Iodine status and thyroid function among Spanish schoolchildren aged 6–7 years: the Tirokid study


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Abstract

Iodine deficiency is still a worldwide public health problem, with children being especially vulnerable. No nationwide study had been conducted to assess the I status of Spanish children, and thus an observational, multicentre and cross-sectional study was conducted in Spain to assess the I status and thyroid function in schoolchildren aged 6–7 years. The median urinary I (UI) and thyroid-stimulating hormone (TSH) levels in whole blood were used to assess the I status and thyroid function, respectively. A FFQ was used to determine the consumption of I-rich foods.

Abbreviations: AC, autonomous communities; ID, iodine deficiency; IS, iodised salt; P25 and P75, 25th and 75th percentiles; RV, reference values; TSH, thyroid-stimulating hormone; UI, urinary iodine; UIC, urinary iodine concentration.

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Iodine status: Iodine deficiency: Thyroid function: Urinary iodine

I deficiency (ID) is still a worldwide public health problem(1). A wide variety of disorders results from ID, ranging from goitre and psychomotor development retardation up to cretinism in more severe cases. ID is considered the most common cause of preventable mental impairment worldwide(2). Thyroid hormones produced by the mother have a key role in brain development and maturation of the fetus(3), thus, a low concentration of maternal thyroid hormones negatively impacts the health of the fetus. ID could be involved in this situation, and thus it could be prevented by adequate I intake to meet the increased needs during pregnancy. It has been reported that mild or moderate ID during pregnancy is associated with low intelligence quotient (IQ)(4). Likewise, higher prevalence of goitre, lower IQ and increased auditory threshold have been detected among children with insufficient I intake(4,5).

Use of iodised salt (IS) is one of the best methods to adequately fulfill I requirements(2), and has been accessible to the Spanish population, with an I content of 60 mg/kg, since 1983. IS consumption has always been voluntary and, except for some regions, no comprehensive public health programme has been implemented to promote it(6). The WHO considers Spain as a zone with an adequate I intake(7), based on local/regional studies conducted in the late 1990s, early 2000s and later(8,9). However, until now, no national study has been conducted in Spanish schoolchildren. The aforementioned regional studies coincided with a remarkable increase in the consumption of IS and other sources of I, such as dairy products, and described median urinary I (UI) within the World Health Organization(2) range corresponding to an adequate I intake in school-age children (100–199 µg/l). There is no information concerning the I content in food products, with the exception of milk(9) despite the use of flour supplemented with IS by some bakeries.

The assessment of ID disorders is the first step towards achieving the goal of their sustainable elimination. Children are a sensitive population to ID; in fact, data from two independent surveys of micronutrient consumption among European children have shown that maximum and minimum average I intakes were below the reference standard for both sexes in the east and west(10). It was necessary to assess the current status of the Spanish children population, along with its geographical distribution. This would in turn facilitate the implementation of targeted public health campaigns.

Thyroid hormones are involved in somatic growth, neurodevelopment and metabolic pathways, which are essential during childhood(11). In the last few years, thyroid function has been studied in paediatric groups for its relationship with obesity(12) or diabetes type 1(13), but nationwide studies in paediatric populations are not common(14). In spite of the fact that neonatal hypothyroidism screening is universal in Spain, little information is available on the prevalence of thyroid dysfunction among healthy Spanish schoolchildren. The present study also aimed to generate normative data for thyroid function in school-age children in our country.

Methods

Participants and study design

An observational, multicentre and cross-sectional study was conducted in the seventeen regions, autonomous communities (AC), compiling Spain, to assess the I status of children (primary objective), prevalence of UI <100 µg/l and thyroid disorders in schoolchildren aged 6–7 years. Each Spanish AC is divided into one or more provinces. In AC with one single province, the one was selected for the study. In AC with two to four provinces, two provinces were randomly selected, and in AC with more than four provinces three were randomly selected for the study. The capital of each province was selected by default for sampling. In addition, one of the province towns having 2000–20,000 inhabitants was randomly selected. One school in the capital and one in the chosen town were randomly selected, and all schoolchildren of the first grade of primary school, which corresponds to 6–7-year-old children, were recruited (each school had one or two first-grade classes with twenty-five students each). Children from the capital represented the urban population, whereas those from the smaller town represented the rural population. The recruitment was conducted over 2 years (2010 and 2011) during the school year period (October to December and January to June).

Variables assessed

The main objective of the present study was to establish the I status of children by assessing the median UI concentration (UIC) of the population. In addition, parents or legal guardians answered a questionnaire, and blood thyroid-stimulating hormone (TSH) levels of the children were also measured. The parents filled in a questionnaire, which included questions about parents’ birthplace and education, family or child history of goitre or thyroid dysfunction, medical treatments used, I use for wound disinfection in the last month, surgery in the last 6 months and consumption of IS and other I-rich foods. Foods with known high I content were chosen, especially dairy products(9) and sea fish. Some studies have shown that I supplements for egg-laying chickens increase the I content in eggs, which might contribute to increased I intake in the population(15). The FFQ was designed to assess the
frequency of consumption and portion size of I-rich foods: milk (number of glasses per d) and yogurt (number of cups of yogurt per d); eggs (number per week); fish and cheese (times per week of consumption); and IS (used for cooking; yes/no). Parents were requested in the questionnaire to look at the salt package label to check whether it was iodised or not. The consistency of responses over time, of the six questions of our simplified FFQ, was analysed by a test-re-test, which was conducted in forty-one parents of children aged 6–7 years. Concordance rates varied between 0·71 and 0·95. The results were also expressed in ‘daily servings’. A single serving was considered as one glass of milk (200–250 ml) or two yogurt cups (250 ml total). In Spain, the yogurt cups consumed by children have a volume of 125 ml each. In the ‘Results’ section, the frequency of consumption of foods has been categorised according to the recommendations of the Spanish Society of Community Nutrition (16); fish: consumption, ≥3 times/week; egg consumption, 5 units/week; and dairy consumption, ≥2 servings/d.

The primary variable was the median UI of the population. The World Health Organization (29) considers a median value of UI between 100 and 199 µg/l as an adequate I status in schoolchildren, a value <100 µg/l as insufficient (50–99 µg/l, mild ID; 20–49 µg/l, moderate ID; <20 µg/l, severe ID) and values ≥200 µg/l as above requirements (≥300 µg/l, excessive). A 20-ml non-fasting sample of urine was obtained from each participant for UI assessment. Each sample was stored in a portable refrigerator and was subsequently frozen at −20°C. Samples were transported to the Malaga Biomedical Research Institute (Hospital Regional Universitario Carlos Haya, Malaga, Spain) for analysis in a container with dry ice to ensure that they stayed frozen and were stored again at −20°C until processing. The laboratory used the modified Benotti and Benotti method for UI determination. A previous digestion of the urine sample was made with chloric acid, followed by the Sandell–Kolthoff reaction, in which I acts as a catalyst for the reduction of Ce (IV) to Ce (III) by As (III). The intra- and inter-assay CV were 2·01 and 4·53%, respectively. The UI assay is subjected, three times a year, to a programme of external quality assessment for the determination of I in urine by the Spanish Association of Neonatal Screening. We performed the quality control in triplicate and the reference material was Seronorm™ Trace Elements Urine (SERO AS), with a mean ±2 score of 0·3.

TSH was assessed in whole blood dried on Whatman 903 filter paper (Whatman Neoreneel card making and quality released in compliance with the FDA Quality System Regulation) and submitted to a fluoroimmunometric assay (AutoDELFIA™ Neonatal hTSH test kit; PerkinElmer Inc.) at the Newborn Screening Laboratory, Hospital Clinic. The nominal range for TSH was 0·07–1·82 mU/l, with intra- and inter-assay CV of 1·82 and 3·67%, respectively. A drop of blood was collected from the child, with consent from the child and his or her parents, by a finger stick applied directly onto the filter paper. The drop was allowed to dry at room temperature and the filter paper was stored in an envelope at 4°C until it was processed in the laboratory. This sample was obtained at the same time as the urine sample.

Statistical analyses

According to previous studies conducted in local areas of Spain, the prevalence of ID (UI <100 µg/l) was estimated as 20%. The sample size needed, assuming an ID prevalence of 20% and an accuracy of ±1·5%, was 2370 individuals. As it was estimated that 10% of values would be lost for analysis, the sample size required was 3100 individuals.

Categorical variables were described by absolute and relative frequencies, whereas quantitative variables were described by mean values, medians, standard deviations, percentiles 25 (P25) and 75 (P75) and number of valid cases. The Kolmogorov–Smirnov test was used to assess whether the variables followed a normal distribution. Missing data were not included in the analyses and were considered as lost.

The UIC was assessed as a continuous variable in the overall population as well as when assessed as a function of another variable (sex, consumption of I-rich foods, geographical area or parents’ educational level). The comparison of UI among the groups was performed by the median test. All results obtained by the median test were confirmed by Mann–Whitney or Kruskal–Wallis tests, according to the number of groups (two or more). The association between UI, TSH and consumption of I-rich food units was assessed by Spearman’s correlation coefficient.

A binary logistic regression analysis was performed to assess the possible effect of the demographic characteristics and consumption of I-rich foods on UI ≥100 µg/l. The χ² test (or exact Fisher’s test, when necessary) was used for the exploratory analysis of the possible risk factors in the case of categorical variables, and the median test was used in the case of quantitative variables. Those variables with a P value above 0·20 in the corresponding bivariate analyses were pre-selected. In order to select the definitive model, different methods of variable selection, automatic as well as manual, were tried.

In order to calculate the TSH reference values (RV) of our population, only children without known thyroid disease and a UI between 100 and 200 µg/l were considered. Extreme cases and outliers were excluded following the method of Tukey. The remaining sample showed normal distribution of TSH for the calculation of RV. Thus, the RV were obtained according to the recommendations of the International Federation of Clinical Chemistry (37), by calculating the 95% CI for the mean of TSH and standard deviations. The prevalence of thyroid dysfunction was operationally defined as the percentages with 95% CI of children with TSH values above (defined as hypothyroidism) or below (defined as hyperthyroidism) RV. Median TSH has been used for descriptions and comparisons among groups.

The software SPSS version 17 was used for data analyses. The statistical significance level was set at 5%.

Ethical statement

The present study was approved by the Ethics Research Committee of Hospital de Mataró (Barcelona, Spain) functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London. The parents or legal guardians of the children signed an informed consent after full explanation of the purpose and nature of all procedures and before enrolling the child for the study. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008.
Results

Description of the population

A total of 1981 schoolchildren were assessed in eleven AC (Table 1; Fig. 1), covering 74.7% of the entire Spanish population. The study could not be conducted in the other six AC (Canary Islands, Cantabria, Galicia, La Rioja, Murcia and Valencia) because of administrative constraints.

Table 1 shows the socio-demographic characteristics of the population, along with their geographical distribution. In all, 52% were male and over half lived in rural areas. In total, 85% or more of both parents were from Spain and 2% were uneducated.
median UI (189 (P25–P75 128–254) μg/l) than IS or milk consumption alone (165 (P25–P75 116–227) and 167 (P25–P75 113–225) μg/l, respectively). In cases with no IS consumption and low milk consumption (≤2 glasses/d), the median UI was significantly lower than in cases with IS consumption alone (146 (P25–P75 97–204) v. 165 (P25–P75 116–227) μg/l; \( P < 0.046 \)).

Higher parental education was associated with higher median UI (\( P < 0.001 \)) and implicated higher intakes of IS and milk (\( P \leq 0.0001 \) and \( P = 0.001 \), respectively) (data not shown).

**Thyroid function**

Only fourteen children presented with or had a history of thyroid disorder: ten had known hypothyroidism (0.5%; 95% CI 0.23, 0.92) (three were treated with levothyroxine and the other seven showed slightly elevated TSH concentrations), one hyperthyroidism, one known increase in antithyroglobulin antibodies, one non-specified thyroid gland disorder and one surgery for thyroglossal duct cyst.

The TSH RV in whole blood were 0.07–1.75 mU/l for males, 0.14–1.82 mU/l for females and 0.10–1.78 mU/l for the overall population. Median TSH was 0.90 (P25–P75 0.62–1.28) mU/l and was higher in females than in males (0.98 v. 0.83 mU/l; \( P < 0.001 \)) (data not shown). No correlation was observed between UI and TSH concentrations. The prevalence of thyroid dysfunction is shown in Table 4.

Family history of hypothyroidism (first-, second- or third-degree relatives) was present in 9.3% of children (161/1729). In these cases, the median TSH (0.99 v. 0.89 mU/l; \( P = 0.036 \)) and the prevalence of hypothyroidism (14.9 v. 6.8%; \( P = 0.001 \)) were significantly higher than in those children with no family history. No correlation was found between prevalence of elevated TSH and median UI in any geographical area. TSH > 3.56 mU/l, twice the upper limit of the reference value (ULRV), was detected in nine cases (0.52%; 95% CI 0.23, 0.98).

**Discussion**

This is the first population-based study conducted to assess the UI in schoolchildren of Spain as a whole. The median UI observed (173 μg/l) indicates an adequate I-related nutrition in schoolchildren, according to World Health Organization criteria. This fact reflects the substantial change in I intake experienced by the Spanish population since the introduction of IS in 1983, evolving from being an ID endemic region to a population with adequate median UI in the late 1990s.

This median UI is higher than that of the Spanish adult population (117.2 μg/l), as it has been described in other...
Our results showed that 10% of children had UI >300 µg/l (Fig. 2). Although these data do not necessarily identify a population with I excess, they prompt for close monitoring of the iodisation of salt and of other putative food products. UI also showed seasonal variations from spring (higher UIc) to winter (lower UIc), as previously observed in other studies [25], which might be explained by the variability of I content in milk throughout the year [9].

Boys showed significantly higher median UI than girls, which could be justified by the higher energy and I intake in boys [21]; however, the results from the FFQ of boys and girls did not differ significantly.

Nevertheless, a very recent study [26] has warned about how hydration status can interfere with UIc values even in large surveys. This factor must be considered as well as urine volume or body surface area when we consider differences between children and adults, boys and girls or seasonal variations.

This is the first time that TSH RV were assessed in Spanish schoolchildren. Our survey detected high prevalence of elevated TSH (7.6% overall). Previous studies conducted in two Spanish provinces showed similar data [29,30] which could be explained by the use of adult TSH RV, with a lower upper limit than those of the children [31]. However, in our study, we used RV calculated in the study’s population itself. The study by Lazar et al. [32], conducted in 121 000 children aged 6 months to 16 years, showed lower prevalence of elevated TSH (3.3%) than our study, although similar prevalence of cases with TSH concentrations compatible with clinical hypothyroidism. The recent study by Johner et al. [33] has shown an association between higher I intakes and a shift in TSH towards higher levels in children. According to those authors, the high prevalence of elevated TSH observed in our population should not be considered as a higher risk for (subclinical) hypothyroidism. Furthermore, the improved I status in children can be a plausible explanation for a physiological variant, corresponding to an euthyroid situation, with slightly elevated TSH [35]. Only cases with initial highly elevated TSH levels show greater risk of cases with in our population should not be considered as a higher risk for (subclinical) hypothyroidism. Furthermore, the improved I status in children can be a plausible explanation for a physiological variant, corresponding to an euthyroid situation, with slightly elevated TSH [35]. Only cases with initial highly elevated TSH levels show greater risk of cases with initial highly elevated TSH levels show greater risk of
### Table 3. Effects of different variables on ioduria (Numbers and percentages)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Children</th>
<th>UI &lt;100 μg/l</th>
<th>Correlation: food consumption/UI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Median UI (μg/l)</td>
<td>$P^†$</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>816</td>
<td>181</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Female</td>
<td>741</td>
<td>154</td>
<td>0.240</td>
</tr>
<tr>
<td>I disinfection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1477</td>
<td>85:2</td>
<td>0.743</td>
</tr>
<tr>
<td>Yes</td>
<td>256</td>
<td>14:8</td>
<td>0.001*</td>
</tr>
<tr>
<td>Iodised salt</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>521</td>
<td>30:2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Yes</td>
<td>1197</td>
<td>69:8</td>
<td>0.16</td>
</tr>
<tr>
<td>Fish consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 times/week</td>
<td>1018</td>
<td>59</td>
<td>0.405</td>
</tr>
<tr>
<td>≥3 times/week</td>
<td>706</td>
<td>41</td>
<td></td>
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<td>Glasses of milk</td>
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<tr>
<td>&lt;2 glasses/d</td>
<td>611</td>
<td>35:2</td>
<td>&lt;0.001*</td>
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<td>≥2 glasses/d</td>
<td>1123</td>
<td>64:8</td>
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<td>Yogurt consumption</td>
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<tr>
<td>None</td>
<td>76</td>
<td>4:5</td>
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</tr>
<tr>
<td>≥1 cup/d</td>
<td>1621</td>
<td>95:5</td>
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<td>Dairy servings§</td>
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<tr>
<td>&lt;2 servings/d</td>
<td>324</td>
<td>18:8</td>
<td>&lt;0.001*</td>
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<tr>
<td>≥2 servings/d</td>
<td>1416</td>
<td>81:3</td>
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<tr>
<td>Cheese consumption</td>
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<tr>
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<tr>
<td>≥1/week</td>
<td>1405</td>
<td>83:3</td>
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<td>Eggs consumption</td>
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<td>&lt;3/week</td>
<td>967</td>
<td>55:5</td>
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<tr>
<td>≥3/week</td>
<td>775</td>
<td>45:5</td>
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<tr>
<td>Rural</td>
<td>937</td>
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<tr>
<td>Urban</td>
<td>813</td>
<td>46:5</td>
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<td>Parents education</td>
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<tr>
<td>Uneducated</td>
<td>23</td>
<td>1:2</td>
<td>&lt;0.001*</td>
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<tr>
<td>Primary education</td>
<td>348</td>
<td>17:6</td>
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</tr>
<tr>
<td>Secondary education</td>
<td>752</td>
<td>38:1</td>
<td></td>
</tr>
<tr>
<td>Higher education</td>
<td>850</td>
<td>42:9</td>
<td></td>
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UI, urinary I.
* Significant $P$ values.
† Median test.
‡ $χ^2$ Test.
§ Dairy serving (one): one glass of milk (200–250 ml) or two yogurt cups (250 ml total).
study is that the labelling of IS was not checked by the investigators, although parents were requested to do so. Some other limitations are the lack of anthropometric and anti-TPO antibodies data, variables that might have had some influence on TSH results, and of thyroxine, which would have helped to better assess thyroid dysfunction. Likewise, the analysis of creatinine in urine could have reduced the variability involved in casual urine sampling. Despite these limitations, the present study is the first one conducted on the 1 status of a representative sample of schoolchildren in Spain. I-rich foods are the most important determinants of UI. The enkik study conducted in 5534 individuals (2–24 years old) representative of the Spanish population showed that the intake pattern along the school years (2–5 and 6–9 years) regarding dairy products and fish was very homogeneous. Thus, the UI data of our 6–7-year-old children are probably similar to that of the extended group aged 2–9 years.

In conclusion, 30 years after the initiation of voluntary consumption of IS, I intake is adequate, but IS intake at home is not the only source in recent years. Although the potential contribution of processed foods to the 1 status in Spanish schoolchildren might be currently lower than that in other European countries, it is necessary to monitor and control the iodisation of foods, as a putative risk of I deficit or excess in the future might exist, while it is necessary to promote the consumption of IS to achieve the WHO target of 90% of household consumption. Prevalence of TSH above RV is high, and although in most cases it will most likely spontaneously normalise, more studies are needed to determine whether these elevated TSH concentrations are associated with an increase in TA.

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The authors declare that there are no conflicts of interest.

References


Spanish children are iodine sufficient