Cow’s milk consumption increases iodine status in women of childbearing age in a randomized controlled trial.

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Abbreviations: ANOVA, Analysis of variance; CRM, Certified reference material; CV, Co-efficient of variance; FT3, Free triiodothyronine; FT4, Free thyroxine; GPx, Glutathione peroxidase; ICP-MS, Inductively Coupled Plasma-Mass Spectrometry; IQR, Interquartile Range; IQR, Intelligence quotient; ISO, International Organization for Standardization; RNI, Reference Nutrient Intake; LRNI, Lower Reference Nutrient Intake; SELENOP, Selenoprotein P; SPSS, Statistical Package for Social Sciences; TMAH, Tetramethylammonium hydroxide; T4, Thyroxine; T3, Triiodothyronine; TSH, Thyroid stimulating hormone; TUL, Tolerable Upper Limit; TxRF, Total reflection x-ray fluorescence; UIC, Urinary iodine concentration; WHO, World Health Organization.
ABSTRACT

Background  Recent evidence has highlighted the prevalence of mild-to-moderate iodine deficiency in women of childbearing age and pregnant women, with important public health ramifications owing to the role of iodine, required for thyroid hormone production, in neurodevelopment. Cow’s milk contributes the greatest amount to iodine intakes in several countries.

Objective The objective of this study was to investigate the effect of increased cow’s milk consumption on iodine status, thyroid hormone concentrations and selenium status.

Methods A 12 week, randomized-controlled trial was conducted in 78 low-moderate milk consuming (<250ml/d) healthy women (18-45 years). The intervention group were asked to consume 3L of semi-skimmed milk per week, while the control group continued their usual milk consumption (baseline median (IQR): 140 (40-240) mL/d). At baseline, week 6 and week 12 participants provided a spot-urine sample [urinary iodine concentration (UIC); creatinine] and a fasting blood sample [thyroid hormone concentrations; serum total selenium; selenoprotein P]. This study was registered at ClinicalTrials.gov Study (Ref: NCT02767167).

Results At baseline, the median (IQR) UIC of all participants was 78.5 (39.1-126.1)µg/L. Changes in the median UIC from baseline to week 6 (35.4 vs. 0.6 µg/L; P=0.014) and week 12 (51.6 vs. -3.8 µg/L; P=0.045) were significantly greater in the intervention group compared with the control group. However, despite being higher within the intervention group at weeks 6 and 12, the change in the iodine:creatinine ratio from baseline was not significantly different between groups at either week 6 (P=0.637) or 12 (P=0.178). There were no significant differences in thyroid hormone concentrations or selenium status between groups at any time point.

Conclusions The present study has demonstrated that the consumption of additional cow’s milk can significantly increase UIC in women of childbearing age. These results suggest that cow’s milk is a potentially important dietary source of iodine in this population group.

KEYWORDS: Iodine; women of childbearing age; milk; randomized-controlled trial; iodine deficiency; selenium
INTRODUCTION

Iodine is an essential micronutrient required for the production of the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), which are critical for human growth and neurodevelopment [1, 2]. Selenium is required for the conversion of T4 to the metabolically active T3 hormone and is thus considered a co-nutrient in the study of thyroid function [3]. Iodine requirements are greater in pregnancy owing to an increased rate of thyroid hormone synthesis, renal clearance and iodine transfer to the fetus [4, 5]. The consequences of poor iodine status during pregnancy range from reduced education [6] and intelligence quotient (IQ) [7], observed with mild deficiency, to the irreversible mental retardation of cretinism linked with severe deficiency [8, 9]. The World Health Organization (WHO) therefore recommends 250µg/d for pregnant and breastfeeding women and the recommended nutrient intake (RNI) for adults, including women of childbearing age, is 150µg/d [10].

Urinary iodine concentration (UIC) is measured to assess iodine nutrition in a population with up to 90% of dietary iodine excreted in the urine. A median UIC of less than 100µg/L in the general population is indicative of iodine deficiency [10], whilst in pregnancy a median UIC below 150 µg/L indicates deficiency in the group [10]. Ensuring women enter pregnancy with sufficient iodine stores in the thyroid is an issue of significant public health importance. Yet deficiency remains a concern worldwide and there are numerous reports of insufficient iodine status (UIC ≤100µg/L) amongst women of childbearing age in industrialized countries, including the US where iodized salt is available [11-15].

Milk and dairy foods provide the greatest contribution to total dietary iodine intakes in the US, Denmark, Norway, the UK and Ireland [16-20], with milk contributing 33% of total intakes in the UK adult population [21]. It has been suggested that a general decline in milk consumption in the UK may be contributing to the increased prevalence of mild-to-moderate iodine deficiency observed there.
in recent years [12, 22, 23]. Observational evidence has consistently shown milk consumption to be positively associated with iodine intake and status, including in the US [11, 24-28]. However, no randomized-controlled trial has yet examined the effect of pasteurized cow’s milk consumption on iodine status. The possibility of increasing iodine intake by increasing milk consumption, an inexpensive and widely-available foodstuff, in a population group vulnerable to iodine deficiency is warranted.

The primary aim of this study was to investigate the effect of increased cow’s milk consumption on iodine status, as measured by UIC. The secondary aims were to investigate the effect of milk consumption on thyroid hormone concentrations and selenium status.

METHODS

Subjects and study design

The study was a 12 week, randomized controlled dietary intervention in 78 healthy women of childbearing age conducted at Ulster University, Coleraine between July and December 2015. Recruitment targeted females aged between 18 and 45 years, whose milk consumption was reported to be habitually low-moderate (consumption of ≤250ml of milk per day). Exclusion criteria included smokers, those with a history of thyroid or gastrointestinal conditions, use of thyroid medication, lactose intolerance, milk allergy, non-milk consumption, vegan diet and those consuming dietary supplements containing iodine or selenium within the 3 months prior to the study. Further exclusion criteria included women who were pregnant, breastfeeding or planning to become pregnant during the study and those who were deemed peri- or post-menopausal. Written informed consent was obtained from eligible participants. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by
the Ulster University Research Ethics Committee (Ref: REC/15/0042). The study was registered on ClinicalTrials.gov (Ref: NCT02767167).

The *a priori* sample size was calculated using GPower version 3.1 [29] with UIC as the primary outcome between groups. A total sample size of 70 was required to detect (or not) an increase of 30% from a population median of UIC (80.1μg/L); changing iodine status from inadequate to sufficient (*α*=0.05, *β*=0.80, effect size=0.30) [26]. A further 10% was added to account for study attrition.

An independent clinical trials manager randomly allocated eligible participants to intervention or control group using MINIM randomization software [30]. Randomisation was stratified by median baseline milk consumption (< or > the median intake of 120mL/d) with an allocation ratio of 1:1 to ensure groups were balanced with respect to their habitual milk consumption. Participants in the intervention group were provided with 3 L of pasteurized semi-skimmed milk weekly and were required to consume this evenly across the week (approximately 430mL/d) as they wished (i.e. as a glass of milk, in a milkshake, or in tea or coffee), whilst continuing with their usual dietary habits and lifestyle. Prior to the analysis of milk used in the intervention study, it was anticipated that consumption of 430mL/d of intervention milk would provide an additional 133μg/d iodine (92% of the UK RNI (140μg/d) [31] based on an expected milk iodine concentration of 310μg/L [32]. Those in the control group were not provided with additional milk and were asked to continue with their usual milk consumption, dietary habits and lifestyle. Milk used in the intervention was purchased at retail and was therefore of similar composition to that habitually consumed by participants prior to intervention, and by the control group, throughout the intervention period. Each participant completed an appointment at baseline (week 0), week 6 and week 12 when anthropometric measurements, dietary intake data, urine and blood samples were collected.
Blood & urine samples

Participants provided a non-fasted 10 ml spot-urine morning sample at weeks 0, 6 and 12. Samples were collected in sterile tubes free from preservatives. Aliquots of each sample were taken and samples were stored at -80°C until batch analysis at the end of the study. A 30ml fasting blood sample was collected at weeks 0, 6 and 12 by a trained phlebotomist. Blood samples were processed within 4 hours of collection. Serum aliquots were obtained by centrifuging whole blood at 1370 x g for 15 minutes at 4°C. Prepared aliquots were stored at -80°C until batch analysis at the end of the study.

Anthropometric measurements

Standing height (cm) was measured to the closest 0.1cm at baseline using a calibrated stadiometer (SECA, Hamburg, Germany). Weight (kg) was measured to the nearest 0.1kg without heavy clothing or footwear at each time point using calibrated scales (SECA, Brosch Direct Ltd, Peterborough, UK). Body mass index (BMI) was calculated as weight (kg) / height² (m²). All measurements were made by the same researcher using the same equipment across all time points.

Dietary intake

At each time point, participants completed a 24hr dietary recall which was analyzed using Nutritics (Dublin, Ireland). The 24hr dietary recalls were used to estimate milk intakes in order to assess compliance with the intervention.

Biochemical measurements

UIC was measured in spot-samples by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) at University Hospital Southampton [7]. Iodine status of the group was classified according to WHO criteria, where a median UIC of <100µg/L indicates risk of iodine deficiency [10]. All samples were measured in duplicate and the accuracy of results was verified with a certified reference material.
Urinary creatinine (Cr) concentration was also measured at University Hospital Southampton by the Jaffe rate method [7,33]. The iodine-to-creatinine ratio was calculated to adjust for intra-individual variation in urine volume [10, 34, 35].

Serum thyroid hormone concentrations were measured to assess thyroid function. Free concentrations of T3 (FT3), T4 (FT4) and TSH were analyzed using a Roche Cobas 8000 modular analyzer (Biochemistry Laboratory, Northern Health and Social Care Trust, Antrim). Normal reference values used were as follows: thyroid stimulating hormone (TSH), 0.27–4.2mIU/L; FT3, 3.5–6.8nmol/L; FT4, 12.0–22.0pmol/L [36].

Two markers of selenium status were measured by the Charité-Universitätsmedizin Berlin. Serum total selenium concentrations were measured by X-ray fluorescence, using a bench-top total reflection X-ray fluorescence (TXRF) spectrometer (PicofoxS2, Bruker Nano GmbH, Berlin). For the purposes of this study, inadequate selenium status was classified as those with a serum selenium concentration of <70µg/L [37]. We also measured concentrations of serum selenoprotein P (SELENOP), a functional measure of selenium status [38], by a sandwich assay (Selenotest, ICI GmbH, Berlin, Germany).

Milk analysis

Weekly samples of the intervention milk (n= 21) were collected and stored at -20°C until their analysis for iodine and selenium concentrations by LGC Limited (Teddington, Middlesex, UK). For selenium determination, samples were microwave-digested with nitric acid [39] and for iodine determination, samples were oven-digested with tetramethylammonium hydroxide (TMAH) prior to their transport to LGC Limited [39]. Samples were analyzed by inductively coupled plasma MS (Element2; ThermoFisher Scientific, Bremen, Germany) as described previously [40]. The uncertainty of the method for iodine and selenium analysis was calculated as ±10% and ±15% respectively using International Organisation for Standardisation (ISO) 17 025 methodology.
Accuracy of the results was verified using a certified reference material (ERM-BD150; LGC Standards, Teddington, UK).

Statistical analysis

Statistical analysis was undertaken using Statistical Package for Social Sciences (SPSS) for Windows (Version 22.0; IBS SPSS Statistics). Results reported are based on intention-to-treat (last observation carried forward) analysis. Normality was assessed using the Kolmogorov-Smirnov test. The UIC, iodine:creatinine and SELENOP data were not normally distributed and therefore we reported medians and the IQR. Differences between groups were assessed using Independent T-tests for parametric data or Mann-Whitney U-tests for non-parametric data. We used the Wilcoxon Signed Rank Test to examine changes within each group over the intervention. To compare the proportion of participants with low iodine or selenium status (UIC <100µg/L; selenium <70µg/L) between groups the Fishers’ Exact test was used due to small numbers in each group. The one-way ANOVA test was used to test changes in the iodine and selenium concentrations in milk samples over the intervention period. Spearman rank correlations were used to explore the relationship between milk intake and iodine status. A P-value of <0.05 was considered statistically significant throughout.

RESULTS

An overview of the recruitment and flow of participants through the study, according to CONSORT guidelines [41], is outlined in Figure 1. Of the 78 participants who were recruited, five withdrew (n=2 intervention group; n= 3 control group). Intention to treat analysis was conducted for all 78 participants. There were no differences in any characteristic at baseline for participants who withdrew from the study and those who completed the study. Baseline characteristics of intervention (n= 39) and control (n= 39) participants are shown in Table 1. The median (IQR) age of participants at baseline was 26.5 (21.8-33.0) years. There were no significant differences with respect to any subject
characteristic, including UIC ($P=0.393$), iodine:creatinine ratio ($P=0.842$) and daily milk
consumption ($P=0.539$), between the intervention and control groups at baseline. Using data from the
24hr dietary recall, median (IQR) daily milk intakes were estimated to be 140mL/d (40-240),
130mL/d (80-240) and 120mL/d (80-225) in the control group at weeks 0, 6 and 12 respectively. In
comparison, the intervention group consumed an estimated 120mL/d (40-200), 340mL/d (120-425)
and 260mL/d (150-420) at weeks 0, 6 and 12 respectively.

**Iodine status**

UIC was significantly higher in the intervention group after weeks 6 and 12 compared to the control
group ($P=0.005$ and $P=0.026$ respectively) (**Table 2**). Changes in UIC from baseline to weeks 6
(median: 35.4 vs. 0.6µg/L, $P=0.014$) and 12 (51.6 vs. -3.8µg/L, $P=0.045$) were also significantly
greater in the intervention compared to the control group. The iodine:creatinine ratio was higher in
the intervention group than the control group at weeks 6 ($P=0.10$) and 12 ($P=0.14$), but these
differences were not significant. However, the change in iodine:creatinine from baseline to week 6
was almost significantly higher in the intervention compared to the control group (36.7 vs. -4.0µg/L,
$P=0.061$). Within the intervention group, both UIC and iodine:creatinine were significantly greater
at weeks 6 (UIC $P=0.02$; iodine:creatinine $P=0.01$) and 12 when compared to baseline (UIC $P=0.007$;
iodine:creatinine $P=0.004$). However there were no significant differences in either UIC or
iodine:creatinine within the control group when baseline values were compared to week 6 (UIC
$P=0.84$; iodine:creatinine $P=0.94$). When baseline values were compared to week 12 there was no
significant difference in UIC within the control group ($P=0.49$) and although, there appeared to be
greater iodine:creatinine ratio, this was non-significant ($P=0.11$). At baseline, 62% of participants in
the intervention group had a UIC $<100\mu g/L$ which significantly reduced to 38% following the
intervention ($P=0.042$). There was no significant change in the proportion of participants with UIC
$<100\mu g/L$ in the control group between baseline (62%) and week 12 (61%) ($P=0.838$).
For all participants, reported milk intake was significantly positively associated with the iodine:creatinine ratio at baseline ($\rho=0.27; P=0.018$) and non-significantly associated with the UIC ($\rho=0.19; P=0.09$). Following the intervention, the positive association between iodine:creatinine and reported milk intake remained significant for the intervention group ($\rho=0.464; P=0.004$) but not for the control group ($\rho=0.311; P=0.06$). At baseline, those participants with a reported milk intake above the median (>120ml/d) had a significantly higher urinary iodine:creatinine ratio (median (IQR): 91.4 (67.2-117) vs. 70.4 (54.7-110)µg/g; $P=0.04$), with no association with the UIC (72.2 (29.6-111) vs. 91.8 (39.6-149) ($P=0.184$), when compared to those with lower milk intake (≤120mL/d). Reported milk consumption in the intervention group at week 6 was 340mL/d and at week 12, 260mL/d, but there was no significant difference between time points ($P=0.791$).

Thyroid hormones and selenium status

At baseline, 97% ($n=76$), 100% and 96% ($n=75$) of participants had a TSH, FT4 and FT3 concentrations within the normal range, respectively. The milk intervention had no effect on any thyroid hormone parameter, with no significant differences in TSH ($P=0.478$), FT3 ($P=0.938$) or FT4 ($P=0.921$) between groups at week 12. (Table 2). Neither was there a significant difference between groups in serum selenium ($P=0.934$) or SELENOP concentrations ($P=0.540$) at week 12 (Table 3).

The mean serum selenium concentration in all groups at baseline was 77.9µg/L and 27% ($n=21$) of participants had inadequate selenium status (<70µg/L). Of these participants, 17% also had a UIC <100µg/L. There were no significant differences between groups in the proportion of participants with a low serum selenium status (<70µg/L) at baseline ($P=0.31$) or at study completion ($P=0.80$).
Iodine and selenium composition of milk

The median (IQR) iodine concentration of intervention milk was 746 (715-790)µg/L and the median (IQR) selenium concentration of intervention milk was 19.9 (18.6-21.1)µg/L. There was no significant difference in the iodine ($P= 0.15$) or selenium concentration of intervention milk when compared across month of collection ($P= 0.22$) (Supplemental Table 1).

DISCUSSION

To the authors’ knowledge, this is the first randomized-controlled trial to investigate the effect of cow’s milk consumption on iodine status. Results show that, following the provision of an additional 3L/wk, an increased daily consumption of cow’s milk significantly increased UIC compared to the control group who maintained their habitual low-moderate intake of milk. The women of childbearing age in our cohort were classed as mildly iodine deficient at baseline according to their median UIC of 78.5 (39.1-126)µg/L. Previous studies have also reported iodine deficiency amongst women of childbearing age in the UK and Ireland [11, 42, 43]. However data from the recent National Diet and Nutrition Survey (NDNS) suggest that, as a whole, UK women of childbearing age (16 – 49 years) have adequate iodine status with a median (20th, 80th percentile) UIC of 117 (65, 198)µg/L [44]. Despite offering population-wide data, based on just one spot urine sample the NDNS data cannot reliably inform on the prevalence of deficiency in this subgroup and it has been noted that dietary data, collected in the same survey, do not support the suggestion of adequate iodine nutrition [44, 45]. It is therefore possible that a considerable proportion of women in the UK may potentially enter pregnancy with low iodine stores [45]. This is particularly worrisome as recent research has shown that even mild-to-moderate iodine deficiency during pregnancy is associated with lower IQ and reading ability of children aged 8 – 9 years [7]. In the US, where salt iodization is mandatory and population UIC has been monitored since 1971, women of childbearing age are in general thought to
have sufficient iodine status [46, 47]. However fluctuations have been noted over the years and attributed to changes in agricultural practices affecting iodine concentrations of the milk [15, 48].

The present intervention was shown to significantly increase median UIC from week 0 to week 12 in the intervention group and to increase the iodine:creatinine ratio, albeit non-significantly (P>0.05). Similar increases in iodine status have been achieved in a two-week encapsulated seaweed intervention study in a group of women aged 18-50 years [43]. Although seaweed is a source of iodine, its content is highly variable and there is concern that consumption of seaweed in large amounts may lead to intakes above the tolerable upper limit (TUL) [49, 50]. Furthermore, iodized salt is not widely available in countries where salt iodization is not mandatory, such as the UK [51] and therefore is unlikely to provide a reliable source of iodine to vulnerable groups. Previous research in the US has indicated that the iodine concentration of dietary supplements is highly variable (range: 0-300µg/d) [52, 53] and that there is a low consumption of iodine-containing supplements among women of childbearing age [54-56]. Milk is therefore the most important source of iodine in many countries [16, 17, 19, 44], and the current study shows that increasing milk consumption is an effective way of increasing iodine intake as assessed by UIC. We have previously reported that women of childbearing age have poor knowledge of the dietary sources of iodine [57]. Therefore public-health strategies to address iodine deficiency could focus on educating young women on the importance of receiving adequate iodine pre-conception and on raising awareness of cow’s milk as a significant dietary source.

It is estimated that 6 billion people worldwide consume milk and although consumption is higher in developed countries, the gap with developing countries is narrowing [578]. The latest dietary survey data on the UK population data show that 73% of women (aged 19-64 years) are consumers of semi-skimmed milk with a median intake of 95g/d [44]. In general, figures show milk consumption is on the decline, particularly amongst young girls and teenagers who are the most vulnerable to the
consequences of iodine deficiency [59]. One reason may be that this age group perceive milk to be a high-fat food. Conducting qualitative research could provide further insight into the potential barriers to increasing milk consumption in women of childbearing age. This will help to inform education campaigns necessary to highlight the nutritional value of milk to the diet.

Iodine and selenium are known to interact, in that selenium is a constituent of the enzymes that convert T4 to the metabolically active T3 hormone [3]. Previous observational evidence has suggested that selenium deficiency may hinder efforts to address iodine deficiency [60-62]. In the present study, baseline median serum selenium concentrations were in the replete range (70-100µg/L) where most selenoproteins including GPx3 show maximal activity [63]. Although SELENOP is indicative of the functional selenium body pool, there are no established reference ranges [38]. No changes in selenium status, as measured by the two biomarkers, were observed between groups as a result of the increased milk consumption. It is possible that this is owing to participants having sufficient selenium status at baseline coupled with the fact that the intervention milk was estimated to provide a minor amount of selenium at 8.5µg/day (14% of the RNI of 60µg/day for adult women) [31]. In addition, the sample size for this study was calculated for the primary outcome measure, UIC and as such, there was not sufficient power to reliably examine subgroup effects of the milk intervention on selenium status.

In this study we measured UIC from spot urine samples as recommended by WHO as our primary outcome [34]. We also adjusted for creatinine to remove some of the intra-individual variation in urine volume. It was unexpected therefore, that we did not observe a significantly higher iodine:creatinine ratio in the intervention group in conjunction with the UIC. It is possible that this is owing to a combination of reduced compliance, particularly in the second half of the intervention, and the possibility that participants in the control group may have unknowingly increased their milk consumption as a result of taking part in this study.
Thyroid hormone concentrations were measured as a measure of thyroid function. Serum concentrations of thyroid hormones are tightly regulated by TSH from the pituitary gland and are maintained within relatively narrow limits, owing to this tight homeostatic regulation [64]. The milk intervention had no effect on thyroid hormone concentrations, as anticipated, as almost all participants had thyroid hormone concentrations within the reference ranges at each time point. It is believed that a healthy replete adult has a body store of approximately 15-20mg iodine, of which the thyroid stores 70-80%. It is important to consider the possibility that, for individuals with a low iodine status at baseline, the milk intervention may have repleted intrathyroidal iodine stores, and thereby exerted a beneficial impact on thyroid function which was not detectable with our range of biomarkers.

Using data from UK food composition tables, we estimated that the additional 430mL/d of semi-skimmed milk would provide approximately 133µg/d of additional iodine [21]. However, based on the median iodine concentration measured in the intervention milk (n=22 samples purchased in Northern Ireland between July and December 2015) this figure was found to be much greater, at 321 µg/d iodine from the additional milk. It is possible that the values in UK food composition tables are inaccurate owing to a limited analysis of samples, which may have resulted in our projected intake of 133µg/d being underestimated. The overall median milk concentration of 746.1µg/L iodine is considerably higher than that reported in previous studies for semi-skimmed milk [65, 66, 40] and the exact reasons for this are not clear from our data. There were no significant differences in the iodine concentration of intervention milk analyzed over the study period. However, it is widely known that the iodine content of milk is highly variable as a result of variation in agricultural practices, soil content and season [8, 17, 67, 68]. An extensive investigation into the factors contributing to this variation is warranted to more accurately estimate its contribution to population dietary intakes in the UK and Ireland. It must be taken into account that the iodine concentration of the intervention milk in this study is specific to the agricultural practices of this region, and that
different results may be obtained with the consumption of milk of a lower iodine concentration and
indeed, within a different season.

In the present study participants were provided with and asked to consume 430mL/d of milk. Similar
levels of compliance were reported in a previous milk intervention study [69]. However it is possible
that our use of dietary assessment as a measure of compliance is limited; the participants may not
have consumed the same amount of milk each day. However, our results still suggest that it is possible
to improve iodine status, as measured by UIC, through a relatively modest increase in milk intake.
Further research would be important to investigate the response to milk intervention in participants
stratified by baseline iodine status and powered on the iodine:creatinine ratio.

This study has demonstrated that a modest increase in consumption of cow’s milk can increase UIC,
a marker of iodine status, in women of childbearing age, a group vulnerable to the effects of iodine
deficiency. Within the context of a public health strategy designed to reduce the prevalence of iodine
deficiency, an increase in milk consumption could represent an important contribution.

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SMO’K, AJY, LKP, MSM and JJS designed the research; SMO’K and EM conducted the research.
SMO’K, SH, JO’R, DK, CD, DK and LS analysed the data; SMO’K wrote the manuscript; AJY,
LKP, MSM, JJS and SCB contributed to data interpretation and AJY had primary responsibility for
the final content. All authors read and approved the final manuscript.
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Figure legends

Figure 1. CONSORT Flow Diagram depicting the flow of participants through the intervention trial
**Table 1: Baseline characteristics of study participants within each group†**

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 39)</th>
<th>Intervention group (n = 39)</th>
<th>P†</th>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>24 (21-32)</td>
<td>28 (23-33)</td>
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<td><strong>Height (m)</strong></td>
<td>1.7 (1.6-1.7)</td>
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<td><strong>Weight (kg)</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
<td>24.4 (21.7-29.3)</td>
<td>24.6 (22.1-27.9)</td>
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<td><strong>UIC (µg/L)</strong></td>
<td>53.2 (34.8-121)</td>
<td>90.5 (43.7-132)</td>
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<td><strong>Iodine:creatinine (µg/g)</strong></td>
<td>80.4 (58.6-110)</td>
<td>70.3 (59.0-116)</td>
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<td><strong>Serum TSH (mIU/L)</strong></td>
<td>2.1 (1.4-2.5)</td>
<td>1.8 (1.3-2.5)</td>
<td>0.481</td>
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<td><strong>Serum FT3 (nmol/L)</strong></td>
<td>4.9 (4.6-5.3)</td>
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<td><strong>Serum FT4 (pmol/L)</strong></td>
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<td><strong>Serum FT3: FT4 ratio</strong></td>
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<td><strong>Serum selenium (µg/L)</strong></td>
<td>77.6 (63.9-84.5)</td>
<td>77.3 (70.5-89.6)</td>
<td>0.294</td>
</tr>
<tr>
<td><strong>SELENOP (mg/L)</strong></td>
<td>4.6 (3.9-5.0)</td>
<td>4.7 (4.0-5.3)</td>
<td>0.402</td>
</tr>
<tr>
<td><strong>Estimated milk intake (mL/d)</strong></td>
<td>140 (40-240)</td>
<td>120 (40-200)</td>
<td>0.539</td>
</tr>
</tbody>
</table>

Values are medians (IQR), n = 78

IQR= Interquartile Range, BMI= Body Mass Index, UIC= Urinary iodine concentration, TSH= Thyroid stimulating hormone, FT3= Free Triiodothyronine, FT4= Free Thyroxine, SELENOP= Selenoprotein P

† Differences between groups at baseline assessed by Mann Whitney U Test or Independent T-Test
Table 2: Iodine status of study participants at baseline, week 6 and 12

<table>
<thead>
<tr>
<th>Iodine status measure</th>
<th>Time period</th>
<th>Control Group Median (IQR)</th>
<th>Intervention Group Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UIC (µg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.492</td>
<td>57.6 (36.7-124)</td>
<td>90.5 (43.7-132)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.005</td>
<td>67.7 (35.1-117)</td>
<td>114 (66.5-181)*</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.026</td>
<td>82.9 (44.9-139)</td>
<td>118 (80.5-207)*</td>
</tr>
<tr>
<td>Change in UIC (µg/L)</td>
<td>Baseline – week 6</td>
<td>0.014</td>
<td>0.6 (-30.4-24.0)</td>
</tr>
<tr>
<td>Week 6 – 12</td>
<td>0.756</td>
<td>1.9 (-20.3-42.4)</td>
<td>7.9 (-35.4-65.4)</td>
</tr>
<tr>
<td>Baseline – week 12</td>
<td>0.045</td>
<td>-3.8 (-20.9-47.5)</td>
<td>51.6 (-6.8-116)</td>
</tr>
<tr>
<td>Iodine:creatinine (µg/g)</td>
<td>Baseline</td>
<td>0.721</td>
<td>81.7 (59.1-111)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.104</td>
<td>76.9 (53.1-139)</td>
<td>102 (73.9-149)*</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.141</td>
<td>103 (66.9-129)</td>
<td>121 (81.9-165)*</td>
</tr>
<tr>
<td>Change in iodine:creatinine (µg/g)</td>
<td>Baseline – week 6</td>
<td>0.061</td>
<td>-4.0 (-19.2-41.4)</td>
</tr>
<tr>
<td>Week 6 – 12</td>
<td>0.637</td>
<td>4.2 (-16.1-40.1)</td>
<td>13.2 (-28.3-49.6)</td>
</tr>
<tr>
<td>Baseline – week 12</td>
<td>0.178</td>
<td>12.7 (-12.0-53.4)</td>
<td>29.3 (-16.7-83.9)</td>
</tr>
<tr>
<td>Serum TSH (mIU/L)</td>
<td>Baseline</td>
<td>0.481</td>
<td>2.1 (1.4-2.5)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.238</td>
<td>2.3 (1.5-3.0)</td>
<td>1.8 (1.3-2.5)</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.478</td>
<td>1.9 (1.5-2.6)</td>
<td>1.9 (1.4-2.4)</td>
</tr>
<tr>
<td>Serum FT3 (nmol/L)</td>
<td>Baseline</td>
<td>0.898</td>
<td>4.9 (4.6-5.8)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.400</td>
<td>5.0 (4.6-5.8)</td>
<td>5.0 (4.4-5.3)</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.938</td>
<td>5.0 (4.6-5.3)</td>
<td>4.9 (4.4-5.5)</td>
</tr>
<tr>
<td>Serum FT4 (pmol/L)</td>
<td>Baseline</td>
<td>0.346</td>
<td>15.1 (14.0-16.4)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.935</td>
<td>15.1 (13.8-16.7)</td>
<td>14.8 (13.5-17.1)</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.921</td>
<td>15.3 (13.9-16.7)</td>
<td>15.2 (13.4-16.9)</td>
</tr>
<tr>
<td>Serum FT3: FT4 ratio</td>
<td>Baseline</td>
<td>0.430</td>
<td>0.3 (0.3-0.4)</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.480</td>
<td>0.3 (0.3-0.4)</td>
<td>0.3 (0.3-0.4)</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.744</td>
<td>0.3 (0.3-0.4)</td>
<td>0.3 (0.3-0.3)</td>
</tr>
</tbody>
</table>

Values are medians (IQR), n= 78

IQR= Interquartile Range, UIC= Urinary iodine concentration, TSH= Thyroid stimulating hormone, FT3= Free Triiodothyronine, FT4= Free Thyroxine

*Differences between groups at each time point assessed by Mann Whitney U Test or Independent T-Test

*Significantly different from baseline, P<0.05
<table>
<thead>
<tr>
<th>Selenium status measure</th>
<th>Time period</th>
<th>(P^*)</th>
<th>Control Group Median (IQR)</th>
<th>Intervention Group Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum selenium (µg/L)</td>
<td>Baseline</td>
<td>0.294</td>
<td>77.6 (63.9-84.5)</td>
<td>77.3 (70.5-89.6)</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.726</td>
<td>75.9 (66.0-85.7)</td>
<td>77.4 (67.5-87.3)</td>
</tr>
<tr>
<td></td>
<td>Week 12</td>
<td>0.934</td>
<td>78.5 (67.7-85.5)</td>
<td>78.6 (67.0-84.4)</td>
</tr>
<tr>
<td>SELENOP (mg/L)</td>
<td>Baseline</td>
<td>0.702</td>
<td>4.63 (3.86-5.02)</td>
<td>4.61 (3.98-5.27)</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.277</td>
<td>4.61 (3.94-5.17)</td>
<td>4.57 (3.89-5.09)</td>
</tr>
<tr>
<td></td>
<td>Week 12</td>
<td>0.540</td>
<td>4.49 (3.63-5.13)</td>
<td>4.50 (3.81-5.01)</td>
</tr>
</tbody>
</table>

Values are medians (IQR), \(n = 78\)
IQR= Interquartile Range, SELENOP= Selenoprotein P
* Differences between groups at each time point assessed by Mann Whitney U Test or Independent T-Test
Figure 1.
All samples ($n=22$) July ($n=2$) August ($n=4$) September ($n=5$) October ($n=4$) November ($n=4$) December ($n=3$) $P^\dagger$

<table>
<thead>
<tr>
<th></th>
<th>All samples ($n=22$)</th>
<th>July ($n=2$)</th>
<th>August ($n=4$)</th>
<th>September ($n=5$)</th>
<th>October ($n=4$)</th>
<th>November ($n=4$)</th>
<th>December ($n=3$)</th>
<th>$P^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine (µg/L)</td>
<td>746.1 (714.8-789.9)</td>
<td>745.2 (86.2-745.2)</td>
<td>745.2 (710.0-780.3)</td>
<td>723.7 (702.3-752.3)</td>
<td>746.1 (730.9-755.4)</td>
<td>816.1 (780.5-836.2)</td>
<td>717.9 (659.5-717.9)</td>
<td>0.153</td>
</tr>
</tbody>
</table>
| Selenium (µg/L) | 19.9 (18.6-21.0) | 17.3 (16.4-17.3) | 19.4 (17.0-20.6) | 18.8 (17.1-17.9) | 21.4 (21.1-21.8) | 20.3 (19.7-21.2) | 20.7 (19.3-20.7) | 0.223  

Values are medians (IQR)

$^\dagger$Differences between months assessed using one-way ANOVA.

**Supplemental Table 1:** Iodine and selenium concentration of intervention milk by month of purchase