Journal of Trace Elements in Medicine and Biology 44 (2017) 1–7

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

EPIDEMIOLOGY

Arsenic in seafood is associated with increased thyroid-stimulating hormone (TSH) in healthy volunteers – A randomized controlled trial

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ARTICLE INFO

Keywords:
Arsenic
Arsenic in seafood
Thyroid-stimulating hormone
Iodine
Dietary intervention
Randomized controlled trial

ABSTRACT

Background: Exposure to exogenous elements like arsenic (As) may influence thyroid enzymes, thyroid-stimulating hormone (TSH), and the two principal thyroid hormones, free thyroxine (FT4) and free triiodothyronine (FT3), but little is known about how this is related to organic arsenicals, the main form in seafood.

Aim: To investigate whether a high intake of dietary arsenic from seafood can impact thyroid function and thyroid hormones by examining possible associations with changes in TSH, FT4, FT3 and the FT4:FT3-ratio in plasma.

Methods: Thirty-eight healthy subjects were randomized into four groups. During a 14-day semi-controlled dietary study, the subjects ingested daily portions of either 150 g cod, salmon, blue mussels or potato (control). Plasma concentrations of total As, FT3, FT4, TSH and selenium (Se), and urinary concentrations of iodine were monitored.

Results: Plasma concentrations of TSH increased significantly in all seafood groups. The change in plasma As, with different coefficients for each seafood group, was the dominant factor in the optimal multiple regression model for change in TSH (R^2 = 0.47). Plasma Se and iodine were negative and positive factors, respectively. There were also indications of changes in FT4, FT3 and the FT4:FT3 ratio consistent with a net inhibiting effect of As on FT4 to FT3 conversion.

Conclusion: Ingestion of seafood rich in various organic As species was strongly associated with an increase of the TSH concentrations in plasma. Change in TSH was positively associated with total plasma As, but varied with the type of seafood ingested. These findings indicate that organic dietary As, apparently depending on chemical form, may influence thyroid hormones and function.

1. Introduction

Arsenic is one of ten chemicals regarded as a major public health concern by the WHO [1]. Previous studies have reported associations between human arsenic (As) exposure from food, environment or urinary arsenic levels respectively, and thyroid hormones. This includes increased concentrations of thyroid-stimulating hormone (TSH) and changes in free thyroxine (FT4) and free triiodothyronine (FT3) concentrations in plasma/serum [2–5]. Hormone production in the thyroid gland is regulated by thyrotropin-releasing hormone (TRH), secreted from the hypothalamus and stimulating the pituitary gland to synthesize and secrete TSH. TSH stimulates the thyroid gland to capture iodine from the blood to synthesize, store and release T4 and minor amounts of T3, which is the biological active form. In peripheral tissues T4 is converted to T3 by selenium dependent iodothyronine deiodinase enzymes [6]. In plasma, more than 99% of T4 and T3 is bound to transport proteins. The protein bound hormones are in equilibrium with the free forms (i.e. FT4 and FT3) that are available for the tissues.

In experimental studies, inorganic arsenicals, such as arsenate (AsO_4^{3-}), sodium arsenite ($NaAsO_2$) and arsenic trioxide (As_2O_3), have

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http://dx.doi.org/10.1016/j.jtemb.2017.05.004
Received 29 March 2017; Received in revised form 8 May 2017; Accepted 10 May 2017
0946-672X/

been shown to disrupt thyroid function by interfering with normal thyroid hormone metabolism [7,8]. Arsenic trioxide may inhibit thyroid peroxidase activity *in vitro* [9], however, the relevance of this finding under normal dietary conditions is unknown. Thyroid peroxidase (TPO) in the thyroid gland is the major enzyme involved in thyroid hormone synthesis. Inhibition of TPO results in suppressed thyroid hormone levels, which through feedback mechanisms results in increased secretion of TRH and TSH. Human exposure to inorganic arsenic (iAs) through well water has been shown to be associated with increased TSH levels and decreased plasma/serum concentrations of FT3 and FT4 in adults living in the region of Abitibi-temiscamingue, in Quebec, Canada [10]. Additionally, blood concentrations of total As were positively associated with a dose-dependent increase in TSH levels in men living in Michigan [3]. In outdoor workers occupationally exposed to As through inhalation of urban air, As exposure was associated with increased TSH concentrations, and slightly decreased FT3 and FT4 [4]. Although little studied, exposure to As may also interact with the metabolism of selenium (Se) and compromise the supply of Se to Se-dependent iodothyronine deiodinases. In this way As may also indirectly affect thyroid metabolism via T4 conversion to T3. Arsenic has been shown to enhance the excretion of Se [11,12].

Fish and seafood naturally contain relatively high amounts of As, and together with rice and rice products, these foods represent the main contributors to total As intake for humans, particularly in those with relatively low exposure from drinking-water or occupation [13,14]. Arsenic exposure from fish intake may have an impact on thyroid hormones [5]. The dominating As species in seafood is the organoarsenical arsenobetaine, a major species in finfish. Other important organic forms are arsenosugars, which are major forms in shellfish and algae, and arsenolipids, mainly present in oily fish (and consequently fish oils) [14].

Possible effects of As on thyroid metabolism may contribute to, or modify, the beneficial health effects of fish and seafood consumption. Since various seafood species contain different spectrums of arsenicals, it is important to assess the possible impact of seafood As on thyroid hormone metabolism. The content of iAs in most seafood is low, in finfish usually < 0.2 mg/kg dry weight [13,15]. Exceptions are some shellfish and edible algae (e.g. hijiki), that may constitute up to 4.5 mg/kg dry weight and > 60 mg/kg dry weight, respectively [14]. Hence, previous observations on changes in thyroid hormones following exposure to relative high levels of iAs are not comparable with As exposure from fish and seafood, since the organic species dominate in seafood.

The purpose of the present study was to investigate whether three different controlled seafood diets (cod, salmon or blue mussels), naturally rich in various forms of As, were associated with changes in TSH, FT4 and FT3 in healthy volunteers.

2. Subjects and methods

2.1. Study subjects and design

The study design and subjects have been described in detail elsewhere [16,17]. Briefly, 38 healthy (C-reactive protein (CRP) < 10 mg/L) men and women (10 men and 28 women) aged 20–40 years were recruited among students at Akershus University College, Norway. Smokers, pregnant or lactating women, persons habitually consuming seafood more than three times a week, and persons using medical drugs other than contraceptives were excluded. All subjects fulfilled the inclusion criteria and were compliant with the protocol throughout the study. The study was approved by the National Committee for Research Ethics and was carried out in accordance with The Code of Ethics of the World Medical Association. Written informed consent was obtained from all participants.

A randomized controlled parallel-group study was conducted, and the subjects were allocated into four treatment groups by random selection. They were given a semi-controlled diet consisting of 150 g either farmed salmon (*Salmo salar*) (n = 11), cod (*Gadus morhua*) (n = 9), blue mussels (*Mytilus edulis*) (n = 8) or potato (control) (n = 10) served as a daily lunch for 14 days. Fourteen days prior to, and during the study period, the subjects were instructed to avoid seafood consumption (except for the seafood provided in the study) and mushrooms, rice or rice products or to take any dietary supplements (to rule out other possible dietary sources to As). The participants were instructed to consume the entire seafood (or control) meal and to maintain their normal physical activity routines.

2.2. Test diets

A homogenous mixture of filleted cod or salmon was prepared as puddings, cut into cubes and stored at −20 °C. The blue mussels were steamed for 10 min, cleansed from their shells within 10 min, and immediately frozen and stored at −20 °C until food preparation [17]. Potatoes were cooked and cut into cubes. The 14-day semi-controlled diet consisted of a 7-day menu with warm and cold dishes which were provided for two consecutive weeks. All of the ingredients in the dishes were identical for all intervention groups, varying only in the seafood species (or potatoes). The 150 g daily portion size is a realistic portion of seafood. The 14-day semi-controlled diet was served at the University College Monday-Friday, and lunch boxes were provided for the participants to bring home for the weekend [16].

2.3. Blood sampling, biochemical and clinical analysis

Blood samples were collected from fasting subjects (minimum12 h) at the same time (between 8 a.m and 10 a.m) at the start of the intervention period and at the end of the study. Serum was obtained from blood samples using gel tubes, kept at room temperature (RT) for at least 30 min until centrifugation at 1300 × g for 10 min at RT. Plasma was obtained from blood samples using EDTA tubes, kept at RT for at least 30 min until centrifugation at 1300 × g for 10 min at RT, and kept frozen (−70 °C) until analysis. Determination of FT4, FT3 and TSH was performed using routine analytical methods (Fürst Medical laboratory, Norway).

2.4. Analysis of arsenic, iodine and selenium

Total determination of As in food, blood plasma and urine, iodine in food and urine and selenium in blood plasma was carried out using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [18,19]. Determination of iAs in urine was measured with anion exchange-chromatography using ICP-MS coupled to HPLC [17]. Certified reference material was selected with regard to similarity in concentration and matrix to sample material analyzed.

For the determination of total As in food and total As and Se in plasma, subsamples of 0.2 g, 0.8 mL and 0.8 mL, respectively were submitted to microwave-assisted wet digestion using 2.0 mL concentrated nitric acid (Merck, Darmstadt, Germany) and 0.50 mL 30% w/w hydrogen peroxide (Merck) in an Ethos Pro microwave system (Milestone, Sorisole, Italy). Prior to analysis, food and blood samples digests were diluted to a final volume of 25 mL and 10 mL, respectively, with deionised water.

Prior to analysis of total As in urine, the samples were diluted 1 + 4 with 5% HNO_3 and filtered using a 0.45 µm single use syringe and disposal filter (Sartorius MiniSart RC25, Göttingen, Germany). For the determination of iAs in urine, the samples were filtrated and subsequently injected (10 µL) into the HPLC system.

For the determination of iodine in food, subsamples of 0.2 g was added 1 mL 1% tetrametyl ammonium hydroxide (TMAH) and 5 mL deionised water before extraction in a dry oven in 3 h at 90 °C. Prior to analysis, the samples were diluted to a final volume of 25 mL with deionised water and filtered through a 0.45 µm disposal filter. For the

determination of iodine in urine, 0.5 mL urine was diluted in 4.5 mL 1% TMAH and filtered using a 0.45 µm single use syringe and a disposal filter prior to analysis.

Statistical analysis. The SPSS software package (SPSS Inc., Chicago, IL, USA) and R (version 3.2.0, https://cran.r-project.org/) were used for the statistical analyses. For the results reported in Table 2, all individuals acted as their own controls if not otherwise stated, and Wilcoxon Signed Rank test were used to compare within group changes before and after the 14-day intervention period. Mann–Whitney *U* test was used to compare changes between control group and seafood group before and after the 14-day intervention period. No correction was applied for multiple testing, because of the pilot nature of this study. To assess multivariate relationships, linear-normal modelling with analysis of covariance (ANCOVA) was performed, and Akaike's information criterion (AIC) was used to choose the optimal models.

3. Results

3.1. Daily intake of arsenic and iodine from the seafood served during the intervention

The daily intake of iodine and As from the seafood served as lunch during the 14-day period is given in Table 1. In the same table, estimated daily intakes of selenium is given. The amount of iodine ingested was highest in the cod and lowest in the salmon group. Also the intake of tAs was highest among the cod consumers and lowest in the salmon group. The contents of inorganic arsenic (iAs), dimethylarsinate (DMA), methylarsonate (MA), arsenobetaine (AB) and unknown arsenicals (i.a. unidentified peaks in the chromatogram), in addition to the minor arsenicals trimethylarsine oxide (TMAO), dimethylarsenoethanol (DMAE), trimethylarsoniopropionate (TMAP), arsenocholine (AC) and tetramethyl-arsonium ion (TETRA) in the study meals are given in Table 1, and previously reported [16]. Briefly, the intake of iAs was low for the fish eaters, and higher for the blue mussel eaters (accounting for 13 µg daily) (Table 1). The main As compound in the cod and salmon was arsenobetaine, accounting for 73 and 78% of the total As respectively, while the corresponding the fraction of unidentified arsenicals accounted for 25 and 16% respectively. The blue mussel diet consisted of approximately 26% arsenobetaine and 59% unidentified arsenicals (Table 1), thus the relative fraction of unidentified

Table 1
The content of iodine, selenium and arsenicals[a] in the daily seafood[b] lunch (150 g) consumed by healthy volunteers in a randomized controlled trial.

	Salmon	Blue mussels	Cod	Control (potato)
Iodine (µg)	47	308	323	3.0[c]
Selenium (µg)[d]	18	83	21	0
Total As (µg)	180	620	670	3.7
Inorganic As (µg)	3.3	13.0	2.8	4.4
AB (µg)	140	160	490	0.8
DMA (µg)	3.0	15.0	1.3	0.1
MA (µg)	< 0.15	< 0.15	< 0.15	< 0.15
TMAO (µg)	6.0	4.4	3.0	< 0.5
DMAE (µg)	< 0.5	6.6	< 0.5	< 0.5
TMAP (µg)	< 0.5	42	2.9	< 0.5
TETRA (µg)	< 0.5	5.4	1.2	< 0.5
AC (µg)	< 0.5	5.4	3.8	< 0.5
Unknowns (µg)[f]	28	368	170	0

[a] Data on the arsenicals in the diet is previously given in Molin et al. (2012).
[b] A homogenous mixture of farmed salmon and cod fillets was prepared as puddings and stored at −20 degrees before analysis, whereas the blue mussels were steamed, cleansed for shells and single frozen immediately and stored at −20 degrees before analysis.
[c] Not analyzed, estimated using values from Dahl et al. (2004). The iodine content of Norwegian foods and diets. Public Health Nutrition. 7 (4); 569–76.
[d] Estimated using Seafood Data (https://sjomatdata.nifes.no) and Kostholdsplanleggeren (www.kostholdsplanleggeren.no).
[f] Unidentified peaks in the chromatogram and unextracted arsenicals.

Table 2
Plasma concentrations of TSH, FT4, FT3, FT4:FT3 ratio, selenium and total arsenic (tAs); and urinary concentrations of iodine, total arsenic and inorganic arsenic (iAs) in healthy volunteers consuming different seafoods in a randomized controlled trial.

	Cod	Salmon	Blue mussel	Potato (control)
N	9	11	8	10
Female gender	6	11	5	6
TSH[a] baseline (mU/L)	1.59 (0.92, 4.70)	1.75 (0.72, 4.40)	2.54 (1.40, 2.94)	1.51 (0.75, 4.10)
TSH end (mU/L)	2.6 (1.38, 7.80)*,#	2.14 (0.71, 7.60)*	3.16 (1.70, 3.83)*	1.65 (0.92, 4.10)
FT4[b] baseline (pmol/L)	13.2 (11.7, 16.3)	14.0 (11.0, 15.4)	15.1 (12.2, 18.0)	12.8 (10.8, 16.2)
FT4 end (pmol/L)	13.8 (12.2, 16.6)	14.2 (10.9, 17.2)	15.2 (13.3, 18.9)*	13.4 (9.6, 17.2)
FT3[c] baseline (pmol/L)	4.0 (3.6, 4.7)	3.9 (3.1, 5.1)	4.5 (3.9, 4.9)	4.3 (2.6, 5.5)
FT3 end (pmol/L)	3.8 (3.4, 4.8)	4.0 (2.9, 5.1)	3.7 (3.0, 4.7)*	4.2 (2.4, 5.4)
FT4:FT3 ratio baseline	3.2 (2.6, 4.1)	3.6 (2.7, 4.4)	3.6 (2.7, 3.8)	3.1 (2.3, 4.3)
FT4:FT3 ratio end	3.6 (3.0, 4.4)*	4.0 (2.7, 4.4)	4.2 (3.7, 4.7)*	3.2 (1.8, 5.0)
Selenium[d] baseline (µg/L)	64.0 (58.0, 96.0)	71.0 (58.0, 98.0)	69.5 (55.0, 80.0)	69.0 (51.0, 98.0)
Selenium end (µg/L)	69.0 (56.0, 91.0)	68.0 (45.0, 84.0)	71.5 (56.0, 89.0)##	66.0 (45.0, 91.0)**
tAs baseline (µg/L)	0.5 (0.5, 0.5)	0.5 (0.5, 2.1)	0.5 (0.5, 1.2)	0.5 (0.5, 1.4)
tAs end (µg/L)	10.6 (7.8, 30.5)**,###	2.95 (0.5, 4.8)**,###	4.85 (2.5, 7.9)*,###	0.5 (0.5, 2.1)
Iodine baseline (µg/L)	80 (20, 180)	100 (40, 190)	85 (20, 180)	95 (50, 240)
Iodine end (µg/L)	220 (130, 320)**,###	100 (40, 180)	155 (100, 240)#	95 (50, 170)
Iodine baseline (µg/L creatinine mmol/L)	5.9 (1.5, 15.6)	5.7 (1.6, 20.6)	8.5 (1.9, 14.7)	7.5 (4.7, 20.9)
Iodine end (µg/L creatinine mmol/L)	15.1 (12.0, 24.0)**,##	7.8 (2.8, 13.7)	10.8 (4.8, 19.6)#	6.3 (4.7, 15.0)
tAs baseline (µg/L)	14.0 (10.0, 24.0)	15.0 (5.0, 35.0)	15.5 (10.0, 24.0)	15.5 (4.0, 53.0)
tAs end (µg/L)	630.0 (223.0, 1331.0)**,###	72.0 (44.0, 205.0)**,###	255.0 (133.0, 452.0)*,###	9.0 (3.0, 46.0)
iAs baseline (µg/L)	0.23 (0.15, 0.75)	0.37 (0.15, 0.66)	0.22 (0.15, 0.29)	0.27 (0.15, 1.06)
iAs end (µg/L)	0.30 (0.15, 1.17)	0.24 (0.15, 0.59)	1.3 (0.71, 2.43)*,##	0.28 (0.15, 1.32)

[a] Reference: 0.2–4.0 mU/L(Fürst Medical laboratory, Norway).
[b] Reference: 11–23 pmol/L (Fürst Medical laboratory, Norway).
[c] Reference: 3.5–6.5 pmol/L (Fürst Medical laboratory, Norway).
[d] Reference: 0.6–1.8 µmol/L (Fürst Medical laboratory, Norway).
* Within group changes (Wilcoxon Signed Ranks test). *P* values (exact, 2-tailed) $P < 0.05$.
** $P < 0.01$.
# Between potato group (control) and seafood group (Mann–Whitney *U* test). *P* values (exact Sig [2 * (1-tailed sig)]) $P < 0.05$.
$P < 0.01$.
$P < 0.001$.

arsenicals was higher for the mussels diet. Moreover, the concentration of TMAP was almost 15 times higher in the blue mussels than in the fish diets. Details of the plasma and urine concentrations of arsenicals following seafood consumption have been published elsewhere [16,17,20].

3.2. Plasma concentration of TSH, T4, T3 and T4:T3 ratio

3.2.1. Within-group changes

Changes in thyroid hormones were assessed using each individual as its own control. There were significant increases in the plasma TSH concentrations in all seafood groups during the study (Table 2 and Fig. 2). Median plasma concentration of TSH increased 1.2-fold, 1.6-fold, and 1.2-fold in the salmon, cod and blue mussel groups, respectively ($p < 0.05$ for all groups). The plasma concentration of FT3 decreased significantly (1.2-fold) in the blue mussel group ($p = 0.018$). In this group, there was also a minor, but significant increase in FT4 ($p = 0.035$). The FT4:FT3 ratio increased significantly in the cod and the blue mussel group ($p < 0.05$ for both).

3.2.2. Seafood groups compared with control group

The only significant difference between the seafood groups and the control group following seafood intake was observed in an increased TSH concentration in the cod group ($p = 0.017$).

3.3. Plasma concentrations of selenium and total arsenic (tAs) and urinary iodine

3.3.1. Within-group changes

The plasma concentration of selenium decreased significantly in the control (potato) group (Table 2). No changes in plasma selenium were observed in any of the seafood groups. Plasma tAs increased significantly in all seafood groups, with the highest increase in the cod group (21-fold) (Table 2) [16]. The urinary concentration of iodine increased significantly only for the cod group.

3.3.2. Seafood groups compared with the control group

The median increase in plasma tAs was significant in all seafood groups compared with the control group (Table 2).

3.4. Urinary concentrations of tAs and iAs

3.4.1. Within-group changes

The concentration of tAs in spot urine samples increased significantly after two weeks of consumption of salmon, blue mussels and cod, with the highest increase found in samples from the cod consumers, thus reflecting the dietary As consumption (Tables 1 and 2). The concentration of iAs in urine increased close to six times for the blue mussel consumers, whereas no changes were seen in the other groups.

3.4.2. Seafood groups compared with control group

Urinary tAs increased significantly in all seafood groups, whereas urinary iAs was only significantly increased after intake of blue mussels.

3.5. AIC-optimal multivariate models for thyroid parameters

To assess possible complex associations, multivariate linear modelling was applied to the thyroid parameters TSH change (dTSH), FT3, FT4 and FT4:FT3, using a set of candidate explanatory variables including study group, final urinary iodine, final plasma Se, plasma As (absolute concentration and change), urinary and dietary As species, and other thyroid parameters (FT3, FT4 and TSH). Akaike's information criterion (AIC) was used to select optimal multivariate models, and the most significant features of the AIC-optimal multivariate models for thyroid parameters are qualitatively summarized in Table 3.

The most complex and, by far, best fitting model ($R^2 = 0.47$, $p < 0.0006$, R^2 adjusted for number of parameters) was obtained for the change in TSH (dTSH). It included final urinary iodine (positive, $p < 0.15$), FT4 (negative, $p < 0.15$), plasma Se (negative, $p < 0.02$), FT3 (positive, $p < 0.01$) and change in plasma As (dAs; positive, but slope and significance differed among groups). The association between dTSH, adjusted for the other model factors, and dAs is shown in Fig. 1, and the regression equation is found in the figure legend. The slope of the regression line for the cod group was significantly lower ($p < 0.005$) than for the two other seafood groups (Fig. 1). Intake or excretion of iAs, AB (arsenobetaine), DMA (dimethylarsinate) and MA (methylarsinate) was not significantly associated with change in TSH. Whereas the association between AB and DMA intake and dTSH was weakly positive, both the intake and excretion of iAs was negatively associated with dTSH. TSH data for individuals in the four groups are shown in Fig. 2. TSH was approximately constant in the control group, while most subjects experienced an increase in the other three groups.

The factors included in the AIC-optimal multivariate models for the other thyroid parameters were as follows: For final FT3 ($R^2 = 0.18$, $p < 0.015$) it included TSH (positive, $p < 0.01$) and urinary iodine (negative, $p = 0.1$). ANCOVA analysis showed no significant group differences. For final FT4 ($R^2 = 0.20$, $p < 0.015$), plasma Se (negative, $p < 0.03$) was the only common factor. Compared with the control group, the blue mussel group had about 30% higher FT4, ($p < 0.005$), whereas the cod and salmon groups, pooled, had about 15% higher final FT4 ($p = 0.08$) for a given level of Se.

For the FT4:FT3-ratio, the optimal model ($R^2 = 0.24$, $p < 0.015$) included TSH (negative, $p < 0.005$) and study group (different offsets for regression lines). Compared with the control group, the blue mussel group had about 25% higher FT4:FT3-ratio ($p < 0.005$) for a given level of TSH, again with intermediate values for the cod and salmon groups (NS).

The FT4:FT3 ratio increased significantly in the cod and blue mussels groups, but not in the salmon group. The increased FT4:FT3 ratio in the subjects who ingested blue mussels was associated with a 15% decrease in FT3 levels, whereas the increase in FT4 levels, although statistically significant, was minor.

4. Discussion

The main finding of the present study was the 20–60% increase in plasma TSH concentrations in healthy adults after consuming 150 g seafood daily for 14 days. The increase in TSH was proportional to the increase in plasma As, but with different constants depending on the type of seafood ingested. This may be interpreted as reflecting differences between the seafoods in the content of certain arsenicals. The salmon and blue mussel groups regression lines were significantly different from and with much steeper slopes than the cod group. The main arsenical in fin fish like cod and salmon is arsenobetaine (AB), but neither AB nor other species analyzed could explain the effects seen. Although not quantified in the present study, previous studies have identified arsenosugars as major arsenical species in marine algae and have also been found in algae-consuming animals like crustaceans and molluscs, although generally at much lower concentrations [21]. Despite large portions of unextractable As are common in bivalves, not all is possible to identify by common speciation methods. Thus, although reported in blue mussels [22] Whaley-Martin, it is unlikely that the unextractable fraction entirely consist of arsenosugars. To the best of our knowledge, have not been identified in fin fish [23]. On the other hand, arsenolipids such as arsenic-containing hydrocarbons have been identified in various fish oils and extracts of fish oils, accounting for 13–35% of the total As [24]. Salmon oil contains arsenolipids and since this fish species has high lipid levels in muscle tissue it is likely that arsenolipids would be present in the salmon meals consumed in this study. Cod being a lean fish with most of its lipids in the liver [25], is expected to have low levels of arsenolipids in its muscles, which is consistent with the flatter regression line between increases in plasma

Table 3
Summary of AIC-optimal multivariate models for T4, T3, T4:T3 ratio and changes in TSH (dTSH) for healthy volunteers consuming different seafoods in a randomized controlled trial.

	R^2_{adj}	Se	Iodine	dAs	T3	T4	TSH	Group
T4	0.18	−(0.05)						Offset: blue mussel + (0.005)
T3	0.18		−(0.1)				+(0.01)	
T4:T3	0.24						−(0.02)	Offset: blue mussel + (0.004)
dTSH	0.47	−(0.02)	+(0.15)	+(varies by group)	+(0.01)	−(0.15)		dAs slope: cod < blue mussel < salmon (0.01)

AIC (Akaike's information criterion) optimal ANCOVA multiple regression models for T4, T3, T4:T3 ratio and changes in TSH (dTSH). Plus and minus signs for regression coefficients, upper bounds for *p*-values in parentheses. For the T4 and T4:T3 models, slopes were the same for all groups, but offsets differed. Only for the blue mussel group, the offsets were significantly larger than for the control group. For the dTSH model, offsets were the same, but slopes (regression coefficients) for dAs differed significantly from zero in all the study groups. Plots and regression lines for dTSH are shown in Fig. 1.

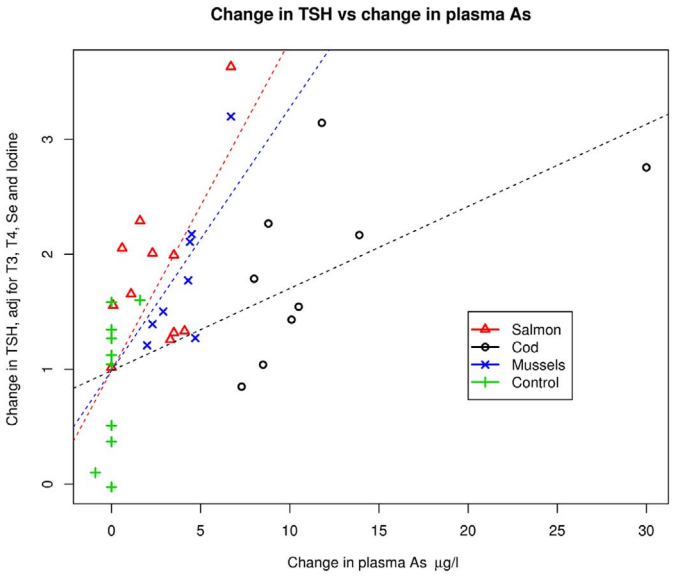

Fig. 1. Changes in TSH versus changes in plasma As during the study. TSH changes are adjusted for plasma Se, urinary Iodine, T3 and T4 according to the AIC-optimal model (R^2_{adj} = 0.47) for changes in TSH (dTSH). The regression equation for TSH change, dTSH, was *dTSH = 0.99 ± 1.32 + 0.04 ± 0.03*Iodine – 0.08 ± 0.06*T4 + 0.45 ± 0.16*T3 – 0.03 ± 0.01*Se + Cg*dAs*, Std. Error = 0.62. Cg, the coefficient of slope for the increase in TSH (dTSH) associated with an increase in plasma As (dAs), varies according to group as follows, P(Cg = 0) in parentheses: Control: 0.53 ± 0.35 (NS), Cod: 0.07 ± 0.02 (0.008), Salmon: 0.29 ± 0.08 (0.0007), Blue mussels: 0.23 ± 0.08 (0.006). The model fit in the figure, adjusted dTSH vs dAs, is R^2_{adj} = 0.46, but this is not directly comparable to the full model because of the large difference in number of parameters.

As and TSH in the cod group.

The median urinary iodine status at baseline in the present study was very low, i.a. 90 (range 20–240) µg/L in the whole study group (n = 38). The urine was sampled after one week of refraining from any seafood, at the end of the run-in period prior to study start. However, already at day -7 the median iodine concentration was at the similar level, at 90 (range 20–400) µg/L (data not shown). According to the WHO recommendations, a cut-off value for iodine deficiency is set at median urinary iodine values below 100 µg/L [26], thus the subjects in our study can be characterized as mildly iodine deficient.

The iodine content was highest in the blue mussel and cod diets, and accordingly only in these two groups did the urinary iodine increase significantly during the 14 days with seafood intake. The study thus confirms the role of lean fish, such as cod, in improving iodine status; since the iodine concentration in this group increased significantly from a median urine concentration of 80–220 µg/L after two consecutive weeks of a daily consumption of 150 g cod. Blue mussels, which provided the same amount of iodine, increased urinary iodine concentrations less than cod. On the other hand, salmon, that had a low iodine content, did not appear to be a good dietary source of iodine, as 14 days of salmon consumption did not have any impact on the urinary iodine levels.

Plasma selenium concentrations of the participants were well below a concentration required for full expression of selenoproteins (> 90–100 µg/L), but well above concentrations asscociated with overt deficiency [27,28].

The optimal multivariate models with change in plasma total As (dTAs) as independent variable with adjustment of covariates explained about 20% of the variation in dependent variables FT4, FT3 and FT4:FT3, but a larger fraction (47%) of the variation in dTSH. While it is not possible to provide a precise attribution of the relative importance of the factors in the model for dTSH, it is clear that dTAs is by far the most important one (Fig. 1). This indicates that As in the diet may play a significant role in thyroid metabolism. We have not been able to identify the metabolites involved, as neither intake nor excretion of iAs, AB and DMA were significantly associated with dTSH in multivariate modelling. Moreover, iAs was a negative factor when dTAs was present in the models.

Our results are consistent with the observation made by Meltzer et al. [5], where individuals ingesting at least three meals of fish weekly for 15 weeks showed a linear association between tAs in plasma and FT4:FT3 ratio [5]. This is one of the few human studies assessing the association between thyroid function and the intake of As-rich seafood. However, due to the design of that study, it was not possible to perform a more detailed analysis of the observed effect in relation to arsenic species. It is noted that in the study by Meltzer and co-workers [5], initially, blood As correlated negatively both with FT3 and FT4, and that Se supplementation seemed to counteract the effect of dietary As from fish/seafood.

Observational studies have found similar results, indicating that As could impact thyroid metabolism. Jain (2015) reported from a large cross-sectional study, NHANES 2007–2010, the association between urinary As levels and thyroid hormones (n = 4855) and found that high levels of the urinary metabolite dimethylarsinate (DMA) was significantly associated with lower levels of FT3, total T3 and total T4 [2]. Interestingly, the only seafood group in the present study where a significant reduction in FT3 was found, was in the blue mussel group. We have previously reported [20] that, when comparing the different groups with respect to urinary DMA excretion relative to total amount As excreted in urine, the blue mussel group was the group with highest with DMA excretion, accounting for approximately 24% of total As. In comparison, the DMA excretion accounted for 9.2% and 1.4% in the salmon and cod groups, respectively [20]. Regarding TSH, Jain found that a high DMA level was associated with higher TSH levels, but only in men [2]. In a cross-sectional study in men aged 18–55, Meeker et al. reported that plasma tAs levels were associated with a dose-dependent increase in covariate-adjusted TSH [3]. Likewise, in a cross-sectional study from Canada (n = 304) (adjusted for covariates such as dietary sources of As), total iAs in well water (14.2 µg/L) was positively associated with TSH, and negatively associated with FT3 and FT4 in adults [10]. A similar association between thyroid hormones and exposure to As from polluted urban air was found in an Italian study (n = 185); also here they found a positive correlation between urinary tAs and TSH and a negative correlation between urinary tAs and T4 and

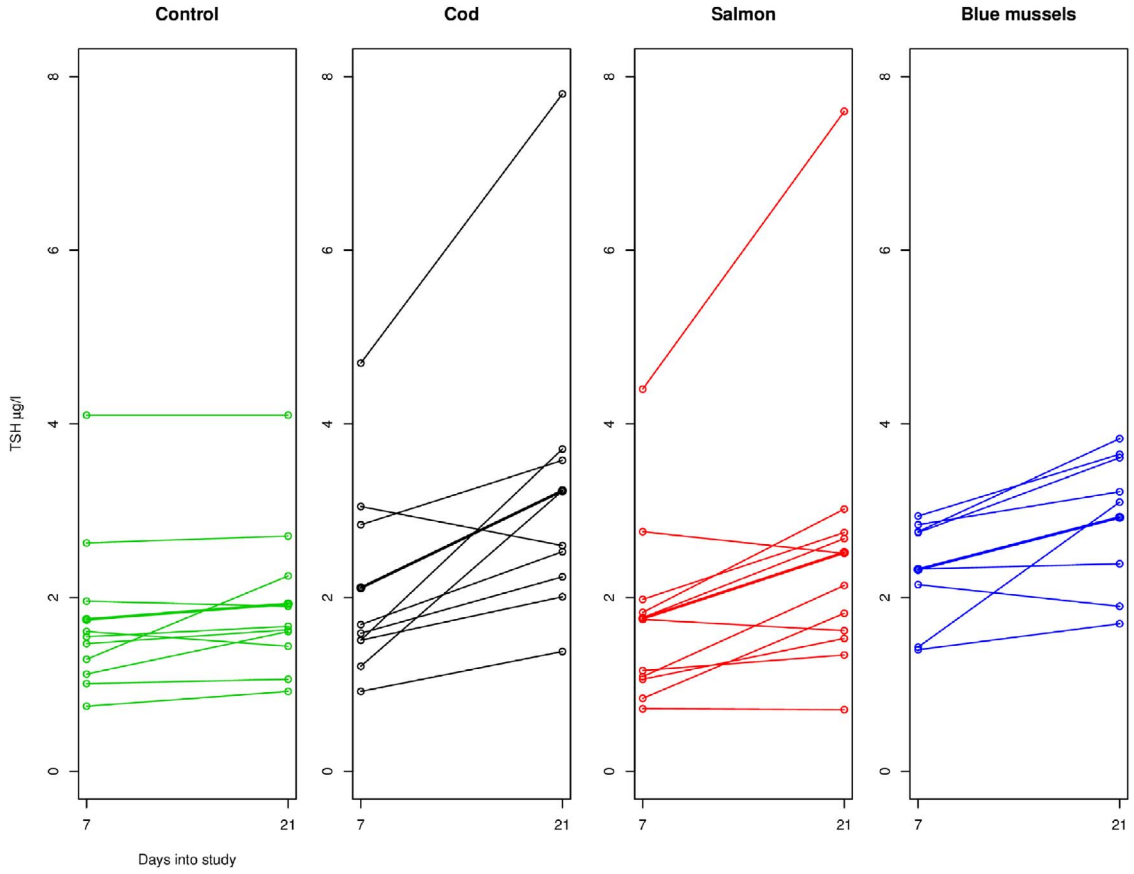

Fig. 2. Individual changes in TSH (dTSH) during the study in the control group and the three study groups.

T3 [4].

Arsenic is known to modify the metabolism and toxicity of selenium as it i.a. promotes the excretion of Se and may also bind to Se [11]. However, the changes in FT4:FT3 ratio in the present study are probably not entirely due to arsenic-selenium interaction. The expected inverse relationship between plasma Se and FT4, because of impaired selenium-containing deiodinase-mediated conversion of T4 to T3 was indeed observed. But plasma Se was also negatively associated with the increase in TSH levels, which itself may result in less conversion of T4–T3.

5. Conclusion

Dietary As from seafood appeared to influence thyroid hormones, particularly by stimulating an increase in plasma TSH levels. The association with change in TSH levels varied with type of seafood consumed indicating that the chemical forms of As are important. Further studies are needed to elucidate the mechanisms by which different organic As from seafood may impact thyroid function and thyroid hormone metabolism.

Acknowledgement

We appreciate the financial support from the Norwegian Research Council (project number 142468/140) and Oslo and Akershus University College of Applied Sciences. We gratefully thank the volunteers in the study and the analytical work (arsenic and iodine) by Siri Bargård and Berit Solli at the National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway.

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