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Biomarkers and Fatty Fish Intake: A Randomized Controlled Trial in Norwegian Preschool Children

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ABSTRACT

Background: Biomarkers such as omega-3 (n–3) PUFAs, urinary iodine concentration (UIC), 1-methylhistidine (1-MH), and trimethylamine *N*-oxide (TMAO) have been associated with fish intake in observational studies, but data from children in randomized controlled trials are limited.

Objectives: The objective of this exploratory analysis was to investigate the effects of fatty fish intake compared with meat intake on various biomarkers in preschool children.

Methods: We randomly allocated (1:1) 232 children, aged 4 to 6 y, from 13 kindergartens. The children received lunch meals of either fatty fish (herring/mackerel) or meat (chicken/lamb/beef) 3 times a week for 16 wk. We analyzed 86 biomarkers in plasma ($n = 207$), serum ($n = 195$), RBCs ($n = 211$), urine ($n = 200$), and hair samples ($n = 210$). We measured the effects of the intervention on the normalized biomarker concentrations in linear mixed-effect regression models taking the clustering within the kindergartens into account. The results are presented as standardized effect sizes.

Results: We found significant effects of the intervention on the following biomarkers: RBC EPA (20:5n–3), 0.61 (95% CI: 0.36, 0.86); DHA (22:6n–3), 0.43 (95% CI: 0.21, 0.66); total n–3 PUFAs, 0.41 (95% CI: 0.20, 0.64); n–3/n–6 ratio, 0.48 (95% CI: 0.24, 0.71); adrenic acid (22:4n–6, −0.65 (95% CI: −0.91, −0.40), arachidonic acid (20:4n–6), −0.54 (95% CI: −0.79, −0.28); total n–6 PUFAs, −0.31 (95% CI: −0.56, −0.06); UIC, 0.32 (95% CI: 0.052, 0.59); hair mercury, 0.83 (95% CI: 0.05, 1.05); and plasma 1-MH, −0.35 (95% CI: −0.61, −0.094).

Conclusions: Of the 86 biomarkers, the strongest effect of fatty fish intake was on n–3 PUFAs, UIC, hair mercury, and plasma 1-MH. We observed no or limited effects on biomarkers related to micronutrient status, inflammation, or essential amino acid, choline oxidation, and tryptophan pathways. The trial was registered at clinicaltrials.gov (NCT02331667). *J Nutr* 2021;151:2134–2141.

Keywords: fatty fish, biomarkers, targeted metabolomics, 1-methylhistidine, mercury, polyunsaturated fatty acids, omega-3, preschool children

Introduction

Although fish is an integral component of many dietary recommendations (1–4), the mechanisms underlying the associations between fish intake and disease are not fully understood (5, 6). The benefits linked to fish intake are often attributed to the omega-3 (n–3) PUFAs, EPA (20:5n–3) and DHA (22:6n–3). Fish is, however, a good source of other nutrients, such as essential amino acids, choline, vitamins, and minerals (7, 8). These nutrients can, individually or in combinations, contribute to some of the positive effects associated with fish intake. Conversely, fish is also a major dietary source of contaminants such as dioxins, mercury, and arsenic (9). These contaminants can also play a role in the diet-disease relation and can counteract the potential benefits of the dietary nutrients contained in fish.

A challenge in nutritional epidemiology is the reliability and validity of dietary assessments. Typically, dietary assessments are reliant on memory-based and self-reported intake, such as FFQs. These methods have limitations such as under- and overreporting and insufficiencies in food composition databases (10). Biomarkers are more objective and independent of recall and self-report constraints. Therefore, the use of biomarkers could improve dietary assessments, and in turn contribute to

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a better understanding of the health effects associated with dietary intake of various foods (11). It should, however, be noted that nutritional biomarkers can reflect both metabolism and dietary intake.

Both EPA and DHA are suitable and well recognized as biomarkers of fatty fish intake and n–3 PUFA supplementation (12). It is of interest to study other potential biomarkers of fish intake. Several studies have explored biomarkers linked to the intake of various food items (13–15). For instance, the amino acid, 1-methylhistidine (1-MH) is derived from the dipeptide anserine, which is found to varying extents in meat, poultry, and fish (16). After digestion, anserine is typically catabolized into β-alanine and 1-MH (17). The human body does not synthesize or reuse 1-MH for protein synthesis (17), underscoring 1-MH's potential as a biomarker of dietary intake. Fish consumption has also been associated with other biomarkers including urinary iodine concentration (UIC), trimethylamine *N*-oxide (TMAO), creatinine, acetylcarnitine, furan fatty acids, and persistent organic and inorganic pollutants (18).

Furthermore, studies investigating biomarkers of dietary intake have tended to be limited by their observational design, and relatively few studies have used a randomized control trial (RCT) approach. In the present study, using targeted metabolomics, we measured the effect of fatty fish intake compared with meat intake on the concentration of several biomarkers using samples from a 16-wk RCT in Norwegian preschool children. The concentrations of these biomarkers were normalized and standardized to compare the intervention effects.

Methods

Study design

Data from a 2-armed RCT, the Fish Intervention Studies-KIDS (FINS-KIDS), were examined. A detailed description of the study design and method has been published elsewhere (19). The primary objective of the original study was to examine the effect of fatty fish intake compared with meat intake on cognitive function measured by the general intellectual ability test, the Weschler Preschool and Primary Scale of Intelligence, third edition (WPPSI-III). In this exploratory substudy, we examined the effect of fatty fish intake on biomarkers in plasma, serum, RBCs, hair, and urine. The procedures were in accordance with the Declaration of Helsinki. Before study start, the participants' caregivers provided signed, informed consent. The trial was approved by the Regional Committees for Medical and Health Research Ethics North (2014/1396) and registered at clinicaltrials.gov (NCT02331667).

The study is based on data from a randomized controlled trial funded by the Norwegian Seafood Research Fund (FHF) (grant number 900842) after vetting by a grant review committee appointed by the Research Council of Norway (project number 222648). BSS was funded through grants from Innlandet Hospital Trust (IHT). FHF and IHT were not involved in the design of the study, collection, analyses, or interpretation of data or in the manuscript writing.
Data described in the manuscript, code book, and analytic code will be made available upon request pending application to the corresponding author.
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Supplemental Tables 1–9 are available from the "Supplementary Data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
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Abbreviations used: AA, arachidonic acid; AdA, adrenic acid; FAME, fatty acid methyl ester; FDR, false discovery rate; HDH, Haraldsplass Diakonale Hospital; IMR, Institute of Marine Research; RCT, randomized controlled trial; TMAO, trimethylamine *N*-oxide; UIC, urinary iodine concentration; WPPSI-III, Weschler Preschool and Primary Scale of Intelligence, third edition; 1-MH, 1-methylhistidine; 25(OH)D_3, 25-hydroxycholecalciferol.

Enrollment and randomization

The study was performed from January to June 2015 in Bergen, Norway. Seventeen kindergartens were invited to participate in the trial, and children were recruited from the 13 kindergartens that agreed to participate. The inclusion criteria were as follows: children aged 4–6 y with sufficient Norwegian language to perform the cognitive tests, and sufficient Norwegian language in the caregivers to answer the online questionnaires. Exclusion criteria were any known food allergies. In total, 314 children were assessed for eligibility, and 232 children were individually randomly assigned in a 1:1 ratio to either the intervention (fish group) or control (meat group), stratified by sex (**Figure 1**). A researcher produced a computer-generated independent allocation sequence and randomization lists for each kindergarten using Microsoft Excel. The treatment allocation was concealed so that the study staff could not know the group identity before a child had been enrolled into the study. The process of randomization was controlled by another researcher. Research assistants from the Institute of Marine Research (IMR) enrolled the participants. These individuals were not informed about the group identity upon enrollment.

Intervention

The children in the fish group received 50–80 g fatty fish (herring or mackerel), whereas the meat group received the same amount of chicken, lamb, or beef 3 times a week for 16 wk. Both groups received identical side dishes. The food was prepared and delivered to the kindergartens by a catering company (Sætt + Salt A/S). Research assistants, not otherwise involved in the study, were present during the lunch meals to reduce risk of children sharing food across the groups and estimate the compliance. The compliance was evaluated by weighting the food using identical digital scales (Digital Glass Kitchen Scale; Soehnle) before and after the meals. Analyses detailing the composition of the lunch meals and the nutrient contents of the provided meals have previously been published (19).

Background diet

The participants' caregivers responded to a validated and modified version of an online FFQ (20). The FFQ evaluated the dietary intake of meat, fish, dairy products, eggs, fruits, and vegetables during the preceding 3 mo. The frequency response options were: never, less than once per month, 1 to 3 times per month, once or twice per week, or 3 or more times per week. The FFQ also obtained information regarding consumption of dietary supplements, with response options as follows: never, 1 to 3 times per month, 1 to 3 times per week, 4 to 6 times per week, or daily. For estimation of weekly dietary intake (**Table 1**), we converted the frequency response data to numeric data by using methods based on a previously validated seafood index (21). The online questionnaire also captured data on demographics, such as children's weight, height, parental education, and family income.

Sample size calculations

The sample size calculation was based on the expected effect of the WPPSI-III scores. For this study, assuming adequate blood samples from 200 participants (100 per group), we had 80% power to detect a standardized effect size of 0.4 at a significance level of 0.05.

Blood sampling and analytical methods

Blood sampling at baseline and end of the study was performed in the kindergartens by 2 biomedical scientists who were blinded to the group identities. Venous blood was collected in BD Vacutainer K2E 7.2-mg vials (Becton, Dickinson and Company) for plasma and RBC preparation, and in BD Vacutainer II Advance for serum preparation. In cases where venipuncture was problematic, capillary blood was collected from the fingertip with an ACCU-CHEK Safe-T-Pro Plus lancet (Roche Diagnostics) into BD Microtainer Blood Collection Tubes (Becton, Dickinson and Company). The blood samples were centrifuged (1000 × *g* at 20°C for 10 min) within 30 min of sampling, transferred to Cryotubes (Nunc), and transported on dry ice to storage at −80°C until analyses. The analyses of RBC fatty acid composition were carried out at IMR. The preparation of the fatty acid methyl ester (FAME)

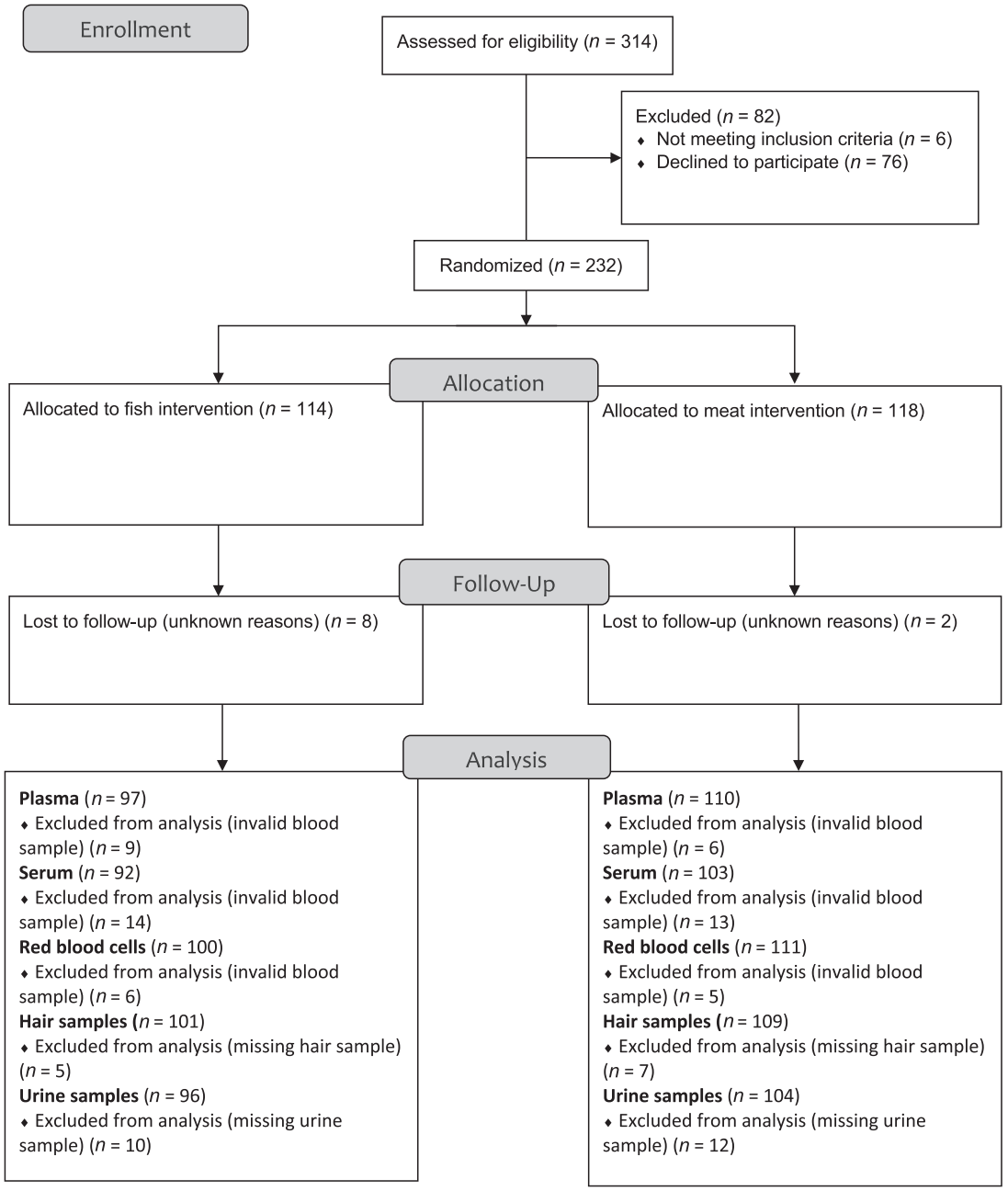

FIGURE 1 Flow chart of the study participants in a randomized controlled trial of fatty fish intake.

is an validated method published elsewhere (22). Briefly, 50 μL of the RBC sample was mixed with 1 mL 0.5M NaOH in methanol, then 2 mL BF3 in methanol, and 2.4 μg of 19:0 internal standard. The mixture was heated at 100°C for 1 h and cooled until it reached room temperature. Aliquots of 1 mL of hexane and 2 mL of H_2O were added, vortex-mixed for 15 s, placed in a centrifuge at 1620 × *g* for 2 min at 20°C, and the hexane phase (containing the FAME) was collected, evaporated under nitrogen, dissolved in hexane, and submitted to gas chromatography analysis at IMR on a Trace GC Ultra gas chromatograph (Thermo Corporation, Waltham, Massachusetts, USA) equipped with a liquid autosampler and a flame ionization detector. Data collection was performed by the Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System. The fatty acid results were expressed as absolute units (milligrams per gram RBC wet weight) (23). The analyses of serum 25-hydroxycholecalciferol [25(OH)D_3], total hair mercury, and UIC were carried out at the IMR, and serum ferritin at Haraldsplass Diakonale Hospital (HDH). Details of these analyses have been described previously (19, 24–26). The effects of the fish intervention on the above-mentioned biomarkers have been presented before, but using a different analytical approach (19, 24). Plasma folate and cobalamin concentrations were determined by microbiological assays based on a chloramphenicol-resistant strain of *Lactobacillus casei* (27) and colistin sulfate–resistant strain of *L. leichmannii* (28), respectively. Kynurenines, trigonelline, B-6, B-1, and B-3 vitamers, choline and its metabolites, creatinine, methylhistidines, arginine, and methylated arginines were analyzed by LC-tandem MS (29, 30), whereas other amino acids, in addition to total homocysteine, total cysteine, and methylmalonic acid were analyzed by GC-tandem MS (31). All plasma samples were analyzed at Bevital Laboratory, Bergen, Norway (www.bevital.no).

Statistical analysis

Baseline information is reported as median (IQR), mean ± SD, or frequency. To investigate biomarker concentrations following 16 wk of

TABLE 1 Baseline characteristics of children randomly assigned to intervention by group[1]

	Fish group ($n = 106$)[2]	Meat group ($n = 116$)
Demographics		
Age, y	5.2 ± 0.6	5.2 ± 0.6
Body weight, kg	20 ± 3.3	20.2 ± 3.0
Body height, cm	113.6 ± 5.9	113.6 ± 6.5
Boys, %	50.0	51.7
Girls, %	50.0	48.3
Parents' education, y	15.4 ± 1.7	15.4 ± 1.6
Family income,[3] NOK		
200,000–749,999, %	27	26
750,000–1,249,999, %	53	61
≥1,250,000, %	20	13
Background diet (FFQ)		
Seafood, dinners/wk	1.8 ± 0.9	1.6 ± 0.9
Egg, numbers/wk	2.6 ± 0.8	2.4 ± 0.9
Chicken, as dinners/wk	1.4 ± 0.9	1.2 ± 0.9
Dairy products, portions/d	1.7 ± 1	1.8 ± 1
Fruits and vegetables, portions/d	2.9 ± 1.3	2.9 ± 1.2
n–3 fatty acid supplements, %	39	38
Other supplements, %	39	39

[1] Values are mean ± SD or percentage. NOK, Norwegian krone.
[2] n = Children randomly assigned to intervention and not lost to follow-up prior to start of intervention (see Figure 1).
[3] Median gross household income in Norway, 2015 = 628 000 NOK (100 NOK = ~10€ or US$11). Source: Statistisk Sentralbyrå. 06,944: Inntekts- og formuesstatistikk for husholdninger 2005–2018 [Income and wealth statistics for households 2005–2018]. Oslo (Norway): Statistics Norway; 2018. Available from: https://www.ssb.no/en/statbank/table/06944/.

fish (intervention) compared with meat (control) intake, linear mixed-effect regression models with random intercepts for kindergartens were conducted for each of the 86 unique outcome variables (biomarkers and indices). The group affiliation (fish or meat) was the exposure. Skewed variables were transformed to meet the requirement of normal distribution of the residuals (e.g., log, square root, inverse) and standardized (mean = 0, SD = 1). The primary analysis followed an intention-to-treat approach. We also conducted a per protocol subgroup analysis, including only children compliant with the dietary intervention (defined as above the median intake for each group). **Figure 2** represents the biomarkers analyzed by IMR [25(OH)D_3, fatty acid composition, total hair mercury, and UIC] and HDH (serum ferritin); these are the biomarkers that have been presented before using different analytical approaches (19, 24–26). **Figure 3** shows the biomarkers analyzed at Bevital, categorized as B-vitamins, choline pathway, tryptophan pathway, amino acids, inflammation, and others. P values ≤0.05 were considered statistically significant. We also adjusted the P values by calculating the false discovery rate (FDR) using the Benjamini–Hochberg procedure to adjust for multiple comparisons (32). The FDR was set to 0.2 because of the experimental character of this study. Moreover, we repeated the regression models adjusting for baseline biomarker concentration, age, sex, and family income. Statistical analyses were performed using statistical software (Stata SE 16.1; StataCorp), and forest plots were produced using the ggplot2 package in R version 3.6.0 with R studio IDE (www.rstudio.com).

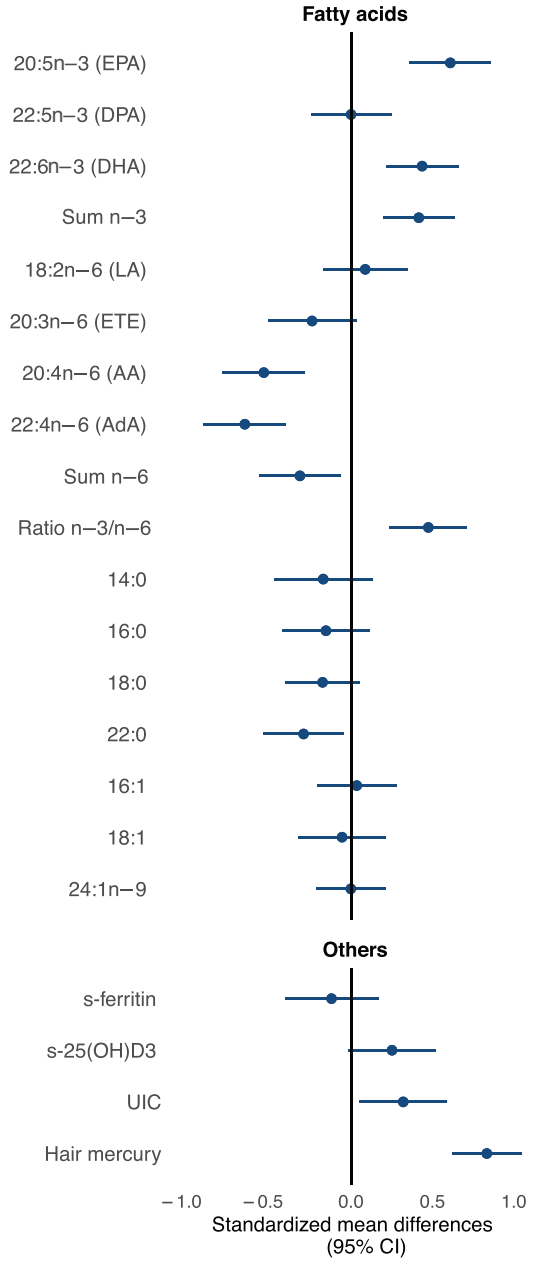

FIGURE 2 Standardized mean difference in RBC fatty acids, serum ferritin, serum vitamin D, urinary iodine concentration, and hair mercury concentration in the preschool children postintervention. The graph shows effect sizes and 95% CIs of the transformed concentrations of the different biomarkers calculated by mixed-effect linear models with random intercepts for kindergartens, without adjustments for covariates and multiple comparisons. Estimates on the right side of the vertical line indicate a higher postintervention concentration of the biomarkers in the fish group compared with the control. n = 139–210; see Supplemental Table 3 for exact n for each individual biomarker. AA, arachidonic acid; AdA, adrenic acid; DPA, docosapentaenoic acid; ETE, eicosatrienoic acid; LA, linoleic acid; s-25(OH)D3, serum 25-hydroxycholecalciferol; UIC, urinary iodine concentration.

Results

Of the 232 children enrolled at baseline, 114 were randomly allocated to the fish group and 118 to the meat group. The enrolment took place from December 19, 2014 to February 9, 2015. Ten children were lost to follow-up. The numbers of missing or unusable plasma, serum, RBC, hair, and urine samples are given in Figure 1. Baseline characteristics are shown in Table 1, and baseline and postintervention biomarker concentrations are shown in **Supplemental Tables 1–4**. We show

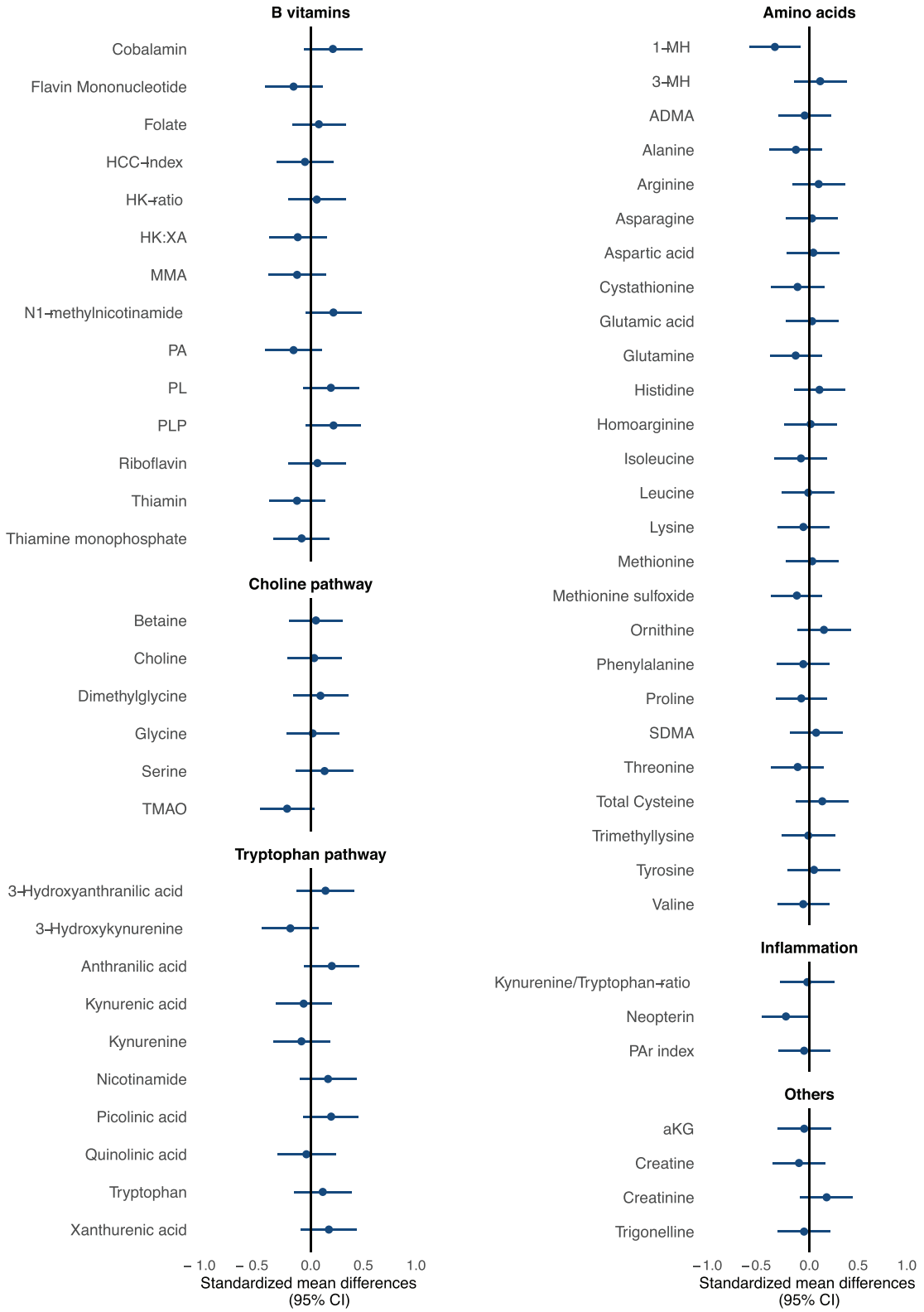

FIGURE 3 Standardized mean difference in plasma biomarker concentration in the preschool children postintervention. The graph shows effect sizes and 95% CIs of the transformed plasma concentrations of the different biomarkers, calculated by mixed-effect linear models with random intercepts for kindergartens, without adjustments for covariates and multiple comparisons. Estimates on the right side of the vertical line indicate a higher postintervention concentration of the biomarkers in the fish group compared with the control. $n = 205–207$; see Supplemental Table 4 for exact n for each individual biomarker. ADMA, asymmetric dimethylarginine; aKG, α-ketoglutarate; HCC index: 10,000 × [homocysteine (Hcy) divided by cysteine and (by) creatinine]; HK-ratio, 3-hydroxykynurenine divided by the sum of kynurenic acid, anthranilic acid, xanthurenic acid plus 3-hydroxyanthranilic acid; MMA, methylmalonic acid; PA, 4-pyridoxic acid; PAr index, 4-pyridoxic acid divided by the sum of pyridoxal 5´-phosphate plus pyridoxal; PL, pyridoxal; PLP, pyridoxal 5´-phosphate; SDMA, symmetric dimethylarginine; TMAO, trimethylamine *N*-oxide; XA, xanthurenic acid; 1-MH, 1-methylhistidine; 3-MH, 3-methylhistidine.

the adjusted end-study differences between these biomarkers in **Supplemental Tables 5** and **6**.

The forest plots (Figures 2 and 3) illustrate the standardized mean differences of the biomarker concentrations between the fish and meat group at end of study. Estimates on the right side of the vertical lines and with positive values indicate a higher postintervention concentration of the biomarkers in the fish group compared with the meat group. We observed significant differences in effect size for the following biomarkers and indices: RBC EPA (20:5n–3), 0.61 (95% CI: 0.36, 0.86); DHA (22:6n–3), 0.43 (95% CI: 0.21, 0.66); total n–3 PUFAs, 0.41 (95% CI: 0.20, 0.64); n–3/n–6 ratio, 0.48 (95% CI: 0.24, 0.71); adrenic acid (AdA; 22:4n–6), −0.65 (95% CI: −0.91, −0.40), arachidonic acid (AA; 20:4n–6), −0.54 (95% CI: −0.79, −0.28); total n–6 PUFAs, −0.31 (95% CI −0.56, −0.06); UIC, 0.32 (95% CI: 0.052, 0.59); hair mercury, 0.83 (95% CI: 0.05, 1.05); plasma 1-MH, −0.35 (95% CI: −0.61, −0.094) (Figures 2 and 3). We also observed a tendency of higher neopterin, 0.24 (95% CI: −0.0069, 0.48) and TMAO, 0.22 (95% CI: −0.0322, 0.403) in the fish group compared with the meat group (Figure 3). In the adjusted analyses, children in the fish group had a lower serum ferritin concentration than controls (Supplemental Table 5). Including only children compliant with the dietary intervention—defined as above total median (IQR) intake of fish [2120 g (1360–2700)] or meat [2750 g (2100–3290)]—did not considerably change the results (**Supplemental Tables 7** and **8**). FDR corrections for multiple comparisons did not notably change these results (**Supplemental Table 9**).

Discussion

In this RCT, we examined the effect of fatty fish intake compared with meat on various biomarkers in Norwegian preschool children. Fatty fish intake 3 times weekly for 16 wk significantly affected circulating fatty acid composition, UIC, hair mercury, and plasma 1-MH. When adjusting for baseline biomarker concentrations, age, sex, and family income, we observed higher plasma neopterin and a lower serum ferritin in children in the fish group compared with those in the meat group. Fatty fish intake had no effect on other biomarkers related to choline and tryptophan pathways, inflammation, or other micronutrients and amino acids.

The higher concentrations of EPA, DHA, and thus also total n–3 PUFAs in the fish group were as expected given the known concentrations of these essential fatty acids in herring and mackerel (33). Our results concur with other RCTs intervening with fatty fish in children and adolescents (34–36). In 2 of the latter studies, fatty fish intake also resulted in a decreased concentration of the n–6 PUFAs AA (34, 35) and AdA (35).

Fatty fish has a lower iodine concentration than lean fish but is still a good source of iodine (37). UIC mainly reflects recent dietary intake, and in iodine-sufficient individuals >90% of consumed iodine will be excreted via urine within 1–2 d (38). The day-to-day variation, in addition to intra- and interindividual variation in UIC, limits the use of UIC as a biomarker of dietary intake at an individual level (39). In this study, the postintervention data collection started within a week after the last study meal was consumed. Despite these limitations in UIC as a biomarker on an individual level, we found a significantly higher postintervention UIC in the fish group.

One of our most notable findings was the mercury concentration in the hair samples. As previously reported, hair mercury concentration was greater in the fish group compared with the control (24). However, the concentration did not lead to an increase in the number of subjects exceeding the US Environmental Protection Agency's reference dose, a dose level at which no adverse effects are expected to occur over a lifetime exposure (24). Nor did the fish mercury exposure from the intervention exceed the tolerable weekly intake set by the European Food Safety Authority. One of the major concerns about mercury intake is its effect on neurodevelopment. The increase in mercury in the present study, however, was not associated with a decline in cognitive function (24). Fish is a major source of mercury due to bioaccumulation and biological magnification of methylmercury (40). Our study confirms that increased intake of fish increases the accumulation of mercury in the body.

Moreover, we observed a lower postintervention plasma concentration of 1-MH in the fish group compared with the meat group, which is in contrast to 2 other fish intervention trials (41, 42). It should be noted, however, that another study found the plasma concentration of 1-MH to be more strongly associated with chicken intake than fish intake (42). In our study, we measured the effect of fatty fish intake (herring and mackerel), whereas participants in the aforementioned study consumed lean fish (haddock). Serum and urinary 1-MH have also been suggested to be a stronger biomarker of fatty fish (salmon) intake than lean fish (cod) in another fish intervention trial (41). Previously, and in part due to the challenges in distinguishing between poultry and fish intake, 1-MH has been suggested as an overall biomarker of animal protein intake rather than a unique biomarker for a specific animal-derived food item (18).

We observed lower serum ferritin concentrations in the fish group compared with the meat group in the adjusted analyses. The iron content in meat, especially lamb and beef, is somewhat higher than in herring and mackerel, and could explain the negative effect of fish intake on this marker of iron status (43). Moreover, there could be differences in the bioavailability of iron in meat and fish. Compared with fish, red meat has a higher percentage of heme iron, which is a more bioavailable source of iron than nonheme iron (44). This difference was previously demonstrated in a study evaluating the effect of animal proteins on iron bioavailability, where the bioavailability of iron was lower when ingested with fish compared with beef (45). It should be mentioned, however, that the content of iron in the study meals was not analyzed in the present study and the meat group consumed a larger amount of the study meals compared with those in the fish group.

The higher postintervention concentrations of neopterin, a marker of inflammation and immune cell activation, in the fish group compared with the meat group in the adjusted analyses was unexpected given the assumed anti-inflammatory effects of n–3 PUFAs. We also observed a tendency toward increased circulating TMAO in the fish intervention group ($P = 0.087$). TMAO has been suggested as a biomarker of fish intake (18). One explanation for the weak effect of fish intake in our study could relate to circulating compared with urinary concentrations of TMAO. The associations of TMAO and fish intake are mainly reported in studies assessing biomarkers in urine shortly after fish intake (42, 46, 47). The metabolism of TMAO in humans is poorly understood, but it has been suggested that TMAO is easily absorbed in the gastrointestinal tract and ∼95% is excreted via urine within

24 h after consumption (48). In line with this, changes in TMAO concentration were more evident in urine than in serum following increased cod consumption in a previous Norwegian RCT (41). In the aforementioned study, the changes in TMAO were also more notable in the cod group compared with the salmon group (41), suggesting that the type of fish (fatty compared with lean) could also be an important consideration when evaluating the effect of fish intake on TMAO concentrations.

No or only marginal differences were observed in other biomarkers such as amino acids and micronutrients. This could be due to the fact that both fish and meat are good sources of essential amino acids and other nutrients. A different control diet, for example, only chicken, only red meat, or a full vegetarian diet, could have led to a different result. Further, irrespective of intervention group, the children's background diet generally complied with the Norwegian dietary recommendations, with the exception of fruit, vegetable, and fish intake (19). In addition, as previously reported, the study population had adequate iodine status (25), and few children had dietary deficiencies of cobalamin (49) and vitamin D (26) at baseline. Results could therefore be different for populations in which micronutrient deficiencies are common.

Moreover, many biomarkers are influenced by nondietary factors such as endogenous synthesis. For instance, mackerel and herring are considered good sources of vitamin D. Even so, in the current study, we did not observe a difference in serum 25(OH)D_3 concentration postintervention. Explanation for the nonsignificant differences between the groups could be the relatively high consumption of cod liver oil in both groups (Table 1), and the fact that sun exposure is a major source of vitamin D (50). The timing of the intervention, starting in winter and ending in early summer, could contribute to increased vitamin D concentrations in both groups.

This study has some limitations. Nonfasting blood samples were collected at different times of the day and this could have implications for biomarkers, which are affected by not only acute dietary intake but also natural biological fluctuations. Moreover, the sample size was based on the main objective of this study, which was to evaluate the effect of fatty fish intake on cognitive function. The study could accordingly be underpowered for detection of changes in certain biomarkers. Furthermore, we could not measure some biomarkers' concentrations in many of the study children because of insufficient biological material. We believe that this loss to follow-up was random, that is, it affected both the intervention and the control group equally. This nondifferential misclassification affected our statistical power but probably did not bias our effect estimates. Finally, this is an exploratory study with several outcomes. Despite the RCT design and adjusting for several outcomes, the fact that our analyses are not hypothesis-driven implies a risk of identifying spurious effects.

Nonetheless, a key strength of our study is the randomized intervention design and relatively large sample size. In addition, we analyzed a wide range of potential biomarkers using targeted analytical methods.

In conclusion, consumption of fatty fish 3 times per week for 16 wk resulted in higher concentrations of hair mercury, RBC n–3 PUFAs, UIC, and lower plasma 1-MH concentrations. There were limited effects on other biomarkers related to micronutrient status, inflammation, amino acid, choline oxidation, and tryptophan pathways.

Acknowledgments

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The authors' responsibilities were as follows—JØ, MWM, and IK: designed and conducted research; PMU and AM: analyzed the blood samples; BSS and TAS: performed statistical analysis and had primary responsibility for the final content; and all authors: read and approved the final manuscript.

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