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ORIGINAL ARTICLE

Effects of administration of iron, iodine and simultaneous iron-plus-iodine on the thyroid hormone profile in iron-deficient adolescent Iranian girls

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Objective: To investigate whether iron supplementation can improve thyroid hormone function in iron-deficient adolescent girls.
Design: A double-blind randomized intervention study.
Setting: The study was performed from 2002 through 2003 in the Islamic Republic of Iran.
Subjects: 103 iron-deficient non-anaemic girls who fulfilled all inclusion criteria were included, and 94 subjects successfully completed the study.
Interventions: Patients were randomly assigned to one of four groups and treated with a single oral dose of 190 mg iodine plus 300 mg ferrous sulphate 5 times/week ($n=24$), 300 mg ferrous sulphate 5 times/week ($n=23$), a single oral dose of 190 mg iodine ($n=25$), or a placebo ($n=22$) for 12 weeks.
Results: All groups were comparable at baseline. After the intervention, there was a significant increase in ferritin and transferrin saturation in the iron + iodine group (17.6 vs 8.7 μg/dl, and 18.8 vs 7.2%, respectively, $P<0.001$ for both) and in the iron group ($P<0.001$ for both). Urinary iodine doubled in the iron + iodine group and in the iodine group ($P<0.001$ for both). Thyroid indices tT4, tT3 and T3RU increased and reverse RT3 decreased in the iron + iodine group (10 vs 8.9 μg/dl, $P<0.001$; 143 vs 138 μg/dl, $P<0.05$; 32.3 vs 28.4%, $P<0.001$ and 24.8 vs 44.2 ng/dl, $P<0.001$, respectively) and in the iron group. These two groups did not differ for any of the four indices, but both differed significantly from the iodine and placebo groups.
Conclusions: Our results indicate that improvement of iron status was accompanied by an improvement in some indices of thyroid hormones.
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Introduction

Iron deficiency anaemia is the most prevalent nutritional deficiency worldwide. It is a major public health problem with adverse consequences, especially for women and children. More than 2 billion people, mainly young women and children, are iron-deficient (WHO/UNICEF/UNN, 1998). Over 90% of affected individuals live in developing countries (ACC/SCN, 1997). As shown by national reports on anaemia in Iran, the prevalence of iron deficiency among 15 to 49-years-old-females is 39% (Salehian and UNICEF, 1995).

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Adolescence is characterized by a large growth spurt and the acquisition of adult phenotypes and biologic rhythms. During this period, iron requirements increase dramatically in both boys and girls as a result of the expansion of the total blood volume, the increase in lean body mass and the onset of menses in young females. The consequences of iron deficiency are more serious for women. In older children and adults, it reduces the work capacity and output and impairs immune function (WHO, 1997a), and is also known to be associated with reduced reproductive capacity (WHO, 1997b). Iron deficiency can be defined as occurring when the body's iron stores become depleted and a restricted supply of iron to various tissues becomes apparent (Bothwell *et al*., 1979), and it results in the depletion of iron-dependent intracellular enzymes participating in many metabolic pathways (Dallman *et al*., 1978). Studies in animals and humans have shown that iron deficiency with or without anaemia impairs thyroid hormone metabolism. Nutritional iron deficiency has been shown to significantly lower the circulating levels of both thyroxine and triiodothyronine in rats (Chen *et al*., 1983; Beard *et al*., 1984; Brigham and Beard, 1995), and to reduce conversion of T4–T3 (Dillman *et al*., 1980). Iron deficiency also impairs thyroid metabolism in human studies. Martinez-Torres and co-workers (1984) reported 10% lower T3 levels in human subjects with moderate to severe iron deficiency anaemia and iron deficiency without anaemia when compared to iron–replete subjects, and Beard and his co-workers showed that in iron-deficient subjects, serum T3 and T4 levels significantly decreased (1990). The two initial steps of thyroid hormone synthesis are catalysed by thyroperoxidase, a haem-containing enzyme (Dillman *et al*., 1980; Martinez-Torres *et al*., 1984; Beard *et al*., 1990a, b, 1998). Severe iron deficiency may lower thyroperoxidase activity and interfere with the synthesis of thyroid hormones (Hurrell, 1997). In addition, iron deficiency results in increased sympathetic activity, as evidenced by increased plasma and urinary catecholamine concentrations (Dillmann *et al*., 1979; Groeneveld *et al*., 1985), increased turnover rates of norepinephrine in sympathetically innervated tissues, and decreased tissue norepinephrine content (Beard and Tobin, 1987; Beard *et al*., 1990b). The study of Smith and co-workers (1994) has confirmed findings on iron deficiency, where increased sympathetic nervous system activity (Dillmann *et al*., 1979; Groeneveld *et al*., 1985) was coupled with overt hypothyroidism (Beard *et al*., 1989). On the other hand, iron deficiency may significantly reduce circulating levels of T4-5′deiodinase in rats (Beard *et al*., 1989), the enzyme responsible for the conversion of T4–T3, resulting in diminished conversion of T4–T3 (Dillman *et al*., 1980).

Therefore, the aim of present study was to ascertain the effects of the administration of iron, iodine and iron + iodine on the thyroid hormone profile in iron-deficient girls. It was conducted from 2002–2003 in southern Iran, where iron deficiency is quite prevalent (Djazayery, 2000). The research hypotheses were that (1) improvement of iron status in iron-deficient subjects can lead to an improvement in the thyroid function test, and (2) that iron supplementation will not enhance the effect of iodine supplementation when iron and iodine are administered simultaneously. The relationship between iron status and thyroid hormones concentrations at baseline has been described previously (Eftekhari *et al*., in press).

Materials and methods

Study area

The trial was carried out from winter 2002 through summer 2003 in the province of Lar in southern Islamic Republic of Iran (800 meters above sea level), an area in which iron deficiency is prevalent.

Study subjects

First, 714 subjects were selected by stepwise random sampling among 2038 female students in grades 1–4 at high schools for girls in the Lar and its vicinity. Second, 431 iron-deficient girls were identified within this sample (serum ferritin $<12\,\mu$g/l and transferrin saturation $<16\%$ (Cook and Skikne, 1989)). Finally, 103 subjects who fulfilled all of the inclusion criteria were chosen. Criteria for case inclusion were: (a) the absence of any systemic disease, including anaemia (haemoglobin <12 mg/dl); (b) serum albumin within the normal range: 3.5–5.5 g/dl; (c) urinary iodine $>100\,\mu$g/l; (d) body mass index >19 kg/m^2; (e) age within the range of 14–18 years.

The number of subjects who did not meet these criteria were 203 for a (presence of anaemia), 23 for b, 17 for c, 59 for d, and 26 for e. Of 103 girls who enrolled, 94 completed the intervention and 9 dropped out of the study. Among the latter, three girls disliked the tablets and 6 girls complained about side effects (constipation, gastric discomfort, and headache).

Subjects were given an oral and a written explanation of the study, including its benefits and procedures, and at the start of the study, the subjects' parents were asked to read and sign an informed consent document. Eligible participants were randomly allocated to 4 treatment groups. Participants and investigators were unaware of group assignment.

The study protocol and ethical aspects were approved by the ethics committee of the Research Council of the Dean of Research Affairs of the Tehran University of Medical Sciences.

Intervention

This was a double blind randomised placebo-controlled trial comparing supplementation for 12 weeks with iron, iodine, iron plus iodine, or placebo. Tablets containing 300 mg of ferrous sulphate (i.e. 60 mg/day of elemental iron) were

obtained from the Daroupakhsh Company (Tehran, Iran) and were given at a dose of 1/day (5 days/week). A single oral dose of 190 mg of iodine in the form of a lipiodol capsule (ethyl esters of iodized fatty acids from poppy seed oil; 38% of lipiodol weight is iodine, Guerbet, France) was used for iodine supplementation (Zimmermann *et al*., 2000b).

The supplements and placebos looked identical and were specially prepared for this study by the Daroupakhsh and Guerbet companies. The selected subjects were randomly assigned in a double-blind fashion to one of four groups.

The iron + iodine-treated group received a single oral dose of 190 mg of iodine in the form of lipiodol plus 300 mg ferrous sulphate 5 times/week. The iron-treated group received a single oral dose of lipiodol placebo capsule plus 300 mg of ferrous sulphate 5 times per week. The iodine-treated group received a single oral dose of 190 mg of iodine lipiodol capsule plus iron placebo 5 times/week, and the control group received a one single lipiodol placebo capsule and iron placebo tablets 5 times a week.

Participants were asked not to take any vitamins or iron supplements during the trial. The tablets for each girl were packed in small, labelled plastic bags. Student were asked not to drink tea or coffee when taking the tablets, which might have inhibited iron absorption and were advised to take them after meals in order to reduce the side effects of ingested tablets. Researcher and mothers supervised ingestion of the supplements. If any girl reported unpleasant side effects of ingested tablets during the study, she was encouraged to continue the study but to take the supplement with a snack.

Background characteristics and food consumption assessment

Demographic data, menstruation, any concurrent illness history, medication, and vitamin and mineral supplementations were collected by interviews at baseline. Anthropometry and food consumption were collected twice, at baseline and at the end of supplementation 12 weeks later. Body weight was measured to the nearest 0.1 kg using a Seca 713 scale while subjects were minimally clothed. Height was determined using measuring tape while subjects were without shoes, and body mass index was calculated by dividing weight (kg) by squared height (m^2). The food consumption pattern was evaluated by a 24-h dietary recall questionnaire. Macro and micronutrient components were calculated by using Food Processor Software modified by incorporating the Iranian food table.

Biochemical assessment

At the beginning and end of the 12-week supplementation period, 10 ml of fasting venous blood samples were drawn from the arm. Blood was collected in two tubes; 2 ml were placed in the EDTA tube for measurement of haemoglobin and haematocrit and 8 ml in another tube for determination of serum albumin, TIBC, iron, ferritin, selenium, total and free thyroxine, total and free triiodothyronine, thyrotropin, T3RU and reverse triiodothyronine. In addition, urine samples were collected from the same subjects and on the same occasion as blood sampling for measurement of urinary iodine. Haemoglobin was measured using the cyanomethaemoglobin method (Dacie and Lewis, 1975), while serum iron (Ruutu, 1975), TIBC (Ceriotti and Ceriotti, 1980) and albumin (Doumus *et al*., 1971) were measured by the colorimetric method (Zist Chimie company lot. no.11–514, lot. no. 12–515 and lot. no. 10–502, respectively). Transferrin saturation was determined by dividing the serum iron concentration by the total iron binding capacity and multiplying by 100. Serum ferritin, tT4, tT3, TSH, fT4, fT3, T3RU and rT3 were determined by radioimmunoassay (Henry, 1996), using commercially available kits (Belgium ZenTech for rT3 and American DSL for the rest). Selenium was measured by the atomic absorption method (Van Dael *et al*., 1995), and urinary iodine estimation was carried out using the digestion method (Dunn *et al*., 1993).

Serum albumin was used as an indicator of malnutrition. Plasma selenium was measured because a low level of this trace element may have a negative effect on iodine metabolism, and urinary iodine was measured as an indicator of iodine deficiency.

Statistical analyses

The normality of distributions was checked for all variables. Differences between groups during supplementation were tested using paired *t*-tests, and variables not normally distributed were compared using the Wilcoxon test. Differences between groups at the beginning and end of the study were tested using analysis of variance (ANOVA). Adjustment for differences in baseline covariates and changes in variables during the study were performed by analysis of covariance using general linear models. Data are expressed as mean and standard deviation (s.d.) unless otherwise noted, and statistical significance is defined as $P<0.05$.

All statistical analyses were computed using SPSS version 11 for Windows (SPSS Inc., Chicago, 2001).

Results

The number of individuals included in the study was 103. After the start of the study, nine individuals (<9% of population) had to be excluded due to non-adherence, poor compliance or low tolerance to the medication. Thus, there were 24 individuals in the iron + iodine group, 23 in the iron group, 25 in the iodine group, and 22 in the control group who completed the study. All groups were well matched in different variables before intervention. Characteristics of study subjects before and after intervention are represented in Table 1. At both time points, body mass index and serum albumin were within the normal range, thus indicating a population free of undernourished girls.

Table 1 Characteristics of study subjects at baseline and after 12 weeks supplementation ($n=94$)

Variable	*Group*	*Baseline*[a]	*12-weeks*
Age (year)	Iron + iodine	15.9 ± 1.4[b]	15.9 ± 1.4
	Iron	16.0 ± 1.5	16.2 ± 1.5
	Iodine	15.0 ± 1.0	15.0 ± 1.2
	Control	16.0 ± 1.5	16.0 ± 1.5
Weight (kg)	Iron + iodine	51.5 ± 5.7	51.0 ± 6.0
	Iron	50.6 ± 5.0	50.5 ± 5.0
	Iodine	50.0 ± 5.0	50.0 ± 4.0
	Control	51.0 ± 4.0	51.0 ± 4.0
Height (cm)	Iron + iodine	155 ± 6	155 ± 6
	Iron	154 ± 5	154 ± 5
	Iodine	155 ± 5	156 ± 4
	Control	158 ± 4	158 ± 4
BMI (kg/m^2)	Iron + iodine	21.5 ± 1.8	21.3 ± 1.9
	Iron	21.2 ± 1.5	21.0 ± 1.7
	Iodine	20.7 ± 1.0	20.5 ± 1.3
	Control	20.5 ± 1.4	20.4 ± 1.6
Albumin (g/dl)	Iron + iodine	3.6 ± 0.17	3.6 ± 0.16
	Iron	3.6 ± 0.15	3.6 ± 0.17
	Iodine	3.6 ± 0.15	3.6 ± 0.18
	Control	3.5 ± 0.17	3.6 ± 0.15
Haemoglobin (g/l)	Iron + iodine	12.5 ± 0.4	14.3 ± 0.4*,†,§
	Iron	12.5 ± 0.4	14.2 ± 0.6*,†,§
	Iodine	12.4 ± 0.3	12.9 ± 0.5*
	Control	12.6 ± 0.2	12.9 ± 0.3*
Ferritin (μg/l)	Iron + iodine	8.7 ± 1.4	17.6 ± 0.9*,†,§
	Iron	8.9 ± 1.1	16.8 ± 1.5*,†,§
	Iodine	9.0 ± 0.8	10.8 ± 0.7*
	Control	9.3 ± 0.6	11.0 ± 0.3*
Transferrin	Iron + iodine	7.2 ± 0.8	18.8 ± 5.5*,†,§
Saturation (%)	Iron	7.1 ± 0.7	20.6 ± 8.6*,†,§
	Iodine	7.3 ± 0.8	9.5 ± 1.0*
	Control	7.4 ± 0.7	9.6 ± 0.8*
Selenium (μg/dl)	Iron + iodine	26 ± 6	27 ± 7
	Iron	29 ± 7	28 ± 6
	Iodine	28 ± 7	27 ± 5
	Control	28 ± 9	27 ± 8
Urinary iodine (μg/l)	Iron + iodine	120 (101–230)[c]	280 (140–410)*,†,§
	Iron	130 (100–210)	120 (100–270)
	Iodine	130 (100–280)	260 (140–380)*,†,§
	Control	110 (100–210)	120 (100–240)

[a]There were no significant differences in baseline characteristics between groups.
[b]Mean ± s.d.
[c]Median; range in parentheses.
*Significantly different from before intervention, $P<0.001$.
†Significantly different from iron-treated group at 12 wk, $P<0.001$.
‡Significantly different from iodine-treated group at 12 wk, $P<0.001$.
§Significantly different from control group at 12 wk, $P<0.001$.

We used two iron-status indicators to confirm iron deficiency at baseline and to monitor the response to iron supplementation. Haematological indices for iron status confirmed that all subjects were iron deficient at the beginning of the study. After 12 weeks of intervention, the iron-treated groups (iron + iodine and iron groups) had significantly higher mean haemoglobin, transferrin saturation and serum ferritin concentrations, compared to baseline. Haemoglobin increased by 14.4 and 13.6%, respectively, compared to 4.0 and 2.4% for iodine and control groups, respectively. Ferritin increased by 102 and 89%, respectively, compared to 20 and 18% for the latter groups, respectively. Transferrin saturation raised by 161 and 190%, respectively, compared to 30 and 29% for iodine and control groups. All three indicators were significantly higher than in iodine and controls groups after supplementation ($P<0.001$ for all comparisons, Table 1).

The level of urinary iodine remained adequate throughout the study in subjects consuming only iodized salt (i.e. iron and control groups), i.e., as shown in Table 1, the level of urinary iodine revealed a population without iodine deficiency. The additional iodine given as lipiodol increased urinary iodine concentrations significantly in iron + iodine and iodine-treated groups (by 133 and 100% respectively, $P<0.001$), and created a significant difference between the four groups at the end of the study ($P<0.001$, Table 1). Plasma selenium levels indicated that deficiency in this micronutrient was not a problem among study subjects.

Table 2 shows the analysis of 24-h dietary intakes of subjects. There was no significant difference between groups at the beginning of the study. However, concerning the intakes of energy, carbohydrate, vitamin A and vitamin C, there were significant changes during the study (for all $P<0.001$). Thyroid parameters are shown in Table 3. Changes in the level of TSH during the study and differences between groups at the end of the study were not significant. Levels of tT4 in iron + iodine and iron-treated groups increased significantly (+11.5 and 10.5%, respectively, $P<0.001$), resulting in significantly higher levels than in both the iodine and control groups (Table 3).

The direction of change in tT3 was similar to that of tT4, in that the level of tT3 in iron + iodine and iron groups at the end of study showed a significant rise (+3.5 and 4%, respectively, $P<0.05$), and final levels were significantly greater than in iodine and control groups (Table 3). Levels of fT3 showed no significant change during the study and no difference between groups at the end of the study. Although levels of fT4 in the iron + iodine and iron groups showed a significant rise at the end of the study (+8.6 and 9.6%, respectively, $P<0.001$ for both), final levels showed no significant difference compared with the iodine and control groups. The rT3 concentration in the iron + iodine and iron-treated groups at the end of the study showed a significant difference in comparison to initial values (−43.9 and −47.2%, respectively, $P<0.001$) and to other groups (Table 3). The level of T3RU indicated that at the end of study, this index rose significantly in the iron + iodine and iron-treated groups (+14 and 19%, respectively, $P<0.001$), and final levels were significantly higher in comparison to other groups (Table 3).

Discussion

The present study explored the hypothesis that iron deficiency might impair thyroid metabolism, as previously reported in animal and human studies (Dallman *et al*., 1978,

Table 2 Food consumption pattern (24-h recall) of study subjects at baseline and after 12 weeks supplementation ($n=94$)

Nutrient	*Group*	*Baseline*[a]	*12-weeks*
Energy (Kcal/day)	Iron + iodine	1716 ± 130[b]	1828 ± 56*
	Iron	1712 ± 129	1801 ± 61*
	Iodine	1708 ± 116	1778 ± 79*
	Control	1772 ± 174	1829 ± 110*
Protein (g/day)	Iron + iodine	72 ± 16	73 ± 11
	Iron	71 ± 14	70 ± 6
	Iodine	72 ± 19	71 ± 10
	Control	72 ± 13	72 ± 8
Carbohydrate (g/day)	Iron + iodine	247 ± 26	272 ± 12*
	Iron	244 ± 21	268 ± 10*
	Iodine	241 ± 22	265 ± 14*
	Control	254 ± 26	273 ± 9*
Fat (g/day)	Iron + iodine	48 ± 9	49 ± 6
	Iron	50 ± 8	50 ± 7
	Iodine	51 ± 7	48 ± 4
	Control	52 ± 7	50 ± 8
Iron (mg/day))	Iron + iodine	15 ± 2	16 ± 1
	Iron	16 ± 2	15 ± 1
	Iodine	16 ± 2	16 ± 2
	Control	15 ± 2	16 ± 1
Vitamin C (mg/day)	Iron + iodine	27 ± 12	49 ± 12*,†,‡,§
	Iron	31 ± 11	63 ± 6*
	Iodine	28 ± 9	60 ± 4*
	Control	28 ± 9	57 ± 4*
Vitamin A (mg/day)	Iron + iodine	450 ± 58	531 ± 87*
	Iron	471 ± 85	490 ± 66*
	Iodine	474 ± 60	500 ± 40*
	Control	465 ± 74	496 ± 63*

[a]There were no significant differences in baseline nutrient intakes between groups
[b]Mean ± s.d.
*Significantly different from before intervention, $P<0.001$.
†Significantly different from iron-treated group, $P<0.001$.
‡Significantly different from iodine-treated group, $P<0.001$.
§Significantly different from control group, $P<0.001$.

Table 3 Thyroid hormone function at baseline and after 12 weeks supplementation ($n=94$)

Indicator	*Group*	*Baseline*[a]	*12-weeks*
TSH (μU/dl)	Iron + iodine	2.5 ± 0.3[b]	2.5 ± 0.6
	Iron	2.8 ± 0.8	2.7 ± 0.7
	Iodine	2.5 ± 0.8	2.6 ± 0.8
	Control	2.4 ± 0.5	2.3 ± 0.4
TT4 (μg/dl)	Iron + iodine	8.9 ± 0.4	10.0 ± 1.5*,§,††
	Iron	8.5 ± 0.8	9.4 ± 0.9*,§,‡‡
	Iodine	8.6 ± 0.7	8.4 ± 0.9
	Control	8.7 ± 0.8	8.4 ± 0.8
FT4 (pg/dl)	Iron + iodine	10.5 ± 1.0	11.4 ± 0.9†
	Iron	10.3 ± 1.6	11.4 ± 0.8*
	Iodine	11.0 ± 1.1	10.7 ± 1.2
	Control	10.7 ± 1.7	11.1 ± 0.7
TT3 (ng/dl)	Iron + iodine	138.4 ± 19.0	143.0 ± 11.0‡,**,††
	Iron	142.8 ± 17.5	147.6 ± 8.0‡,§,††
	Iodine	130.8 ± 17.8	131.1 ± 12.0
	Control	133.0 ± 16.3	129.5 ± 7.2
FT3 (pg/ml)	Iron + iodine	2.6 ± 0.4	2.5 ± 0.3
	Iron	2.8 ± 0.5	2.7 ± 0.3
	Iodine	2.7 ± 0.4	2.7 ± 0.4
	Control	2.6 ± 0.4	2.6 ± 0.4
RT3 (ng/dl)	Iron + iodine	44.2 ± 3.0	24.8 ± 4.5*,§,††
	Iron	40.9 ± 7.0	21.6 ± 3.7*,§,††
	Iodine	43.7 ± 3.0	42.0 ± 2.6
	Control	41.0 ± 8.0	39.5 ± 5.0
T3RU (%)	Iron + iodine	28.4 ± 2.7	32.3 ± 2.5*,§,††
	Iron	27.5 ± 3.6	32.7 ± 2.0*,§,††
	Iodine	27.2 ± 3.3	28.4 ± 3.3
	Control	27.0 ± 3.8	28.4 ± 3.0

[a]There was no significant differences in baseline parameters between groups.
[b]Mean ± s.d.
Significantly different from before intervention: *$P<0.001$, †$P<0.01$ and ‡$P<0.05$.
Significantly different from iodine-treated group: §$P<0.001$ and **$P<0.05$.
Significantly different from control group: ††$P<0.001$ and ‡‡$P<0.01$.

1980; Chen *et al*., 1983; Beard *et al*., 1984, 1990a, b; Martinez-Torres *et al*., 1984; Brigham and Beard, 1995). Our findings confirm that an improvement in iron status had a positive impact on serum concentrations of thyroid hormones in iron-deficient adolescent girls.

In this study, iron deficiency at baseline was confirmed using combined serum ferritin and transferrin saturation criteria, and at 12 weeks postintervention, iron status was remeasured in all subjects. Haemoglobin and iron indices showed a significant improvement at the end of the study in iron-supplemented groups. These indices also showed a significant improvement in the iodine and control groups, although such improvements were minimal in comparison to the other two groups.

We were concerned about potential fluctuations in iodine intake from iodized salt alone. Therefore, iodized oil was given to ensure that at least one-half of the subjects in the iron-supplemented and control groups would have an ample and steady supply of iodine during the study period. The additional iodine given as iodized oil increased urinary iodine concentrations significantly. Selenium is essential to normal thyroid hormone metabolism (Arthur *et al*., 1990), but there was no evidence of selenium deficiency in study subjects.

Dietary data showed that all four groups had an equivalent diet before the intervention, but as concerns the intake of energy, carbohydrates, and vitamins C and A, there were significant differences between the onset and end of the study. The significant improvement in intake of these nutrients was likely due to several factors. First, detailed information was given to parents regarding correct food consumption during this period of rapid growth, and this may have contributed to changes in feeding patterns at home. Second, seasonal change led to greater availability of fruits and vegetables (especially lemons and oranges in this specific area). Thus, the significant increase in iron status and haemoglobin in iodine and control groups compared with baseline was likely due to an increased intake of vitamin C, an enhancer of iron absorption (Ballot *et al*., 1987).

The current study shows that iron supplementation can cause a significant increase in the concentrations of tT3, tT4,

fT4 and T3RU in the physiologically normal range, with the most significant increase in the tT4 concentration. Extensive data from animal and human studies indicate that iron deficiency impairs thyroid metabolism. The initial steps in thyroid hormone synthesis (iodide incorporation into tyrosine residues of thyroglobulin and covalent binding of the residues) are catalysed by haeme-containing peroxidases. Theoretically, severe iron deficiency could lower thyroperoxidase activity and interfere with thyroid hormone synthesis. Animal studies have documented that weanling rats fed iron-deficient diets have significantly lower T3 and T4 compared to rats fed adequate iron (Chen *et al*., 1983; Beard *et al*., 1984; Brigham and Beard, 1995).

Recently Hess and co-workers (2002) showed that thyroid peroxidase activity is significantly reduced in iron deficiency anaemia. Rats with iron deficiency and moderate iron deficiency anaemia have reduced conversion of T4–T3 (Dillman *et al*., 1980), and lower serum T3 and T4 concentrations compared to controls (Tang *et al*., 1988). On the other hand, iron-deficient rats have significantly lower hepatic 5′-deiodinase activity, with hepatic production of T3 reduced to only 46% of that in controls (Beard *et al*., 1990b) reduced turn over of serum T3 and blunted the TSH response to exogenous TRH (Beard *et al*., 1989). Few studies in humans have been carried out on this subject. Results of those studies show that in adults, iron deficiency is accompanied by reduced serum T4 and T3 as compared to healthy controls (Dillman *et al*., 1980; Martinez-Torres *et al*., 1984; Brigham and Beard, 1995). In addition, Zimmermann *et al* (2000a) and Hess *et al* (2002) showed that iron supplementation improved the efficacy of iodized salt in goitrous children with iron deficiency. In contrast, the study of Tienboon and Unachak (2003) showed that there was no statistical difference in thyroid hormones in iron-deficient-anaemic children prior to resolution of anaemia as compared to after its resolution.

In our study, despite increases in tT3 and tT4 concentrations, the TSH concentration was unaffected by iron supplementation. Zimmermann's study showed that 15 and 30 weeks after iodine supplementation, median TSH values were significantly lower in non-anaemic goitrous children than in anaemic goitrous children (2000a). On the other hand, Beard showed that iron deficiency blunts the thyrotropic response to exogenous TRH (1989). Scrimshaw has reported that in iron-deficient women, no variation in the level of TSH secretion is observed in response to cold stress (1984). The pituitary production of TSH is under complex feed-back control from intracellular T3, in addition to control from TRH and other neurohormonal modulations (Spira and Gordon, 1986).

Our study provides support for the contention that the decrease in rT3 is related to changes in iron status. As a result of iron supplementation and improved iron status, the plasma level of rT3 showed a significant decrease in the iron + iodine and iron groups and a significant difference compared to iodine and control groups. In most organs, the plasma concentration of T3 determines the binding of T3 to nuclear receptors and its metabolic activities (Larsen *et al*., 1981; Silva and Larsen, 1986). Beard *et al*. showed that, in rats, T3 disposal from the plasma pool and irreversible loss from the system were significantly slowed down in iron deficiency (1989). Moreover, Bianco and Silva demonstrated that peripheral deiodination in iron deficiency was associated with decreased utilization or the disappearance of T3 from the plasma pool (1987). Iron deficiency decreased plasma concentrations of T3 and T4 and increased *in vitro* hepatic rT3 deiodination suggesting that iron-deficient animals tend to metabolize thyroid hormone via a deactivating pathway (Smith *et al*., 1993). Presumably, a small fraction of T4 was converted to T3 and a larger proportion metabolized to a physiologically inactive metabolite, rT3. It is not yet clear how iron deficiency exerts its effects on deiodinase activity. Kaplan and Utiger have shown that outer ring deiodinase activity is not affected by either ferrous or ferric ions in an *in vitro* incubation method (Kaplan and Utiger, 1978). This, of course, does not rule out the possibility that iron needs to be incorporated into the enzyme during synthesis.

The effect of iron treatment on the thyroid profile might have been greater if the follow-up had been longer. We did not extend the study past 12 weeks because we sought to limit the delay in iron supplementation of the iron-deficient adolescents in the placebo and iodine groups.

In summary, concomitantly with an improvement in iron status in iron-supplemented subjects, there was a significantly improvement in thyroid hormone levels and greater final levels compared with the placebo group. Thus, the results support our first research hypothesis, i.e. that improvement of iron status in iron-deficient subjects can lead to an improvement in thyroid function tests. There was no significant difference in thyroid hormone indices between the iron and the iron + iodine groups, i.e., as expected, iron supplementation did not enhance the effect of iodine supplementation in this population for which we showed that iodine deficiency was not a problem (probably due to widespread consumption of iodized salt). Finally, the combined supplementation with iron and iodine had a significantly greater effect on thyroid function than iodine supplementation which was not effective.

Thus, public health interventions to improve the consumption of iron should be recommended in this setting. A study by Zimmermann *et al*. (2000a) showed that there are synergistic effects between simultaneous iron and iodine deficiencies, which indicate that the best approach to their prevention or treatment would be a simultaneous approach to both deficiencies. Thus, rather than iron supplementation, combined fortification of salt with iron and iodine may be of value in geographical areas in which iodine deficiency is a problem. Several recent intervention studies have pointed to the efficacy and safety of this procedure (Diosady *et al*., 2002; Zimmermann *et al*., 2002, 2004a, b).

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