Low level seaweed supplementation improves iodine status in iodine-insufficient women

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Emilie Combet[1*], Zheng Feei Ma[1], Frances Cousins[1], Brett Thompson[1], Michael E. J. Lean[1]

[1] Human Nutrition, School of Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, UK

*** Corresponding author:** Emilie Combet, Human Nutrition, School of Medicine, College of Medical, veterinary and Life Sciences, University of Glasgow, New Lister Building, Alexandra Parade, Glasgow, G31 2ER, UK.

Email: Emilie.CombetAspray@glasgow.ac.uk

Phone: +44 141 201 8527

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Abstract

Iodine-insufficiency is now a sustained issue in the UK and other European countries, due to low intakes of dairy and seafoods (especially where iodine fortification is not in place). Here, we tested commercially-available encapsulated edible seaweed (Napiers Hebridean Seagreens® *Ascophyllum nodosum* species - NaHS) for its acceptability to consumers, iodine bioavailability and the impact of a 2-week long daily supplementation on iodine levels and thyroid function. Healthy non-pregnant women of childbearing age, self-reporting low dairy and seafood consumptions, with no history of thyroid or gastro-intestinal disease were recruited. Seaweed iodine (712 µg, in 1g seaweed) was modestly bioavailable at 33% (IQR 28-46) of the ingested iodine dose, compared to 59% (IQR 46-74) for potassium iodide (n=22). After supplementation (2 weeks, 0.5g seaweed daily, n=42), urinary iodine excretion increased from 78 µg/L (IQR 39-114) to 140 µg/L (IQR 103-195), $p<0.001$. Thyroid stimulating hormone increased from 1.5 mUI/L (IQR 1.2-2.2) to 2.1 mUI/L (IQR 1.3-2.9) ($p<0.001$) with two subjects exceeding the normal range after supplementation (but normal free thyroxine). There was no change in other thyroid hormones levels after supplementation. The seaweed was palatable and acceptable to consumers as a whole food or as an ingredient, and effective as a source of iodine in an insufficient population. Incorporation in staple foods would provide an alternative to fortification of salt or other foods with potassium iodine.

Keywords: Iodine, women, seaweed, *Ascophyllum nodosum*, bioavailability, thyroid function, childbearing age

Introduction

Iodine is essential for the synthesis of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) which play a key roles in metabolism, and are vital for a growing fetus, for normal growth and brain development [1]. While hypothyroidism complicates some pregnancies [2], it does not preclude hypothyroid women to become pregnant [3], and iodine intake is crucial during the period surrounding child-bearing. When the iodine intake is below the recommended intake (250 µg/day in pregnancy [4] although a new threshold value of 200 µg/day has been proposed [5]), adequate secretion of the thyroid hormones may still be achieved by physiological adaptation. Modifications of thyroid and pituitary activities increases thyroid stimulating hormone (TSH) secretion, which enhances production of T_3 relative to T_4 and rapid iodine turnover [6], but fetal supply and placental transfer remain low. For epidemiological purposes, iodine insufficiency is defined as a population, or subgroup, with a median urinary excretion (UIC) less than 100 µg/l for non-pregnant adults, and below 150 µg/L for groups of pregnant women[4]. While iodine fortification of common foods is widespread, it is not provided in all countries. There is no requirement for iodine fortification of foods in UK, and iodine fortification is unusual. There is growing concern that subclinical iodine deficiency may be emerging in post-industrial countries previously assumed to be iodine sufficient and there is currently very little evidence about the need for specific dietary advice, or for iodine fortification / supplementation targeted towards these two key vulnerable groups: young women and their infants.

With dairy and seafoods as main dietary source of iodine [7], the UK has been considered iodine replete. Areas with historical endemic goitre ('Derbyshire neck') no longer see clinical dietary hypothyroidism, in what was hailed an accidental public health success, following change to farming practice and supplementation of dairy herds [8]. However, a recent survey of

British schoolgirls has highlighted mild iodine deficiency with median urinary iodine concentrations of 80 µg/L [9]. Similar results were found in a Scottish survey of women of childbearing age [10]. Although few people have frank iodine deficiency and hypothyroidism, a low or marginal intake presents a potential hazard in pregnancy due to the increased demand placed on maternal thyroid function [11]. This level of iodine insufficiency in the population is sufficient to impair intellectual development of future generations. Bath *et al.* showed that low maternal iodine status in pregnancy (individual iodine-to-creatinine ratios below 150 µg/g in spot samples) was associated with decreased cognitive functions in the ALSPAC cohort of 1040 children from the south of England [12]. While there is no lack of availability of dietary iodine in these regions [13], the explanation may be that many of the young female population commonly exclude fish and/or dairy products from their diets, for social or other reasons, leading to either low or marginal iodine intakes [14].

Seaweeds used to feature as cheap and natural traditional foods in the British diet [15] until recently. European standards have later come in to ensure suitability as a human food. Despite this, it is still rather neglected throughout Europe, with little data available on the range of seaweed products for sale in the UK or Europe (Norman et al. in 1988 studied mainly a range of kelp tablet, citing laverbread and Nori seaweed sheet as other seaweed products available [16]. Data on its consumption are lacking, despite the fact that it is a rich source of iodine, with wide variation between species (from 16 to 8165 μg/g) [17].

This study aimed to investigate the potential of seaweed as a safe and acceptable option for dietary iodine supplementation, specifically answering the following research questions:

1) What is the bioavailability of iodine from an encapsulated edible seaweed (Seagreens® *Ascophyllum nodosum* species), in a group of asymptomatic non-pregnant women reporting to consume low amounts of iodine-rich foods?

2) What is the impact of daily consumption of the encapsulated seaweed on iodine levels and thyroid function, in the same group of women?
3) Is the encapsulated seaweed acceptable for consumers (taste / use)?

Material and Methods

Seaweed supplement

Each capsule contained 0.5g Seagreens *Ascophyllum nodosum* (Napiers Hebridean Seagreens Capsules - NaHS), equivalent to 356 µg iodine (suppliers information based on measurements from independent UKAS accredited laboratories). NaHS is a dried and milled seaweed, sourced in Scotland and produced to distinct human food seaweed™ standards (patents pending) ensuring the safety, quality, sustainability and consistency of the products. All products are rigorously monitored during harvesting, drying and milling, and analyzed independently by UKAS accredited laboratories for nutritional composition, contaminants and heavy metals.

In vitro iodine bioavailability assays

The *in vitro* determination of the bioavailability of iodine in seaweed is based on the simple simulation of gastric and intestinal digestion according to the method developed by Romaris Hortas *et al.* [18].

Digestion was carried out in triplicate. In brief, powdered NaHS (0.5 g) was added to distilled water (20mL) and the pH was adjusted to 2.0 with a 6M hydrochloric acid. Fresh gastric solution (0.15 g, pepsin 6.0% (w/v) dissolved in 6.0M HCl) was added to the flask, prior to incubation (37°C in a shaking bath at 150 rpm for 120 minutes). Digestate aliquots (0.5 mL)

were transferred to -20°C prior to iodine determination. The digestate pH was neutralized with NaOH (pH 7.5). Dialysis bags filled with 0.15N PIPES (20 mL) were placed inside each flask, along with intestinal digestion solution (pancreatin 4.0% (m/v) and bile salts 2.5% (m/v) dissolved in 0.1M sodium hydrogen carbonate, 5mL). The flasks were incubated at 37°C in a shaking water bath at 150 rpm for 120 min. The enzymatic reaction was stopped by immersing the flasks in an ice water bath. The dialysis bags were removed and residual or non-dialyzable fraction (remaining slurries in the flasks) were transferred to polyethylene vials and separately weighed. Aliquots (1.5 mL) from the dialysate (20 mL) and non-dialysate fractions (25 mL) were transferred to - 20°C prior iodine determination.

Colonic fermentation of the digestate was carried out as previously described [19] to test whether iodine was trapped in the seaweed matrix after digestion. Briefly, faecal samples (16g) from three healthy volunteers were homogenized with a blender (30 s) in fermentation buffer (50 mL) to make a 32% faecal slurry. An aliquot (5 mL) of the non-dialyzable fraction of the intestinal digestate was added to faecal slurries (50 mL). The bottle was purged with OFN (1 min) and sealed and incubated in a shaking water bath at 37°C and 60 stroke/min. Samples were taken at t= 0h, 2h, 4h, 6h and 24h to measure pH and were immediately stored at -20°C prior to iodine determination.

Human iodine bioavailability experimental design

The study was approved by the University of Glasgow Medical Veterinary and Life Sciences College Ethics committee. All participants provided written informed consent.

Healthy women aged 18-46, self-reporting as low-iodine consumers, were recruited locally using via posters and word-of-mouth, to take part in cross-over iodine bioavailability study. Those with existing thyroid or gastro-intestinal conditions, taking medication other than the contraceptive pill or smoking were excluded, as well as pregnant or lactating women and

136 those planning to conceive. Those taking dietary supplements containing iodine were also
137 excluded. Appropriate sample size for bioequivalence / bioavailability studies vary between
138 12 and 24 subjects. According to Hauschke et al., 20 participants are required for standard 2 x
139 2 cross-over studies, with a bioequivalence range of 0.8-1.25, using a conservative 20%
140 coefficient of variation (with ±=0.05, ² =0.80) [20].

141 Height, weight, waist circumference and blood pressure were measured after recruitment.
142 Usual dietary intake was determined using an iodine-specific food frequency questionnaire [21].
143 Participants were allocated at random to treatment order (potassium iodine (KI) or seaweed
144 first) and were asked to avoid all iodine-rich foods (dairy and seafood) for the duration of the
145 study. Prospective food diaries were kept for the duration of the study. The iodine content of
146 participants diet was determined by entering all foods in a dietary assessment software
147 (Windiets 2005, Robert Gordon University) using appropriate food composition tables [22]. A
148 7-day wash out period between each leg of the cross-over intervention. Participants were
149 asked to replicate their diet during the second leg of the study.

150 All urine passed on Day 1 (baseline 24h urines) was collected. On Day 2, participants
151 received either a seaweed supplement (NaHS, 1 g) or potassium iodide (KI) supplement
152 (equivalent iodine content; 712 µg) to be taken fasted with a breakfast of white toast and a
153 glass of water. Urine was collected for 24 hours, in fractions for the periods 0-2h, 2-5h, 5-8h,
154 8-20h and 20-24h.

155 **Seaweed supplementation study – experimental design**

156 Healthy women aged 18-50, self-reporting as low-iodine consumers, were recruited locally
157 using via posters and word-of-mouth, to take part in cross-over seaweed supplementation
158 study. Those with existing thyroid or gastro-intestinal conditions, or taking medication other
159 than the contraceptive pill were excluded, as well as those taking iodised dietary

supplements. None had taken part in the bioavailability study. The supplementation study was approved by the University of Glasgow Medical Veterinary and Life Sciences College Ethics committee. All participants provided written informed consent. The a priori sample size was calculated in G Power (Kiel University, Germany) using UIC as a primary outcome for mean difference between two groups using the Wilcoxon signed-Rank test for matched pairs, assuming a logistic parent distribution. A sample size of n=42 was calculated, to detect (or not) an increase from the current population UIC for the target group (median 75ug/L, calculated mean 94 µg/L, standard deviation 80 µg/L [10]) to a sufficient UIC (100 µg/mL), equivalent to a ~14% increase in UIC, and an effect size of 0.47, with ±=0.05, ² =0.80).

Participants' height, weight, waist circumference and blood pressure were measured at the beginning and end of the supplementation period. Usual dietary intake was determined using an iodine-specific food frequency questionnaire [21]. During the run-in period, participants were asked to keep a 4-day weighed food diary. Urine was collected for 24 hours on Day 4. On day 5, participants were supplied with a stock of supplements, and instructed to consume one capsule of NaHS daily (0.5 g per day, equivalent to an intake of 356 µg/d of iodine) for 14 days, while following their usual diet. A fasted venous blood sample was collected, and the total volume of the urine collection measured. At the end of the supplementation period, participants replicated the diet recorded on the 4-day weighed diary (Days 16-19), and collected 24-hour urine on the last day of supplementation (Day 19). A final fasted venous blood sample was collected (Day 20). All urine and plasma samples were aliquoted and stored at -80˚C until analysis. Compliance was checked by counting the number of capsules remaining in the container supplied to volunteers.

Urinary iodine measurements

Urinary iodine and iodine concentration in digestates were analysed using the colorimetric Sandell-Kolthoff reaction adapted for the 96-well microtiter plate, as described by Ohashi *et al.* [23], using a custom-made sealing cassette. Sample were measured in triplicates (CV% <10%).

Thyroid function tests

Thyroid stimulating hormone (TSH), thyroglobulin (Tg), triiodothyronine (T_3 and fT_3) and thyroxine (T_4 and fT_4) were measured in plasma in duplicates using immunoassays (ELISA assays, Astra biotech GmBh, Luckenwalde, Germany).

Acceptability of the supplement

Participants filled a self-administered questionnaire focusing on habitual frequency of consumption of seaweed products (6-point Likert scale, “daily” to “never”), opinions on taste (3 statements, 5-point Likert scales, “strongly agree” to “strongly disagree”), after-taste (1 statement, 5-point Likert scales, “strongly agree” to “strongly disagree”) and overall acceptability of seaweed as a food or ingredient (3 statements, 5-point Likert scales, “strongly agree” to “strongly disagree”). Open questions were used to gather information on taste, after taste, and views on seaweed as an ingredient in foods.

Statistical analyses

Data were expressed as mean ± SD or as median and inter-quartile range (IQR) depending on normality, which was checked using the Shapiro-Wilks test. Categorical data (Likert scale) was described using the mode and IQR. Significance was implied at $p<0.05$. Wilcoxon signed-Rank test for matched pairs or paired t-test was used to assess the difference between

paired groups depending on their data distribution, while the Mann-Witney U-test or independent t-test was used to compare unrelated samples. Analysis was carried out using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

In vivo bioavailability study

Healthy females (n=22), median age 24.5 (IQR 22-34) were recruited and completed the bioavailability study. Socio-demographic and anthropometric details for the group are summarized in Table 1.

Dietary iodine intake was low (below 55 µg/day) throughout the bioavailability study period, for each study arm (Table 2). The baseline median UIC, for the 24 hours preceding the study, was 40 µg/L (IQR 24-66) prior to seaweed intake and 31 µg/L (IQR 19-71) prior to KI intake. Correcting for total urine volumes, this was equivalent to 50 µg/24h (IQR 40-82) preceding seaweed intake, and 48 µg/24h (IQR 32-86) preceding KI intake.

Urinary iodine output, in µg.L-1.h-1 is presented in Figure 1, with cumulated iodine excretion in µg presented in Figure 2. The peak iodine excretion time occurred earlier for KI (0-2h) compared to the seaweed (2-5h). The amount of iodine excreted over the 24h period following ingestion was greater ($p<0.001$) following KI intake (421 µg, IQR 328-526) compared to seaweed intake (239 µg, IQR 199-352).

Participants were grouped according to habitual iodine intake, as either sufficient (n=7) or insufficient (n=13). The dose of iodine excreted in urine was calculated based on the iodine load of the NaHS capsule / KI plus the dietary iodine intake of day 3 (Table 2). The dose of

iodine excreted was significantly higher ($p<0.001$) following KI intake (59%, IQR 46-74) than seaweed intake (33%, IQR 28-46). This was true for both subgroups ($p=0.009$ and $p=0.017$ for insufficient and sufficient group, respectively). However, while the dose of iodine excreted after KI was higher in the sufficient group (73% vs. 46%, $p=0.036$), there was no difference between groups after seaweed ingestion (46% vs 31%) (Table 3).

In vitro bioavailability assays

After digestion in the simulated gastric compartment, only 9.9±0.1% of the iodine present in the sample was available and in solution. After digestion in the simulated intestinal compartment, 4.9±0.1% of the initial iodine dose present was recovered in the dialysis bag, with a further 5.0±0.0% in the non-dialysable fraction. This indicates that approximately 90% of the iodine was still trapped in the seaweed matrix at that point and consistent with the cumulated dose excretion in urine during the in vivo bioavailability study (up to 5h post ingestion), which was approximately 12% of the dose ingested (IQR 7-15). After faecal fermentation of an aliquot of the non-dialysable fraction, 51.2±10.4% of the iodine present was available, and in solution.

Impact of seaweed supplementation on urinary iodine

A total of 42 healthy females of childbearing age took part in the 2-week supplementation study. The demographic, anthropometric and dietary profiles of participants are presented in Table 4.

At baseline, median UIC was well below the cut-off for sufficiency (100 µg/L) at 78 µg/L (IQR 39-114). The group average iodine intake was 110 µg (IQR 73-141), with 31 participants with an intake below the recommended intake of 140 µg/day. Subsequently, individuals were classified as having iodine-sufficient (>140 µg) or insufficient intake (<140

µg) based on their habitual iodine consumption as estimated by the FFQ. There was no difference in weight, BMI, waist circumference between the subgroups with sufficient or insufficient iodine intake at baseline.

After supplementation, median UIC increased significantly to 140 µg/L (IQR 103-194) ($p<0.001$). This increase in UIC differed between sufficient and insufficient group (+23 µg/L, IQR 17-66 for the sufficient group, +97 µg/L, IQR 57-132 for the insufficient group; $p=0.041$) and was only statistically significant in participants with insufficient habitual iodine intake ($p<0.001$). The total amount of iodine excreted over 24 hours was however significantly increased for both insufficient, from 93 µg/day (IQR 60-109) to 262 µg/day (IQR 198-301), $p<0.001$, and sufficient groups, from 138 µg/day (IQR 73-157) to 214 µg/day (IQR 75-343 µg/day), $p<0.041$. Neither weights nor waist circumferences changed during the supplementation study.

Impact of seaweed supplementation on thyroid function

The thyroid function tests are presented in Table 5. At baseline, Tg and fT3 levels were different between iodine sufficient and insufficient subgroups ($p=0.047$ and $p=0.048$, respectively). Tg values were within the Tg reference range in healthy adults (3 - 40 µg/L) but higher than the proposed cut-off for iodine sufficiency (10 µg/L).

TSH levels were within the normal range (0.4 – 4.5 mUI/L) [24] for all but one participant, who had a borderline TSH level of 5.72 (but normal fT4 levels).

There was no significant change in the thyroid hormones T3, T4, fT3, fT4 following supplementation, or Tg (with values remaining over 10 µg/L) [25]. There was however a significant increase in TSH, from a median 1.5 mUI/L (IQR 1.2-2.2) to 2.1 mUI/L (IQR 1.3-2.9) ($p<0.001$). This increase was significant in both insufficient and sufficient groups ($p=0.027$ and $p=0.006$, respectively), but more marked in those with sufficient habitual iodine

intake (p=0.044). Serum TSH did exceed the normal range for two participants (7.3 and 8.0 mUI/L) with fT4 still within the normal range. While fT3 levels did not significantly change for the whole group, those in the insufficient group had a decrease after supplementation (p=0.048).

Seaweed consumption and acceptability of the supplement

Participants in the bioavailability and supplementation studies answered a side questionnaire on seaweed consumption (combined n=63). They had very rarely been exposed to seaweed as a foodstuff, with 19% never having consumed it knowingly; 60% of participants had consumed it as sushi, on a monthly basis (18%) or less often (37%). Less than half (40%) of participants had consumed whole seaweed (less than twice a year). Most had never consumed lava bread (90%), nor seaweed as a tablet (92%) or a capsule (87%). The main reasons for the low consumption was lack of opportunity (mentioned by 64% of participants), and lack of appeal (54%).

Participants agreed that the taste of the supplement was acceptable when swallowed as a capsule (mode 5, median 4, IQR 3-5) and disagreed that there was an unpleasant after-taste (mode 2, median 2, IQR 2-4) or that the capsule were difficult to swallow (mode 1, median 2, IQR 1-2). Supplementation study participants who had added the seaweed to foods (n=24) neither agreed nor disagreed on the acceptability of its taste as an ingredient (mode 3, median 3, IQR 3-3) or its ease of use for cooking (mode 3, median 3, IQR 3-4).

Participants agreed that encapsulated seaweed is a good way to include seaweed in the diet (mode 4, median 4, IQR 4-5). Preferred ways to consume seaweed included encapsulated (71%), as an ingredient in food (33%) or as a whole food (19%). Most (67%) saw the potential use of seaweed as a food ingredient as a positive. The main reasons where assumed health benefits and extra nutrients (35%) and flavour enhancement (24%). A minority (7%)

held negative view on seaweed as an ingredient, with taste the main concern (75%). The rest were either unsure or with no opinion.

Discussion

This study showed that asymptomatic young women with diets low in seafoods and dairy products do indeed display biochemical evidence of quite marked iodine deficiency. It then shows how an acceptable/palatable commercially available seaweed product can boost the iodine intake of a group of mostly iodine-insufficient women, without deleterious impact on thyroid function. Even in an iodine-sufficient population (UIC above 100 µg/L), the consumption of this product (or product of similar quality and traceability) would not be contraindicated because the urinary iodine levels attained would not exceed 500 µg/L.

Daily intake of an encapsulated seaweed (NaHS) was effective at raising the UIC of a group of females after a two-week supplementation period with a slight increase in the TSH levels after seaweed supplementation. Our results are in agreement with Teas *et al.* who supplemented iodine-replete healthy post-menopausal women with *Alaria esculenta* capsules for 7 weeks (475 µg iodine/day) [26] and Clark *et al.* (kelp, 1 g iodine/day for 6 weeks) [27]. The TSH levels remained within the normal range for all but two participants, with no change observed for the thyroid hormones, whereas Clark *et al* observed a decrease in total T3 after supplementation. Tg values remained higher than the proposed 10 µg/L cut-off for iodine insufficiency [25], even after the supplementation, which might be indicative of a lag period for Tg values to fall within iodine sufficiency range after achieving iodine sufficient status.

The iodine contained in NaHS was bioavailable, although to a lesser extent (30%) than previously reported by Aquaron (90-100% for iodine-sufficient women, and 62-85% for

iodine-insufficient women over 48-hours) [28] or Teas (60% for iodine-sufficient women over 48-hours) [26]. This may be directly related to our shorter (24-hour) urine collection, and the type of seaweed used in the other studies (*Gracillaria verrucosa, Laminaria hyperborea* and *Alaria esculenta).* Incomplete collections are also a possible explanation. We showed a difference in excretion between those with either sufficient or insufficient iodine intake, as previously described [28]. This is consistent with the generally-held understanding that most of the iodine will be excreted in urine if iodine stores are replete. *In vitro* digestion confirmed limited release of the iodine from the seaweed matrix in the first gastric and intestinal phases of simulated digestion. We showed that colonic fermentation of seaweed is important to free iodine from the seaweed matrix, with mechanism relying on fermentation of the polysaccharide matrix [29] or metabolism of organic iodine [18]. Therefore, the seaweed matrix may delay iodine absorption (compared to KI), with iodine released from the food over a longer period. Impact of further processing such as cooking needs to be taken in consideration if seaweed is used as an ingredient, as it would lead to partial loss via evaporation [30; 17].

Several studies reported that iodine insufficient populations were diagnosed with iodine-induced hyper- or hypothyroidism following high iodine intake [31; 32; 33; 34], however, a two-week iodine supplementation with up to 500 μg/d had no impact on thyroid function tests in euthyroid subjects [35]. Upper tolerable limit of iodine intake in healthy individuals have been defined as 1.1 mg/d in the United States and 600 µg/d in the European Union [36; 37]. While epidemiological evidence has linked high daily seaweed/iodine intake with higher thyroid cancer risk in Japan [38], this observation is not supported by experimental studies in rats with chronic high iodine intake (up to 1g/L in drinking water) [39]. The thyroid gland can adapt to excessive iodine intake after initial diminution in the excretion of thyroid hormone due to the Wolff-Chaikoff effect. This effect was demonstrated to have a longer lasting suppression of

345 the thyroid gland in those ingesting excess seaweed [40]. Restricting the seaweed intake was
346 able to reverse iodine-induced goiter and transient hypothyroidism [41].

347 Reports of widespread iodine insufficiency in Britain and other Europeans countries, the
348 renewed interest in iodine nutrition and the lack of iodine prophylaxis in the UK represent an
349 opportunity for seaweed as a foodstuff. Iodine insufficiency results from low intake of dairy
350 (especially milk, which consumption has been steadily decreasing since 1975 [42]), and seafood
351 (which consumption is low in the UK population at 37g/day [43]). Iodised salt is the main
352 method of iodine prophylaxis worldwide but there is still a concern, among clinical and
353 public health professionals, that attributing a positive, health promoting characteristic to salt
354 may blunt the public health effort toward salt reduction in relation to the prevention of
355 cardiovascular diseases. A recent join WHO/ICCIDD meeting debated this topic, to synergise
356 salt reduction and iodine fortification agendas [44]. With table salt usage falling in the UK
357 following successful public health campaigns, it may be contradictory to portray salt as a
358 vehicle for iodine. Viable alternatives to increase iodine status include fortification of staple
359 foods with seaweed, which was previously successfully incorporated in a nutritionally-
360 balanced pizza, designed in the context of health-by-stealth improvement of ready meals.
361 Seaweed addition enabled to reduce the sodium content of the product, while improving
362 nutritional content, without compromising the taste or appearance[45]. Given that iodine is
363 extensively stored in the thyroid, it can safely be consumed intermittently, which makes
364 seaweed use in a range of foods attractive, and occasional seaweed intake enough to ensure
365 iodine sufficiency.

366 Seaweed consumption in most Western cultures has been low, due to low availability in the
367 market and poor consumer awareness regarding potential health benefits [46]. The benefits of
368 incorporating seaweed isolates into the habitual diet goes further than addressing iodine

deficiency, with impact of seaweed consumption on serum oestradiol, reduction of the glycemic response to a carbohydrate load, and increased satiety via lowered gastric emptying. These aspects may be relevant to the development of functional foods for weight management [47; 48; 49; 50; 51]. Incorporation in bread had no impact on taste or appearance [46]. Trade price are such that the additional cost per loaf would be minimal considering that seaweed is iodine-rich and that little would be required.

The contaminants and heavy metal content of seaweed is sometimes a concern, especially in retailed products with poor traceability and limited compositional analysis, as consumption may expose the consumer to heavy metals such as organic / inorganic arsenic [52]. Water quality is important for seaweed quality, and France is the only European country with specific regulations for the use of seaweeds as vegetables [30]. The seaweed used in this study (NaHS) was grown in Scottish Grade A Pristine water (SEPA/SNH evaluation) and produced to Human Food Seaweed™ standards (patents pending). Compositional analysis, carried out on every batch, showed no contaminants and heavy metals below threshold levels. This is important if seaweed will become a more commonly used ingredient in processed foods.

In conclusion the answers to the research questions behind this study are:

1) Iodine bioavailability from the encapsulated seaweed was low in the group of women studied. The seaweed matrix may be a key factor for this low bioavailability.
2) Daily consumption of 0.5g of NaHS increased urinary iodine level to 140 µg/L for the group. TSH increased slightly, within the normal range for all but two participants. Increase in TSH level may be linked to iodine-induced hypothyroidism, especially in those with replete iodine stores, although no change to thyroid hormones levels were observed [40].

3) Participants indicated that the encapsulated seaweed had an acceptable taste, was easy to use, and were positive about seaweed use as an ingredient.

The study conclusions would have been strengthened with a randomised controlled crossover study design, longer exposure time and reassessment of iodine status and thyroid function after the end of the intervention, but that would demand an impractical duration of high tolerance from volunteers. It would be of value to repeat the biochemical aspects in different subject groups. The influence of the seaweed matrix on bioavailability will be an important factor to consider if seaweed is incorporated in cooked and uncooked staple foods. A large-scale survey needs to take place to properly investigate attitudes to seaweed utilisation in processed foods and cuisine in general.

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Figure legends

Figure 1: Urinary iodine excretion in µg/L/h over 24h, after ingestion of a dose of 712µg iodine, from KI (■) or NaHS (○).

Figure 2: Cumulated iodine output in µg over 24h, after ingestion of a dose of 712µg iodine, from KI (■) or NaHS (○).

Table 1: Characteristics of the bioavailability study participants (n=22)

		Median	IQR
Demographic & anthropometric details	Age (yrs)	24.5	22-34
	Height (cm)	165	163-167
	Weight (kg)	60	56-70
	Waist (cm)	71	66-77
	BMI (kg/m^2)	22	20-24
Usual diet	Milk (mg/day)	131	92-236
	Other dairy (mg/day)	115	81-172
	Seafood inc. fish (mg/day)	24	13-29
	Daily iodine intake (µg/day)	127	87-142
		Count (n)	**(%)**
Ethnicity	White British	6	27%
	White Europeans	4	18%
	Other ethnicities	12	55%
Body composition	Overweight (BMI>25)	3	14%
	Obese (BMI>30)	1	5%
Iodine intake	Daily iodine intake >140 µg/day	7	33%
	Daily iodine intake <140 µg/day	14	67%

Table 2: Daily dietary iodine intake (µg) according to study arm

Study arm	***Day 1***		***Day 2***		***Day 3***	
	median	IQR	median	IQR	median	IQR
NaHS - KI	54	32-84	45	29-65	39	28-64
KI - NaHS	53	33-58	48	26-91	38	25-65

Table 3: Percentage iodine dose excreted, according to habitual iodine intake (sufficient & insufficient)

	Seaweed		**KI**	
	median	IQR	median	IQR
insufficient (n=13)	31% [a]	6-14	46% [b]	40-72
sufficient (n=7)	46% [a]	33-49	73% [b]	64-77
All (n=22)	*33% [a]*	*28-46*	*59% [b]*	*46-74*

[a,b] significantly different change (" pre-post supplementation) between groups at p<0.05

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550 **Table 4: Characteristics of the participants in the 2-week supplementation study (n=42)**

		Median	IQR
Anthropometric and demographic information	Age (yrs)	27.0	22-37
	Height (cm)	164	162-168
	Weight (kg)	62	57-71
	Waist (cm)	72	67-82
	BMI (kg/m2)	23	21-26
Usual diet	Milk (mg/day)	180	79-259
	Other dairy (mg/day)	71	37-159
	Seafood inc. fish (mg/day)	20	8-38
	Daily iodine intake (µg/day)	110	70-139
		Count (n)	**(%)**
Ethnicity	White British	25	60%
	White Europeans	9	21%
	Other ethnicities	8	19%
Body composition	Overweight (BMI>25)	10	24%
	Obese (BMI>30)	4	10%
Iodine intake	Daily iodine intake >140 µg/day	11	26%
	Daily iodine intake <140 µg/day	31	74%

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Table 5: Iodine status and thyroid function pre and post supplementation in participants meeting the daily iodine recommendation (n=11) or not (n=31). Data are presented as median (IQR).

	All (n=42)							Insufficient (n=31)							Sufficient (n=11)						
	Pre		Post			Δ		Pre		Post			Δ		Pre		Post			Δ	
UIC (µg/L)	78	(39-114)	140	(103-194)	***	72	(23-129)	50	(38-99)	149	(105-194)	***	97	(57-132) [a]	104	(85-122)	139	(106-200)		23	(17-66) [b]
UIC (µg/24h)	94	(61-142)	248	(177-305)	***	147	(82-190)	93	(60-109)	262	(198-301)	***	149	(102-195)	138	(73-157)	214	(75-343)	*	76	(25-167)
TSH (mUI/L)	1.5	(1-2)	2.1	(1-3)	***	0.5	(0-1)	1.4	(1.1-2.2)	1.9	(1.2-2.8)	*	0.4	(-0.1-0.8) [a]	1.7	(1.4-2.2)	2.7	(2.2-3.2)	**	0.8	(0.6-1.3) [b]
Tg (µg/L)	21.8	(16.8-32.1)	20.6	(17-30.1)		-1.0	(-3.6-2.5)	26.6	(17.2-35)	24.0	(17.6-31.8)		-1.7	(-3.8-3.1)	17.2	(12-22.9)	15.8	(14.7-20.2)		-0.4	(-1.7-1.8)
T3 (nmol/L)	1.9	(1.7-2.2)	1.9	(1.7-2.2)		-0.1	(-0.2-0.1)	1.9	(1.7-2.3)	2.0	(1.7-2.1)		-0.1	(-0.3-0.1)	1.9	(1.8-2)	1.9	(1.7-2.3)		-0.1	(-0.2-0.1)
T4 (nmol/L)	86.9	(75.6-97.4)	86.0	(75.9-102.1)		2.3	(-3.2-11.7)	89.9	(77.9-101.1)	86.9	(75-110.8)		-0.3	(-4.6-8.1)	80.8	(72.9-85)	83.8	(79.3-98.3)	*	2.9	(1.3-14.9)
fT3 (pmol/L)	5.5	(3.3-7.7)	4.4	(2.9-6.7)		-0.2	(-1.2-0.4)	4.1	(3.1-7)	3.3 *	(2.9-6.6)		-0.3	(-1.3-0.1)	6.8	(5.8-8.2)	6.8	(5.6-8.1)		0.0	(-0.5-1)
fT4 (pmol/L)	13.8	(12.4-15.6)	14.4	(12.4-15.9)		0.4	(-0.6-1.1)	13.9	(12.1-15.6)	14.5	(12.3-15.6)		0.4	(-0.5-0.9)	13.5	(12.9-15.4)	14.3	(12.8-16.5)		0.2	(-1.0-1.7)

” difference between parameters measured pre and post supplementation

* p<0.05, ** <p<0.01, *** p<0.001 pre vs post supplementation

[a,b] significantly different change (” pre-post supplementation) between groups at p<0.05