

A dose-response crossover iodine balance study to determine iodine requirements in early infancy^{1,2}

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ABSTRACT

Background: Optimal iodine intake during infancy is critical for brain development, but no estimated average requirement (EAR) is available for this age group.

Objective: We measured daily iodine intake, excretion, and retention over a range of iodine intakes in early infancy to determine the minimum daily intake required to achieve iodine balance.

Design: In a dose-response crossover study, we randomly assigned healthy infants ($n = 11$; mean \pm SD age 13 ± 3 wk) to sequentially consume over 33 d 3 infant formula milks (IFMs) containing 10.5, 19.3, and $38.5 \mu\text{g I}/100 \text{ kcal}$, respectively. Each IFM was consumed for 11 d, consisting of a 6-d run-in period followed by a 4-d balance period and 1 run-out day.

Results: Iodine intake (mean \pm SD: 54.6 ± 8.1 , 142.3 ± 23.1 , and $268.4 \pm 32.6 \mu\text{g/d}$), excretion (55.9 ± 8.6 , 121.9 ± 21.7 , and $228.7 \pm 39.3 \mu\text{g/d}$), and retention (-1.6 ± 8.3 , 20.6 ± 21.6 , and $39.8 \pm 34.3 \mu\text{g/d}$) differed among the low, middle, and high iodine IFM groups ($P < 0.001$ for all). There was a linear relation between daily iodine intake and both daily iodine excretion and daily iodine retention. Zero balance (iodine intake = iodine excretion, iodine retention = $0 \mu\text{g/d}$) was achieved at a daily iodine intake of $70 \mu\text{g}$ (95% CI: 60, $80 \mu\text{g}$).

Conclusion: Our data indicate the iodine requirement in 2- to 5-month-old infants is $70 \mu\text{g/d}$. Adding an allowance for accumulation of thyroidal iodine stores would produce an EAR of $72 \mu\text{g}$ and a recommended dietary allowance of $80 \mu\text{g}$. This trial was registered at clinicaltrials.gov as NCT02045784. *Am J Clin Nutr* 2016;104:620–8.

Keywords: iodine, infants, iodine requirement, iodine balance, iodine intake, iodine deficiency

INTRODUCTION

Iodine is an essential component of the thyroid hormones, and hypothyroidism caused by iodine deficiency early in life can irreversibly impair neuromotor development (1–3). The thyroxine production rate during early infancy is $\sim 5\text{--}6 \mu\text{g} \cdot \text{kg body weight (BW)}^{-1} \cdot \text{d}^{-1}$,⁷ higher than at any other life stage (4). Infants are born with minimal thyroidal iodine stores (5) and are entirely dependent on dietary iodine, making them particularly vulnerable

to low iodine intakes (1, 5–8). However, data on the daily dietary iodine requirement at this critical age is limited (1, 6, 9–11).

The Institute of Medicine of the US National Academy of Sciences recommends an adequate intake (AI) of iodine of $110 \mu\text{g/d}$ during the first 6 mo of infancy (12). The AI was set based on the median breast milk iodine concentration of $146 \mu\text{g/L}$ measured in a small study of US women in the 1980s, a period when overall iodine intake in the US was excessive (12, 13), and may therefore be set too high.

The cutoff for the median urinary iodine concentration (UIC), the exposure biomarker recommended by WHO to assess iodine status in infants, is $100 \mu\text{g/L}$ (14, 15), but there is little evidence to support this cutoff in infancy. Assuming that the mean daily urine volume in early infancy is $\sim 500 \text{ mL}$ (16, 17) and iodine bioavailability is $\sim 92\%$ (12), a median UIC of $100 \mu\text{g/L}$ would extrapolate to a mean daily iodine intake of $\sim 55 \mu\text{g}$, half the current AI. The discrepancy between the present intake recommendations and the WHO UIC cutoff makes monitoring of population iodine status at this age problematic because interpretation of the 2 measures disagrees.

Assessment of iodine intakes in populations should be based on the estimated average requirement (EAR) and not on the AI (10, 12, 16, 18). Quantitative data are urgently needed to define an EAR in infancy, and this has been highlighted as a research priority (10, 11). Moreover, a well-defined EAR would allow

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² Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁷ Abbreviations used: AI, adequate intake; AIC, Akaike information criterion; BW, body weight; EAR, estimated average requirement; fT4, free thyroxine; IDA, isotope dilution analysis; IFM, infant formula milk; LOD, limit of detection; MC-ICP-MS, multicollector inductively coupled plasma mass spectrometry; MEM, mixed effects model; PP, polypropylene; RDA, recommended dietary allowance; TMAH, tetramethylammonium hydroxide; TSH, thyroid-stimulating hormone; UIC, urinary iodine concentration.

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WHO to set an evidence-based cutoff for the median UIC to be used in monitoring iodine status in infant populations.

The balance study technique is a classic design used to define nutrient requirements. By assessing total nutrient intake and excretion, nutrient retention can be calculated, and the daily intake required to maintain the existing body pool can be determined (19, 20). The EARs for iodine in 1–3- and 4–8-y-old children are based on data from small iodine balance studies (12, 21, 22), and the technique has been used in infants to assess balance for other nutrients (23–28).

Switzerland has a carefully monitored iodized salt program with high household coverage that has assured iodine sufficiency in the Swiss population for many decades (29, 30). The iodine intake in formula-fed weaning infants in a Swiss national study was adequate (30).

The overall objective of the study was to determine the daily iodine intake required to achieve metabolic balance in full-term healthy infants in an iodine-sufficient area. Therefore, we assessed the iodine intake, excretion, and retention over a range of iodine intakes in 2- to 5-mo-old formula-fed Swiss infants. The primary outcome of the study was iodine retention (micrograms per day and micrograms per kilogram BW per day) in iodine-sufficient infants, derived from the difference in measured iodine intake and iodine excretion.

METHODS

Study design

We conducted a randomized, double-blind, dose-response balance study in which exclusively formula-fed infants were assigned to consume an infant formula milk (IFM) containing low, medium, and high iodine content (see below). The 3 IFMs were fed sequentially in random order with a crossover design, each for 11 d over a total study period of 33 d. Each of the three 11-d periods consisted of 6 run-in days followed by a 4-d balance period and 1 run-out day to avoid overlap with the new IFM period. The study was home-based.

Randomization and masking

Using spreadsheet software (Excel; Microsoft Office 2010), we randomly assigned the participants to 1 of 6 possible sequences of administration of the 3 IFMs (block size of 3). The 3 IFMs were packed in identical cans labeled with color codes that did not indicate the type of formula or concentration of iodine. Subjects, investigators, and sponsors were masked to formula assignment.

Subjects

We enrolled a convenience sample of 11 infants from Zurich, Switzerland, and conducted the study between January 2015 and August 2015. Healthy infants were included in the study if they fulfilled the following criteria: 1) 2–4 mo old at entry; 2) exclusively formula-fed; 3) singleton born at full-term (in pregnancy week 38–42); 4) normal birth weight (≥ 2500 g); 5) euthyroid: not hypothyroid [defined as thyroid-stimulating hormone (TSH) ≥ 4.2 mU/L and free thyroxine (fT4) < 15 pmol/L] or hyperthyroid (defined as TSH < 0.1 mU/L and fT4 > 30 pmol/L); 6) normal iron status (ferritin 37–220 $\mu\text{g/L}$) and hemoglobin (95–135 g/L); 7) no infection/inflammation (C-reactive protein < 10 mg/L); 8) no known family history of thyroid disease; 9) no overt gastric reflux; 10) currently not receiving any breast milk, solid food, or iodine-containing dietary supplements; 11) having received infant formula

based on cow milk protein already before the study and showing no symptoms indicating cow milk allergy; 12) no maternal or infant exposure to iodine-containing contrast agent or iodine-containing medication (for the mother, within the last year); and 13) maternal residence in Switzerland ≥ 1 y before delivery and since delivery. Mothers were encouraged to breastfeed their infants and were only asked to participate in the study if they could not or did not intend to breastfeed.

Sample-size calculations were based on the best available estimate of the minimum number of data points required to construct a regression model of iodine retention as a function of iodine intake. In the absence of data on iodine retention in infants, we used the data from an iodine balance study in 18–30-mo-old children and estimated an R^2 of 0.365 for the intake/retention regression (21, 31, 32). We used G*Power (version 3.1.3) (33) for sample-size calculation. A squared correlation coefficient of 0.365 can be detected at $P < 0.05$ with 80% power and an effect size (f^2) of 0.30 with 29 intake/retention observations. Our aim was thus to recruit a minimum of 10 infants to achieve 30 observations (means \pm SDs calculated from 4 balance days \cdot infant $^{-1} \cdot$ dose $^{-1}$).

Ethical permission for the study was obtained from the Ethics Committee of the Canton Zurich (KEK-ZH-Nr. 2014-0271) and from the Ethics Committee of the ETH Zurich (EK 2013-N-21). Parents gave informed written consent before participation in the study and received a symbolic reimbursement at the end of the trial. The study was registered at clinicaltrials.gov as NCT02045784.

Study diet

The study IFM contained 3 concentrations of iodine based on the AI (110 $\mu\text{g/d}$) for 0–6-mo-old infants and aimed to supply 55% (60 $\mu\text{g/d}$), 100% (110 $\mu\text{g/d}$), and 200% (220 $\mu\text{g/d}$) of the AI/d, respectively. The iodine fortification levels were set at 10.5 $\mu\text{g}/100$ kcal, 19.3 $\mu\text{g}/100$ kcal, and 38.5 $\mu\text{g}/100$ kcal, respectively. Iodine was added to the IFM powder as potassium iodide (DSM Nutritional Products Ltd.). The actual iodine content of the ready-to-drink IFM was measured and confirmed by multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) (see below) (34).

The IFM was specifically produced for the study by HOCHDORF Swiss Nutrition Ltd. by use of an iodine-free vitamin and mineral mix supplied by DSM Nutritional Products Ltd. It was a cow milk-based formula (proteins 1.4 g/100 mL, whey/casein 60/40). The study IFM conformed to the commercial premium infant formula for 0–6 mo by the same producer (Hero Baby 1: brand owner, Hero Ltd.), except for the iodine content. The total nutrient composition, as well as the tested iodine contents of the study IFMs, complied with European Community directives on infant formula and follow-on formula (minimum 10 μg I/100 kcal and maximum 50 μg I/100 kcal) (35).

Study procedures

Recruitment was done through posters at local health clinics and pediatric practices. We asked the parents and their infants to attend a clinic appointment before the start of the study, during which we explained the study protocol and obtained written informed consent from the parents. We measured each infant's length and weight and obtained venous blood samples from the infants.

During home visits, we provided the IFM, along with bottled water and detailed instructions for the IFM preparation. At the beginning of each 11-d study sequence, we supplied the new IFM to the home and the remaining IFM of the last 11-d sequence was collected. At the end of the study, we collected all unused materials and measured the infants' weight.

During each of the three 4-d balance periods (4×24 h), the parents recorded all IFM consumed by the infant in a daily diary. The parents were asked to weigh the ready-to-drink infant formula bottle before and after each feeding using the provided calibrated scales (Kern EMB1200-1, KERN) ($d = 0.1$ g). Parents used single-use bibs, which were weighed before and after each feeding to assess formula milk spillages. An IFM sample was collected from each feeding. Samples were cooled immediately in the participant's refrigerator and collected by the study team on a consecutive day. For each study day, one daily pooled IFM sample was generated by the study team from equal parts of the IFM samples collected from each individual feeding of that study day and stored at -25°C at the Human Nutrition Laboratory of the ETH Zurich until analysis.

During each 4-d balance period (4×24 h), all soiled diapers and cleaning tissues were collected. Parents were asked to record any losses of urine or feces in the infants' clothing or bedding in a daily diary. Parents were also instructed not to bath their baby during the balance days to avoid potential losses of urine in the bath water. All diapers, cleaning tissues, and baby lotions were provided to the participants for the whole study period. All materials used in the study were tested for iodine contamination before the study.

Assessment of iodine intake

The IFM consumption during each 4-d balance period was assessed from the weight of the IFM bottles, which was recorded by the parents before and after each feeding. The iodine intake per feeding and over 24 h was calculated from the amount of IFM consumed and the measured iodine concentration in the collected IFM samples.

The iodine concentration in the IFM samples was analyzed by MC-ICP-MS by use of isotope dilution analysis (IDA) with ^{129}I and tellurium for mass bias correction (34). Sample preparation was done by use of tetramethylammonium hydroxide (TMAH; Tama) for iodine extraction at 90°C for 180 min. Whole milk reference powder (NIST SRM1549a whole milk powder; National Institute of Standards and Technology) was used as an external control. The mean \pm SD iodine concentration of the standard reference material ($n = 13$) was 3342 ± 51 $\mu\text{g/kg}$, well in agreement with the certified acceptable range (3040–3640 $\mu\text{g/kg}$). The CV was 2% ($n = 13$).

Assessment of iodine excretion

Total daily iodine excretion in urine and feces was assessed by complete collection of all soiled infant diapers and cleaning tissues during each 4-d balance period. The iodine content was analyzed after alkaline extraction by MC-ICP-MS by use of IDA with ^{129}I and tellurium for mass bias correction.

Iodine extraction.

Iodine was extracted from the used diapers by use of microwave-assisted extraction with TMAH. The outer plastic and unsoiled outer

edges of each diaper were removed. The diaper was then cut in small pieces, the sample weight was recorded, and the pieces transferred into a blender (Kenwood BL770). Ultrapure water (≥ 18.2 M Ω cm, Barnstead E-Pure, Thermo Scientific) was added to the blender to achieve a total weight of 1000 g, and the exact weight was noted. The diaper pieces were homogenized. An aliquot of 1 g of the homogenate was sampled into a microwave extraction system Teflon vessel (Multiwave GO, Anton Paar), and the exact weight was noted. Iodine was extracted by adding 1.0 mL TMAH (25%) (Tama pure-AA TMAH 25%) to each vessel, followed by 6.0 mL of ultrapure water. The Teflon vessels were placed into the microwave extraction system, and the samples were heated up to 180°C within 10 min. The temperature was then kept at 180°C for 10 min, before the samples were cooled down to 40°C . The Teflon vessels were removed from the system and carefully opened in a laminar flow hood. The content was transferred into graduated 50-mL disposable polypropylene (PP) tubes with screw cap (Sarstedt). Each vessel was rinsed with 2.0 mL of ultrapure water, and the rinse water was added to the respective 50-mL PP tubes, resulting in a final volume of 10 mL with a TMAH concentration of 2.5%. To each sample, 50 μL of a 10 mg/L tellurium spike solution (AppliChem) and 50 μL of a 1.3 $\mu\text{g/g}$ ^{129}I spike solution (NIST SRM 4949C ^{129}I radioactivity standard, Standard Reference Material 4949C; National Institute of Standards and Technology) were added, as recently described (34). The tubes were vortexed before overnight sedimentation at room temperature. On the next day, the samples were centrifuged (Mistral 1000, MSE) for 10 min at 3500 g at ambient temperature. By using 20-mL syringes (BD Discard II, Becton Dickinson), the supernatants were filtered through disposable syringe filters (Chromafil GF/RC-45/25, pore size: 1.0/0.45 μm , Macherey-Nagel) into graduated 15-mL PP tubes with screw cap (Semadeni) and stored at 4°C until analysis.

Iodine analysis.

The iodine content in the samples was measured by MC-ICP-MS (Finnigan NEPTUNE high resolution double focusing MC-ICP-MS; Thermo Scientific) by applying IDA with ^{129}I for quantification and tellurium for mass bias correction (34). Each run in the microwave extraction system consisted of 10 diaper samples, a blank sample (ultrapure water), and a control diaper sample for quality control.

Quality control.

Control diapers were prepared by adding 10 mL of laboratory specific control urines with UICs of 2173 and 4850 $\mu\text{g/L}$ to infant diapers. The UIC of the control urines was measured by the Pino et al. (36) modification of the Sandell-Kolthoff method. Our laboratory is certified by the Program to Ensure the Quality of Urinary Iodine Procedures (Centers for Disease Control and Prevention) and participates successfully in its quarterly external validation. The control diapers were measured in the same way as the collected infant diapers. The expected iodine content in the control diapers was 21.7 and 48.5 μg , respectively. Measured mean \pm SD iodine content was 21.4 ± 2.3 μg ($n = 41$) and 44.0 ± 2.0 μg ($n = 88$), equivalent to mean \pm SD recoveries of $99\% \pm 11\%$ and $91\% \pm 4\%$ with a CV of 11% and 5%, respectively.

The method was validated with respect to recovery, variability, and limit of detection (LOD) ahead of the human study. Laboratory-specific urine control samples with a UIC of 25 $\mu\text{g/L}$ were spiked with a 100 mg/L iodide solution from analytic grade potassium

iodide (Riedel de Haën, Sigma-Aldrich). The spiked urine control samples (40 mL) were applied to infant diapers to obtain a final iodine content in the diapers of 17 μg ($n = 2$), 25 μg ($n = 3$), 38 μg ($n = 2$), 46 μg ($n = 3$), 68 μg ($n = 2$), 93 μg ($n = 3$), and 119 μg ($n = 2$), respectively. The iodine concentrations of the spiked urine samples were verified by use of the Pino et al. (36) modification of the Sandell-Kolthoff method. The diapers were processed with spiked urine, and their iodine content was analyzed, as described above. The overall mean \pm SD iodine recovery for all control diaper samples was $98\% \pm 7\%$.

The interassay variation was determined by preparing and analyzing 3 individual diapers with 4 different iodine contents (25, 46, 93, and 180 μg /diaper). The intra-assay variation was determined by analyzing triplicates from one diaper for each iodine level. The interassay variability was 0.6% ($n = 12$) and the intra-assay variability was 0.9% ($n = 12$). The LOD of the method was determined by analyzing 10 unused diapers. The mean \pm SD iodine content was 0.7 ± 0.6 μg /diaper, resulting in a LOD of 2.4 μg iodine/diaper.

Biochemical measurements

Serum ferritin was measured by ELISA by use of Orgentec ferritin kits (ORG 5FE; Orgentec). TSH and fT4 were measured by use of Tosoh AIA-600 II Automated Immunoassay Analyzer (TSH and fT4 Assays; Tosoh). Hemoglobin was analyzed by use of Sysmex XN-1000 automated hematology analyzer (Sysmex). C-reactive protein was analyzed by rate turbidimetry by use of Beckman UniCel Dx C 600 (Beckman Coulter).

Statistical analysis

We used EXCEL (2010; Microsoft), IBM SPSS Statistics software (version 22; IBM) and R statistical programming environment (version 3.2.3) (37) by use of the packages nlme (38) and ggplot2 (39) for data processing and analysis. The primary outcome measure was iodine retention, calculated for each infant and balance day as the difference between measured daily iodine intake and measured daily iodine excretion. One data point for iodine intake, excretion, and retention (one balance day of subject ID 9 for the high dose) was removed as an outlier based on visual inspection, leaving 131 data points for data analysis. Mean \pm SD iodine intake, excretion, and retention were calculated for each infant from data obtained for the 4 balance days for each of the 3 doses and the individual mean value thus calculated. Data on iodine intake, excretion, and retention are expressed in $\mu\text{g}/\text{d}$, as well as in $\mu\text{g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$, with the latter for consistency with other infant balance studies (25–28).

Data were examined for normality by use of the Shapiro-Wilk test. All data were normally distributed. Data on iodine intake, excretion, and retention also fulfilled the condition of sphericity (for the 3 doses), as tested by Mauchly's test. All data are presented as means \pm SDs. Associations between the subject characteristics at baseline and iodine intake, iodine excretion, and iodine retention were assessed by using Pearson's correlation coefficient (r). Differences in iodine intake, excretion, and retention among the 3 doses were compared by repeated-measure ANOVA by use of Bonferroni correction for multiple comparisons.

We assessed the dose-response relation between daily iodine intake and iodine excretion as well as between daily iodine intake and iodine retention by fitting mixed effects models (MEMs) for the

micrograms per day and micrograms per kilogram per day data, with individual daily iodine intake as fixed factor and participant as random factor. Baseline hemoglobin concentration correlated with iodine retention for all 3 iodine doses and was thus also included as a fixed factor in the MEMs. Each subject provided 4 data points (daily values from 4 balance days) per parameter and per dose. Based on visual inspection of the locally weighted scatter plot smoothing lines, we tested linear models for the relation between intake and excretion and linear, as well as logarithmic models for the relation between intake and retention. The residuals were tested for normality. The final model selection was based on a likelihood ratio test, i.e. the Akaike Information Criterion (AIC). The model was also evaluated by using goodness-of-fit plots and Pearson's correlation coefficient for the observed and predicted data. Significance was set at $P < 0.05$.

The final MEMs were used to obtain predicted iodine excretion and predicted iodine retention (micrograms per day and micrograms per kilogram per day) for each individual intake ($n = 131$). Mean \pm SD predicted excretion and retention of 4 balance days were calculated per infant and per dose. The agreement between observed and predicted values for iodine excretion and retention was evaluated by use of Pearson's correlation coefficient. The predicted data were analyzed in the same way as the measured excretion and retention (see above).

Zero balance (iodine intake = iodine excretion, iodine retention = 0 $\mu\text{g}/\text{d}$) was obtained from the MEM of iodine intake compared with iodine retention micrograms per day and micrograms per kilogram per day, the former used to estimate a proposed EAR). We compared the MEM of iodine intake compared with iodine retention to a linear model of iodine intake compared with iodine retention (the latter does not account for repeated measures) and derived the SD of the iodine retention.

RESULTS

The measured mean \pm SD iodine content in the ready-to-drink low, medium, and high iodine IFM was 6.1 ± 0.5 , 16.4 ± 1.8 , and 30.0 ± 2.3 $\mu\text{g}/100$ g, respectively ($n = 44$ in each group). In total, 11 infants (5 girls and 6 boys) were screened and enrolled, and all completed the study. All infants were of Caucasian descent, except one infant whose father was of Asian origin; the mothers represented 5 different European nationalities. No adverse events were reported during the trial.

Baseline characteristics of the participants are shown in **Table 1**. At baseline, 2 infants showed isolated mild elevated TSH ($4.2 < \text{TSH} < 5.1$ mU/L), and 7 infants showed isolated mild low fT4 ($15 > \text{fT4} > 11.8$ pmol/L). However, we confirmed a normal total triiodothyronine value for the 3 infants with the lowest fT4 ($11.8 < \text{fT4} < 13.3$ pmol/L) (data not shown). There were 3 infants who had borderline low iron stores ($37 > \text{plasma ferritin} > 21.9$ $\mu\text{g}/\text{L}$), but the hemoglobin concentration was normal in all infants. For all 3 iodine doses, there were no significant associations between baseline characteristics and iodine intake or iodine excretion, except for age at study start and iodine excretion for the low iodine dose ($r = -0.789$, $P = 0.004$). Baseline hemoglobin concentration was correlated with iodine retention for all 3 iodine doses ($r = 0.608$ – 0.666 , $P = 0.025$ – 0.047).

Iodine intake, iodine excretion, and iodine retention expressed as $\mu\text{g}/\text{d}$ and $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for the low, medium, and high iodine IFM are shown in **Table 2**. Individual data for the 11 study infants are provided in **Supplemental Table 1**. Daily

iodine intake, iodine excretion, and iodine retention differed among all 3 doses ($P < 0.001$ for all comparisons). We observed a linear dose-response association between iodine intake and iodine excretion (Figures 1A and 2A) and between iodine intake and iodine retention (Figures 1B and 2B). In positive balance, infants excreted a constant proportion of 87% of the ingested iodine and retained a constant proportion of 13% (Table 2).

The predicted dose-response relation between daily iodine intake and excretion obtained from the MEM analysis agreed well with the measured data. The locally weighted scatter plot smoothing line is comparable with the predicted linear model fit obtained from the MEM analysis (Figures 1A and 2A). The same was observed for the daily iodine intake and iodine retention (Figures 1B and 2B). We observed a strong correlation between the observed and predicted data for iodine excretion ($r = 0.919$, $P < 0.001$ for the micrograms per kilograms per day data) and iodine retention ($r = 0.637$, $P < 0.001$, for the micrograms per kilograms BW per day data), indicating satisfactory performance of the model to predict the iodine excretion and iodine retention from iodine intakes (data not shown). Hemoglobin was identified as a covariate for the MEM analysis and improved the overall model fit for iodine excretion (AIC 1270 compared with 1273, $P = 0.0162$ for the micrograms per day data; AIC 765 compared with 769, $P = 0.0168$ for the micrograms per kilograms BW per day data) and iodine retention (AIC 1270 compared with 1274, $P = 0.0163$ for the micrograms per day data; AIC 765 compared with 769, $P = 0.0167$ for the micrograms per kilograms BW per day data).

The predicted iodine excretion can be described as a function of iodine intake by the following equations:

$$\text{Iodine excretion } (\mu\text{g/d}) = 0.77 \times \text{iodine intake } (\mu\text{g/d}) - 1.48 \times \text{hemoglobin (g/L)} + 180.51 \quad (1)$$

$$\text{Iodine excretion } (\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = 0.76 \times \text{iodine intake } (\mu\text{g/kg/d}) - 0.21 \times \text{hemoglobin (g/L)} + 25.80 \quad (2)$$

The relation between iodine intake and predicted iodine retention is expressed by the following equations:

TABLE 1
Subject characteristics¹

Variable	Value (n = 11)
Girls:boys	5:6
Vaginal delivery:Cesarean section	5:6
Birth weight, kg	3.58 ± 0.43 (2.85–4.21)
Gestational age, wk	40 ± 1 (38–42)
Age, ² wk	13 ± 3 (9–19)
Body weight, ² kg	6.30 ± 0.61 (5.32–7.84)
Body weight at study end, kg	7.33 ± 0.77 (6.33–9.41)
TSH, ² mU/L	3.0 ± 1.2 (1.1–5.1)
fT4, ² pmol/L	14.8 ± 2.1 (11.8–18.7)
Hemoglobin, ² g/L	111 ± 7 (99–122)
Plasma ferritin, ² μg/L	65.3 ± 36.3 (21.9–133.0)

¹Values are ratios or means ± SDs (ranges) (all such values). fT4, free thyroxine; TSH, thyroid-stimulating hormone.

²At study start.

TABLE 2

Observed and predicted daily iodine intake, iodine excretion, and iodine retention for the 11 infants¹

	IFM		
	Low iodine	Middle iodine	High iodine
Observed, μg/d			
Iodine intake	54.6 ± 8.1 ^a	142.3 ± 23.1 ^b	268.4 ± 32.6 ^c
Iodine excretion	55.9 ± 8.6 ^a	121.9 ± 21.7 ^b	228.7 ± 39.3 ^c
Iodine retention	−1.6 ± 8.3 ^a	20.6 ± 21.6 ^b	39.8 ± 34.3 ^c
Predicted, ² μg/d			
Iodine intake	n.a.	n.a.	n.a.
Iodine excretion	57.6 ± 11.3 ^a	124.9 ± 16.5 ^b	222.6 ± 23.0 ^c
Iodine retention	−3.3 ± 11.3 ^a	17.5 ± 14.1 ^b	47.0 ± 14.2 ^c
Observed, μg · kg ^{−1} · d ^{−1}			
Iodine intake	8.0 ± 1.0 ^a	21.3 ± 2.1 ^b	39.6 ± 4.3 ^c
Iodine excretion	8.2 ± 1.1 ^a	18.3 ± 2.7 ^b	33.2 ± 5.6 ^c
Iodine retention	−0.4 ± 1.4 ^a	2.9 ± 3.1 ^b	6.2 ± 4.4 ^c
Predicted, ³ μg · kg ^{−1} · d ^{−1}			
Iodine intake	n.a.	n.a.	n.a.
Iodine excretion	8.5 ± 1.8 ^a	18.7 ± 1.7 ^b	32.6 ± 2.9 ^c
Iodine retention	−0.6 ± 1.5 ^a	2.6 ± 1.8 ^b	7.0 ± 2.2 ^c
Observed, ⁴ % of intake			
Iodine intake	n.a.	n.a.	n.a.
Iodine excretion	104.5 ± 16.2 ^a	87.8 ± 15.5 ^b	87.0 ± 12.6 ^b
Iodine retention	−4.5 ± 16.0 ^a	11.9 ± 15.5 ^b	13.0 ± 12.6 ^b

¹Values are means ± SDs. The differences between the IFMs were evaluated using repeated-measure ANOVA with Bonferroni correction. Values with different superscript letters were significantly different ($P \leq 0.001$). IFM, infant formula milk; MEM, mixed-effects model; n.a., not applicable.

²Predicted excretions were calculated from the observed intakes by use of MEM: iodine excretion (μg/d) = 0.77 × iodine intake (μg/d) − 1.48 × hemoglobin (g/L) + 180.51. Predicted retentions were calculated from the observed intakes by use of MEM: iodine retention (μg/d) = 0.23 × iodine intake (μg/d) + 1.48 × hemoglobin (g/L) − 180.51.

³Predicted excretions were calculated from the observed intakes by use of MEM: iodine excretion (μg · kg^{−1} · d^{−1}) = 0.76 × iodine intake (μg · kg^{−1} · d^{−1}) − 0.21 × hemoglobin (g/L) + 25.80. Predicted retentions were calculated from the observed intakes by use of MEM: iodine retention (μg · kg^{−1} · d^{−1}) = 0.24 × iodine intake (μg · kg^{−1} · d^{−1}) + 0.21 × hemoglobin (g/L) − 25.78.

⁴Derived from μg/d data.

$$\text{Iodine retention } (\mu\text{g/d}) = 0.23 \times \text{iodine intake } (\mu\text{g/d}) + 1.48 \times \text{hemoglobin (g/L)} - 180.51 \quad (3)$$

$$\text{Iodine retention } (\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = 0.24 \times \text{iodine intake } (\mu\text{g/kg/d}) + 0.21 \times \text{hemoglobin (g/L)} - 25.78 \quad (4)$$

Iodine balance (iodine intake = iodine excretion, iodine retention = 0 μg/d) was derived from the MEM based on the micrograms per day data (Figure 1B) and was achieved at an iodine intake of 70 μg/d (95% CI: 60, 80 μg/d). This result is in agreement with the value derived from the MEM based on the micrograms per kilograms BW per day data, i.e., 10.6 μg · kg^{−1} · d^{−1} (95% CI: 9.2, 12.0 μg · kg^{−1} · d^{−1}) (Figure 2B).

Use of a linear model yielded the same iodine intake at null balance as the MEM and did not change the data interpretation. The

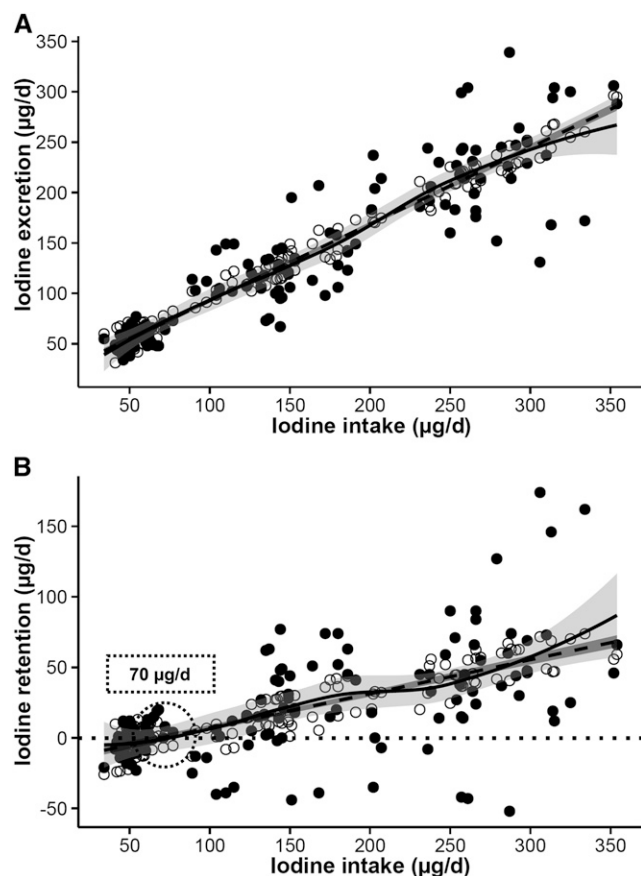


FIGURE 1 Association between total daily iodine intake and excretion ($\mu\text{g}/\text{d}$) (A) and total daily iodine intake and retention ($\mu\text{g}/\text{d}$) (B). Filled data points are measured values. Each subject provided 4 data points (daily values from 4 balance days)/dose ($n = 131$). The filled line and light gray area represent the locally weighted scatter plot smoothing line and the corresponding 95% CI for the observed data. The dashed line and dark gray area represent the fitted linear MEM and the corresponding 95% CI. (A) Iodine excretion ($\mu\text{g}/\text{d}$) = $0.77 \times \text{iodine intake } (\mu\text{g}/\text{d}) - 1.48 \times \text{hemoglobin (g/L)} + 180.51$. (B) Iodine retention ($\mu\text{g}/\text{d}$) = $0.23 \times \text{iodine intake } (\mu\text{g}/\text{d}) + 1.48 \times \text{hemoglobin (g/L)} - 180.51$. Open data points are predicted data obtained from the MEM ($n = 131$). The dotted line in (B) represents the zero balance line. MEM, mixed effects model.

SD of the iodine retention at null balance obtained from the linear model was $3.75 \mu\text{g}/\text{d}$.

DISCUSSION

This study suggests that 2- to 5-mo-old infants require a minimum daily iodine intake of $70 \mu\text{g}/\text{d}$. At an average BW of 6.7 kg at 4 mo of age (40), this corresponds to a daily iodine intake of $10.4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, comparable with the observed minimum intake level of $10.6 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ derived from the micrograms per kilogram BW per day data. Intakes below this level will likely result in negative balance, i.e. iodine depletion, and eventually hypothyroidism caused by insufficient iodine to maintain normal thyroid hormone synthesis. In positive balance, infants excreted a constant proportion of 87% of ingested iodine, slightly lower than what has been estimated for adults [90–92% (12, 41–43)]. The iodine retention was linear, and no down-regulation of the retention rate (13%) was observed at intakes up to $300 \mu\text{g}/\text{d}$. This

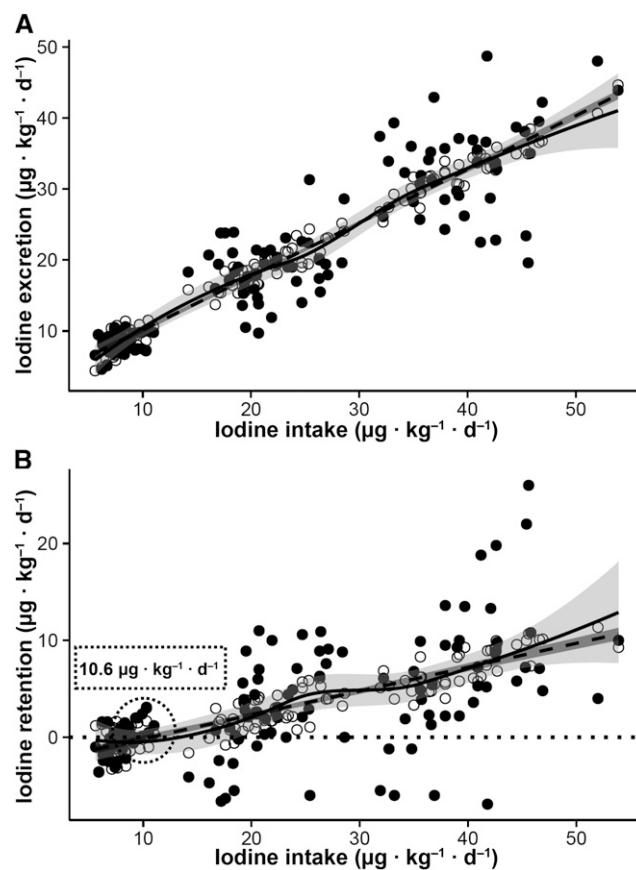


FIGURE 2 Association between daily iodine intake and excretion adjusted for body weight ($\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (A) and daily iodine intake and retention adjusted for body weight ($\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (B). Filled data points are measured values. Each subject provided 4 data points (daily values from 4 balance days)/dose ($n = 131$). The filled line and light gray area represent the locally weighted scatter plot smoothing line and the corresponding 95% CI for the observed data. The dashed line and dark gray area represent the fitted linear MEM and the corresponding 95% CI. (A) Iodine excretion ($\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) = $0.76 \times \text{iodine intake } (\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) - 0.21 \times \text{hemoglobin (g/L)} + 25.80$. (B) Iodine retention ($\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) = $0.24 \times \text{iodine intake } (\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) + 0.21 \times \text{hemoglobin (g/L)} - 25.78$. Open data points are predicted data obtained from the MEM ($n = 131$). The dotted line in (B) represents the zero balance line. MEM, mixed effects model.

suggests that the infant thyroid accumulates significant amounts of iodine when intakes exceed the minimum requirement.

Balance studies determine the intake required to maintain existing body pools (19, 20). Healthy childhood growth and development is characterized by gradual accumulation of thyroidal iodine stores; this stored iodine can be used to produce thyroid hormones during periods of low dietary iodine intake. Therefore, to build thyroidal iodine stores, an additional allowance should be added to the minimum iodine requirement for balance. Data on the thyroidal iodine content in infants are scarce (5, 44–46). An autopsy study of full-term newborns in an iodine-sufficient area reported a mean (\pm SD) thyroidal iodine content of $292 \mu\text{g}$ (± 47) (5, 47), a plausible amount considering the thyroid gland in newborns weighs $\sim 1 \text{ g}$ (4). Iodine-sufficient adults store ~ 5 – 15 mg of thyroidal iodine (48–51). Assuming a mean thyroidal iodine store of $300 \mu\text{g}$ at birth, 15 y accumulation time, and a mean thyroidal iodine store of 10 mg in iodine-sufficient adults, the daily amount of dietary iodine needed to build up thyroid stores is $\sim 1.77 \mu\text{g}/\text{d}$. Rounding up and adding this allowance to the

minimum daily iodine intake requirement of 70 μg suggests an EAR candidate of 72 μg for 2- to 5-mo-old infants.

Assessment of iodine intakes in populations should be based on the EAR (12, 16). To compare our data with the present intake recommendations, a recommended dietary allowance (RDA) must be estimated. This is conventionally done by adding 2 SDs to the EAR (12). The estimated SD of the iodine retention at null balance was 3.75 $\mu\text{g}/\text{d}$ (by use of the linear model). Adding 2 SDs to the intake at null balance (70 $\mu\text{g}/\text{d}$) plus 2 $\mu\text{g}/\text{d}$ to account for iodine stores results in an estimated RDA of 80 $\mu\text{g}/\text{d}$. This RDA candidate is lower than the AI of 110 μg defined by Institute of Medicine and the current recommended nutrient intake of 90 μg defined by WHO.

The EAR candidate of 72 $\mu\text{g}/\text{d}$ for infants is slightly higher than the current EAR of 65 $\mu\text{g}/\text{d}$ for 1–3- and 4–8-y-old children (12, 21, 22) but similar to the current EAR of 73 $\mu\text{g}/\text{d}$ for 9–13-y-old children (12). However, it is sharply higher than the requirements for children in these age groups when expressed per kg BW. Based on our proposed EAR and a mean BW of 6.7 kg at 4 mo of age (40), the iodine requirement during early infancy would be 10.7 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. In contrast, it would be 5.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 1–3-y-old children [based on the EAR of 65 $\mu\text{g}/\text{d}$ and a mean BW of 11.9 kg at 2 y (40)], 3.2 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 4–8-y-old children [based on the EAR of 65 $\mu\text{g}/\text{d}$ and a mean BW of 20.4 kg at 6 y (40)], and 2.3 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 9–13-y-old children [based on the EAR of 73 $\mu\text{g}/\text{d}$ and a mean BW of 31.6 kg at 10 y (40)]. This decrease in the iodine requirement per kg BW during childhood is likely explained by a decrease in thyroid hormone requirements per kg BW during this same period. In infants, thyroxine production rates are estimated to be $\sim 5\text{--}6 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, decreasing to $\sim 2\text{--}3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in 3–9-y-old children and to $\sim 1.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in adults (4). Therefore, our proposed iodine requirement for infants, expressed in $\mu\text{g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$, may be used to extrapolate intakes adapted for varying BWs during early infancy (0–6 mo) but should not be extrapolated to older children.

Our findings provide, to our knowledge, the first rigorously defined data to estimate the minimum UIC threshold, indicating adequate iodine nutrition in infant populations. Based on an iodine intake of 72 $\mu\text{g}/\text{d}$ (our estimate of the EAR in 2–5-mo-old infants), a mean urinary iodine excretion rate of 87% [as measured in this study and assuming negligible fecal iodine excretion (52)], and an estimated mean urine volume of 0.5 L/d at this age (16, 17), the corresponding UIC is $\sim 125 \mu\text{g}/\text{L}$. In UIC distributions from population surveys that have been adjusted for within-subject variability, the percentage of infants with a UIC below this threshold are likely to have deficient iodine intakes (53). Our findings also suggest the current median UIC cut-off of 100 $\mu\text{g}/\text{L}$ to define iodine sufficiency in infant populations may be too low (14, 15). However, more studies investigating the association between UIC, thyroid function, and other health outcomes are needed to define the optimal UIC range corresponding to adequate iodine nutrition during infancy.

To our knowledge, this study is the first iodine balance study in euthyroid, iodine-sufficient, term infants. Iodine status during pregnancy is adequate in Switzerland (30). All infants were formula-fed before the study, and commercial IFM sold in Switzerland must comply with the national (54) and European (35) regulations and supply 57–286 μg I/d. The IFMs consumed

by the participating infants before the study supplied 102–128 μg I/d. Delange et al. (31, 32) conducted an iodine balance study in a mixed group of preterm and full-term infants in Belgium, at a time when the country was iodine-deficient (55). In that study, the infants' mean \pm SEM iodine intake was $20.0 \pm 1.9 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, close to our middle dose, and excretion was $12.7 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ($11.4 \pm 1.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in urine and $1.3 \pm 0.1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in feces). The mean \pm SEM iodine retention was $7.3 \pm 1.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, equivalent to 37% of the iodine intake, which is 3 times the iodine retention observed for the same dose in our study (12%). However, because Delange's study was done in an iodine-deficient area, it likely overestimated iodine retention because additional iodine is needed to restore depleted iodine stores (56). Also, the study results should be interpreted with caution because all samples were measured by use of the Sandell-Kolthoff method, a technique that, depending on the digestion step, may be inadequate for iodine determination in complex sample matrices (34).

The present study has both strengths and weaknesses. The balance study method generally neglects the metabolism from different body compartments of the nutrient under study (19). However, because the main iodine pool is concentrated in the thyroid and only a smaller proportion is incorporated in thyroid hormones or is present as circulating serum iodine, we feel that the balance study technique is a valid approach for iodine (12). It has also been argued that balance should be estimated from a steady state of habitual intake and that the study duration in balance studies may be too short to obtain a new balance at a different intake (19). However, our data show constant rates of iodine excretion and retention at the 2 higher doses, supporting a rapid steady state and justifying the use of our data as the basis for dietary intake recommendations. Another potential weakness of the balance study technique is the tendency to underestimate nutrient excretion and consequently overestimate nutrient retention because of incomplete collections and because losses from sweat, saliva, and skin are not measured (20, 23). To overcome these shortcomings, we developed and validated an innovative whole-diaper collection method to collect both urine and feces, which minimizes losses and increases the comfort for participating infants compared with conventional collection techniques (23, 24, 57). We assumed iodine losses from sweat and skin to be insignificant (58). Although the iodine content of saliva may be high [$\sim 370\text{--}520 \mu\text{g}/\text{L}$ in adults (59)], most of the iodine secreted in the saliva is recycled and taken up by the intestine (60), so we assumed salivary iodine losses relative to the total iodine pool were minimal. We did not account for regurgitation of IFM, but the losses were minimal.

Accurate and precise analytic methods are key factors for determining exact nutrient balances (19). All our analytic methods showed high precision and low CVs. We used ICP-MS, the gold standard for iodine analysis, and all of our analyses were performed and controlled by use of external quality controls. Another strength of our study is the crossover design. We corrected the dose-response data for the correlation of repeated measures within individuals. Although we studied only 11 infants, the variability was low: the CV for the intake at null balance was only 11%, compared with 43% in the Belgian study (31, 32). For multiple comparisons between the 3 iodine doses, we used the Bonferroni correction to reduce the rate of false positive associations. Even with this conservative correction, the differences in intake, excretion, and retention across doses were significant for all

comparisons. Only formula-fed infants were enrolled in the study in order to assess dose-response relations over a range of intakes. However, we believe that our results can also be applied to breast-fed infants: iodine bioavailability is likely comparable for breast milk and IFM because most iodine in breast milk is present as iodide (as for IFM), and only a minor proportion is organically bound (61, 62). Our data suggest an estimated breast milk iodine concentration of $\geq 92 \mu\text{g/L}$ [assuming ingestion of 0.78 L/d (12, 63)] to meet the infants' daily iodine requirement. Consequently, present regulations for minimum iodine levels in IFM may be set too low and require revision (35, 64). Baseline hemoglobin was a predictor of iodine retention and was included as factor in the models, but the effect of this covariate on iodine excretion and retention was small.

In conclusion, this is the first study, to our knowledge, to provide experimental data on iodine retention and iodine balance in iodine-sufficient infants. The data provide a scientific evidence base for the establishment of an EAR for iodine during the first 6 mo of life. However, further studies linking iodine intake and iodine status to health and development outcomes are needed to assess the favorable intake level and the tolerable upper intake level for iodine in infants.

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The author's responsibilities were as follows—SD, MBZ, JB, and MA: designed the research; SD and TD: conducted the balance study; MBZ, CB, and MA: supervised the study; SD, VG, and MA: analyzed data; SD and MA: wrote the paper and had primary responsibility for final content; and all authors: read, edited, and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

- Zimmermann MB. Iodine deficiency. *Endocr Rev* 2009;30:376–408.
- Zimmermann MB. The role of iodine in human growth and development. *Semin Cell Dev Biol* 2011;22:645–52.
- Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. *Eur J Endocrinol* 2004; 151:U25–37.
- Brown RS. Disorders of the thyroid gland in infancy, childhood and adolescence. In: De Groot LJ, editor. *Endotext*. South Dartmouth (MA): Endocrine Education Inc; 2012.
- Delange F. Screening for congenital hypothyroidism used as an indicator of the degree of iodine deficiency and of its control. *Thyroid* 1998;8:1185–92.
- Ares S, Quero J, Morreale de Escobar G. Iodine nutrition and iodine deficiency in term and preterm newborns: iodine nutrition in newborns. In: Preedy VR, Burrow GN, Watson R, editors. *Comprehensive handbook of iodine: nutritional, biochemical, pathological and therapeutic aspects*. Burlington (MA): Elsevier Academic Press; 2009. p.1477–85.
- Delange F. Iodine requirements during pregnancy, lactation and the neonatal period and indicators of optimal iodine nutrition. *Public Health Nutr* 2007;10:1571–80.
- Theodoropoulos T, Braverman LE, Vagenakis AG. Iodide-induced hypothyroidism: a potential hazard during perinatal life. *Science* 1979; 205:502–3.
- Azizi F, Smyth P. Breastfeeding and maternal and infant iodine nutrition. *Clin Endocrinol (Oxf)* 2009;70:803–9.
- Swanson CA, Zimmermann MB, Skeaff S, Pearce EN, Dwyer JT, Trumbo PR, Zehaluk C, Andrews KW, Carriquiry A, Caldwell KL, et al. Summary of an NIH workshop to identify research needs to improve the monitoring of iodine status in the United States and to inform the DRI. *J Nutr* 2012;142:1175S–85S.
- Trumbo PR. Evidence needed to inform the next dietary reference intakes for iodine. *Adv Nutr* 2013;4:718–22.
- Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, molybdenum, nickel, silicon, vanadium and zinc. Washington (DC): National Academy Press; 2001.
- Gushurst CA, Mueller JA, Green JA, Sedor F. Breast milk iodide: reassessment in the 1980s. *Pediatrics* 1984;73:354–7.
- World Health Organization, United Nations Children's Fund, International Council for the Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd ed. Geneva (Switzerland): World Health Organization; 2007.
- Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. *Public Health Nutr* 2007;10:1606–11.
- World Health Organization, Food and Agriculture Organization of the United Nations. Vitamin and mineral requirements in human nutrition. 2nd ed. Geneva (Switzerland): World Health Organization; 2004.
- Goellner MH, Ziegler EE, Fomon SJ. Urination during the first three years of life. *Nephron* 1981;28:174–8.
- Food and Nutrition Board, Institute of Medicine. Dietary reference intakes: applications in dietary assessment. Washington (DC): National Academy Press; 2000.
- Mertz W. Use and Misuse of Balance Studies. *J Nutr* 1987;117:1811–3.
- Baer DJ, Fong AKH, Edd RD, Novotny JA, Oexmann MJ. Compartmental modeling, stable isotopes, and balance studies. In: Dennis B, ed. *Well-controlled diet studies in humans: a practical guide to design and management*. Chicago (IL): American Dietetic Association; 1999.
- Ingenbleek Y, Malvaux P. Iodine balance studies in protein-calorie malnutrition. *Arch Dis Child* 1974;49:305–9.
- Malvaux P, Beckers C, De Visscher M. Iodine balance studies in nongoitrous children and in adolescents on low iodine intake. *J Clin Endocrinol Metab* 1969;29:79–84.
- Ziegler EE, Fomon SJ. Methods in infant nutrition research: balance and growth studies. *Acta Paediatr Scand Suppl* 1982;299:90–6.
- Cooke RJ, Perrin F, Moore J, Paule C, Ruckman K. Methodology of nutrient balance studies in the preterm infant. *J Pediatr Gastroenterol Nutr* 1988;7:434–40.
- Sievers E, Schleyerbach U, Schaub J. Magnesium balance studies in premature and term infants. *Eur J Nutr* 2000;39:1–6.
- Sievers E, Oldigs HD, Dörner K, Kollmann M, Schaub J. Molybdenum balance studies in premature male infants. *Eur J Pediatr* 2001;160:109–13.
- Dörner K, Schneider K, Sievers E, Schulz-Lell G, Oldigs HD, Schaub J. Selenium balances in young infants fed on breast milk and adapted cow's milk formula. *J Trace Elem Electrolytes Health Dis* 1990;4:37–40.
- Giles MM, Fenton MH, Shaw B, Elton RA, Clarke M, Lang M, Hume R. Sequential calcium and phosphorus balance studies in preterm infants. *J Pediatr* 1987;110:591–8.
- Zimmermann MB, Aeberli I, Torresani T, Burgi H. Increasing the iodine concentration in the Swiss iodized salt program markedly improved iodine status in pregnant women and children: a 5-y prospective national study. *Am J Clin Nutr* 2005;82:388–92.
- Andersson M, Aeberli I, Wust N, Piacenza AM, Bucher T, Henschen I, Haldimann M, Zimmermann MB. The Swiss iodized salt program provides adequate iodine for school children and pregnant women, but weaning infants not receiving iodine-containing complementary foods as well as their mothers are iodine deficient. *J Clin Endocrinol Metab* 2010;95:5217–24.
- Delange F, Bourdoux P, Chanoine JP, Ermans AM. Physiopathology of iodine nutrition during pregnancy, lactation, and early postnatal life. In: Berger H, editor. *Vitamins and minerals in pregnancy and lactation*. New York: Raven Press; 1988.
- Delange F. Requirements of iodine in humans. In: Delange F, Dunn JT, Glinioer D, editors. *Iodine deficiency in Europe: a continuing concern*. New York: Plenum Press; 1993. p. 5–15.
- Faul F, Erdfelder E, Lang AG, Buchner AG. *Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175–91.

34. Dold S, Baumgartner J, Zeder C, Krzystek A, Osei J, Haldimann M, Zimmermann MB, Andersson M. Optimization of a new mass spectrometry method for measurement of breast milk iodine concentrations and an assessment of the effect of analytic method and timing of within-feed sample collection on breast milk iodine concentrations. *Thyroid* 2016;26:287–95.
35. Commission of the European Communities. Commission directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending directive 1999/21/EC. Official Journal of the European Union; 2006. L401/1–L401/33. Version current 22 December 2006. Internet: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0141&from=EN> (accessed 03 July 2016).
36. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. *Clin Chem* 1996;42:239–43.
37. R Core Team. A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2014.
38. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. *nlme*: linear and nonlinear mixed effects models. R package version 3.1–118, 2014.
39. Wickham H. *ggplot2*: elegant graphics for data analysis. New York: Springer, 2009.
40. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva (Switzerland): World Health Organization; 2006.
41. Vought RL, London WT. Iodine intake excretion and thyroïdal accumulation in healthy subjects. *J Clin Endocrinol Metab* 1967;27:913–9.
42. Nath SK, Moinier B, Thuillier F, Rongier M, Desjeux JF. Urinary excretion of iodide and fluoride from supplemented food grade salt. *Int J Vitam Nutr Res* 1992;62:66–72.
43. Jahreis G, Hausmann W, Kiessling G, Franke K, Leiterer M. Bioavailability of iodine from normal diets rich in dairy products: results of balance studies in women. *Exp Clin Endocrinol Diabetes* 2001;109:163–7.
44. Delange FM, Dunn JT. Iodine deficiency. In: Braverman LE, Utiger RD, editors. *The thyroid: a fundamental and clinical text*. Philadelphia (PA): Lippincott Williams & Wilkins; 2005. p. 264–88.
45. Bakker B, Vulsma T, de Randamie J, Achterhuis AM, Wiedijk B, Oosting H, Glas C, de Vijlder JJ. A negative iodine balance is found in healthy neonates compared with neonates with thyroid agenesis. *J Endocrinol* 1999;161:115–20.
46. Ogborn RE, Waggener RE, Vanhove E. Radioactive-iodine concentration in thyroid glands of newborn infants. *Pediatrics* 1960;26:771–6.
47. Delange F, Bourdoux P, Laurence M, Peneva L, Walfish P, Willgerodt H. Neonatal thyroid function in iodine deficiency. In: Delange F, Dunn JT, Glinioer D, editors. *Iodine deficiency in Europe: a continuing concern*. New York: Plenum Press; 1993. p. 199–209.
48. Zaichick VY, Choporov YY. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. *J Radioan Nucl Ch Ar* 1996;207:153–61.
49. Milakovic M, Berg G, Eggertsen R, Nystrom E, Olsson A, Larsson A, Hansson M. Determination of intrathyroidal iodine by x-ray fluorescence analysis in 60-to 65-year olds living in an iodine-sufficient area. *J Intern Med* 2006;260:69–75.
50. Zabala J, Carrion N, Murillo M, Quintana M, Chirinos J, Seijas N, Duarte L, Bratter P. Determination of normal human intrathyroidal iodine in Caracas population. *J Trace Elem Med Biol* 2009;23:9–14.
51. Hays MT. Estimation of total body iodine content in normal young men. *Thyroid* 2001;11:671–5.
52. Laurberg P, Andersen SL. Nutrition: breast milk: a gateway to iodine-dependent brain development. *Nat Rev Endocrinol* 2014;10:134–5.
53. Zimmermann MB, Hussein I, Al Ghannami S, El Badawi S, Al Hamad NM, Abbas Hajj B, Al-Thani M, Al-Thani AA, Winichagoon P, Pongcharoen T, et al. Estimation of the prevalence of inadequate and excessive iodine intakes in school-age children from the adjusted distribution of urinary iodine concentrations from population surveys. *J Nutr* 2016;146(6):1204–11.
54. Das Eidgenössisches Departement des Innern (EDI). Verordnung des EDI über Speziallebensmittel 817.022.104. 23. Version current 4 February 2014. [cited 2016 Jul 3]. Available from: <https://www.admin.ch/opc/de/classified-compilation/20050168/201402040000/817.022.104.pdf>.
55. Delange F, Van Onderbergen A, Shabana W, Vandemeulebroucke E, Vertongen F, Gnat D, Dramaix M. Silent iodine prophylaxis in Western Europe only partly corrects iodine deficiency; the case of Belgium. *Eur J Endocrinol* 2000;143:189–96.
56. Delange F, Wolff P, Gnat D, Dramaix M, Pilchen M, Vertongen F. Iodine deficiency during infancy and early childhood in Belgium: does it pose a risk to brain development? *Eur J Pediatr* 2001;160:251–4.
57. Liaw LC, Nayar DM, Pedler SJ, Coulthard MG. Home collection of urine for culture from infants by three methods: survey of parents' preferences and bacterial contamination rates. *BMJ* 2000;320:1312–3.
58. Smyth PP, Duntas LH. Iodine uptake and loss-can frequent strenuous exercise induce iodine deficiency? *Horm Metab Res* 2005;37:555–8.
59. Ford H, Johnson L, Purdie G, Feek C. Effects of hyperthyroidism and radioactive iodine given to ablate the thyroid on the composition of whole stimulated saliva. *Clin Endocrinol (Oxf)* 1997;46:189–93.
60. Kogai T, Brent GA. The sodium iodide symporter (NIS): regulation and approaches to targeting for cancer therapeutics. *Pharmacol Ther* 2012;135:355–70.
61. Dorea JG. Iodine nutrition and breast feeding. *J Trace Elem Med Biol* 2002;16:207–20.
62. Fernández-Sánchez LM, Bermejo-Barrera P, Fraga-Bermudez JM, Szpunar J, Lobinski R. Determination of iodine in human milk and infant formulas. *J Trace Elem Med Biol* 2007;21:10–3.
63. da Costa TH, Haisma H, Wells JC, Mander AP, Whitehead RG, Bluck LJ. How much human milk do infants consume?: data from 12 countries using a standardized stable isotope methodology. *J Nutr* 2010;140:2227–32.
64. US Food and Drug Administration. CFR - Code of Federal Regulations - Title 21 - Part 107 Infant Formula (21CFR107). Version current 1 April 2015. [cited 2016 Jul 3]. Available from: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=107&showFR=1>.