DETERMINATION OF IRON & IODINE ABSORPTION FROM
IRON AND IODINE DOUBLE-FORTIFIED SALT

by

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A thesis in conformity with the requirements
for the Degree of Master of Science
Graduate Department of Nutritional Sciences
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DETERMINATION OF IRON & IODINE ABSORPTION FROM IRON AND IODINE DOUBLE-FORTIFIED SALT

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Abstract

A table salt, fortified with iron (Fe) and iodine (I), would be useful in areas where Fe and I deficiencies coexist, however, interactions between the two minerals prevent their simultaneous use as fortificants. A method has been developed to coat I with dextran (dex) such that Fe and I do not interact. Our objective was to determine the absorption of Fe and I from this salt when provided in meals designed to significantly inhibit or enhance Fe-absorption. Subjects with normal haematologic status (n=16) ingested the two meals containing 5 g of table salt with 50 μg of I as potassium iodide and 1 mg of Fe (ferrous fumarate labeled with $^{59}$Fe) per g of salt. Measured by whole-body counting, Fe-absorption from the enhancing meal (8.8 ± 1.8 %) was significantly higher than Fe-absorption from the inhibiting meal (1.7 ± 0.8 %) (p<0.0001). Urinary excretion of iodine baseline and post-ingestion (48 hr before & after) were not significantly different (10.9 ± 1.5 μg/dl vs. 13.03 ± 1.1, p<0.47), nor was it affected by the two meals. We conclude that dex coated-I was well absorbed and that Fe was also well absorbed but influenced by the composition of the meal.
ABSTRACT

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LIST OF ABBREVIATIONS

IDA  Iron Deficiency Anemia
IDD  Iodine Deficiency Disorders
Hb   Hemoglobin
Enhcd Enhanced
Inhbtd Impaired
Uncrctd Uncorrected
Rf-dcrtd Corrected based on reference-dose
Se-frcrdt Corrected based on serum ferritin
WBC  Whole-Body Counting
IDRC International Development and Research Center
SHMP Sodium hexametaphosphate
WHO  World Health Organization
UNICEF United Nations Children’s Fund
ICCIDD International Council for Control of Iodine Deficiency Disorders
EDDI Ethylenediamine dihydroiodide
GRAS Generally Recognized As Safe
FDA Food and Drug Administration
USP United States Pharmacopoeia
IOM Institute Of Medicine
MDIS Micronutrients Deficiency Information System
NIN National Institute of Nutrition
CHAPTER 1

Introduction
1. INTRODUCTION

1.1 Background

Anemia and goiter are the two major nutrition related disorders, affecting more than 1/3 of the world population with serious consequences on mental and physical development. The manufacture and distribution of a salt fortified with both iodine and iron has been suggested as an inexpensive, effective and efficacious means to prevent both iodine and iron deficiencies. However, stability and bioavailability have remained as two major concerns in the manufacture of such a salt.

When iron and iodine are both added to the salt, the iodine is converted to elemental iodine, which can evaporate, and thus is rapidly lost. Earlier trials by Burgie et al. had indicated that the iodine moiety of the double-fortified salt was unstable because of the loss of iodine due to evaporation and catalytic oxidation of I⁻ to I₂ in the presence of ferrous ions and air oxygen (Burgie et al. 1986). Iron is also readily oxidized to the ferric form, which has a lowered bioavailability, an unpleasant taste and unsightly, yellowish brown or rust colour.

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 (Narasinga Rao, 1994). The forming of a physical barrier (i.e. encapsulating) between the iodine compound, and iron would stabilize the systems.
Encapsulating agents could include such stabilizers as sodium hexametaphosphate, dextrin, or even purified salt.

Successful application of sodium hexametaphosphate (SHMP) as an agent to stabilize the double-fortified salt was recently reported in India (Narasinga Rao, 1994). As a chelating agent, SHMP keeps the iron soluble and prevents its interaction with iodine. However, recent extensive trials performed by Dr. Diosady's team at the Department of Chemical Engineering, University of Toronto, concluded that dextran coated iodine in combination with ferrous fumarate results in a more stable compound. In order to prevent the evaporation of iodine, and the iodine oxidation in the presence of ferrous ion, the iodine moiety of the double-fortified salt was dextran encapsulated. Diosady et al. demonstrated that the physical isolation of iodine results in acceptable iodine retention under the worst expected storage and distribution conditions.

It has been recognized that the control of iron balance in normal man resides with the absorptive rather than excretory process. Radio-isotope studies of single meals have demonstrated that meal composition also has a major impact on nonheme iron absorption. Factors such as ascorbic acid markedly increase nonheme iron absorption whereas inhibitory components such as polyphenols or phytates impair its assimilation to a similar extent (Bothwell et al. 1989, Hallberg 1981). Depending on the balance between the enhancers and inhibitors as well as the body iron stores, absorption may vary as much as 20-fold in the same individual (Cook et al. 1991).

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Magnesium chloride, is a hygroscopic impurity often found in unpurified salt which dramatically increases the moisture content of the salt and results in immediate loss of more than 90% of the added iodine.
A problem with any measurement of iron absorption from groups of subjects is the inter- and intra-subject variabilities in absorption. In order to eliminate the difference among subjects' iron status, we obtained an independent measure of their capacity to absorb iron via the ingestion of a reference-dose of inorganic ferrous salt. In the present study, dietary absorption of nonheme iron was measured by means of whole-body counting (WBC) technique. Whole-body counting is one of the most sensitive and reliable methods of measuring iron absorption. As all the retained radioactivity is measured with WBC, the dose of the administered radioactivity can be substantially reduced in comparison with other methods, such as red cell incorporation of radioiron.

Urinary excretion of iodine reflects the plasma level of inorganic iodine which in turn reflects absorbed iodine (Vought & London, 1967). Inorganic compounds such as those present in iodized salt are reported to be completely absorbed and excreted in urine, with very little lost in feces. Hence urinary excretion of iodine was measured in the current study.

1.2.1. Objective

The objective of current study was to determine the absorption of iron and iodine from table salt in healthy human volunteers ingesting high and low iron-available meals.

1.2.2. Hypothesis

i) Iodine and iron supplied by the double-fortified salt is absorbed by healthy human subjects.
ii) Absorption of iron from a meal containing inhibitors of iron absorption will be lower than from a meal containing enhancers of iron absorption.

1.2.3. Study Significance

It is hoped that the result of this project will provide information to the International Development and Research Centre (IDRC), and other international agencies, in their efforts to eradicate iodine and iron deficiencies in at-risk areas of the world.
CHAPTER 2

REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

2.1. Iron

Iron is a reactive metal that is rarely found free in nature. Iron chemistry is complex, primarily because of its dual valency and reactivity with oxygen. These include electron transfer, the transport, storage, and activation of oxygen, nitrogen fixation, detoxification of activated oxygen species, and deoxyribonucleotide synthesis from ribonucleoside diphosphates (Worwood 1980, Bezkorovainy 1980). Iron is, however, a potentially toxic element, being a catalyst in the development of highly toxic free oxygen radicals (Owen et al. 1987). Biological systems have therefore developed several ways of limiting the entry of iron into the body and converting any absorbed iron into a bound "safe" form. In mammals, serum iron is bound to transferrin, and most body iron is present as porphyrin complexes (hemoglobin, myoglobin, and heme-containing enzymes). Iron is stored as ferritin and hemosiderin.

2.1.1. Iron Absorption

Regulatory control of iron status occurs as a result of absorption and bioavailability. It has been postulated that iron absorption is influenced by the rate of tissue iron uptake and by the size of labile iron pool in various body tissues (Cavill et al. 1975). The amount of iron in the body stores is the major factor controlling the absorption of iron from the gastrointestinal tract. Even a small change in iron stores, as reflected by serum ferritin values, is accompanied by reciprocal alteration in iron

There are two types of iron compounds in the diet with respect to the mechanism of absorption, heme iron (derived from hemoglobin and myoglobin) and nonheme iron (derived mainly from cereals, fruits, and vegetables). The absorption of these two kinds of iron is influenced differently by dietary factors. The absorption of nonheme iron draws the most interest as it forms the main part of the dietary iron intake. Its absorption in meals varies widely due to the marked effect of both dietary factors and the iron status of the subjects. When a comparison was made of the absorption of heme and nonheme iron from meals, a significant correlation was found between the absorption of the two kinds of iron. However, a much greater fraction of the heme iron was absorbed (37%) than the nonheme iron (5%), (Bjorn-Rasmussen et al. 1974).

After heme iron enters the intestinal cells it is rapidly degraded by heme deoxygenase (Raffi et al. 1974), and the released iron enters the common intracellular iron pool. Subsequent mucosal handling of this iron appears to be identical to that of inorganic iron (Cook, 1990). The duodenum and the proximal portion of the upper intestine are the two major sites of iron absorption in the intestinal tract. However, the elimination is almost entirely through the colon (Robschit-Robbins 1929).

Four major factors govern the amount of iron absorbed from an iron-fortified food. These are the iron fortification compound used, the amount of iron added, the presence of enhancers or inhibitors of iron absorption in the meal, and the iron status of the consumer (Fomon & Zlotkin, 1992). Wider application of isotopic techniques during the 1950’s and
1960's led to the realization that the bioavailability of ingested iron may be more important than total intake. It was also found early on that one food could interact with the absorption of iron from another food (Layrisse et al. 1968). An important conclusion from these isotope studies was that the bioavailability of iron in a meal is not the sum of the absorption of iron from the single foods contained in a meal, but rather a net effect of all food items, and their constituents, increasing or decreasing the nonheme iron absorption (Cook et al. 1969). The Indian National Institute of Nutrition in 1975 showed that dietary factors play an important role in the development of iron deficiency in Indian subjects. The same study demonstrated that, although most habitually consumed diets in different regions of India showed seemingly adequate amounts of iron (20-30 mg, NIN, India, 1975), data from isotope studies using the whole-body counting technique indicated that in nonanemic adult males, only 1 to 5% of this iron was absorbed (Narasinga Rao, 1978).

During the past two decades, application of the extrinsic-tag method for measuring nonheme-iron absorption has led to the identification of a large array of dietary factors affecting iron absorption in humans (Reddy et al. 1991). It was concluded that in the context of the US diet, the role of enhancers was less important than the role of inhibitors of nonheme iron absorption (Cook et al. 1991). Cook et al. (1976) reported that chelates in the diet may have a major effect on the assimilation of polyvalent transitional cations such as iron. It was shown that certain chelates such as ascorbic acid enhance iron absorption by forming iron ascorbate complexes at low pH which remains soluble at the high pH of the duodenum and donate their iron to mucosal cells. Other chelating
compounds, including polyphenols (containing alkyl groups), phosphates, carbonates, and oxalates were found to have the opposite effect on biavailability. Their effect is usually due to the formation of polymers. They can either enhance or reduce iron absorption depending on the stability constant, solubility at the intraluminal pH of the intestinal tract, and the ability of the iron-complex to penetrate the mucosal barrier.

While beef, lamb, pork, chicken, liver and fish substantially raise the rate of nonheme iron absorption, milk, cheese, and eggs do not increase and may decrease iron availability (Cook & Monsen 1976). Tannic acid in tea (Disler et al. 1975, Hallberg & Cook 1979), phosphitin of egg yolk (Rossander et al. 1975, Monsen & Cook 1979, Hurrell et al. 1988), phytates present in bran and nuts (Macfarlane et al.1988), and finally polyphenols present in legumes and leafy vegetables (Tustawiroon et al. 1991), clearly suppress absorption when present in sufficient amounts.

Of especial interest is the observation that meat has about the same promoting effect on the absorption of heme and nonheme iron (Martinez et al. 1971). However, the absorption promoting effect of meat is dose related (Layrisse et al. 1968, Hallberg 1979).

2.1.2. Iron Deficiency Anemia (IDA)

Anemia is traditionally defined according to age- and sex-related “cut-off points” of hemoglobin values (Bothwell et al. 1979). Iron deficiency anemia refers to an anemia that is associated with additional laboratory evidence of iron depletion as a result of one or more of the following test results; low serum ferritin concentration, low transferrin saturation, or an elevation in the erythrocyte portophyrin level (Earl & Woteki, 1993).
Iron deficiency is present when body iron is diminished (Cook et al. 1986, Charlton et al. 1982). The presence of iron deficiency implies neither the degree nor the presence of anemia. Thus, an individual may be iron deficient without manifesting iron deficiency anemia, the converse, however, does not occur (Pollitt et al. 1976).

The consequences of iron deficiency anemia have traditionally focused on anemia which reduces maximum oxygen consumption and maximum work performance (Basta et al. 1979, Edgerton et al. 1979). Severe anemia is generally accepted as a health hazard. Among functions that may be impaired are work capacity (Viteri et al. 1974), immune function (Sirkanita et al. 1976, Joynson et al. 1972), and learning ability (Webb et al. 1973). Severe anemia during pregnancy increases morbidity and mortality and is associated with an increase risk of low birth weight infants (National Institute of Nutrition, Annual Report, India, 1980).

There is now considerable evidence that mild iron deficiency even without anemia is associated with significant health consequences. The greatest impact of iron deficiency is in growing children, who develop defects in attention span leading to learning and problem solving difficulty (Rajomaki et al. 1979, Beilby et al. 1992, Leggett et al. 1990, Witte 1991). This learning deficit may have lasting consequences by limiting an individual’s subsequent achievement. Iron supplementation has been shown, to increase growth velocity and decrease level of the morbidity in anemic children (Coenen et al. 1991). Earlier studies of Pollitt et al. (1989) also show that schoolchildren with iron deficiency have poorer cognitive function, which is only partly improved by iron treatment.
2.1.3. Iron Requirements

According to Arthur and Isbister, a review of previous studies of anemia indicates iron deficiency is almost never due to dietary deficiency in an adult in Western society. This is because the average person requires very little iron intake to replace that lost through sweat, menstruation, and urine, and also because as much as 90% of the iron needed for the formation of blood cells is derived from recycling senescent red blood cells (Hoffbrand 1981). Estimates of iron requirements are based on obligatory losses, which include those from exfoliation of cells from internal and external surfaces, urine, sweat and menstrual fluids, in addition to iron needed for growth and during pregnancy (Green et al. 1968). Menstruation increases median iron requirements to about 1.4 mg daily (2.4 mg in 90% of females) and pregnancy raises this further to more than 5 mg daily in the second and third trimesters (Bothwell, 1995). Fifty percent of women would maintain normal hemoglobin values if they absorbed 1.2 mg daily (0.8 basal plus 0.4 mg menstrual losses), while a figure of 2.2 mg daily would be required to cover 95% of menstruating women (FAO/WHO Joint Report, 1988).

2.1.3. Measurements of Iron Status

Conventional laboratory indices of iron status include serum iron, transferrin / total iron-binding capacity, transferrin saturation and ferritin. Although each of these measurements has merit, no single determination gives a reliable index of iron status (Cazzola et al. 1992, Burns et al. 1990). Iron deficiency without anemia is diagnosed on the basis of a combination of biochemical indicators of iron status in which the hemoglobin
concentration remains within the normal range. Although no single indication of iron status is diagnostic of functional iron deficiency, a low serum ferritin concentration indicates that iron reserves are depleted (Earl & Woteki, 1993). Low serum ferritin values indicate iron deficiency but high values do not necessarily mean increased body stores. Inflammation, liver disease, hemolytic malignant diseases and hemolytic anemia may increase serum ferritin concentration out of proportion to the size of the body store (Lifschitz et al. 1974). The serum ferritin correlation in healthy adults is proportional to the size of body iron stores (Addison et al. 1972, Jacobs et al. 1972, Cook et al. 1974), and changes in the serum ferritin value correlate with changes in the size of the stores (Siimes et al. 1974).

2.1.4. Iron Toxicity

There are two diseases associated with an excess of iron in the body. In 
haemochromatosis immense deposits of inorganic iron are found in the liver, pancreas, skin and other parts of the body (Muir et al. 1914, Sheldon, 1935). In polycythaemic ruba vera the number of red cells and the amount of hemoglobin are very greatly in excess of normal. So much so, that the amount of iron in the blood may be doubled (Widdowson, 1937). In addition to iron loading disorders (eg. haemochromatosis), there have been recent disturbing claims based on epidemiological data suggesting that subjects with only modestly raised iron stores are at greater risk of developing malignancy (Stevens et al. 1988) and ischemic heart disease (Salonen et al. 1992).
2.2. Iron Supplementation

Administration of therapeutic doses of iron is recommended as a short term measure to correct anemia in situations when it is necessary to raise Hb levels quickly. However, when anemia is less severe, and the main objective is to improve iron balance over a longer period of time and to prevent the development of anemia in at risk populations, fortification of foods with iron has been suggested as a practical measure (WHO Technical Report Series, 1972). Supplementation involves the giving of iron in medicinal form. This is often the only feasible approach when there is a large iron requirement and a relatively short time span, as occurring during pregnancy (Baker et al. 1979, International Nutritional Anemia Consultative Group 1979, WHO Technical Report Series, 1975). Meanwhile, fortification is an approach which can be applied to large population groups at low cost. It has the advantage that the identification and cooperation of actual and potential deficient individuals is not a prerequisite as it is with supplementation.

2.2.1. Criteria for the Selection of Vehicles for Iron Fortification

Iron fortification has been considered as one of the practical approaches for the prevention and the control of iron deficiency anemia in the population. Several factors need to be considered in the choice of vehicles for iron fortification:
i) The vehicle must be consumed in sufficient amount by the target groups in the population. Ideal vehicles in most countries from this point of view are salt and flour where the variability in consumption is only about twofold.

ii) The fortified vehicle should remain stable and palatable after fortification.

iii) The distribution of the fortification in the vehicle should not change during storage (i.e. no sedimentation).

2.2.2. Salt as a Vehicle for Iron Fortification

Common table salt (NaCl) is considered to be a suitable vehicle for iron fortification satisfying all the criteria of an ideal vehicle because: a) salt is consumed by all segments of the population, rich or poor, perhaps somewhat more by the poor; and, b) salt consumption lies within a narrow range of 12-20 g/day, with an average intake of 15g/day (Narasinga Rao, NIN Report, India).

2.2.3. Criteria for the Selection of Iron Sources

The identification of a suitable iron compound to be used with salt which will meet the twin criteria of stability during storage and satisfactory bioavailability has been a technological challenge. There are several factors that must be considered in choosing a fortifying compound (WHO, 1975; Cook and Reusser, 1983). The following are examples of properties to keep in mind:

a) Relative bioavailability of the compound

b) Reactivity of the compound to cause discoloration or any changes in flavor or odor.
c) Stability of the compound with storage and food preparation.

d) Compatibility with other nutrients

The bioavailability of nonheme iron is governed by its solubility in the lumen of the proximal small bowel. Freely water-soluble sources, such as ferrous sulfate and ferrous gluconate, exchange with the common nonheme Fe pool in a fortified meal. Unfortunately, these highly soluble Fe compounds also cause unacceptable organoleptic changes when added to stored food (Hurrell, 1984). Other iron sources such as ferrous fumarate and ferrous succinate are slowly soluble in water but readily soluble in dilute acids such as gastric juice. These compounds provide an attractive compromise as they appear to be sufficiently unreactive to avoid organoleptic problems during storage. Hurrel et al. (1989) indicated that ferrous fumarate is a suitable Fe source for food fortification. Ferrous fumarate, Fe(COO--CH=CH--COO), is a reddish orange to reddish brown powder that is odorless and almost tasteless. It contains about 33 % Fe, and has a similar bioavailability to ferrous sulfate in both rat and human studies, (Clydesdale & Wiemer, 1985).

2.3. Radioisotope Studies

The introduction of radioisotopes made it possible to label single food items biosynthetically with radioiron (Moore et al, 1951). It became possible via this method to measure iron absorption from a single composite meal (Hallberg et al, 1972). Meanwhile, the accuracy of the extrinsic tag method is mainly based on the validity of the assumption that a complete isotopic exchange occurs between the extrinsic tag and the main part of the nonheme iron compounds in the diet (Bjorn-Rasmussen et al. 1972, Cook et al. 1972).
When single foods biosynthetically labeled with radioiron (intrinsic tracer) were carefully mixed with a trace amount of iron salt labeled with another radioiron isotope (extrinsic tracer), the observation was made that the absorption of the two tracers, from such double labeled foods was almost identical (Hallberg et al. 1979).

$^{59}\text{Fe}$ is one of the isotopes usually employed in extrinsic tag studies. It can readily be detected by means of its energetic gamma ray (1.1 and 1.3 MeV) and by its beta emission (Emax 0.27 and 0.46 MeV). This use of radioisotopes of iron was first described by Hahn (Hahn et al. 1943). Since then, highly accurate techniques of measuring iron absorption have been developed such as, whole-body counting. Presently, whole-body counting of orally absorbed radioiron is accepted as the reference method of iron absorption (Bothwell et al. 1979). The method permits accurate iron studies using 1μci of $^{59}\text{Fe}$ or less, thus reducing radiation exposure of the patients to a minimum (Price et al. 1962). Use of the incorporation of oral and intravenous tracers into erythrocytes has been shown to have a close correlation with whole-body counting (Lunn et al. 1967, Werner et al. 1983), however, the use of only oral tracer data was reported to give a poorer correlation because of the uncertainty of blood volumes and radioiron utilization in new erythrocytes (Werner et al. 1983). It was observed by Werner et al. that the use of a double radioisotope technique with post-absorption serum measurements had the best correlation with whole-body counting.

Despite such precise measurements, absorption studies have been difficult because of large differences in absorption among normal subjects and in the same subject with repeated testing (Kuhn et al. 1968, Brise et al. 1962). The largest variable is the
difference between individuals. While mucosal response can be standardized by comparing the absorption of food iron against a common reference standard, day to day variation in the same subject are still appreciable (Cook et al.). Layrisse et al. (1969) showed that the differences in iron status among different subjects can be eliminated by obtaining an independent measure of their capacity to absorb iron. They introduced the model of using a reference-dose of inorganic radioiron given at a physiologic level under the standardized conditions to each subject. The absorption of iron from various foods will then be expressed as the ratio of food iron absorption to reference-dose absorption (Magnussen et al. 1981). There is a high correlation between the iron absorption from meals and absorption from the reference-doses (Bjorn-Rasmussen et al, 1976). The slope of a regression line between the two absorption measurements (meals / reference-doses) is an index of the bioavailability of the nonheme iron in a meal (Bjorn-Rasmussen et al. 1976). The most suitable type of iron compound to be employed as a reference standard is probably a ferrous iron salt, since this represents the final common pathway by which all forms of dietary iron with the exception of hemoglobin are assimilated (Cuhn et al. 1968).

Serum ferritin can also be used as an alternative to the reference-dose absorption. However, serum ferritin is only an indirect measure of an individual’s ability to absorb iron, and extraneous factors such as minor infections may affect iron absorption and serum ferritin in opposite directions. Reference-doses therefore are preferable and should be used whenever possible (Hallberg 1980)
2.4. Iodine

Iodine is an essential micronutrient for all animal species, including humans (Hetzel and Maberly 1986). It is an integral part of the thyroid hormones, thyroxin and triiodothyronine. These hormones are required for normal calogenesis, thermoregulation, intermediary metabolism, protein synthesis, reproduction, growth, development, hematopoiesis and neuromuscular function (Fisher and Carr 1974). The ocean is the world's major source of iodine. In coastal area, seafood, water, and even iodide-containing sea mist are dependable iodide sources. Iodine is present in food and water predominantly as iodide and, to a lesser degree, bound to amino acids.

2.4.1. Iodine Metabolism

Iodine is rapidly and almost completely absorbed and transported to the thyroid gland for the synthesis into the thyroid hormones, to salivary and gastric glands, and to kidneys for excretion into gastrointestinal tract and urine (Silva 1985). Iodinated amino acids are well absorbed as such, although more slowly and less completely than iodide. However, a proportion of their iodide may be lost in the feces in organic combination. The remainder is broken down and absorbed as iodine (Keating et al. 1949). Other forms of inorganic iodine are reduced to iodine prior to absorption (Cohn 1932).

Iodine metabolism and thyroid function are closely linked, since the only known role of iodine is in the synthesis of thyroid hormones. In order to ensure an adequate supply of hormones, the human thyroid must trap about 60 μg of iodine daily (Underwood
This is primarily achieved irrespective of the plasma level, by adjustment of the thyroidal iodine clearance rate, so that when the plasma iodide decreases the thyroidal clearance increases, with the actual iodide uptake remaining more or less constant (Underwood 1971). Adaptation to iodide deficiency thus occurs by increasing the thyroidal iodide clearance rate. Such functional overactivity of the iodide-trapping mechanism is usually associated with an increase in the gland mass, or goiter, but in mild iodine deficiency the biochemical manifestation of the deficiency, namely low plasma iodide and urinary iodine excretion and high thyroidal iodine clearance and radioiodine uptake, have been demonstrated without any obvious goiter (Wayne et al. 1964).

Since only the thyroid gland and the kidneys are in competition for plasma iodide, the ultimate accumulation of iodine by either of these organs, as measured by the 24-hr or 48-hr thyroidal uptake or urinary excretion, or both, is simply a reflection of this competition and not a measure of the level of the function of either thyroid or kidneys. If kidney function were completely absent, virtually 100 per cent of administered iodine would eventually accumulate in the thyroid gland (Berson 1956).

2.4.2 Iodine Requirements

The requirement of iodine in adults must be at least equal to the daily amount of hormonal iodine degraded in the peripheral tissues and unrecovered by the thyroid (40 to 100 μg/day). An extra margin of safety is needed to meet increased demands that may be imposed by natural goitrogens under certain conditions. Balance studies have found that intakes ranging from 44 to 162 μg/day are sufficient to maintain positive balance (WHO,
MDIS Working Paper #1, 1993). As a significant degree of neurological development occurs within weeks of conception, and especially during the first month of fetal growth, it is imperative that all women of reproductive age have adequate iodine stores, not only women who are pregnant (WHO, MDIS Working Paper #1, 1993).

2.4.3. Iodine Deficiency Disorders (IDD)

Iodine Deficiency Disorder as a term, was introduced in 1983 and has since become generally accepted. It (IDD) refers to the wide spectrum of effects of iodine deficiency on growth and development (Hetzel 1983). Iodine deficiency disorders continue to threaten the health and well being, and the social and economic productivity and advancement, of several hundred million people throughout the developing world. Recent evidence indicates a wide spectrum of disorders resulting from severe iodine deficiency. These iodine deficiency disorders include: goiters at all ages, endemic cretinism, (characterized most commonly by mental deficiency, deaf-mutism and spastic diplegia), and lesser degrees of neurological defect related to fetal iodine deficiency; impaired mental function in children and adults (associated with reduced levels of circulating thyroxin); increased still birth, and prenatal infant mortality (Hetzel 1987). Impairment of nervous system development and function is the most important consequence of iodine deficiency. The term “endemic cretinism” is traditionally used to describe this condition, and usually includes deaf-mutism, mental retardation, and a characteristic spastic or rigid neuromotor disorder. Endemic cretinism also includes an insult to the nervous system which is irreversible; however, in an iodine deficiency there
may be aspects of neurological dysfunction related to hypothyroidism which are reversible with treatment (Delonge 1986). Studies in man have been complemented by studies in animals which have established the effects of iodine deficiency on the brain. These studies have confirmed that the effects of iodine deficiency are mediated through the thyroid gland secretion of thyroid hormones, thyroxin (T4) and triiodothyronine (T3), (Hetzel et al. 1993).

It is known that thiocynate, a naturally occurring goitrogen * resulting from chronic consumption of poorly detoxified cassava, aggravates the effects of iodine deficiency (Gitan et al. 1986). The principal goiotrogens identified are thiocynates and isothiocynates; polyphenols (biflavonoids); phenolic derivatives (resorcinol and others); phathalic acid derivatives; and possibly calcium and lithium. Natural goitrogens, such as those found in cabbage and cassava, have been implicated in the pathogenesis of goiter in some parts of the world (Matovinovic, 1983).

2.4.4. Iodine Toxicity

Ever since the Canadian federal law obliged the table salt producers to add 76 μg of iodine per gram of salt, endemic goiter has ceased to be a problem in Canada. On the contrary, iodine excess appears to be a growing problem. Excessive intakes of iodine can cause enlargement of the thyroid gland, just as deficiency can. This goiter-like condition can be so severe as to block the airways in infants and cause suffocation (Whitney & Rolfes 1993).

* (GOY-troh-jen), a thyroid antagonist found in food; causes toxic goiter.
The iodine intake of Canadians is in excess of 1000 μg/day, based on the analysis of a representative diet, with 60% coming from table salt and 25% from dairy products (Fischer and Giroux 1987a). Much of the iodine present in milk comes from ethylenediamine dihydroiodide (EDDI), added to feed to prevent foot rot in cattle (Berg and Padgitt 1985), and from improper use of iodophor sanitizers (Dunsmore and Wheeler 1977).

### 2.4.5. Assessment of Iodine / Thyroid Status

The two basic clinical techniques in the measurement of goiter are a) inspection and palpation, and b) ultrasonography. On the other hand, before an individual develops goiter in response to iodine deficiency, other important physiological changes occur. These changes can be detected through the use of biochemical indicators, including serum thyroid hormones, TSH, and urinary iodine levels (WHO, MDIS Working Paper #1, 1993).

Since all the iodide secreted into the gastrointestinal tract is reabsorbed, the main excretory route for the inorganic form of iodine is urine. Although losses in the milk of lactating women and losses in sweat in hot climate can be considerable, urinary excretion is a reliable indicator of iodine status under most circumstances.

### 2.4.6. Iodine Deficiency Prevention

Substantial evidence is now available that iodine deficiency disorders can be prevented by iodization programs (Hetzel BS, Dunn JT, Stanbury JB, 1987). A verity of foods such as salt, bread, sweets, milk, sugar, and water have been used as vehicles for
iodine. However, over the past 50 years, iodized (or iodinated) salt has been the mainstay of iodine deficiency prophylaxis (Hetzel 1987). Iodinated salt is the most practical, effective, and satisfactory means for correction of iodine deficiency. Ever since Marine and Kimball demonstrated the efficacy of iodine prophylaxis in the control of endemic goiter, widespread iodination of edible salt, to ensure physiological intakes of iodine, has been the continued practice in almost all developed countries. Salt is one of the few commodities consumed by all sections of the community irrespective of social or economic level. It is consumed at approximately the same level throughout the year by all normal adults. Compared to other food commodities, whose production is dispersed, the production of salt is limited to fewer production centers. Recent studies have shown that in many remote villages, salt is one of the few commodities that comes from outside the village. All these factors make salt one of the most effective vehicles for dietary supplementation of micronutrients (Vekentash Mannar, 1985).

The process of salt iodination aims to mix salt with a prefixed quantity of iodine to ensure the desired dosage of iodine in the salt. The techniques for iodination include dry mixing, drip feeding, submersion, and spray mixing, of which the latter is the most widely used (Vekentash Mannar, 1985). Iodine is normally introduced as a compound such as potassium iodide, potassium iodate, or calcium iodate (Venkatesh Mannar, 1987). The choice of method of salt iodination, as well as the iodine fortification compound, depends on conditions prevailing in a particular geographic location.
2.5 Double-Fortified Salt

When salt is used as a vehicle for iron fortification, and the iron compound used is an iron salt, it is necessary to keep the pH of the salt low to prevent discoloration and formation of poorly available ferric hydroxides. The bioavailability of iron from such acidified salts may then be good. When iron and iodine are both added to the salt, the iodine is converted to elemental iodine, which can evaporate at low pH. Earlier trials by Burgie et al. had indicated that the iodine moiety of the double-fortified salt was unstable because of the loss of iodine due to evaporation and catalytic oxidation of I to I₂ in the presence of ferrous ions and air oxygen (Burgie et al. 1986). Iron is also readily oxidized to the ferric form, which has a lowered bioavailability, an unpleasant taste and unsightly, yellowish brown or rust colour.

Despite the apparent chemical incompatibility of iron and iodine, previous published reports have shown that it is possible to stabilize iodine on salt in the presence of iron (Narasinga Rao, 1994). Chemical compounds used as stabilizers were orthophosphoric acid, and sodium hexametaphosphate. These compounds make a complex with iron which keeps iron soluble (i.e. in ferrous state), (Narasinga Rao & Vijayasarathy, 1975). Successful application of sodium hexametaphosphate (SHMP) as an agent to stabilize the double-fortified salt was recently reported in India (Narasinga Rao, 1994). As a chelating agent, SHMP keeps the iron soluble and prevents its interaction with iodine. However, SHMP may have undesired effects on the bioavailability of other minerals in the meal.
An alternative approach to the use of a stabilizer is to create a physical barrier between the iodine and the iron. Dextran has been found to be an excellent encapsulating agent. It prevents the evaporation and oxidation of iodine in the presence of ferrous iron and is, itself, an inert compound.

Subsequent in vitro and in vivo tests on rats, conducted at the laboratory of Dr. Rao, the Department of Nutritional Sciences, University of Toronto, showed that the encapsulated iron and iodine were bioavailable. Thus, the next logical step in the series of these investigations around the double-fortified salt was to determine the in vivo bioavailability of the salt in humans.
CHAPTER 3

MATERIALS & METHODS
3. MATERIALS & METHODS

3.1. Experimental Design

3.1.1. Subjects

Sixteen volunteers (8 males, 8 females) ranging in age from 21 to 53 were studied. All were in good health. Their iron status was unknown at the beginning of the study. Written, informed consent was obtained for each volunteer before the study started and all experiments were approved by the Hospital for Sick Children Research Ethics Board.

3.1.2. Procedure

Volunteers were randomly assigned to a meal designed to have low or high iron bioavailability, (Table 3.1). Each subject received both meals. The high iron bioavailability meal designed to enhance iron absorption maximally, contained > 90 g of meat and sufficient fruit, citrus juice or fresh vegetables to provide >100 mg vitamin C. The subjects were not allowed to drink coffee or tea with this meal. Eggs or foods with high content of bran were not permitted. 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 were permitted with this meal. The low bioavailable meal contained bran cereal and dairy products. At least one cup of tea or coffee was taken with this meal. We added to each meal 5 g of $^{59}$Fe-labeled table salt containing 50 μg iodine as potassium iodide and 1 mg of iron as $^{59}$Fe-labeled ferrous...
<table>
<thead>
<tr>
<th>Food</th>
<th>Wt (g)</th>
<th>Total Iron (mg)</th>
<th>Heme Iron (mg)</th>
<th>Nonheme Iron (mg)</th>
<th>Ascorbic Acid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Meat-containing high availability</strong> <em>(34 g protein, 415 KCal)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef-vegetable stew</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef, lean, raw, 1/4 pound</td>
<td>114</td>
<td>2.6</td>
<td>1.1</td>
<td>1.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Tomato paste 3 ounces</td>
<td>114</td>
<td>2.6</td>
<td></td>
<td>2.6</td>
<td>37</td>
</tr>
<tr>
<td>Chopped onions 1/4 cups</td>
<td>57</td>
<td>0.15</td>
<td></td>
<td>0.15</td>
<td>3.5</td>
</tr>
<tr>
<td>Bell pepper, diced, 1/2 ounce</td>
<td>14</td>
<td>0.16</td>
<td></td>
<td>0.16</td>
<td>16</td>
</tr>
<tr>
<td>Garlic cloves, minced, 2 cloves</td>
<td>5</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Peaches canned, 1/2 cup</td>
<td>114</td>
<td>0.45</td>
<td></td>
<td>0.45</td>
<td>3</td>
</tr>
<tr>
<td>Orange juice 1/2 cup</td>
<td>128</td>
<td>0.6</td>
<td></td>
<td>0.6</td>
<td>36.3</td>
</tr>
<tr>
<td>Total iron</td>
<td></td>
<td>6.5</td>
<td></td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid 98 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>98.1</td>
</tr>
<tr>
<td><strong>2. Nonmeat, low availability meal</strong> <em>(22g protein, 730 KCal)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navy beans, cooked 1/2 cup</td>
<td>95</td>
<td>2.6</td>
<td></td>
<td>2.6</td>
<td>0</td>
</tr>
<tr>
<td>White rice, cooked 1/2 cup</td>
<td>114</td>
<td>0.9</td>
<td></td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Whole wheat bread 1 piece</td>
<td>30</td>
<td>0.7</td>
<td></td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Margarine, 1 tablespoon</td>
<td>14</td>
<td>0.0</td>
<td></td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Apple slices 1/2 cup</td>
<td>55</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Walnut, black, raw tablespoon</td>
<td>8</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Almond, raw, 1 tablespoon</td>
<td>8</td>
<td>0.4</td>
<td></td>
<td>0.4</td>
<td>trace</td>
</tr>
<tr>
<td>Yogurt, skim milk, 1 cup</td>
<td>226</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Total iron</td>
<td></td>
<td>5.3</td>
<td></td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid 3 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3.1 The composition of high and low bioavailable meals
fumarate per gram of salt, (this amount of salt represents 1/3 of the estimated maximum daily salt intake). In order to avoid any interaction with iron-absorption results, and also to prevent any false reading in urinary iodine excretion, subjects were asked to stop supplements of iron and vitamin C, throughout the study.

3.1.3. Protocol

Twenty four-hour urine samples were collected on the two days prior to each of the two test meals for baseline iodine excretion, and also for two 24-hour periods following each test meal. All test meals and the reference iron dose were given between 08:00-10:00 after a 10-hour fast. Subjects were randomly assigned to receive each of the test meals. Four hours after the ingestion of each meal, a whole-body count was performed on each subject to establish a baseline value for the ingested radioiron isotope (Schiffer et al. 1962). Two weeks later, when all unabsorbed radioactivity was completely excreted (Bothwell, Charlton, Cook, and Finch 1979), subjects were counted for the second time to determine retained radioactivity. This sequence was repeated for each of the two test meals and the reference-dose of iron, Table 3.2.

---

* Previous trials indicate that a reasonably consistent result can be obtained if the initial count is delayed for 4 hours.

b Counts at baseline (4 hours) / Counts at day 14 X 100
Table 3.2. Study Design

16 Healthy Subjects

Randomization for HBM or LBM

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hr Urine Sample</td>
<td>★</td>
<td>★</td>
<td>★</td>
<td>★</td>
<td></td>
</tr>
<tr>
<td>High Bioavailable. Meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Bioavailable. Meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Sample (finger prick)</td>
<td>★</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-Body Counting</td>
<td></td>
<td></td>
<td>★</td>
<td>★</td>
<td></td>
</tr>
</tbody>
</table>

The same cycle was repeated for the second test meal, and also for the reference-dose of inorganic iron, with the exception that no urine collection was necessary for the third cycle. Each subject received both meals as well as the reference-dose of iron.

★ HBM = High Bioavailable Meal
★ LBM = Low Bioavailable Meal
3.1.4. Test Dose of Inorganic Iron

Each subject (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.

3.1.5. Test for Palatability and Taste of the Double-Fortified Salt

Following the ingestion of each test meals, our subjects were provided with a questionnaire, testing their opinion regarding the taste and palatability of the meals in which the salt was added to.

3.2. Preparation of Double-Fortified Salt

3.2.1. Iron & Iodine Sources

We prepared $^{59}$Fe-labeled ferrous fumarate from ferrous sulfate manufactured by Mandel Scientific Company Ltd. (Guelph, Ontario) based on Fomon’s method in our laboratory (Fomon et al. 1989). Then, $^{59}$Fe-labeled ferrous fumarate was diluted with cold ferrous fumarate in a 1000:1 ratio. A test of purity for this radioiron was performed according to USP National Formulary, 1995. Aliquots of ferrous fumarate were checked for purity and subsequently were physically mixed with the iodized salt. The fortification of NaCl with iodine was performed in the laboratory of Dr. Diosady at 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 of salt and iodine at 50 μg/g of salt. The iron dosages were based on iron content of ferrous fumarate after it was tested for its purity.

3.3. Measurements

Using the facilities of the Medical Physics Laboratories at the Toronto Hospital, iron absorption was measured by means of whole-body counting technique. In these measurements we used an energy band of (0.4-1.4 Mev) and a sensitivity of 1.2×10⁻⁸. The whole-body counting equipment consisted of 8 NaI crystal detectors (4 upper & 4 lower ones) positioned above and beneath a bed, all located inside a 3×2×2 m³ room. For each subject we obtained a numerical value for the number of counts at baseline (four hours post-ingestion) and 2 weeks later. The baseline counts were multiplied by a correction factor of 0.806 to correct for the radioiron decay after two weeks.

Urinary iodine concentration was determined using the method of Dunn et al. 1993. Urine was digested with chloric acid under mild conditions and iodine was determined by its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenious acid, as described by Sandell & Kalthoff (Robbins & Rall, 1967, and Sandell & Kalthoff, 1937). Percentage iodine absorption was calculated using the data from the

---

* We used the decay equation of \( A = A_o \cdot e^{-kt} \) to calculate the correction factor of 0.806, Where \( A \) is the remaining activity after two weeks, \( A_o \) is the original amount of activity, \( (e) \) is the natural logarithm, \( (t) \) is the time period, and finally, \( (k) \) is the rate of decay.
second 24-hr urine collections prior to the ingestion of each test meal and the immediate 24-hr urine collections post-ingestion of the test meal.

Plasma ferritin was determined by RadioImmunoAssay (RIA) kit purchased from Ramco Laboratories Inc., Houston, Texas.

Hemoglobin concentration was determined by the Cyanomethemoglobin method (recommended by the International Committee for Standardization in Hematology). Drabkin’s reagent needed for this measurement was purchased from BDH Ltd., Toronto, Ontario.

3.4. Statistical Analysis

3.4.1. Sample Size Determination

From previous work by Cook et al. 1991, the estimated mean iron absorption from meals designed to maximize and minimize iron absorption is $8 \pm 3.9$ (X ± SD) and $3.2 \pm 1.5$ %. We expected the differences in iron absorption from the labeled salt to be in the same range. Using mean and variance data from Cook et al, with a type I error of 5 %; type II error of 20 %; and a one-tailed test, conventional sample size estimates yielded 16 subjects.

3.4.2. Data Analysis

Paired t-test was used to compare absorption of iron and iodine from the two types of meals to determine whether the mean log absorption ratio differed from zero (Cook et al.

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*a* Post-ingestion 24-hr urinary iodine excretion, minus baseline 24-hr urinary iodine excretion, divided by baseline urinary iodine excretion, times hundred.
1969). Since the distribution of iron absorption values are skewed, values for the percent absorption were converted to logarithms for statistical analysis. The result were reconverted to antilogarithms to recover the original units.

3.4.3. Correction for Interindividual Variation

Because of marked influence of iron status on absorption, comparison of individual dietary absorption values with the two different meals was converted to a common reference point (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 subjects). An alternate method used as a reference to compare individual absorption values is based on serum ferritin concentration since ferritin bears a close inverse relationship to iron absorption (Cook et al. 1991, Magnusson et al. 1981, and Cook et al. 1974). Dietary absorption in each subject was corrected to a value corresponding to a serum ferritin of 40 g/l by using the following equation:

$$\log Ac = \log Ao + \log Fo - \log 40$$

Where $Ac$ is corrected dietary absorption, $Ao$ is observed absorption, and $Fo$ is observed serum ferritin.
CHAPTER 4

RESULTS
4. RESULTS

4.1. Iron Absorption

Individual data on hemoglobin, and serum ferritin, as well as iron and iodine absorption from the composite meals (designed to impair or enhance iron availability from the meals) are presented in Table 4.1.

Our study included 8 male and 8 female subjects. The subjects had a mean age of 34.4 years (range 21 to 53 years old). Because the distribution of the absorption figures was skewed, further analyses were made by means of logarithmic transformation of the individual values for serum ferritin, as well as the reference-dose absorption, Figure 4.1.

When the acceptability of the salt was tested 93% of our subjects found the double-fortified salt agreeable in terms of taste and palatability. Mean absorption "uncorrected" from the iron enhancing meal was significantly higher than the iron inhibitory one (8.84 ± 1.8% vs. 1.7 ± 0.8, P < 0.0017). Similarly mean absorption, after correction based on an individual's absorption of a reference dose of inorganic iron or correction based on iron stores, were also significantly higher with the enhancing meal (36.7 ± 3.4% vs. 6.3 ± 3.1, P < 0.0001 based on absorption of reference dose; 10.4 ± 2.4 vs. 2.2 ± 0.9, P < 0.0069, based on iron stores). There was a significant negative correlation between log serum ferritin and the absorption from reference-dose of inorganic iron (r = -0.35, P < 0.0003), Figure 4.2.

The mean hemoglobin and serum ferritin concentration of male subjects were 147.4 ± 1.9 g/l and 76.6 ± 14 μg/l, respectively. Whereas, the mean hemoglobin and
serum ferritin concentration of female subjects were $127 \pm 7.35$ g/l and $37.1 \pm 7.7$ μg/l, respectively. Within the 7-week period of this study there were no significant changes in hemoglobin or serum ferritin status of our subjects, Table 4.1. Two female subjects with the lowest hemoglobin and serum ferritin values (Hbs; 102.4 & 90.4 g/l, and serum ferritin concentrations; 5.5 & 3 μg/l) had the highest rates of iron absorption from each meal, table 4.1. Although there were differences in Hb values between individual subjects, mean Hb concentration prior to study (135 ± 5.3 g/l) and after the completion of study (134 ± 5.2 g/l) were not significantly different. Serum ferritin and Hb concentration were within the expected range for 14 subjects in our study, Table 4.2. The two female subjects had their reference-dose absorption of the inorganic iron outside the two standard deviations (65.2 & 48.4 %) from the mean reference-dose absorption for the rest of the group (9.96 ± 1.7 %), therefore excluded from the data analysis.
<table>
<thead>
<tr>
<th>N</th>
<th>Hb (g/l)</th>
<th>Serum Ferritin (μg/l)</th>
<th>Reference Dose Abs. (%)</th>
<th>⁵⁹Fe Absorption (%)</th>
<th>Urinary Iodine Excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Enhanced Meal</td>
</tr>
<tr>
<td>1</td>
<td>(f)</td>
<td>134</td>
<td>19</td>
<td>14</td>
<td>13.1</td>
</tr>
<tr>
<td>2</td>
<td>(m)</td>
<td>146</td>
<td>160</td>
<td>162</td>
<td>8.5</td>
</tr>
<tr>
<td>3</td>
<td>(f)</td>
<td>127</td>
<td>84</td>
<td>78</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>(f)</td>
<td>150</td>
<td>19</td>
<td>17</td>
<td>17.1</td>
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<tr>
<td>5</td>
<td>(m)</td>
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<td>154</td>
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<tr>
<td>6</td>
<td>(m)</td>
<td>145</td>
<td>49</td>
<td>39</td>
<td>8.7</td>
</tr>
<tr>
<td>7</td>
<td>(f)</td>
<td>149</td>
<td>20</td>
<td>118</td>
<td>5.9</td>
</tr>
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<td>8</td>
<td>(f)</td>
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<td>44</td>
<td>35</td>
<td>13.6</td>
</tr>
<tr>
<td>9</td>
<td>(m)</td>
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<td>119</td>
<td>118</td>
<td>8.1</td>
</tr>
<tr>
<td>10</td>
<td>(m)</td>
<td>144</td>
<td>42</td>
<td>41</td>
<td>13.3</td>
</tr>
<tr>
<td>11</td>
<td>(m)</td>
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<td>31</td>
<td>26.5</td>
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</tr>
<tr>
<td>12</td>
<td>(f)</td>
<td>125</td>
<td>72</td>
<td>55</td>
<td>6.9</td>
</tr>
<tr>
<td>13</td>
<td>(m)</td>
<td>N/A</td>
<td>26</td>
<td>26</td>
<td>6.9</td>
</tr>
<tr>
<td>14</td>
<td>(m)</td>
<td>148</td>
<td>38</td>
<td>56</td>
<td>5.3</td>
</tr>
<tr>
<td>15</td>
<td>(f)</td>
<td>107</td>
<td>6</td>
<td>5</td>
<td>65.2</td>
</tr>
<tr>
<td>16</td>
<td>(f)</td>
<td>88.6</td>
<td>92.3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>X ± SEM</td>
<td>135 ± 5.3</td>
<td>134 ± 5.3</td>
<td>59 ± 14</td>
<td>58 ± 14</td>
</tr>
</tbody>
</table>

Uncorrected = Uncorrected iron absorption
RF-derd = Corrected based on reference-dose absorption
Se-derd = Corrected based on serum ferritin concentration
* Numbers 15 & 16 are excluded from our data analysis

Table 4.1 Table of results from the double-fortified salt investigation
Figure 4.1. Mean Serum Ferritin Concentrations
(transformed data to obtain normality)
Figure 4.2. Correlation between iron stores and absorption of the reference-dose of inorganic iron
4.2. Urinary Iodine Excretion

Table 4.3. shows baseline and post-ingestion urinary excretion of iodine. Each 24-hr baseline value is the mean of two urine collections before each test meal. Also, each 24-hr post-ingestion value is the mean of two urine collections after each test meal. Although there was a trend towards higher values in the first post-ingestion collection (13.03 ± 1.1 vs. 10.9 ± 1.5, \( p < 0.47 \)) compared to the mean of the baseline values, the differences were not statistically significant. By the second day after the meal, the urinary iodine values were similar to the baseline values. Also, the absorption of iodine from the high iron bioavailability meal was not significantly different from the low iron bioavailability meal, 40.7 ± 7.2 and 63 ± 22.6 % \( P < 0.41 \), respectively, (Table 4.1).
Table 4.2 Serum Ferritin and Hemoglobin Concentrations in Male and Female Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Ave. Age</th>
<th>Hb g/l Initial</th>
<th>Hb g/l Final</th>
<th>Serum Ferritin µg/l Initial</th>
<th>Serum Ferritin µg/l Final</th>
<th>Ref. Abs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>8</td>
<td>27.1</td>
<td>147.4 ± 1.9</td>
<td>132.0 ± 8.8</td>
<td>93.6 ± 23.4</td>
<td>91.6 ± 21.6</td>
<td>10.1 ± 2.6</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>44.3</td>
<td>127.0 ± 7.2</td>
<td>123.8 ± 7.1</td>
<td>33.5 ± 12.32</td>
<td>28.7 ± 9.3</td>
<td>21.6 ± 8.0</td>
</tr>
</tbody>
</table>

* All values are Mean ± SEM
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Urinary Iodine Excretion $\mu g/dl$ First 24-hrs (before the ingestion)</th>
<th>Urinary Iodine Excretion $\mu g/dl$ Second 24-hrs (before the ingestion)</th>
<th>Urinary Iodine Excretion $\mu g/dl$ First 24-hrs (after the ingestion)</th>
<th>Urinary Iodine Excretion $\mu g/dl$ Second 24-hrs (after the ingestion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(f)</td>
<td>11.26</td>
<td>14.85</td>
<td>13.04</td>
<td>14.76</td>
</tr>
<tr>
<td>2(f)</td>
<td>10.16</td>
<td>9.71</td>
<td>13.17</td>
<td>13.42</td>
</tr>
<tr>
<td>3(m)</td>
<td>14.61</td>
<td>13.88</td>
<td>16.07</td>
<td>13.19</td>
</tr>
<tr>
<td>4(f)</td>
<td>8.73</td>
<td>7.13</td>
<td>9.78</td>
<td>7.14</td>
</tr>
<tr>
<td>5(f)</td>
<td>10.53</td>
<td>1.99</td>
<td>8.03</td>
<td>6.02</td>
</tr>
<tr>
<td>6(m)</td>
<td>4.63</td>
<td>4.32</td>
<td>8.32</td>
<td>3.91</td>
</tr>
<tr>
<td>7(m)</td>
<td>14.38</td>
<td>22.02</td>
<td>17.71</td>
<td>16.56</td>
</tr>
<tr>
<td>8(f)</td>
<td>10.15</td>
<td>11.81</td>
<td>12.24</td>
<td>12.22</td>
</tr>
<tr>
<td>9(f)</td>
<td>20.60</td>
<td>15.23</td>
<td>17.18</td>
<td>21.59</td>
</tr>
<tr>
<td>10(f)</td>
<td>3.41</td>
<td>7.95</td>
<td>11.77</td>
<td>13.1</td>
</tr>
<tr>
<td>11(m)</td>
<td>20.5</td>
<td>20.53</td>
<td>22.79</td>
<td>23.37</td>
</tr>
<tr>
<td>12(m)</td>
<td>6.85</td>
<td>6.18</td>
<td>10.24</td>
<td>8.65</td>
</tr>
<tr>
<td>13(m)</td>
<td>10.67</td>
<td>16.3</td>
<td>17.1</td>
<td>15.06</td>
</tr>
<tr>
<td>14(f)</td>
<td>4.18</td>
<td>8.46</td>
<td>13.09</td>
<td>5.08</td>
</tr>
<tr>
<td>15(m)</td>
<td>7.79</td>
<td>3.68</td>
<td>4.56</td>
<td>4.62</td>
</tr>
<tr>
<td>16(m)</td>
<td>9.4</td>
<td>11.59</td>
<td>13.35</td>
<td>9.84</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>10.49 ± 1.27</td>
<td>10.98 ± 1.46</td>
<td>13.03 ± 1.12</td>
<td>11.78 ± 1.45</td>
</tr>
</tbody>
</table>

Table 4.3  Baseline and Post-ingestion Urinary Excretion of Iodine
**Figure 4.3** Baseline (BL) & Post-ingestion (PI) Urinary Excretion of Iodine μg/dl
CHAPTER 5

DISCUSSION
5. DISCUSSION

5.1 Discussion

Iron deficiency anemia and iodine deficiency disorders remain major problems in many parts of the world, and their prevention through supplementation and fortification programs are an urgent priority. Most fortification trials have failed in the eradication of iron/iodine deficiencies, thus, the double-fortified salt provides a promising opportunity to overcome the deficiency of these two most important micronutrients.

The double-fortified salt investigated in the present study, appears to be quite satisfactory with respect to bioavailability of iron and iodine. In the present study, mean individual iron-absorption figures “uncorrected”, from the “enhancing meal” and the “inhibiting meal” were 8.8 ± 1.8 and 1.7 ± 0.8 %, respectively, (P < 0.0017). The mean urinary excretion of iodine (representing iodine absorption) was not significantly different from the baseline excretion values in our subjects (P<0.47). Also, the difference between the iodine absorption from the test meals (i.e. the enhancing and the inhibiting meals) was not significant (p < 0.41). The addition of the double-fortified salt to the test meals did not alter the flavor or the palatability of the meals.

There are two methodological aspects of the present study that deserve comment. 1) Our findings agree with the previous work done by Cook and co-workers, demonstrating the influence of inhibitors and enhancers on iron absorption from composite meals (Cook et al. 1991). 2) The addition of the dextran-coated iodine did not compromise the apparent absorption of iodine from the double-fortified salt.
Results from the current study confirm previous observations that other food items in a meal enhance or inhibit iron absorption from that meal. With the "enhancing" meal which was designed to maximize the iron absorption, the uncorrected iron absorption values were five-fold higher than those for the "inhibitory" meal. It should be noted, however, that the enhancing and the inhibiting meals used in the present study were likely an exaggeration of the type of meals ingested in geographical locations where meat is scarce. The inhibiting meal in the current study contained no meat, and minimum fruits, vegetables or vitamin C. Thus in a non experimental setting one would expect average iron absorption in an iron replete population to be between the two extremes used in the current study.

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 a non-iron sufficient subject when 10-15 g of salt is consumed per day. If we attempt to relate our study results to Canadian RNI's for iron we come up with the following condition. Assuming a worst case scenario represented by the iron-inhibiting meal, a salt intake of 15 g/day for men, and a mean absorption of 1.7 % (average iron absorption of male subjects), men would absorb 0.3 mg of elemental iron. The total iron requirement for men is 1.1 mg/day, thus, iron fortified-salt would provide 23 % of the requirements. For women assuming a salt intake of 10 g/day and a mean absorption of 3.1 % (average iron absorption of female subjects), the total absorption would be 0.31 mg. In this case, the iron fortified-salt would provide only 18 % of the requirements.
From the perspective of at risk populations in developing countries where this initiative is primarily aimed at, there are several essential factors to be considered. First, an average salt intake of 10-15 g/day has been reported from populations in these countries (Narasinga Rao, 1994), with more salt ingested by men, due to more meal consumption, as compared to women. Secondly, a typical meal in most such populations is a mixture of “enhancers and inhibitors” of iron absorption. Therefore in such populations, iron absorption between the two mean values is expected. Also, at risk individuals have poor iron status, therefore, a higher absorption from the iron fortified-salt is expected among these people. And finally, loss of blood due to parasitic infections would result in an increase in iron absorption from the fortified salt. Based on the described scenario and the results from the present study, we would expect an effectively higher iron absorption in areas affected by iron deficiency anemia.

There are a number of reasons for suggesting that dextran-coating of iodine is the preferred method of protecting iodine from oxidation. Earlier trials by Burgi et al. had indicated that the iodine moiety of the double-fortified salt was unstable because of the net loss of iodine due to evaporation and catalytic oxidation of I to I2 in the presence of ferrous ions and air oxygen (Burgi et al. 1986). Although successful application of sodium hexametaphosphate (SHMP) to stabilize the double-fortified salt has been demonstrated (Narasinga Rao 1994), dextran, unlike SHMP, is an inert carbohydrate which is safe to use in any diet. Sodium hexametaphosphate is a chelating agent which may have undesired effects on the bioavailability of other minerals in the meal. In addition, extensive tests conducted by Diosady et al. demonstrated that physical isolation
of iodine by dextran encapsulating, results in acceptable iodine retention under the worst storage and distribution conditions. As the process of iodine oxidation ($\Gamma$ to $I_2$ in the presence of ferrous ion) occurs at a much more rapid pace in humid conditions, the importance of a physical separation (e.g. dextran coating of iodine) is even more important. Finally, the method of preparation of the double-fortified salt with dextran-coated iodine is very simple and inexpensive especially with large-scale production.

In the medical literature there are at least two methods describing the "correction" for the difference in iron status between individual subjects in research studies examining iron absorption. One method is based on the individual serum ferritin concentration, while the other uses the absorption of a reference-dose of inorganic iron. In the present study, the correction for dietary iron absorption, based on individual serum ferritin status and reference-dose of iron absorption, were comparable. However, the correction based on serum ferritin status may be less reliable since only two ferritin values were available from each subject in our study. Cook et al. suggested that it is important to obtain at least 5 serum ferritin assessments to obtain a reliable mean ferritin value (Cook et al. 1991), Figure 4.4.

In the past Bjorn-Rasmussen and co-workers demonstrated a high correlation between iron absorption from meals and from reference-doses (Bjorn-Rasmussen et al. 1976). Also, a high correlation between iron absorption and measures of iron stores such as bone marrow homosiderin and serum ferritin, was reported by others, (Cook et al. 1974, Disler et al. 1976, Walters et al. 1975, Charlton et al. 1977, Heinrich et al. 1977, and Bezwoda et al. 1979). In our current investigation of the double-fortified salt we
observed a significant correlation \( r = -0.35, P < 0.0003 \) between the reference-dose absorption of inorganic iron and individual serum ferritin stores, Figure 4.2. Before the exclusion of the two iron deficient females from our data analyses, this correlation coefficient was \( r = 0.76 \) (similar value was reported by Cook et al. 1991). This was not unexpected since the absorption of iron is known to increase under conditions in which tissue iron is reduced, with more iron being absorbed by iron deficient subjects and less by subjects with iron overload (Magnusson et al. 1981).

Magnusson et al. initially described the use of a reference-dose of iron absorption value to improve the comparison of iron absorption values between different subjects. The absorption of iron from each meal was expressed as the ratio food iron absorption/reference-dose of inorganic iron (ferrous fumarate) absorption. In a study conducted by Magnusson et al., the distribution of absorption measurements in 96 normal men showed that only 3 men had an absorption exceeding 40 % from the reference-doses (Magnusson et al. 1981). Magnusson noted an inverse correlation between reference-dose absorption and serum ferritin values (eg. 20 % absorption with a serum ferritin of 60 \( \mu g/l \) to 70-80 % absorption corresponding to a serum ferritin of 12 \( \mu g/l \)). In the current study only the two iron deficient subjects (not included in our data analysis), demonstrated a reference-dose absorption exceeding 40 % (48 & 65 %), with corresponding serum ferritin concentrations of 3.5 and 3 \( \mu g/L \), respectively. Considering the fact that the mean absorption from the reference-dose of inorganic iron in our study subjects (excluding the two subjects previously mentioned) was 9.96 % (representing an iron replete population), it is most appropriate not to use either of the two methods of corrections, as the
"uncorrected" values are a better reflection of the results from this iron replete, homogeneous group of volunteers.

Daily urinary excretion of iodine closely reflects iodine intake, and has been used as a measure of iodine status in many large scale nutrition surveys (Gibson RS, 1991). Follis et al. showed that the percentage of 131-I uptake by the thyroid of a given individual is inversely correlated with urinary iodine excretion (Follis et al. 1962, 1964). This relationship, together with the observations that the largest fraction of dietary iodine is excreted via the kidneys and that urinary iodine excretion is lowest in areas of endemic goiter (Follis et al. 1964), makes urinary iodine measurement a valuable test to assess iodine status. The urinary cutoff points to assess the severity of iodine deficiency, by measuring iodine concentration in urine samples, are categorized by WHO/UNICEF/ICCIDD as: deficient, urinary iodine concentration < 0.79 μmol/L *, and severely deficient, urinary iodine concentration < 0.16 μmol/L. The mean urinary excretion of iodine in our subjects, before the dietary intervention was 0.87 ± 0.1 μmol/L (or 10.98 ± 1.46 μg/dl). Urinary iodine excretion was equivalent or higher after the test meals, thus we believe that dextran-coating does not negatively influence the absorption of iodine.

5.2. Conclusion

Our major objective was to determine the absorption of iron and iodine from fortified salt in healthy human subjects ingesting high and low iron-bioavailable meals. We

* Dividing by 0.079 converts μmol/L to μg/L.
demonstrated iron absorption within the expected range from the fortified salt. Based on the significant difference between the absorption from the two different test meals, our hypothesis that the iron absorption from the inhibitory meal would be lower than the enhancing meal was confirmed. Our results indicated that the iodine from the fortified salt is readily available and absorbable as iodine from the iodized salt. Thus, we conclude that dextran-coated iodine and iron are well absorbed from double-fortified table salt.

5.2.1. Future Studies

Having established that the iodine and iron are well absorbed, community trials to determine efficacy of the fortified salt in treating and preventing iodine deficiency and preventing iron deficiency are needed. A study has been proposed to begin in Ghana (Skyere West district) to accomplish this goal. It aims to provide evidence of the efficacy of the double-fortified salt and advisability or otherwise of its use to combat iron and iodine deficiencies.
Uncorrected iron absorption
Corrected based on reference dose
Corrected based on serum ferritin

Figure 4.4 Comparison of dietary iron absorption from the two test meals "uncorrected" & after "correction" for inter-subject variabilities based on the two different methods.
CHAPTER 6

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6. REFERENCES


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