but compensated for this hiatus by consuming the assigned amount of fatty fish for 2 extra weeks at the end of the study (total study duration for this participant was 6.5 months). After considering these deviations, the amount of consumed salmon and mackerell consumed by the fatty fish group was estimated at ~90 g/day (~630 g/week).

Participants in the group assigned to nut consumption also reported full compliance during the study period with the exception of one participant who reported consuming ~60% of the assigned nuts between visit 8 and 9. The amount of ingested nuts was estimated at ~30 g/day in the nuts group.

Clinical and laboratory procedures

Body weight was measured using the same calibrated digital scale to the nearest 0.1 kg. Waist circumference was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured at the widest portion of the buttocks. Blood pressure was measured using the Omron 705IT (Omron HEALTHCARE, Kyoto, Japan) after the participant rested quietly in a sitting position for at least 5 min alone in a quiet room. The mean of 3 measurements spaced 1 min apart was calculated at screening, randomization, and 3 month and 6 month visits.

Blood samples were obtained following a minimum of a 10-hour fast. Analyses of blood samples were performed at Oslo University Hospital Clinical Chemistry Laboratory/Ullevål. Lipids were measured using enzymatic colorimetric methods, while apolipoprotein B was determined using an immunoturbidimetric method. Serum glucose was measured using hexokinase. HbA1c was measured using ion-exchange quantitative high performance liquid chromatography. C-reactive protein (CRP) was determined with a particle enhanced turbidimetric assay. C-peptide and insulin concentrations were analyzed at the hormone laboratory of OUS/Aker, using a non-competitive electro-chemiluminescence immunoassay (ECLIA) (Modular E170 Cobas e601 kit Roche Diagnostics). The Homeostasis Model Assessment insulin resistance index (HOMA-IR) was calculated to estimate insulin resistance [25].

To further estimate insulin sensitivity a euglycemic hyperinsulinemic clamp was performed at the Diabetes Research Laboratory at Oslo University Hospital Aker in a subset of participants, 10 subjects in the control and 10 patients in the fatty fish group at randomization and 6 months. The hyperinsulinemic euglycemic clamp was performed after an overnight fast. A fixed dose of insulin 40 $mU/m^2/min^{-1}$ was infused, and glucose 200 mg/mL was adjusted to maintain plasma glucose levels at 5.0 mmol/L for 150 min (euglycemia). Insulin sensitivity was reported as glucose infusion rate during the last 30 min of the clamp. Prior to the clamp, weight and fat-free mass were measured with a Tanita Body Composition Analyzer BC-418MA (Tokyo, Japan).

Assessment of circulating POPs concentrations

POPs were measured in 200 μl of serum at the National Institute for Health and Welfare, Kuopio, Finland. The following POPs were measured: polybrominated diphenylethers (PBDEs) 47, 99 and 153; polychlorinated biphenyls (PCBs) including dioxin-like (118, 156) and non-dioxin-like (74, 99, 138, 153, 170, 180, 183, 187) compounds; and organochlorine pesticides including dichlorodiphenyltrichloroethane (p,p′DDT), dichlorodiphenyldichloroethylene (p,p′DDE), hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), pentachlorobenzene (PeCB), hexachlorobenzene, *trans*-nonachlor, and oxychlordane. The detection rate for PeCB, α-HCH, γ-HCH, oxychlordane and all PBDEs was <75% thus, these analytes were excluded from further statistical analyses.

A full description of the analytical method has been published previously [26]. Limits of quantification for POPs were 5–40 pg/ml. In each batch of samples a control serum sample from the National Institute of Standard and Technology, Standard Reference Material (SRM) 1958 for POPs was included (n = 13). An in-house produced low level control sample was prepared by 1–9 dilution of SRM 1958 with new born calf serum (NBCS) and also included in each batch of samples (n = 13). Average concentrations for POPs from SRM 1958 were 87–111% of the certified/indicative concentrations. Coefficients of variation (CVs) were 1.4–4.4% for SRM 1958 and 1.8–8.9% for diluted SRM 1958. The Environmental Health Unit participates to AMAP inter laboratory comparison (Ring Test for Persistent Organic Pollutants in human serum, National Institute of Public Health, Quebec, Canada) where 16 of the target POPs from 3 serum samples are reported for each round. During the last 2 years results from all samples for all POPs have been acceptable ($|z|<2$). Accuracy of the results for individual compounds from individual samples varied from 77–121% of the assigned values.

Assessment of POPs in fish samples

Four samples of farmed salmon from the producers suppling the fish in the study were analyzed at the National Institute for Health and Welfare, Kuopio, Finland for POPs content utilizing the same method used for serum samples.

Outcome measures

The primary outcome endpoint was changes in concentrations of POPs including organochlorinated pesticides (5 compounds), dioxin-like PCBs (2 compounds) and non-dioxin-like PCBs (8 compounds). Secondary outcomes were changes in weight, BMI, waist circumference, blood pressure and heart rate, and other cardiometabolic risk factors including concentrations of total cholesterol, HDL- and LDL-cholesterol, triglycerides, apolipoprotein B, glucose and HbA1c as well as C-reactive protein, insulin and C-peptide, HOMA-IR and clamp.

Statistical analysis

Power calculation was attempted, however, given the lack of previous data was exploratory. Differences in hexachlorobenzene (HCB) between the highest and lowest tertile were about 3-fold in the Nurses' Health Study, while differences in total polychlorinated biphenyls (PCBs) between the highest and lower tertile were about 4-fold [27]. Another study showed about a doubling of levels between the highest and lowest quartiles [28]. In a 6-month period a possibly clinically relevant change in POPs may be a 15–20% increase – this is also the difference in PCBs between representative and high consumers in Norway [29]. Based on the median HCB level in the Nurses' Health Study [27] of about 30 ng/g lipids, a difference of a mean of 36 ng/g lipids between groups with a standard deviation of about 9 ng/g lipids would require 36 participants in each group, with power set at 80% and alpha set at 5%. Our intention was to include 40 participants in each group to allow for dropouts for a total of 120 participants. 1 participant in the nut group had β-HCH and p,p'DDE values that were outliers (4228, 7071 pg/ml, respectively). These values were removed but no substantial changes were seen in results after this exclusion.

Analyses followed the intent-to-treat principle with the last value carried forward for dropouts, with additional complementary analyses of the per protocol population. These analyses did not differ substantially, and the intent-to-treat analyses are presented. Independent Student's t-test was performed comparing fatty fish group and nut group with controls. Variables that were not normally distributed were presented as median and 25th and 75th percentile and changes were analyzed using the Mann–Whitney test. Data was analyzed using IBM SPSS Statistics for Windows version 21 (SPSS Inc., Chicago, IL). The significance level was set at $p < 0.05$.

Results

Of the total of 131 participants (56 men and 75 women) randomized, 120 completed the study (Fig. 1). Age ranged between 37 and 69 years, and BMI ranged between 25.5 and 38.2 kg/m^2. Screening characteristics, medication use and laboratory analyses are presented in Table 1. Antihypertensive medications were adjusted by the study physician or the participant's general practitioner during the study in 12 participants. Of these, 4 participants changed dosage (3 increased dosage - 1 in the nut group, 2 in the control group) and 1 participant in the fatty fish group reduced dosage. Two stopped medication (1 in the fatty fish group and 1 in the nut group). One participant in the fatty fish group started medication and 5 changed type of anti-hypertensive treatment (3 in the nut and 2 in the control groups, respectively). In addition, 1 participant in the control group started statin treatment.

Weight, BMI, waist and hip circumferences, systolic and diastolic blood pressure and heart rate remained unchanged and did not differ between the 3 groups (Table 2). Among cardiometabolic risk markers only HDL-cholesterol increased in fatty fish group in comparison to controls (+0.1 mmol/L, CI (0.05–0.2), $p = 0.005$). Other markers, including insulin, HOMA-IR index and c-peptide did not differ between groups. Change in glucose infusion rate did not differ between fatty fish group and controls ($p = 0.7$, data not shown).

Table 3 shows dietary intakes at screening and 6 months. In the group assigned to fatty fish, the energy percentage from protein increased while percentages from

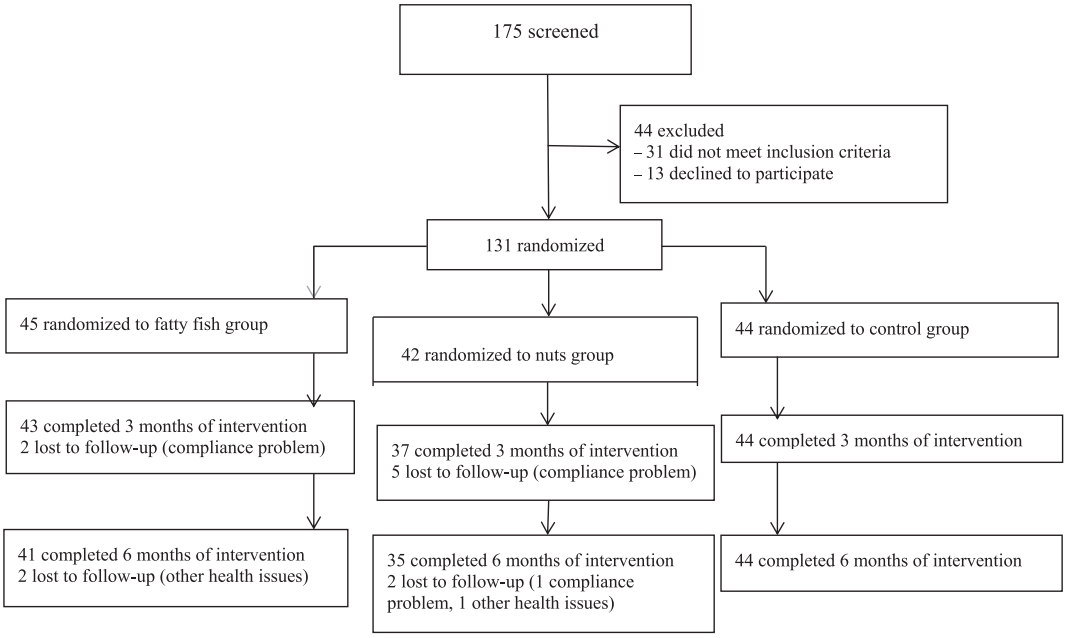

Figure 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram for participants' allocation into study arms.

Table 1 Screening characteristics. Mean (SD) values or percentages are shown, except for alcohol consumption, shown as median (25th, 75th percentile).

	Fatty fish diet (n = 45)	Nuts diet (n = 42)	Controls (n = 44)
Females, n (%)	26 (57.8)	24 (57.1)	25 (56.8)
Age, years	55.9 (6.6)	58.0 (5.2)	55.0 (6.9)
Education attained			
Primary school, n (%)	1 (2.2)	–	1 (2.3)
High school, n (%)	17 (37.8)	9 (21.4)	11 (25.0)
University, n (%)	27 (60.0)	33 (78.6)	32 (72.7)
Alcohol consumption (units/week)	2 (0, 5)	3 (1, 7)	3 (0, 5)
Body weight, kg	94.7 (13.4)	94.4 (13.5)	92.9 (15.1)
BMI, kg/m^2	31.1 (2.8)	31.7 (3.1)	31.2 (2.9)
Waist circumference males, cm	116 (10)	114 (9)	112 (7)
Waist circumference females, cm	108 (8)	109 (7)	108 (8)
Systolic blood pressure, mmHg	136 (15)	137 (13)	134 (13)
Diastolic blood pressure, mmHg	85 (10)	86 (9)	83 (7)
Heart rate/minute	68 (9)	70 (9)	66 (9)
Medications			
Use of antiplatelet agents, n (%)	6 (13.3)	5 (11.9)	3 (6.8)
Use of antihypertensives, n (%)	18 (40.0)	20 (47.6)	15 (34.1)
Use of lipid lowering drugs, n (%)	9 (20.0)	7 (16.7)	7 (15.9)
Laboratory values, fasting			
Total cholesterol, mmol/L	5.5 (1.1)	5.6 (1.1)	5.7 (1.2)
HDL-cholesterol, mmol/L	1.4 (0.3)	1.5 (0.4)	1.5 (0.4)
LDL-cholesterol, mmol/L	3.7 (0.9)	3.7 (1.0)	3.8 (1.1)
Triglycerides, mmol/L	1.5 (0.8)	1.4 (0.6)	1.5 (1.0)
Glucose, mmol/L	5.7 (0.5)	5.6 (0.5)	5.8 (0.7)
Metabolic syndrome, n (%)	31 (69)	19 (45)	22 (50)

carbohydrates and fat decreased in the fatty fish group compared to controls (Table 3). On the other hand, the energy percentages of total, mono- and polyunsaturated fat increased and the energy percentage from carbohydrates decreased in the group assigned to nuts compared to controls.

Neither of the intervention groups showed significant difference in the circulating concentrations of selected POPs (organochlorine pesticides including HCB, β-HCH, *trans*-nonachlor p,p'DDT, p,p'DDE, dioxin-like PCBs (118, 156) and non-dioxin-like PCBs (74, 99, 138, 153, 170, 180, 183, 187)) compared to controls after 6 months (Table 4).

Analyses of POPs concentrations in fish samples are presented in Table 5.

Discussion

This randomized controlled clinical trial found no change in circulating concentrations of 15 POPs during 6 months of high dietary intakes of fatty fish compared to avoidance of fatty fish in the diet among persons at risk of cardiometabolic disorders. In addition to comparison with a control group avoiding fatty fish, we included a group consuming nuts and avoiding fatty fish. Likewise no differences were found between the group consuming fatty fish compared to nuts group (data not shown). Cardiometabolic risk markers did not change in the groups consuming fatty fish or nuts compared to the group asked to avoid both food groups, with the exception of concentrations of HDL-cholesterol that increased in the group consuming fatty fish.

The study used a randomized controlled design to avoid bias and a randomization procedure with stratification for gender and BMI. Participants were approximately equally divided according to gender and selected in regard to the presence of overweight or obesity and at least 1 other cardiometabolic risk factor. They thus represented a sample of the population most likely to show possible cardiometabolic risks of eating fatty fish. During the trial there was a low level of dropouts overall (8%). Furthermore, participants showed good compliance with the assigned dietary group, as shown by compliance checks at each visit and the FFQ results.

We are unaware of any published similar randomized interventional studies that explored POPs concentrations in overweight and obese individuals in regard to fatty fish intakes. However, these types of studies may not be ideal to study the effect of diet on POPs as the main factor determining changes in serum concentrations of POPs during a relative short period of 6 months would be the dynamics of POPs storage and release. While body weight was carefully monitored and stayed stable, a number of factors may influence these dynamics mostly over longer periods of time.

The POPs that we measured are classified as organochlorine pesticides and dioxin-like and non-dioxin-like PCBs. These POPs have been connected to cardiometabolic disturbances both in cross-sectional and prospective studies [30]. We recently published a cross-sectional study showing that high concentrations of the same organochlorine pesticides and dioxin-like and non-dioxin-like PCBs, as measured in the current study, more than

Table 2 Changes in clinical characteristics and cardiometabolic markers between baseline and six months. Mean (SD) values are shown, except for C-reactive protein, insulin and c-peptide concentrations for which median (25th, 75th percentile) values are shown.

	Fatty fish diet (n = 45)				Nuts diet (n = 42)				Controls (n = 44)
	Baseline	6 months[a]	Change	P[b]	Baseline	6 months[a]	Change	P[b]	Change
Weight, kg	95.0 (13.9)	94.7 (14.4)	−0.3 (3.3)	0.4	94.3 (14.1)	94.8 (14.1)	0.4 (2.6)	0.7	0.2 (2.4)
BMI, kg/m^2	31.1 (2.8)	31.0 (3.2)	−0.1 (1.0)	0.4	31.5 (2.8)	31.7 (2.9)	0.2 (1.0)	0.5	0.1 (0.8)
WC[d] male, cm	116 (9)	116 (10)	0.5 (3.2)	0.9	114 (9)	115 (6)	0.9 (3.2)	0.7	0.4 (4.0)
WC female, cm	108 (8)	110 (9)	0.5 (3.4)	0.6	109 (8)	109 (9)	1.0 (4.0)	0.4	−0.1 (4.5)
SBP, mmHg	133 (12)	130 (12)	−4 (11)	1.0	135 (14)	131 (16)	−4 (9)	1.0	−4 (14)
DBP, mmHg	83 (7)	81 (7)	−1 (7)	0.8	85 (9)	83 (10)	−2 (5)	1.0	−2 (6)
Heart rate/min	68 (10)	67 (10)	−1 (7)	0.4	71 (11)	71 (8)	0 (8)	0.8	0 (8)
Cardiometabolic markers									
Total cholesterol mmol/L	5.3 (1.0)	5.4 (1.1)	0.0 (0.5)	0.01	5.5 (1.0)	5.5 (1.1)	0.0 (0.5)	0.1	−0.3 (0.5)
HDL-cholesterol, mmol/L	1.4 (0.3)	1.5 (0.4)	0.1 (0.2)	0.005	1.5 (0.3)	1.5 (0.4)	0.0 (0.1)	0.7	0.0 (0.2)
LDL-cholesterol, mmol/L	3.5 (0.8)	3.4 (0.9)	−0.1 (0.5)	0.3	3.7 (0.9)	3.7 (0.9)	0.0 (0.5)	0.06	−0.2 (0.4)
Triglycerides, mmol/L	1.6 (1.1)	1.5 (1.5)	−0.1 (0.7)	0.7	1.5 (0.6)	1.6 (0.8)	0.1 (0.5)	0.2	−0.1 (0.6)
Apolipoprotein B, g/L	1.1 (0.2)	1.1 (0.2)	0.0 (0.1)	0.1	1.1 (0.2)	1.1 (0.3)	0.0 (0.2)	0.2	0.0 (0.2)
Glucose mmol/L	5.6 (0.5)	5.6 (0.4)	0.0 (0.4)	0.3	5.7 (0.4)	5.8 (0.5)	0.1 (0.4)	0.8	0.1 (0.4)
HbA1c, %	5.3 (0.3)	5.6 (0.4)	0.0 (0.1)	0.1	5.3 (0.3)	5.3 (0.4)	0.0 (0.2)	0.6	0.0 (0.2)
C-reactive protein, mg/L	1.7 (0.8–3.8)	1.6 (0.7–3.8)	0.0 (−0.5–0.4)	0.3	1.6 (1.0–3.5)	2.4 (0.9–4.9)	0.0 (−0.4–0.9)	0.3	0.4 (−0.1–0.9)
Insulin, pmol/L[c]	68 (51, 114)	85 (58, 127)	8 (−15, 25)	0.7	75 (55, 95)	83 (62, 116)	4 (−22, 21)	0.9	3 (−13, 21)
C-peptide, pmol/L	887 (665, 1173)	990 (847, 1243)	91 (−50, 222)	0.08	893 (807, 1052)	959 (802, 1209)	25 (−133, 188)	0.6	4 (−88, 93)
HOMA-IR index	1.8 (0.9)	1.8 (1.4)	0.0 (1.1)	0.7	1.9 (0.8)	1.8 (1.0)	−0.1 (0.7)	0.8	0.0 (0.8)

[a] Values for 6 and 5 participants, respectively, were carried forward from baseline in the fish and nut groups.
[b] T-test compared each of fish and nut groups to controls.
[c] Insulin and c-peptide values (5 missing in fatty fish group, 8 missing in nuts group, 2 missing in controls) Non-parametric Mann–Whitney test compared each of fish and nut groups with controls.
[d] WC waist circumference.

Table 3 Changes in dietary intake between baseline and six months. Mean (SD) values are shown.

	Fatty fish diet (n = 36)				Nuts diet (n = 30)				Controls (n = 37)
	Baseline	6 months	Change	P[a]	Baseline	6 months[a]	Change	P[a]	Change
Study foods									
Fatty fish, g/day	31.6 (20.2)	90.4 (4.3)	58.7 (19.5)	<0.001	33.4 (31.7)	1.0 (2.7)	−32.4 (32.1)	0.8	−30.4 (20.7)
Nuts, g/day	9.6 (14.1)	0.0 (0.2)	−9.6 (14.1)	0.1	6.9 (7.6)	30.0 (−)	23.1 (7.6)	<0.001	−5.2 (7.5)
Macronutrients									
Energy, kJ/day	8866 (2830)	8040 (2334)	−625 (2108)	0.5	8872 (2477)	8598 (1998)	−275 (2149)	0.9	−331 (1745)
Energy, kcal/day	2057 (666)	1914 (556)	−143 (492)	0.5	2110 (588)	2047 (474)	−63 (511)	0.9	−76 (415)
Protein, E%	16.3 (1.9)	18.3 (1.9)	2.0 (2.0)	<0.001	17.0 (2.3)	16.9 (2.2)	−0.1 (2.2)	0.6	0.2 (2.1)
Carbohydrates, E%	44.3 (7.4)	42.5 (6.9)	−1.7 (4.6)	<0.001	46.0 (5.3)	42.5 (7.2)	−3.5 (5.3)	<0.0001	2.6 (4.7)
Fat, E%	36.6 (6.6)	36.2 (5.1)	−0.4 (4.7)	0.008	33.4 (4.1)	37.0 (5.5)	3.6 (5.1)	<0.0001	−3.3 (4.6)
Saturated fat, E%	13.1 (2.0)	12.7 (2.2)	−0.6 (1.8)	0.4	12.1 (2.2)	11.7 (2.2)	−0.3 (2.3)	0.5	0.0 (1.8)
Monounsaturated fat, E%	13.1 (3.4)	12.1 (2.1)	−1.0 (2.4)	0.1	11.7 (1.7)	13.9 (2.4)	2.2 (2.3)	<0.0001	−1.9 (2.7)
Polyunsaturated fat, E%	6.7 (1.9)	6.4 (1.6)	−0.3 (1.9)	0.054	6.1 (1.1)	7.9 (1.4)	1.9 (1.1)	<0.0001	−1.0 (1.3)
Alcohol, E%	2.8 (3.3)	2.7 (3.8)	−0.1 (2.2)	0.3	3.5 (3.0)	3.3 (2.9)	−0.2 (1.8)	0.2	0.4 (2.1)
Fiber, E%	2.8 (0.6)	2.7 (0.7)	−0.1 (0.5)	0.4	2.8 (0.6)	2.9 (0.6)	0.1 (0.5)	0.6	0.0 (0.8)

[a] T-test used to compare each of fatty fish or nut diets versus controls.

Table 4 Changes in POPs between baseline and six months. Mean (SD) values are shown, for change mean (CI) values are shown.

	Fatty fish diet (n = 45)			Nuts diet (n = 42)			Controls (n = 44)
	Baseline	6 months[a]	Change	Baseline	6 months[a]	Change	Change
Organochlorinated compounds (pg/ml)							
HCB	88.4 (33.9)	87.2 (32.8)	−1.2 (−5.4−3.1)	90.6 (32.3)	89.5 (32.5)	−1.0 (−4.3−2.2)	−3.5 (−7.4−0.4)
β-HCH	16.6 (12.6)	16.4 (11.0)	−0.2 (−1.5−1.0)	27.4 (46.1)	26.7 (43.1)	−0.7 (−2.5−1.1)	−1.3 (−2.4−0.1)
trans-Nonachlor	42.5 (26.5)	40.6 (26.1)	−1.9 (−4.8−1,0)	48.9 (38.6)	49.4 (38.3)	0.5 (−3.6−4.7)	−3.2 (−6.2−0.2)
p,p'DDT	8.2 (1.3)	8.2 (1.0)	0.0 (−0.6−0.5)	10.2 (7.8)	9.9 (7.0)	−0.3 (−0.8−0.2)	−0.5 (−1.1−0.1)
p,p'DDE	443.9 (394.1)	414.2 (372.0)	−29.8 (−73.6−14.0)	463.8 (298.5)	476.8 (324.9)	13.0 (−15.7−41.6)	−16.9 (−43.9−10.1)
Dioxin-like polychlorinated biphenyls (pg/ml)							
PCB-118	50.1 (35.1)	46.6 (31.6)	−3.5 (−9.0−2.0)	53.1 (30.3)	52.8 (30.1)	−0.3 (−2.5−1.9)	−1.8 (−4.6−1.1)
PCB-156	36.1 (17.9)	34.7 (18.0)	−1.4 (−3.9−1.2)	37.1 (17.5)	38.2 (18.3)	1.1 (−1.9−4.1)	−1.2 (−3.4−1.0)
Nondioxin-like polychlorinated biphenyls (pg/ml)							
PCB-74	19.4 (13.1)	19.0 (13.4)	−0.4 (−2.1−1.3)	19.9 (14.9)	19.6 (14.7)	−0.3 (−1.3−0.7)	−0.8 (−1.8−0.1)
PCB-99	23.8 (16.8)	22.7 (15.6)	−1.1 (−2.7−0.5)	28.2 (20.9)	28.1 (21.0)	−0.1 (−1.7−1.4)	−1.1 (−2.3−0.1)
PCB-138	167.3 (90.3)	159.4 (84.9)	−8.0 (−20.3−4.4)	177.8 (89.7)	179.9 (91.8)	2.1 (−10.7−15.0)	−6.0 (−15.1−3.0)
PCB-153	294.7 (148.0)	280.7 (142.1)	−14.0 (34.5−6.4)	305.6 (139.1)	313.1 (145.7)	7.5 (−15.2−30.2)	−8.9 (−25.7−7.9)
PCB-170	103.4 (51.0)	98.9 (50.7)	−4.5 (−11.6−2.6)	102.1 (45.9)	105.2 (49.1)	3.1 (5.2−11.4)	−3.2 (−8.9−2.5)
PCB-180	234.6 (113.0)	225.0 (113.2)	−9.7 (−24.6−5.2)	236.0 (106.0)	242.8 (114.4)	6.8 (−11.8−25.3)	−6.1 (−19.0−6.7)
PCB-183	17.4 (10.3)	16.4 (9.4)	−1.0 (−2.4−0.5)	18.5 (10.1)	18.9 (10.7)	0.4 (−1.0−1.8)	−0.8 (−1.8−0.3)
PCB-187	60.2 (32.0)	57.9 (31.7)	−2.3 (−7.0−2.3)	62.1 (28.2)	62.7 (29.2)	0.6 (−3.8−5.0)	−2.0 (−5.5−1.6)

HCB =Hexachlorobenzene, HCH-beta = β-hexachlorocyclohexane, p,p'DDT = dichlorodiphenyltrichloroethane, p,p'DDE = Dichlorodiphenyldichloroethylene, PCB = polychlorinated biphenyl.

[a] Values for 4 and 6 participants in the fish and nut groups, respectively, were carried forward from baseline.

double the odds ratio for metabolic syndrome in obese people [31].

POPs have been associated with a range of cardiometabolic risk factors in particular insulin-resistance and related disorders [32]. We previously showed associations between POPs and cardiometabolic risk factors [31], giving the background for conducting the present study. However, a cross-sectional study conducted among Inuits living in Greenland found that POPs may adversely affect insulin secretion [33], giving support to the notion that POPs may be more prominent in the development of beta-cell dysfunction-type diabetes rather than the insulin-resistance type [34]. Insulin secretion was not studied in the current trial. While most studies did not identify a specific compound, in one study p,p'DDE concentrations were elevated in patients with diabetes [35]. In the current study we did not find changes in HOMA-IR index or in the glucose infusion rate between participants in the fatty fish group versus controls, however, only 10 individuals from each group underwent the clamp procedure.

Table 5 POPs concentrations in fatty fish samples (n = 4). Mean (range) values are shown.

Organochlorinated pesticides (pg/g)	
HCB	781 (386−1034)
β-HCH	65 (25−87)
trans-Nonachlor	485 (220−656)
p,p'DDT	390 (240−543)
p,p'DDE	1926 (855−2632)
Dioxin-like polychlorinated biphenyls (pg/g)	
PCB-118	354 (155−474)
PCB-156	38 (14−52)
Nondioxin-like polychlorinated biphenyls (pg/g)	
PCB-74	63 (27−84)
PCB-99	263 (108−362)
PCB-138	736 (313−981)
PCB-153	917 (387−1273)
PCB-170	108 (40−178)
PCB-180	237 (92−377)
PCB-183	67 (25−113)
PCB-187	284 (117−445)

In contrast to the putative detrimental effects of POPs on cardiometabolic risks, fish oils (or fatty fish) may improve cardiometabolic risk factors. In line with our observation of an increase in HDL-cholesterol in the fatty fish group, systematic review and meta-analysis concluded that consuming fish improved HDL-cholesterol and triglyceride levels [36]. A study conducted in volunteers with impaired glucose metabolism found that fatty fish increased HDL particle diameter and concentrations of lipid components in HDL, possibly boosting the anti-atherogenic properties of HDL [37]. Also, another study found that fresh fish was superior to omega-3 supplements in increasing HDL-cholesterol and positively modifying lipid profiles [38]. It is possible that increase in HDL-cholesterol in the fatty fish compared to the group consuming nuts reflects the lack of fatty fish in the diet of the group consuming nuts. We did not find a reduction in triglyceride concentrations in the fatty fish group, perhaps because participants' baseline levels were only mildly elevated.

Uncertainty remains regarding the associations between fish, n-3 fatty acids and their effects on CVD. If POPs and other pollutants may impair the nutritional benefits of fish [39] using fish oil supplements may be an alternative. A recent meta-analysis attributed the benefits of eating fatty fish as being due to their n-3 fatty acid content [40]. However, it appears that evidence of benefits from n-3 fatty acids supplementation has diminished over time, possibly due to better pharmacological prevention and

treatment of patients with CVD [41]. The type of n-3 fatty acid may play a role. A recent study showed that ingestion of a total of 4 g eicosapentaenoic acid daily in individuals with elevated triglyceride levels lowered cardiovascular death compared with placebo, despite use of statins by all participants [42]. Generally, the majority of dietary factors that may reduce CVD are food groups, rather than isolated nutrients [43], both because of the balance of micronutrients and other substances in foods and substitution of good foods for other, less healthy choices. There is evidence that lean fish could also play a protective role due to other useful nutrients [40].

We found no increase in POPs in the nuts group compared to controls in the current study. A previously cited meta-analysis confirms multiple observations that nut consumption may lower all-cause mortality and CVD [20]. A review from 2018 based on cohort and interventional trials indicates that some nutrients that are richly found in nuts such as L-arginine, some minerals, phytosterols and unsaturated fatty acids could be linked to beneficial health effects of nuts [44]. For example, a review of meta-analyses found that nuts lowered LDL cholesterol [20], a finding that did not attain statistical significance in the current study. A randomized crossover trial found that both fatty fish and walnuts lowered cholesterol and triglyceride concentrations in normal to mildly hyperlipidemic individuals [45]. However, one study showed that nuts and seeds could be rich in PCBs, while other contaminants typical for animal foods are not present in nuts in significant concentrations [46]. An interventional study that compared a hypocaloric vegetarian and conventional diet did not find any reduction in concentrations of POPs in the vegetarian group, compared to conventional diet, possibly due to mobilization of fat stores in response to a decreased calorie intake [47].

Limitations

While the short study period may not be optimal to show changes in serum POPs concentrations, some smaller studies have found changes in serum POPs after short times of follow-up. For example one study reported reduction of POPs after 2 months of vitamin C supplementation [48] and likewise reductions were reported after one year of supplementation with olestra [49]. The validity of such studies is not clear.

A further limitation relates to possible recall bias while filling out the FFQ, as the FFQ covered a year at inclusion and the past 6 months at the end of the study. Notably, the food composition of salmon has been changed from marine-based diet in the early 1990s to a 70% plant based diet at present and that resulted in n-3 fatty acid content of the fish [50]. Contaminants like polycyclic aromatic hydrocarbons (PAHs), which are omnipresent in vegetable oils, are used in aquaculture today [51]. Some findings indicate that development in feed formulations may reduce traditional POPs in salmon, but may increase others, such as PAHs, normally not connected to salmon [52], however, PAH concentrations were not analyzed in the current trial. We did not analyze the content of POPs in the nuts used.

We did not analyze body adipose tissue samples for changes in contamination by POPs. In a previous small and nonrandomized study consumption of 380 g of farmed salmon weekly did not increase concentrations of HCB, p,p'DDE, sum of PCBs in plasma or adipose tissue samples indicating that steady-state levels of POPs were not affected by fatty fish consumption [53].

In the current study protein intakes unavoidably increased modestly in the fatty fish group. A recent study conducted in mice found that the accumulation of POPs in adipose tissue and liver was affected by macronutrient intake, and not the total intake of POPs [54]. A high-fat and high-protein diet resulted in lower deposition of POPs in the adipose tissue and liver than did a low-fat and high-carbohydrate diet. These findings may suggest the importance of controlling macronutrients in dietary studies of POPs. Absolute contents of POPs in diet are not critical in determining the body burden.

Finally, as discussed recently [34] there are disadvantages to RCTs in the study of effects of diet on serum POP concentrations due to fluctuations and non-linear responses. However, an observational study that aimed to study whether fatty fish increased serum POP concentrations would need to elicit dietary data from food frequency questionnaires and more or less reliable data on the content of POPs in the diet as measured many years earlier could be fraught with limitations [34].

Comparisons of POPs content of salmon

We compared results of our analyses of POPs in fish samples from manufacturers who provided fish in the present study (Table 5) to values published by the Norwegian Institute of Marine Research [55]. We found similar values for HCB (mean, 0.78 μg/kg compared to 0.95 μ/kg, respectively), while β-HCH (mean 0.06 μg/kg compared to 0.16 μg/kg) and p,p'DDT (mean 0.4 μg/kg compared to 5.70 μg/kg) values were lower in the fish samples. Analyses for other POPs were not available [55].

Conclusion

Our main findings did not show changes in circulating POP concentrations in persons with overweight or obesity and at least 1 metabolic risk factor after 6 months of eating high amounts of fatty fish (>600 g/week) or nuts compared to avoiding fatty fish and nuts. In addition, intake of fatty fish or nuts was not associated with improved cardiometabolic risks.

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Declaration of Competing Interest

The authors have no conflict of interest to declare. All authors had access to the data and have seen and approved the final submitted manuscript.

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