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Iron Is Well Absorbed by Healthy Adults after Ingestion of Double-Fortified (Iron and Dextran-Coated Iodine) Table Salt and Urinary Iodine Excretion Is Unaffected[1,2]

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ABSTRACT Severe deficiencies of iron (Fe) and iodine (I) affect more than one third of the world's population. A table salt, fortified with I and Fe, would be useful in areas in which anemia and goiter coexist. However, interactions between the two minerals have prevented their simultaneous use as fortificants. A method has been developed to coat I with dextran such that after spraying onto table salt, Fe and I do not interact. Our objective was to determine the absorption of Fe and the urinary excretion of I from table salt when provided in meals designed to significantly inhibit or enhance Fe absorption. Subjects ($n = 16$) ingested Fe-enhancing and Fe-inhibiting meals containing 5 g of table salt with 0.39 μmol dextran-coated I as potassium iodide and 1 mg of Fe (ferrous fumarate labeled with ^{59}Fe) per gram of salt. Subjects also received a reference dose of 3 mg of ferrous fumarate labeled with ^{59}Fe to "correct" for interindividual variation in iron absorption at a later date. Measured by whole-body counting, Fe-absorption from the Fe-enhancing meal (36.2 $\pm$ 12.0%, corrected; 13.5 $\pm$ 13.8% uncorrected) was significantly higher than that from the Fe-inhibiting meal (7.4 $\pm$ 11.3%, corrected; 4.0 $\pm$ 8.4%, uncorrected) ($P < 0.0001$). Urinary excretion of iodine at baseline and postingestion were not significantly different (0.89 $\pm$ 0.5 vs. 1.06 $\pm$ 0.39 μmol/L, $P < 0.47$) and were within the normal range. We conclude that Fe was well absorbed but influenced by the composition of the meal and that urinary excretion of iodine was maintained in the normal range with dextran-coated iodine. J. Nutr. 129: 117–121, 1999.

KEY WORDS: • *iron absorption* • *double-fortified salt* • *dextran-coated iodine* • *humans*

Iron deficiency anemia and iodine deficiency are two major nutrition-related disorders, affecting more than one third of the world's population. Untreated, both result in serious health consequences. Food fortification is recognized as a possible means to prevent micronutrient deficiencies. The fortification of table salt with both iodine and iron has been suggested as an inexpensive and possibly effective and efficacious means to prevent both iodine and iron deficiencies because table salt is inexpensive and universally used. However, ensuring the stability and bioavailability of iron and iodine when used in combination has remained problematic.

There are predictable chemical interactions when iodine and iron are combined. In the presence of ferrous ions and oxygen, the iodine moiety of the double-fortified salt is likely to be unstable due to evaporation and catalytic oxidation of I^- to I_2. Iron is also readily oxidized to the ferric form, which has a lowered bioavailability, an unpleasant taste and an unsightly, yellowish brown or rust color. Despite the apparent chemical incompatibility of iron and iodine, previous published reports indicated that it may be possible to stabilize iodine on salt in the presence of iron using various chelating agents such as sodium hexametaphosphate (SHMP), although these agents are not without their own drawbacks (Rao 1994). The creation of a physical barrier between the iodine compound and the iron would prevent their interaction. In this study, we have used a dextran-coated iodine moiety as a barrier between the two elements. The salt was prepared and used in close proximity to the actual study; thus stability was not an issue. However, dextran-coated iodine in combination with ferrous fumarate has been found to be stable after prolonged storage even under varying harsh environmental conditions (personal communication, L. Diosady, Department of Chemical Engineering, University of Toronto).

The objective of this study was to determine the absorption of iron and urinary excretion of iodine after the ingestion of double-fortified table salt (iron and dextran-coated iodine) with meals designed to inhibit or enhance iron absorption in healthy human volunteers.

MATERIAL AND METHODS

Subjects. Sixteen healthy volunteers (8 men, 8 women) ranging in age from 21 to 53 y were studied at the Hospital for Sick Children in Toronto. Written, informed consent was obtained for each volun-

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TABLE 1

Hemoglobin, ferritin and reference iron absorption at baseline and at the end of the study[1,2]

Subjects	*n*	Age	Hemoglobin		Ferritin		Reference iron absorption
		y	*g/L*		*μg/L*		%
			Initial	Final	Initial	Final	
Men	8	27.1 ± 7.1	147.4 ± 1.9	145.8 ± 8.8	93.6 ± 23.4	91.6 ± 21.6	10.1 ± 2.6
Women	8	44.3 ± 6.2	127.0 ± 7.2	123.8 ± 7.1	33.5 ± 12.3	33.5 ± 12.3	21.6 ± 8.0

[1] Values are means ± SD, $n = 16$. Initial vs. final values did not differ significantly ($P > 0.05$).
[2] The initial and final blood samples and absorption determinations were taken 14 d apart.

teer before the study started and all experiments were approved by the Hospital for Sick Children Research Ethics Board.

Procedures. Baseline serum hemoglobin and ferritin concentrations were used to evaluate the subjects' iron status (**Table 1**). All subjects received both of the test meals (**Table 2**). One meal was designed to enhance iron absorption (high iron-availability meal), whereas the other was designed to inhibit absorption (low iron-availability meal). Assignment to the first meal was by random choice. The meals were provided 14 d apart. All test meals and the reference dose of inorganic iron were given between 0800 and 1000 h after a 10-h fast. The high iron bioavailability meal, designed to maximally enhance iron absorption, contained >90 g of meat and sufficient fruit, citrus juice or fresh vegetables to provide ~100 mg vitamin C. No coffee or tea, eggs or foods with high content of bran were allowed with this meal. The low iron availability meal was modified to maximally inhibit the absorption of nonheme iron. No meat products, and a minimum of fresh vegetables, fruits, and ascorbic acid was permitted with this meal. This meal also contained bran cereal and dairy products, and at least one cup of tea or coffee was consumed. Subjects were prohibited from taking supplements of iron and vitamin C throughout the study.

Table salt (5 g) containing 0.39 μmol (50 μg) iodine as potassium iodide and 1 mg of iron as ^{59}Fe-labeled ferrous fumarate per gram of salt was added to each test meal. This amount of salt represents one half to one third of the estimated daily salt intake in rural Ghana (Lartey 1994). After the ingestion of each test meal, subjects were provided with a questionnaire, requesting their opinion regarding the taste and palatability of the meals. Two weeks after the ingestion of their second meal, subjects (in the fasting state) received a test dose of labeled inorganic iron. The reference dose consisted of 3 mg of inorganic iron as ^{59}Fe-labeled ferrous fumarate in 50 mL water. Immediately before administration, 18.9 mg of ascorbic acid was taken, sufficient to give a 2:1 molar ratio of ascorbic acid to iron (Cook et al. 1991). The total dose of radioactivity in each test meal and the reference dose was 1 μCi (37 kBq).

Two consecutive 24-h urine collections were completed before each of the test meals to determine baseline iodine excretion (**Table 3**). Two consecutive 24-h urine collections were also collected after each of the test meals to determine urinary iodine excretion.

Preparation of double-fortified salt. ^{59}Fe-labeled ferrous fumarate was prepared in our laboratory from ferrous sulfate (Mandel Scientific Company, Guelph, Canada), based on the method of

TABLE 2

Composition of high and low iron-availability meals

Meal	Weight	Total iron	Heme iron	Nonheme iron	Ascorbic acid
	g/meal	*mg*			
High bioavailability meal[1] (Beef-vegetable stew)					
Beef, lean, raw	114	2.6	1.1	1.5	0.27
Tomato paste	85.5	2.5	0	2.5	37
Onions	57	0.15	0	0.15	3.5
Bell pepper	14	0.16	0	0.16	16
Garlic cloves	5	0.10	0	0.10	2
Peaches (canned)	114	0.45	0	0.45	3
Orange juice	128	0.60	0	0.60	36.3
Total iron		6.56	1.1	5.46	
Total ascorbic acid					98.1
Low bioavailability meal[2]					
Navy beans	95	2.6	0	2.6	0
White rice	114	0.9	0	0.9	0
Whole wheat bread	30	0.7	0	0.7	0
Margarine	14	0	0	0	0
Walnuts	8	0.5	0	0.5	0
Almonds	8	0.4	0	0.4	trace
Yogurt (skim milk)	226	0.1	0	0.1	2
Total iron		5.2	0	5.2	
Ascorbic acid					3

[1] The high availability meal which contained 34 g of protein was designed to enhance iron absorption.
[2] The low bioavailability meal which contained 22 g protein was designed to inhibit iron absorption.

TABLE 3

^{59}Fe absorption in subjects after consuming iron-enhancing and iron-inhibiting meals and urinary iodine excretion before and after the meals[1]

	Iron-enhancing meal[2] plus double-fortified salt		Iron-inhibiting meal[3] plus double-fortified salt		*P*-value
^{59}Fe absorption, %					
Uncorrected	13.5 ± 13.8		4.0 ± 8.4		<0.01
Corrected[4]	36.2 ± 12.0		7.4 ± 11.3		<0.01
Urinary iodine	Before	After	Before	After	
Excretion,[5] *μmol/L*	0.87 ± 0.53	1.09 ± 0.52	0.91 ± 0.46	1.02 ± 0.32	>0.10[7]
μmol/24 h[6]	1.06 ± 0.55	1.34 ± 0.54	1.19 ± 0.66	1.26 ± 0.78	>0.10

[1] Values are means ± SD, n = 16.
[2] The high iron bioavailability meal designed to maximally enhance iron absorption contained >90 g of meat and sufficient fruit, citrus juice or fresh vegetables to provide > 100 mg vitamin C. No coffee or tea, eggs or foods with high content of bran were allowed with this meal.
[3] The low iron availability meal was modified to maximally inhibit the absorption of non-heme iron. No meat products, and a minimum of fresh vegetables, fruits, and ascorbic acid was permitted with this meal. This meal also contained bran cereal and dairy products, and at least one cup of tea or coffee was taken with this meal. Subjects were prohibited from taking supplements of iron and vitamin C throughout the study.
[4] Dietary absorption was corrected to a mean reference value of 40% in each subject by multiplying by 40/*R* where *R* is the reference-dose absorption (for each subject).
[5] Urinary excretion of iodine >0.79 μmol/L is associated with normal iodine status (WHO 1993).
[6] The Australian normal range of daily urinary iodine excretion is 0.55–1.10 μmol/24 h (Buttfield and Hetzel 1967).
[7] Comparison is between urinary iodine excretion values from before and after each meal.

Fomon et al. (1989). The ^{59}Fe-labeled ferrous fumarate was checked for purity and subsequently diluted with cold ferrous fumarate in a 1000:1 ratio (USP 1995). The fortification of the table salt was performed by L. Diosady in the Department of Chemical Engineering, University of Toronto. Potassium iodide (1%) was dextrin encapsulated and subsequently spray-dried with the table salt. The double-fortified salt contained iron at 1 mg/g salt and iodine at 0.39 μmol (50 μg)/g salt.

Determination of iron absorption. Using the facilities of the Medical Physics Laboratories at the Toronto Hospital, iron absorption was measured using a whole-body counting technique (Schiffer et al. 1962). Four hours after the ingestion of each test meal, the first count was performed on each subject to establish a baseline value for the ingested radioiron isotope. Two weeks later, when all unabsorbed radioiron was completely excreted, subjects were counted for the second time to determine retained radioactivity (Cook et al. 1970). This sequence was repeated for each of the two test meals and the reference dose of iron.

Because of the marked influence of iron status on absorption, comparison of individual dietary absorption values with the two different meals was converted to a common reference value (Cook et al. 1991). Dietary absorption was corrected to a mean reference value of 40% in each subject by multiplying by 40/*R* where *R* is the reference-dose absorption (for each subject).

Assay methodology. Urinary iodine concentration was determined using the method of Dunn et al. (1993). Apparent iodine absorption was calculated as the difference in urinary excretion before and after the ingestion of the fortified salt and multiplying by 100. Plasma ferritin was determined by RadioImmunoAssay (RIA) kit (Ramco Laboratories, Houston, TX). All ferritin samples from a subject were assayed on the same day (in a single batch) in one 96-well microtiter plate to minimize interassay variation. An external reference standard (Lyphochek Anemia Control: Bio-Rad, Anaheim, CA) was assayed in duplicate on each microtiter plate for the ferritin assay. On the basis of these external controls, the within- and between-assay variations were 7.2 and 15.7%, respectively. Hemoglobin concentration was determined by the Cyanomethemoglobin method. Drabkin's reagent needed for this measurement was purchased from BDH, Toronto, Canada.

Data analyses. Paired *t* test of log absorption ratios was used to compare absorption of iron and urinary excretion of iodine associated with the iron-enhancing and iron-inhibiting meals (Cook et al. 1969). Values with a $P < 0.01$ were considered significant.

RESULTS

Baseline and "end-of-study" data on hemoglobin and serum ferritin, as well as reference iron absorption are shown in Table 1. The mean hemoglobin concentration before (135 ± 5.3 g/L) and after the completion of the study (134 ± 5.2 g/L) was not significantly different. Serum ferritin and hemoglobin concentrations were within the normal range for 14 of the 16 subjects included in the analysis. Two female subjects with the lowest hemoglobin and serum ferritin values (hemoglobin, 102.4 and 90.4 g/L; serum ferritin concentrations; 5.5 and 3.0 μg/L) had the highest rates of iron absorption from each meal.

Iron absorption. Iron absorption after the enhancing and inhibiting meals is shown in Table 3. Mean "uncorrected" absorption from the Fe-enhancing meal was significantly higher than that from the Fe-inhibitory meal. Similarly, mean absorption, after correction based on individual absorption of a reference dose of inorganic iron, was also significantly higher with the Fe-enhancing meal. There was a significant negative correlation between log serum ferritin and the absorption from the reference dose of iron ($r = -0.35$, $P < 0.0003$).

Urinary iodine excretion. Table 3 shows urinary iodine excretion before and after the ingestion of each of the two test meals. Urine was collected for two discrete 24-h periods before and after the meals. Because iodine excretion was similar for each of the two 24-h collections before and after the test meals, the average of the 2 d has been used in the table. There were no significant differences in excretion after each of the two test meals. Urinary excretion of iodine from baseline and postingestion was not significantly different and was within the normal range (Buttfield and Hetzel, 1967).

Taste and palatability. When the acceptability of the salt was tested, 93% of the subjects found the double-fortified salt agreeable in terms of taste and palatability.

DISCUSSION

Iron deficiency anemia and iodine deficiency disorders remain major problems in many parts of the world. Their prevention through supplementation and fortification programs are an urgent priority. Large-scale nutrition surveys have con-

clusively determined that iodine fortification of salt is successful in preventing iodine deficiency (Marine and Kimball 1921, Sooch et al. 1973, Tai et al. 1982, Thilly et al. 1980). Daily urinary excretion of iodine closely reflects iodine intake and has been used as a measure of iodine status (Gibson 1991). The urinary excretion cutoff points used to assess the severity of iodine deficiency were categorized by the Joint WHO/UNICEF/ICCIDD Consultation Report as follows: deficient (urinary iodine concentration < 0.79 μmol/L) and severely deficient (urinary iodine concentration < 0.16 μmol/L) (WHO 1993). The mean urinary excretion of iodine in our subjects, before the ingestion of the dextran-coated iodine via the double-fortified salt, was 0.89 ± 0.5 μmol/L (11.3 ± 6.2 μg/dL), indicating sufficient iodine status. After the ingestion of the double-fortified salt with the test meals, urinary iodine excretion was equivalent or higher. Thus we believe that the dextran-coating did not negatively influence the absorption of iodine. It remains to be demonstrated in a clinical trial whether iodine absorption from a double-fortified salt would also prevent iodine deficiency, but results from the current trial lead us to be optimistic. It is also important to note that iodine excretion was not affected by the composition of the various meals.

Magnusson et al. (1981) initially described the use of a reference dose of iron absorption value to improve the comparison of iron absorption values between subjects of varying iron status (good and poor). Our subjects were relatively homogeneous in terms of their iron status; they were mainly iron-replete individuals. When their iron absorption values from the test meals were corrected on the basis of their absorption of the reference dose of iron, the iron absorption values from the test meals all increased by a factor of 4. Had we recruited individuals with a more wide-ranging iron status, the reference-dose method of "correction" would have equalized individual iron absorption values. However, in this study, we believe that it is more appropriate to use the uncorrected values because they are a better reflection of iron-replete, homogeneous subjects.

For foods fortified with iron, the most important test of efficacy would be the bioavailability of the iron added to the food. Under controlled conditions, the relative bioavailability of ferrous fumarate is comparable to that of ferrous sulfate (Hurrell et al. 1989). Ferrous fumarate, however, is relatively insoluble in a neutral pH environment and thus is more suitable as a fortificant. The absorption of the ferrous fumarate from the double-fortified salt was 13.5 ± 13.8 and 4.0 ± 8.4%, respectively, from the "iron-enhancing" and the "iron-inhibiting" meals. Given the fact that the majority of subjects included in the study had sufficient iron stores and were not anemic, these results may be considered quite positive (i.e., indicative of reasonable iron absorption). Had the same protocol been followed in subjects who were iron depleted, one might reasonably predict significantly higher rates of absorption.

There are two aspects of this study that deserve specific comment. First, our findings agree with the previous work done by Cook and co-workers (1991), which demonstrated the influence of inhibitors and enhancers on iron absorption from composite meals. It should be noted, however, that the enhancing and the inhibiting meals used in this study were likely an exaggeration of the type of meals ingested in geographical locations in which meat is scarce. The inhibiting meal in the current study contained no meat, and minimum fruits, vegetables or vitamin C. Thus, in a nonexperimental setting, one would expect average iron absorption in an iron replete population to be between the two extremes used in this study. Second, the addition of the dextran-coated iodine did not compromise the apparent absorption of iodine from the double-fortified salt.

The fortified salt was designed to provide an amount of iodine adequate to treat and prevent iodine deficiency and an amount of iron sufficient to prevent iron deficiency in an iron-deficient subject when 10–15 g of salt is consumed per day. If we attempt to relate the study results to current Canadian Recommended Nutrient Intakes (RNI) for iron, the following approximations become apparent (Canada Health and Welfare 1990). Assuming a worst case scenario represented by the iron-inhibiting meal, a salt intake of 15 g/d for men and a mean absorption of 1.3% (average iron absorption of our male subjects), men would absorb 0.2 mg of elemental iron per day. The total iron requirement for men is 1.0 mg/d; thus, iron-fortified salt would provide 20% of the requirement. For women, assuming a salt intake of 10 g/d and a mean absorption of 6.8% (average iron absorption of our female subjects), the total absorption would be 0.68 mg. In this case, the iron-fortified salt would provide 45% of the estimated requirement of 1.5 mg/d. From the perspective of at-risk populations in developing countries toward which this initiative is primarily directed, there are several essential factors to be considered. First, an average salt intake of 10–15 g/d has been reported from populations in these countries with more salt ingested by men (due to higher food consumption), compared with women (Lartey 1994). Second, a typical meal in the developing world is a mixture of "enhancers and inhibitors" of iron absorption. Therefore, in such populations, iron absorption between the two mean values is expected. Finally, at-risk individuals have poor iron status due a combination of poor iron intake and blood loss via parasitic infections; therefore, a higher absorption from the iron-fortified salt is expected among these populations. Based on this realistic depiction of the iron status and meal types from populations at risk, we would expect an effectively higher iron absorption from the double-fortified salt in areas affected by iron deficiency anemia.

Although the current study was not designed to examine the effectiveness of double-fortified salt, it is reassuring that the addition of the test salt to the meals did not appreciably alter the flavor or the palatability of the meals. In addition, the method of preparation of the double-fortified salt with dextran-coated iodine is very simple and inexpensive especially with large-scale production.

Our objective was to determine the absorption of iron, as ferrous fumarate, and the urinary excretion of iodine from double-fortified salt, in healthy human subjects ingesting high and low iron-bioavailable meals. We demonstrated iron absorption within the expected range from the fortified salt. Our results, albeit by indirect methods, indicated that some of the iodine from the fortified salt is readily available and absorbable as iodine from the iodized salt. Thus, we conclude that a double-fortified salt may be useful in the prevention of both iron and iodine deficiency. This study was the first step in determining whether double-fortified salt might be an efficacious method to prevent iron and iodine deficiencies, but further studies on iodine absorption should be undertaken.

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