DISS. ETH NO. 15002

Interactions between Iodine and Iron Deficiencies

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of
Doctor of Natural Sciences

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2003

Acknowledgements

I would like to thank the following people who helped make this thesis possible.

My very special thanks go to Michael Zimmermann for his generous advice, support and encouragement. I am sincerely grateful for his contribution and commitment.

I am also very grateful to Richard Hurrell for assigning me this project and for the support and guidance he gave me throughout the thesis.

I would like to thank the present and former members of the Human Nutrition Laboratory of the ETH Zürich; Marie-Helene Balsat, Torsten Bohn, Lena Davidsson, Eberhard Denk, Ines Egli, Meredith Fidler, Martine Hurrell, Sabine Jacob, Adam Krzystek, Diego Moretti, Sabine Renggli, Käthe Santagata, Franziska Staubli, Stefan Storcksdieck, Thomas Walczyk, Monika Wälti and Rita Wegmüller. Thank you for taking your time, whenever I approached you with a question! In particular, I would like to thank Christophe Zeder for his enormous patience and help concerning analytical and technical problems.

In Côte d'Ivoire, I would like to thank Pierre Adou (Institute of National Public Health, Abidjan) and Jean-Baptiste Gbato (Public Health, Danané) for their contribution and commitment in the fieldwork. I am also grateful for the help of many technicians concerning the blood collection.

My thank also to Olivier Girardin, the staff and the students of the Centre Suisse de Recherches Scientifiques in Abidjan Côte d'Ivoire.

My appreciations to Toni Torresani and Luciano Molinari (Children's Hospital, Zürich) for the thyroid hormone analysis and for statistical assistance, respectively. To Carol Flowers (University of Kansas Medical Center, Kansas City, USA) for the analysis of some iron status measurements.

I would like to thank Wolfgang Langhans, Myrtha Arnold and the group of Physiology and Animal Husbandry of the ETH Zürich for their advice and help concerning the rat study.

My thanks also to François Delange for being a co-examiner and for his advice during the national screening in Switzerland.

Thanks to Matthias Hoppler, Karin Lüscher and Sylvia Sommer for their contribution as part of their studies at the ETH Zürich.

My special thanks also to my parents, for their constant support and encouragement.

Finally, I would like to sincerely thank all the participating children and teachers. I am very aware that the project would not have been possible without their participation.

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Abbreviations

BSA	Body surface area
Hb	Hemoglobin
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDA	Iron deficiency anemia
IDD	Iodine deficiency disorders
INACG	International Nutritional Anemia Consultative Group
RDA	Recommended Dietary Allowance
SF	Serum ferritin
T_3	Triiodothyronine
T_4	Thyroxine
TfR	Serum transferrin receptor
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing-hormone
TSH	Thyrotropin, Thyroid-stimulating hormone
Tvol	Thyroid volume
UI	Urinary iodine
UNICEF	United Nations Children's Fund
USI	Universal salt iodization
WHO	World Health Organization
ZPP	Zinc protoporphyrin

Summary

Iodine and iron deficiencies are major public health problems in many developing countries. Both produce a spectrum of disorders, particularly in young children and pregnant women. In countries where iodine deficiency occurs, universal salt iodization (USI) is the recommended long-term strategy to eliminate iodine deficiency disorders (IDD). However, USI does not always completely resolve IDD. The reasons for this are not entirely clear. Deficiencies of iron, selenium, zinc and vitamin A may blunt thyroid metabolism and therefore reduce the effectiveness of USI. The main objective of this thesis was to investigate the interactions between iodine and iron deficiencies. The mechanism of the adverse effect of iron deficiency on thyroid metabolism in rats was investigated. A further objective was to investigate iodine nutrition and thyroid volume (Tvol) changes during iodine repletion with iodized salt, as little is known concerning the impact of iodized salt on changes in Tvol.

In a randomized, double-blind, placebo-controlled trial in western Côte d'Ivoire, goitrous iron-deficient children (n=166) consuming iodized salt were supplemented with iron (60 mg iron/day, 4 days/week for 16 weeks) or placebo. At 0, 1, 6, 12, and 20 weeks, indicators of iron and iodine status were measured. Iron supplementation improved significantly Tvol response to iodized salt. Goiter prevalence was 43% in the iron supplemented group compared to 62% in the placebo group ($P<0.02$). These results indicate that iron supplementation improves the efficacy of iodized salt in goitrous children with iron deficiency. They also suggest that a high prevalence of iron deficiency among children in areas of endemic goiter may reduce the effectiveness of iodine prophylaxis.

However, the mechanism by which iron influenced thyroid metabolism was not clear. We investigated the effect of iron deficiency anemia (IDA) on thyroid peroxidase (TPO) activity and thyroid hormone concentrations in rats, feeding iron-deficient diets containing 3, 7 and 11 μg iron/g diet. Because IDA reduces food intake, three control groups were pair-fed iron-sufficient diets (35 μg/g) to each of the iron-deficient groups and one control group consumed food ad libitum. After 4 weeks of feeding, hemoglobin, thyroxine and triiodothyronine concentrations were significantly lower in the iron-deficient groups compared to the pair-fed groups and the ad libitum control group. By multiple regression, IDA also significantly reduced TPO activity ($P<0.05$). Compared with the ad libitum controls, TPO activity per thyroid determined by the guaiacol assay was decreased by 56%, 45%, and 33% depending on the severity of IDA. These results indicate that iron deficiency sharply reduces TPO activity, and

suggest that decreased TPO activity contributes to the adverse effect of IDA on thyroid metabolism.

Two years after salt iodization in Côte d'Ivoire had been implemented, a cross-sectional study investigated if deficiencies in iron, selenium and vitamin A and/or a high consumption of cassava could explain the persisting high goiter rate of 74% in school children. In primary school children (n=1013), hemoglobin, plasma ferritin, transferrin receptor, erythrocyte zinc protoporphyrin, plasma selenium, plasma retinol, urinary iodine and thiocyanate, serum thyroxine and thyrotropin were measured and regression done to determine associations with increased Tvol by ultrasound. However, despite their high prevalence, neither vitamin A nor selenium deficiency nor urinary thiocyanate predicted goiter. Low plasma ferritin was the only iron status indicator significantly predicting goiter. However, other factors not identified in this study probably act in concert with iron depletion to blunt the thyroid response to iodine prophylaxis.

In a 5-year prospective study, measurements of Tvol by ultrasound, urinary iodine and thyroid hormone concentrations were done each year in rural villages of western Côte d'Ivoire. A significant age shift in the distribution of goiter prevalence was observed. Whereas more 5-9 years old children were goitrous compared to 10-14 year-olds before iodine repletion, goiter prevalence was significantly higher in the older children than in the younger children (52% vs. 19%) four years after the introduction of iodized salt. These results indicate that enlarged Tvol may not completely normalize after iodine repletion.

In parallel to the studies in Côte d'Ivoire, the Swiss iodization program was monitored in a representative national sample of school children. Our findings confirmed earlier reports suggesting that reference criteria at that time recommended by the World Health Organization and the International Council for Control of Iodine Deficiency Disorders were too high. This led to a workshop which resulted in new updated provisional reference values. The generation of new, truly international reference criteria for Tvol by ultrasound in school children is currently underway.

In conclusion, besides providing further information of the impact of salt iodization on thyroid metabolism, findings of this thesis showed that iron deficiency blunts the response of the thyroid to iodized salt and may reduce the effectiveness of USI. In addition, the studies pointed to reduced TPO activity in IDA as a contributory mechanism of the adverse effects of iron deficiency on thyroid metabolism.

Zusammenfassung

In vielen Entwicklungsländern sind Iod- und Eisenmangel bedeutende Gesundheitsrisiken. Beide verursachen eine Vielzahl von Gesundheitsproblemen, vor allem bei Kleinkindern und schwangeren Frauen. In Ländern mit Iodmangel empfiehlt sich die Iodierung von Salz als langfristig effizienteste Strategie, um Iodmangelerscheinungen zu verhindern. Trotzdem können durch Iodmangel verursachte Gesundheitsstörungen dadurch nicht immer vollständig behoben werden. Die Gründe dafür sind nicht eindeutig. Möglicherweise könnte der Mangel an Eisen, Selen, Zink und Vitamin A den Schilddrüsenstoffwechsel hemmen und dadurch die Wirksamkeit der Iodprophylaxe vermindern. Hauptziel dieser Dissertation war die Untersuchung der Interaktionen zwischen Iod- und Eisenmangel. Weiter wurde der Mechanismus der Hemmung des Schilddrüsenstoffwechsels infolge Eisenmangel in Ratten erforscht. Ferner wurde die Iodversorgung sowie die Veränderungen des Schilddrüsenvolumens während der Iodprophylaxe mit iodiertem Salz untersucht, da über die Auswirkungen von iodiertem Salz auf das Schilddrüsenvolumen (Tvol) nur sehr wenig bekannt ist.

Dazu wurde an der Elfenbeinküste eine prospektive, randomisierte, doppel-blinde, Placebo-kontrollierte Studie mit 5- bis 14-jährigen Kindern (n=166) durchgeführt, die gleichzeitig eine Anämie und einen Kropf aufwiesen und täglich iodiertes Salz konsumierten. Der einen Hälfte der Kinder wurden Eisentabletten verabreicht (60 mg Eisen/Tag, 4 Tage/Woche während 16 Wochen), der anderen Hälfte Placebotabletten. Nach 0, 1, 6, 12 und 20 Wochen wurden Indikatoren des Eisen- und Iodstatus sowie das Tvol bestimmt. Durch die Eisensupplementierung wurde der Eisenstatus im Vergleich zur Placebo-Behandlung signifikant verbessert (P<0.05). Nach 20 Wochen betrug die Kropfprävalenz in der Eisen-Gruppe 43%, in der Placebo-Gruppe 62% (P <0.05). Die Resultate zeigen, dass die Eisensupplementierung bei Kindern mit einem Kropf und Eisenmangel die Wirksamkeit von iodiertem Salz verbessert. Zusätzlich deuten sie darauf hin, dass eine hohe Prävalenz von Eisenmangel bei Kindern in Gebieten mit endemischem Kropf die Wirksamkeit einer Iodprophylaxe hemmen kann.

Der Wirkungsmechanismus von Eisenmangel auf den Schilddrüsenstoffwechsel ist allerdings noch unklar. Wir untersuchten den Einfluss von Eisenmangelanämie auf die Aktivität der Schilddrüsen-Peroxidase (TPO) und auf die Konzentrationen der Schilddrüsenhormone in Ratten, denen Futter mit tiefem Eisengehalt (3, 7 und 11 μg Eisen/g) verabreicht wurde. Da Eisenmangelanämie die Nahrungsaufnahme reduziert, wurde pro Eisenmangel-Gruppe je eine Kontrollgruppen restriktiv mit der selben Futtermenge, aber ausreichender Eisenkonzentration (35 μg Eisen/g) gefüttert. Zusätzlich konsumierte eine Kontrollgruppe dieses Futter ad libitum. Nach 4-wöchiger Fütterungsperiode waren die Hämoglobin-, Thyroxin- und Triiodothyroninkonzentrationen in den Eisenmangel-Gruppen signifikant tiefer als in den restriktiv gefütterten Gruppen sowie der ad libitum-Kontrollgruppe. Gemäss multipler Regressionsanalyse verminderte Eisenmangelanämie zudem die TPO-Aktivität signifikant (P<0.05). Verglichen mit der ad libitum-Kontrollgruppe war die TPO-

Aktivität pro Schilddrüse, bestimmt mit der Guaiacolmethode, in Abhängigkeit von der Ausprägung der Eisenmangelanämie um 56%, 45% und 33% reduziert. Diese Resultate weisen einerseits darauf hin, dass Eisenmangel die TPO-Aktivität stark senkt und andererseits, dass die reduzierte TPO-Aktivität zur Hemmung des Schilddrüsenstoffwechsels infolge Eisenmangelanämie beiträgt.

Zwei Jahre nachdem die Salziodierung an der Elfenbeinküste eingeführt wurde, untersuchten wir in einer Querschnittsstudie, ob Mangel an Eisen, Selen und Vitamin A oder ein hoher Konsum von Maniok die verbleibende hohe Kropfprävalenz von 74% in Schulkindern erklären könnte. In 5- bis 14-jährigen Kindern (n=1013) wurden Hämoglobin, Plasma-Ferritin, Transferrin-Rezeptor, Eryhrozyten-Zinkprotoporphyrin, Plasma-Selen, Plasma-Retinol, Iod- und Thiocyanatkonzentrationen im Urin, Serum Thyroxin und Thyreotropin gemessen und mit Hilfe der Regressionsanalyse auf einen möglichen Zusammenhang mit dem Tvol hin untersucht. Trotz ihrer hohen Prävalenz hatten weder Selen-, noch Vitamin A-Mangel, noch die Thiocyanatkonzentration im Urin einen signifikanten Einfluss auf die Kropfprävalenz. Einzig über tiefe Plasma-Ferritinkonzentrationen war es möglich, einen Kropf mit Signifikanz vorauszusagen. Vermutlich beeinflussen weitere, in dieser Studie nicht identifizierte Faktoren zusammen mit Eisenmangel den Schilddrüsenstoffwechsel und verhindern so die volle Wirkung der Iodprophylaxe.

In einer prospektiven Studie in abgelegenen Dörfern der Elfenbeinküste wurden jährlich während 5 Jahren das Tvol mit Ultraschall, die Iodausscheidung im Urin sowie die Schilddrüsenhormonkonzentrationen untersucht. Dabei wurde festgestellt, dass die signifikante Abhängigkeit der Kropfprävalenz vom Alter der Kinder je nach Dauer der Iodprophylaxe unterschiedlich war. Während vor der Einführung von iodiertem Salz weniger 10- bis 14-jährige Kinder als 5- bis 9-jährige Kinder einen Kropf hatten, war die Kropfprävalenz 4 Jahre danach unter den älteren Kindern im Vergleich zu den Jüngeren signifikant höher (52% vs. 19%). Diese Resultate deuten darauf hin, dass Iodprophylaxe mit Salz ein vergrössertes Tvol möglicherweise nicht vollständig zum Normalzustand zu reduzieren vermag.

Parallel zu den Studien an der Elfenbeinküste wurde das Salziodierungsprogramm der Schweiz überprüft. Unsere Befunde bestätigen frühere Studien, die darauf hinwiesen, dass die damaligen Referenzwerte zu hoch waren, die von der Weltgesundheitsorganisation und dem International Council for Control of Iodine Deficiency Disorders empfohlen wurden. Anlässlich eines daraufhin organisierten Workshops wurden neue provisorische Referenzwerte definiert. Neue, für Schulkinder gültige, internationale Referenzwerte für Tvol mit Ultraschall sind zur Zeit in Vorbereitung.

Zusammenfassend ausgedrückt hat diese Dissertation zusätzliche Informationen über die Wirkung von iodiertem Salz auf den Schilddrüsenstoffwechsel geliefert. Zudem konnte gezeigt werden, dass Eisenmangel den Schilddrüsenstoffwechsel hemmt und dadurch die Wirksamkeit von iodiertem Salz verringern kann. Schliesslich konnte die bei Eisenmangel reduzierte TPO-Aktivität in Eisenmangel als ein Faktor identifiziert werden, der zum Mechanismus der Hemmung des Schilddrüsenstoffwechsels bei Eisenmangelanämie beiträgt.

Introduction

In developing countries, the prevalence of multiple, overlapping micronutrient deficiencies is high, particularly in young children and pregnant women. Deficiencies of iodine and iron are major public health problems in Africa and worldwide, and many children are at high risk for both goiter and iron deficiency anemia (WHO *et al.*, 2001). Iodine and iron deficiencies have significant adverse health impacts, and if they occur during fetal life or early infancy, can both cause mental and motor retardation (Delange *et al.*, 2001; Grantham-McGregor & Ani, 2001).

For most countries where iodine deficiency disorders (IDD) are prevalent, the implementation of a sustainable salt iodization program is an effective, long-term solution for IDD. However, iodine fortification programs do not always completely resolve IDD. Although the reasons for this are not entirely clear, it is thought that iodine supplementation may be less effective in certain regions due to the modifying influence of coexisting nutritional deficiencies, such as protein-energy malnutrition, and deficiencies of iron, selenium, zinc and vitamin A (Ingenbleek, 1983; Arthur *et al.*, 1999; Zimmermann *et al.*, 2000; Freake *et al.*, 2001). Iodine deficiency may act alone or in concert with these other nutritional influences to produce IDD.

Previous studies of the Laboratory of Human Nutrition at the Swiss Federal Institute of Technology in Zürich have shown that the therapeutic response to oral iodized oil is impaired in goitrous children with iron deficiency anemia, compared to goitrous children who are not anemic (Zimmermann *et al.*, 2000). This suggests that a high prevalence of iron deficiency anemia among children may limit the effectiveness of iodine intervention programs in regions where these deficiencies coexist.

A possible mechanism that explains this interaction is that iron deficiency may lower thyroid peroxidase activity, an iron-dependent enzyme, and interfere with thyroid hormone synthesis.

Objectives

The general aim of the doctoral program was to gain insight into the interactions and health impact of micronutrient deficiencies, particularly iron and iodine deficiencies. The first objective was to investigate the relationship between iron and iodine deficiencies in Côte d'Ivoire, and to test the hypothesis that clinical and biochemical response to iodine repletion in iron-deficient children with goiter would be improved by concomitant provision of iron. A further objective was to investigate the

mechanism of the adverse effect of iron deficiency on the thyroid gland and thyroid hormone metabolism in rats. In addition, iodine status and thyroid volume during iodine repletion were investigated.

Outline of the thesis

Chapter 1 General introduction and literature review on iodine and iron deficiencies and potential interactions

Chapter 2 A randomized, double-blind, placebo-controlled trial to test whether iron supplementation in goitrous, iron-deficient children would improve their response to iodized salt in western Côte d'Ivoire

Chapter 3 A rat study to evaluate the impact of iron deficiency anemia on thyroid peroxidase activity and thyroid hormone concentration

Chapter 4 A cross-sectional study to investigate whether iron, selenium or vitamin A status predicted persisting increased thyroid volume two years after introduction of salt iodization

Chapter 5 A prospective study over 5 years using thyroid ultrasound to define the longitudinal changes in goiter prevalence in school children after iodized salt was introduced in Côte d'Ivoire

Chapter 6 Determination of thyroid volume by ultrasound of an iodine-sufficient national sample in Switzerland and comparison to the current WHO/ICCIDD reference criteria for thyroid volume

References

Arthur JR, Beckett GJ & Mitchell JH (1999) The interactions between selenium and iodine deficiencies in man and animals. *Nutrition Research Reviews* **12**, 55-73.

Delange F, de Benoist B, Pretell E & Dunn JT (2001) Iodine deficiency in the world: where do we stand at the turn of the century? *Thyroid* **11**, 437-447.

Freake HC, Govoni KE, Guda K, Huang C & Zinn SA (2001) Actions and interactions of thyroid hormone and zinc status in growing rats. *Journal of Nutrition* **131**, 1135-1141.

Grantham-McGregor S & Ani C (2001) A review of studies on the effect of iron deficiency on cognitive development in children. *Journal of Nutrition* **131**, 649S-666S; discussion 666S-668S.

Ingenbleek Y (1983) Vitamin A deficiency impairs the normal mannosylation, conformation and iodination of the thyroglobulin: a new etiological approach to endemic goiter. *Experientia. Supplementum* **44**, 264-297.

World Health Organization, United Nations Children's Fund & United Nations University (2001) Iron deficiency anemia: Assessment, prevention, and control. WHO, Geneva; WHO/NHD/01.3.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. *American Journal of Clinical Nutrition* **71**, 88-93.

Literature Review

Iodine

Iodine deficiency is one of the world's most prevalent nutritional deficiencies. Almost one third of the world's population lives in areas of iodine deficiency. Most of these people are in developing countries, but many in the industrialized countries are also affected (Dunn, 1998). In 1999, the World Health Organization (WHO) estimated that 13% of the world's total population were affected by goiter (WHO *et al.*, 2001a). Although goiter is the most visible indicator, iodine deficiency produces a spectrum of disorders that are termed the iodine deficiency disorders (IDD) (Hetzel, 1983). These include goiter, hypothyroidism, cretinism, congenital anomalies, neurological dysfunction, impaired reproduction, still birth and spontaneous abortion. Iodine is essential for the human body, as it is part of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). These hormones are involved in many different ways in human metabolism and are essential for normal growth and mental and physical development.

Iodine deficiency disorders

Iodine deficiency in the fetus

Iodine deficiency is considered the leading cause of preventable mental retardation (Delange *et al.*, 2001). Severe iodine deficiency during pregnancy can lead to endemic cretinism in the offspring, which is the most serious IDD. Two types of cretinism have been described: 1) neurological cretinism: marked by dominant neurological disorders, extreme mental retardation and a high prevalence of deafmutism, and 2) myxedematous cretinism: marked by severe thyroid insufficiency (Dumont *et al.*, 1994b). Many intermediate forms exist between these two forms of cretinism (Dumont *et al.*, 1994b; Delange, 2000a).

However, endemic cretinism only constitutes the extreme expression of a spectrum of abnormalities in physical and intellectual development and in the functional capacities of the thyroid gland (Delange, 2000a). The degree of severity of iodine deficiency that takes place during pregnancy determines the potential harmful effects on the fetus (Glinoer, 2001). Effects of less severe iodine deficiency during pregnancy on cognitive function later in life are difficult to determine as many confounding factors can complicate the interpretation of the results. However, some studies showed clear evidence of the adverse effects of iodine deficiency during

pregnancy. Fierro-Benitez *et al.* (1988) compared 8 and 15 year old school children of mothers who had received iodized oil during pregnancy to children of a neighboring comparable community whose mothers had not received iodized oil. Statistical significant differences in tests of intellectual function were not found, but results showed distinct differences in maturation of psychomotor function between the two groups. A case-control study in Bangladesh, comparing mental retardation according to maternal history of goiter, found an increased risk of reduced intelligent scores in children of goitrous mothers (Durkin *et al.*, 2000). Haddow *et al.* (1999) tested the neuropsychological development of children whose mothers were hypothyroid during pregnancy. Although none of the children were hypothyroid as newborns, their full-scale intelligence quotient scores at the age of 7 to 9 years were 7 points lower than those of the matched controls. These results indicate that maternal hypothyroidism has adverse effects on the child's development even without immediate clinical manifestation. As thyroid hormones are transferred from mother to fetus, both before and probably even after the onset of fetal thyroid function (Glinoer & Delange, 2000), maternal thyroid sufficiency might therefore be most important in early pregnancy.

Increased thyroid hormone requirements during pregnancy can be met in iodine sufficiency. When iodine is restricted or deficient, adequate physiological adaptation is difficult to achieve and can therefore lead to adverse pregnancy outcomes. Potter (1980) reviewed the effects of maternal hypothyroidism on reproductive outcomes concluding that rates of abortion, stillbirth, and preterm birth are higher among hypothyroid women compared to euthyroid women. Another review has described a greater incidence of obstetric complications and fetal abnormalities associated with maternal hypothyroidism (Lazarus & Kokandi, 2000). The relative risk of multiple miscarriages and stillbirths was twice as high for iodine-deficient women in Senegal compared to non-deficient women (Dillon & Milliez, 2000). A randomized controlled trial in Zaire observed slightly, but not significantly, higher mean birth weights among neonates of iodine supplemented mothers (Thilly *et al.*, 1994). In a study in Algeria, the rate of prematurity, stillbirths and abortions in the groups receiving iodine supplements before or during pregnancy was reduced when compared to the untreated group, whereas placental and birth weights were significantly higher (Chaouki & Benmiloud, 1994). Although there are clearly other factors influencing pregnancy outcome, based on the available data Dunn & Delange (2001) concluded that correction of iodine deficiency *per se* substantially decreases neonatal mortality.

Iodine deficiency in the neonate and during infancy

In contrast with adult data, which have shown that the iodine stores of the thyroid are not affected by iodine deficiency unless severe iodine deficiency is present, iodine content in the thyroid of newborns depletes with milder iodine deficiency (Delange, 2000b). Alterations of thyroid function in newborns have been reported from less severe endemic areas, even when thyroid function in adults was normal (Sava *et al.*, 1984). Therefore, newborns are particularly sensitive to the effect of iodine deficiency. The most important effect of iodine deficiency on the brain takes place during fetal life and early infancy at the time of maximum growth rate of the brain (Hetzel, 1994). However, it is difficult to distinguish between the effects of gestational iodine deficiency and postnatal iodine deficiency responsible for any observed intellectual deficits. In a study in China, the effects of iodine supplementation during pregnancy and early life were studied and compared to older children who had not previously received iodine. Children treated prenatally had fewer neurologic abnormalities, increased head growth, and an improved developmental quotient compared to children who were treated during neonatal period (Cao *et al.*, 1994). Compared with untreated children, iodine supplementation during the third trimester and during the newborn period was associated with a trend toward higher development scores, although it did not improve neurologic development (Cao *et al.*, 1994).

Child survival is also threatened by iodine deficiency. DeLong *et al.* (1997) added KIO_3 to irrigation water in western China over several years. In three treated villages, infant mortality decreased to half the average of the previous years. In a study in Indonesia, iodine supplementation with oral iodized oil of 6 weeks old infants reduced the relative risk of death during the first 2 months by 72% compared to placebo controls (Cobra *et al.*, 1997). Investigation from other countries confirm these findings and support that the correction of iodine deficiency decreases infant mortality (Dunn & Delange, 2001).

Iodine deficiency in childhood and adulthood

Iodine deficiency in childhood and adulthood causes goiter. Although some studies have shown larger thyroid volumes (Tvol) in girls than in boys (Delange *et al.*, 1997; Foo *et al.*, 1999; Djokomoeljanto *et al.*, 2001), others have found no gender difference in Tvol (Vitti *et al.*, 1994; Xu *et al.*, 1999). A meta-analysis of 18 studies on mental development in endemic goiter areas (17 severe and 1 mild) showed that non-cretin and clinically euthyroid individuals had a mean loss of 13.5 intelligence

quotient points compared to controls from nearby iodine-sufficient areas after correction of iodine deficiency by iodized oil (Bleichrodt & Born, 1994). However, most children growing up in an iodine-deficient region, were also exposed to iodine deficiency during fetal life. Therefore, it is not known to what extent lasting effects of maternal iodine deficiency are responsible for any intellectual deficits and what effect individual iodine deficiency during childhood contributes. Studies that examined to what extent the damage of mild iodine deficiency on the cognitive function is reversible have reported controversial results. A randomized iodine supplementation trial among goitrous children (5-12 y) in Bolivia observed no significant change in intelligent scores (Bautista *et al.*, 1982). In contrast, in school children in Malawi iodine supplementation significantly improved mental and psychomotor performance (Shrestha, 1994).

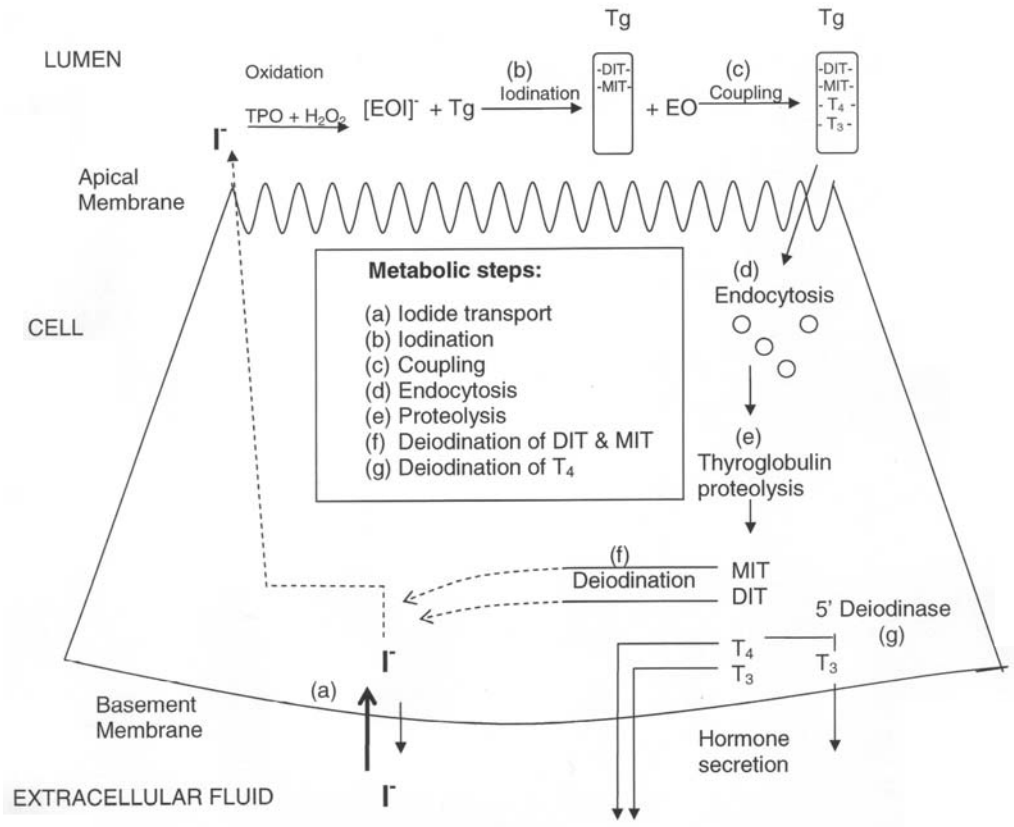
Beside the public health impact, iodine deficiency has also an adverse economic effect. As hypothyroid people move more slowly, think less clearly, require more sleep, and respond sluggishly to stimuli compared to euthyroid people, they are less efficient in many tasks (Dunn, 1994). This can impair significantly work productivity, and consequently, can handicap the economy by reducing work output.

Thyroid hormone synthesis

Iodine enters the thyroid follicle cells as inorganic iodide and is transformed through a series of metabolic steps into the thyroid hormones T_4 and T_3. The major external influence on this system is thyrotropin (TSH). The Na^+/I^- symporter mediates the first and key step in the process of supplying iodide to the gland in transporting iodide against the electrochemical gradient across the thyroid's basal membrane into the cytoplasm of the follicular cells (Carrasco, 2000). Besides the inorganic iodide transported from the serum into the thyroid, some iodide derives also from deiodination of organic iodine compounds within the gland. Iodide must first be oxidized to a higher oxidation state before it can act as an effective iodinating agent. Only H_2O_2 is sufficiently potent to oxidize iodide (Corvilain *et al.*, 1991). At the apical membrane thyroid peroxidase (TPO) catalyzes the iodination of tyrosyl residues of thyroglobulin (Tg) producing either monoiodotyrosine or diiodotyrosine (Taurog, 1970; Hosoya *et al.*, 1971). Two residues of diiodotyrosine then couple within Tg to form T_4, or one diiodotyrosine and one monoiodotyrosine to form T_3. This coupling reaction is also catalyzed by TPO (Taurog, 2000). The mature iodinated Tg molecule is stored in the colloid. About one-third of Tg's iodine is in T_4 and T_3, the remainder being in the inactive precursors, monoiodotyrosine and diiodotyrosine (Dunn & Dunn,

2000). Prior to secretion from the thyroid, T_4 and T_3 must be released from peptide linkage within Tg. Tg retrieved by micropinocytosis passes first through the endosome system, where proteolysis and hormone release is initiated, then into lysosomes, where the process is completed (Dunn & Dunn, 2000). T_4 is the main secretory product of the thyroid and is then deiodinated to its biologically active metabolite T_3. Seventy to 90% of the daily production of T_3 originates from extrathyroidal deiodination from T_4, with the rest derived from the thyroid. Not all internalized Tg undergoes proteolysis. Some is recycled back to the follicular lumen, apparently by a selective process targeting immature Tg molecules (Dunn & Dunn, 2001).

Figure 1: Summary diagram of major steps in thyroid hormone biosynthesis and secretion (modified from Taurog, 2000)



[EOI]- : enzyme-bound hypoiodite; EO : form of TPO initiating the coupling reaction
MIT: Monoidodtyrosine; DIT: Diiodotyrosine

Iodine metabolism in iodine deficiency

When iodine intake is abnormally low, adequate secretion of thyroid hormones may still be achieved by marked modifications of thyroid activity (Delange, 2000b). Iodine deficiency leads to increased TSH stimulation, increased iodine uptake, rapid iodine turnover, and enhanced production of T_3 relative to T_4. However, the response of rats to iodine-deficient diets can be markedly affected by the strain of rat (Okamura *et al.*, 1981a) and by nutritional factors other than the iodine content of the diet (Okamura *et al.*, 1981b). These results suggest that both hereditary and nutritional factors may be involved in the variable responses of humans to iodine deficiency.

Increased stimulation by thyrotropin

TSH is the primary factor that regulates the function of thyroid follicular cells and, ultimately, thyroid hormone secretion. In a classic negative feedback system, thyroid hormone inhibits the synthesis of TSH directly at the pituitary level and indirectly at the hypothalamic level by reducing the secretion of thyrotropin-releasing-hormone (TRH) (Cohen *et al.*, 2000). Elevated serum TSH levels have been reported repeatedly but not systematically in humans with chronic iodine deficiency (Delange *et al.*, 1971; Patel *et al.*, 1973; Chopra *et al.*, 1975). It has been suggested that the iodine-deficient thyroid is more sensitive to TSH (Bray, 1968; Brabant *et al.*, 1992), but the biochemical mechanism for this increased sensitivity are unknown (Pisarev & Gärtner, 2000). The lack of systematic correlation between goiter and TSH levels indicates that differences in the duration of elevated TSH levels and in thyroid responsiveness to TSH, as well as other factors, may determine whether goiter develops (Dumont *et al.*, 1992).

Increase in iodine uptake

The most important adaptation of the thyroid to an insufficient iodine supply is to increase the trapping of iodine. The accumulation in the thyroid of about 100 µg per day must be ensured (Delange, 2000b). To preserve existing iodine stores, the amount of iodine excreted in the urine must be reduced to a level corresponding to the level of iodine intake. A linear proportionality between iodine excretion and iodine intake within the physiological range has been shown by Vought & London (1967). As long as the iodine intake remains above a threshold of about 50 µg/day, the absolute uptake of iodide by the thyroid remains normal and the organic iodine

content of the thyroid remains within the limits of normal (i.e. 10-20 mg), despite a decrease in the serum iodine concentration (Delange, 2000b).

Alterations in the thyroid metabolism

In rats fed iodine-deficient diets serum T_4 concentrations are greatly reduced, and most of the T_3 in the circulation arises directly from the thyroid (Abrams & Larsen, 1973). This occurs not only through increased thyroidal biosynthesis of T_3, but also through deiodination of T_4 by the greatly increased levels of deiodinase in the activated gland (Pazos-Moura *et al.*, 1991). The shift to increased T_3 secretion and serum T_3:T_4 ratios may play an important role in the adaptation to iodine deficiency because T_3 is the most active thyroid hormone and requires less iodine for synthesis (Greer *et al.*, 1968). Similarly, it has been shown that the monoiodotyrosine/ diiodotyrosine ratio is increased in iodine deficiency (Ermans *et al.*, 1963a). However, insufficient iodination of Tg appear to be responsible for reduced efficiency of thyroid hormone synthesis (Dumont *et al.*, 1995).

Thyroid enlargement

The thyroid gland has a unique structure and is the largest of the organs that functions exclusively as an endocrine gland (Capen, 2000). The basic unit of cellular organization in the mature thyroid is the thyroid follicle. This consists of a lumen filled with viscous colloid and is surrounded by a single layer of epithel cells enclosed by a basement membrane (Pintar, 2000). The basic process in the transformation of the normal thyroid to a goiter is the generation of new thyrocytes and follicles (hyperplasia) in addition to increasing cell volume (hypertrophy). Besides TSH, other thyroid growth-stimulatory factors are thought to be of importance in the increased follicular cell replication. As mentioned before, however, the results on the association between elevated TSH levels and thyroid enlargement are not consistent. In rats, it has been shown that during goiter development TSH mainly induces hypertrophy, whereas intracellular iodine content mainly regulates thyroid hyperplasia (Stübner *et al.*, 1987). Whether TSH stimulation or intrathyroidal iodine depletion is more important for thyroid growth is difficult to determine (Pisarev & Gärtner, 2000) and probably depends on the severity of iodine deficiency.

Whereas in the early days goiter was considered as an adaptation to iodine deficiency, there is now no doubt that the large colloid goiter is a maladaptation (Delange *et al.*, 2001). Theoretically, the optimal thyroid response to iodine deficiency

would be an increase in thyroid blood flow, in iodide trapping capacity and in iodination rate, and rather low Tg content in a much reduced colloid space (Dumont *et al.*, 1995). However, endemic goiter is often large and filled with colloid. The low iodine and the high Tg concentration lead to a lesser iodination of Tg. Increased hydrolysis of large amounts of this protein is necessary to achieve normal secretion. This, excessive hydrolysis and deiodination of released iodotyrosines floods the thyrocyte iodide compartment and results in a leak of iodide (Ermans *et al.*, 1963b). In consequence, the urinary iodine (UI) loss will be enhanced and lead to an aggravation of iodine deficiency creating a vicious cycle with further dilution of luminal iodide versus Tg (Dumont *et al.*, 1995). According to Dumont *et al.* (1995) the ideally adapted thyroid would grow by a factor of no more than 2, and be comprised of an increased number of small follicles.

Etiology of iodine deficiency

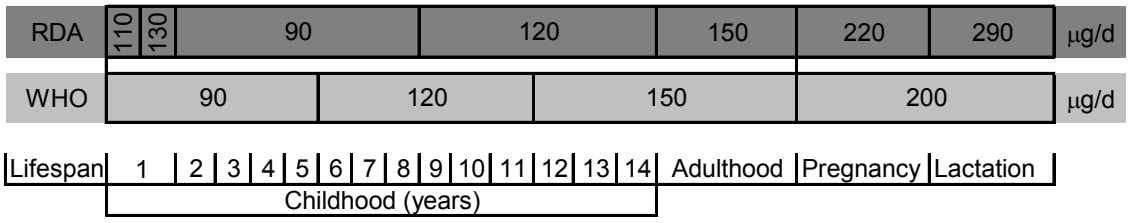
Recommended daily iodine intake

The recommendations for daily iodine intakes by WHO, United Nations Children's Fund (UNICEF) and International Council for Control of Iodine Deficiency Disorders (ICCIDD) (WHO *et al.*, 2001a) and the Recommended Dietary Allowance (RDA) for iodine (Institute of Medicine, 2002a) are equal for adolescents and adults (Figure 2). However, RDAs are higher for early infancy, pregnancy and lactation than the recommendations by WHO/UNICEF/ICCIDD. Whereas the recommendations by WHO/UNICEF/ICCIDD are based on the former RDA's by the Food and Nutrition Board of the National Academy Sciences in the United States (National Research Council, 1989), new Dietary Reference Intakes including RDAs have been published recently (Institute of Medicine, 2002a). The WHO/UNICEF/ICCIDD recommendation for lactating women is based on the assumption that an increment of 50 μg/day is needed to cover the daily iodine requirement of the infant (National Research Council, 1989) resulting in a recommended daily iodine intake of 200 μg. In contrast, the new RDA for lactation adds the iodine loss in human milk (114 μg/day) to the estimated average requirement of an adult women (95 μg/day). The RDA for iodine is calculated by adding twice the coefficient of variation of 20%, resulting in 290 μg/day for pregnancy (Institute of Medicine, 2002a). The recommended daily iodine intake for infants is 50 μg (WHO *et al.*, 2001a), as the Food and Nutrition Board of the National Academy Sciences in the United States (National Research Council, 1989) assumed that the amount of iodine in the milk of Northern American women was

presumably much greater than the needs of their infants. The new Dietary Reference Intakes for infants, however, do not include RDAs, but give the Adequate Intake of 110 µg/day for 0-6 months old and 130 µg/day for 7-12 months old infants, instead (Institute of Medicine, 2002a).

The Tolerable Upper Intake Level is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals and is set for iodine at 1100 µg/day for adults (Institute of Medicine, 2002a). Studies have shown that elevated TSH concentrations is one of the first effects of iodine excess (Roti & Vagenakis, 2000). Although an elevated TSH concentration may not be a clinically significant adverse effect, it is an indicator for increased risk of developing clinical hypothyroidism and was therefore chosen as the critical adverse effect on which to base the Tolerable Upper Intake Level for iodine (Institute of Medicine, 2002a).

Figure 2: Daily iodine intake recommended by WHO, UNICEF and ICCIDD (WHO *et al.*, 2001a) and RDAs (Institute of Medicine, 2002a) for different life stages



Demography of iodine deficiency

Numerous studies have established the epidemiological link between insufficient iodine intake through food and water with the development of endemic goiter. Iodine-deficient areas are mainly characterized by soil from which iodine has been leached by glaciation, heavy rainfall and flood (Hetzel, 1993). Populations in these areas depending on food locally grown, consequently get iodine-deficient. However, the greater availability of methods assessing iodine deficiency has demonstrated that IDD occur in many areas where none of these conditions were found (WHO *et al.*, 2001a). The fact that significant iodine deficiency has been found in regions where IDD have been considered to be eliminated by prophylactic programs (WHO *et al.*, 2001a), support the assumption that other nutritional factors may influence the prevalence and severity of iodine deficiency. Besides goitrogenic foods, other

nutritional interactions, such as protein-energy malnutrition (Gaitan *et al.*, 1983) and micronutrient deficiencies (Boyages, 1993; Zimmermann & Köhrle, 2002) may modify the response to iodine prophylaxis. These potential nutrient interactions will be discussed in a later section.

Goitrogenic factors

Agents that cause thyroid enlargement are known as goitrogens. They may cause goiter by acting directly on the thyroid gland, but they can also act indirectly by altering the regulatory mechanisms of the thyroid gland and the peripheral metabolism and excretion of thyroid hormones (Gaitan, 1990). Potential goitrogenic substances or their precursors are widespread in vegetables of the Brassica family (Fenwick & Heaney, 1983). More important, however, are the naturally occurring goitrogens cyanoglucosides in several staple foods such as cassava, maize, bamboo shoots, sweet potatoes, and lima beans (Gaitan, 1990). After ingestion, these glucosides release cyanide, which is detoxified to thiocyanate. Thiocyanate is a powerful goitrogenic substance as it is an anion with the same molecular size as iodine. It inhibits thyroid accumulation of iodide and, at higher doses, competes with iodide during protein binding (Wollman, 1962). Thiocyanate has been shown to compete with iodine, as it serves as a substrate for TPO and therefore inhibits the iodination of tyrosyl residues of Tg (Michot *et al.*, 1980; Virion *et al.*, 1980). In contrast, thiocyanate stimulates the coupling reaction (Virion *et al.*, 1980). As iodine also is a substrate for the Tg iodination and a stimulatory ligand for the coupling reaction, these studies suggest that thiocyanate binds to the same regulatory site as iodine but has a slightly different affinity (Michot *et al.*, 1980). As reviewed by Delange (2000b), several studies have shown that cassava plays a role in the etiology of endemic goiter together with iodine deficiency. The goitrogenic effect of the cassava is determined by the ratio between dietary iodine intake and thiocyanate. Other staple foods also contain goitrogens such as phenolic compounds. Their inhibiting effects on TPO activity have been found in millet (Gaitan *et al.*, 1989; Sartelet *et al.*, 1996) and in babassu, which is a staple food in Brazil (Gaitan *et al.*, 1994). The regular consumption of these staple foods may contribute to the genesis of endemic goiter in areas of iodine deficiency.

Indicators to assess iodine deficiency

Target group

To assess iodine status in a region, it is recommended to choose a population group using the following criteria: vulnerability, representativeness and accessibility. Applying these criteria, the most useful target groups are school-age children because of their high vulnerability and easy access (WHO *et al.*, 1994). Moreover, school-age children are often affected of other health concerns such as other micronutrient deficiencies. However, a major drawback of school-based surveys in developing countries is that children not attending school are not represented. This possibly leads to biased prevalence estimates (WHO *et al.*, 2001a).

Urinary iodine

Once the need for thyroidal iodine has been met, excess iodine is excreted by the kidney (Hetzel, 1993). About 90% of iodine intake within the physiological range is eventually excreted in the urine (Vought & London, 1967; Nath *et al.*, 1992). The median UI in casual samples is currently the most practical indicator to assess recent dietary iodine intake. It is important to consider that iodine excretion of individuals varies over the day and between days, but this variation can be evened out by a big enough sample size. Therefore, the iodine concentration in spot urine samples of children and adults provides an adequate assessment of a population's iodine nutrition (WHO *et al.*, 2001a).

Table 1: Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations in school-aged children (WHO *et al.*, 2001a).

Median urinary iodine [µg/L]	Iodine intake	Iodine nutrition
<20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Optimal
200-299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
>300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

Many analytical techniques are used to measure UI. Most methods depend on the catalytic action of iodide on the reduction of the ceric ion to the cerous ion in the presence of arsenious acid. This reaction is called Sandell-Kolthoff reaction, in which samples first have to be digested with ammonium persulfate or chloric acid. In adequate iodine nutrition, the median UI concentration should be at least 100 µg/L, with less than 20% of values below 50 µg/L (Table 1) (WHO *et al.*, 2001a). The recommended critical threshold of 100 µg/L has been recently confirmed in a study which evaluated the UI in iodine-replete populations because the frequencey of UI concentrations <50 µg/L was found to be far lower than the previously assumed value of 20% (Delange *et al.*, 2002).

Thyroid size

The prevalence of goiter is another indicator for assessing the extent of iodine deficiency in a population (WHO *et al.*, 1994). For years, palpation has been the single method available for defining Tvol. In 1994, a new two-grade classification system was proposed by WHO/UNICEF/ICCIDD. It defined goiter as any enlarged thyroid that is palpable (Grade 1) or visible (Grade 2) (WHO *et al.*, 1994). This simplified the use of palpation even more. However, in areas of mild IDD where the prevalence of visible goiter is low, sensitivity and specificity of palpation are poor, and misclassification can be as high as 40% (Gutekunst & Teichert, 1993; Vitti *et al.*, 1994; WHO, 1994; Zimmermann *et al.*, 2000d). Under these conditions ultrasonography is more reliable. The higher sensitivity of ultrasonography becomes even more important when the impact of iodine prophylaxis is monitored, as the Tvol are expected to decrease over time. It is a safe, non-invasive specialized technique. Using portable ultrasound equipment, it can be performed in the field and, using a generator, even under conditions without electric current.

In thyroid ultrasound longitudinal and transverse scans are preformed to measure the depth (d), the width (w) and the length (l) of each lobe. Brunn *et al.* (1981) have measured volume of 25 thyroids by real-time ultrasound in cadavers and compared with direct measurements obtained by submersion in a water quench. They have found that the best calculated volume of the thyroid lobe is obtained by using a corrected formula for a rotation ellipsoid (Brunn *et al.*, 1981):

$$\text{Thyroid volume V [ml]} = 0.479 \cdot d \cdot w \cdot l$$

Tvol is the sum of the volumes of both lobes. The volume of the isthmus is not included (WHO *et al.*, 2001a). Tvol measurements in children should be presented as a function of age, sex, and body surface area (BSA). Using the Tvol relative to BSA has the advantage when applied in countries with a high prevalence of child growth retardation and in countries where the age of a child is not known with certainty. BSA is calculated as following, where W is weight in kg and H is height in cm (DuBois & DuBois, 1916):

$$\text{Body surface area } [m^2] = W^{0.425} \cdot H^{0.725} \cdot 71.84 \cdot 10^{-4}$$

The normative values proposed by Gutekunst & Teichert (1993) were the most commonly used, until in 1997, WHO and ICCIDD adopted a new Tvol references (WHO & ICCIDD, 1997). These references emerged from a large European study, using data from a subgroup of 3474 children born or living in areas where iodine intake is normal (Delange *et al.*, 1997). However, in the following years, several reports have suggested that the WHO/ICCIDD reference criteria may be too high (Foo *et al.*, 1999; Xu *et al.*, 1999; Hess & Zimmermann, 2000; Djokomoeljanto *et al.*, 2001). In a workshop organized by WHO and ICCIDD where interobserver and interequipment variation in ultrasound Tvol was evaluated, it was suggested that a systematic bias of one examiner has led to an overestimation of the current reference criteria (Zimmermann *et al.*, 2001a). As this examiner had generated all ultrasound measurements in the European study, a correction factor for the systematic difference of this operator was estimated. When applied to the WHO/ICCIDD reference data, it sharply reduced the discrepancy between the WHO/ICCIDD criteria and those from other iodine-sufficient children around the world (Zimmermann *et al.*, 2001a). Subsequently, the WHO/ICCIDD criteria were withdrawn (WHO *et al.*, 2001a). It has been suggested that the corrected, updated references replace the WHO/ICCIDD criteria for interpretation of ultrasound Tvol data among school-age children until data from further studies become available (Zimmermann *et al.*, 2001b). Besides the debate on Tvol reference criteria during the last 2-3 years, there is also a controversy on the usefulness of Tvol as an indicator in determining the impact of universal salt iodization programs (USI). Little is known on how long it takes for the goiter to disappear or if thyroid enlargement is completely reversible at all. In rats, iodine supplementation abolishes not only hypertrophy, but also hyperplasia of the glands and restores normal function and regulation (Stübner *et al.*, 1987). In school children, Tvol by ultrasound had not changed 395 days after treatment with 120, 240, 480 mg of oral iodized oil, whereas in the groups receiving 960 mg oral iodine or an

intramuscular injection of 480 mg iodine Tvol was decreased by –29% and –23%, respectively (Benmiloud *et al.*, 1994). In a study in 6-12 yr old children in Côte d'Ivoire, oral iodized oil containing 200 mg iodine reduced mean Tvol by ultrasound by –35% and –41% after 30 and 50 weeks, respectively (Zimmermann *et al.*, 2000a). Thirteen months after oral administration of potassium iodide solution, 8.7 mg every 2nd week or 29.7 mg every month, mean Tvol measured by ultrasound had decreased in both groups to values comparable with those in iodine-sufficient areas (Todd & Dunn, 1998). However, there is not much data on the effect of iodized salt on the reduction of thyroid enlargement. Jooste *et al.* (2000) found 1 year after mandatory iodization of salt in South Africa no difference in goiter rate by palpation in school children. In a randomized trial in school children in China, Tvol by ultrasound decreased to normal after 18 months of salt iodized at 25 ppm (Zhao *et al.*, 1999).

It is now recognized that once USI is phased in, the prevalence of low UI will fall faster than the prevalence of goiter (Sullivan & May, 1999). After some period when USI has been achieved, the prevalence of low UI and goiter will again be in agreement indicating no IDD. Until then, the use of Tvol as an indicator might be of limited value. However, it is unknown how long this adaptation takes. Therefore, it is important that the results on thyroid size be interpreted cautiously to judge the success of USI (Sullivan & May, 1999) unless iodized salt has been available for a long period.

Besides the need for new international reference criteria for Tvol by ultrasound, there is a need to further investigate the impact of USI on the thyroid gland.

Blood constituents

Determining serum concentrations of the thyroid hormones, T_4 and T_3, is usually not recommended for monitoring iodine nutrition (WHO *et al.*, 2001a). It is argued that, even though in iodine deficiency, serum T_4 is typically lower and serum T_3 is higher than in normal population, the overlap is large enough to make these tests not practical for ordinary epidemiological purposes. However, the aim of any USI is the prevention of adverse effects of iodine deficiency. Normalization of the thyroid function is therefore the major goal and its assessment advisable.

Similar recommendations are given for TSH concentration. As the difference between iodine-deficient and iodine-sufficient population groups is neither great nor consistent, much overlap occurs between individual TSH values. Therefore, the blood TSH concentration in school-age children and adults is not a practical marker for iodine deficiency, and its routine use in school-based surveys is not

recommended (WHO *et al.*, 2001a). However, neonatal TSH screening is considered very useful in assessing IDD status of a population. An elevated TSH level in neonates and infants is of concern because it indicates inadequate thyroid hormone concentration during the crucial stage of brain development. Consequently, TSH concentrations reflect the risk of damage to the developing brain and subsequent impairment of intellectual development (Delange *et al.*, 2001). However, it is only recommended as an indicator for iodine deficiency if a national program already exists (WHO *et al.*, 2001a).

Another blood constituent which can serve as surveillance indicator is Tg. Tg is the most abundant protein of the thyroid and provides a matrix for the synthesis of the thyroid hormones and a vehicle for the subsequent storage (Dunn & Dunn, 2000). A small amount of Tg is secreted into the blood circulation by a mechanism which is still unclear (Chopra & Sabatino, 2000). Abnormal serum Tg concentrations result from abnormalities in Tvol, excess thyroidal stimulation, or physical thyroid damage (Spencer, 2000). Tg rises in individuals with an insufficient iodine intake and it normalizes before Tvol has decreased (WHO *et al.*, 1994). Tg has been shown to correlate well with other indicators of iodine deficiency (Missler *et al.*, 1994; Knudsen *et al.*, 2001; van den Briel *et al.*, 2001; Zimmermann *et al.*, in press). However, a major limitation to the use of Tg in IDD monitoring are assay-dependent factors influencing Tg measurement reliability which include lack of a standard reference material, poor sensitivity of some assays, and poor interassay precision (Torrens & Burch, 2001).

Iodization programs

Iodized oil

The two main strategies to correct iodine deficiency are supplementation and fortification. Iodization of salt, irrigation water, drinking water or bread are possibilities to fortify with iodine (Bürgi & Helbling, 1996). DeLong *et al.* (1997) showed that iodine supplementation of irrigation water of wheat in areas of severe iodine deficiency decreases neonatal and infant mortality. However, besides salt fortification neither of the other strategies have been used in large scale. Although salt fortification has been the ultimate goal (WHO *et al.*, 1999), the use of iodized oil is recommended when immediate iodine supplementation is needed during the implementation of USI. The most frequently used iodized oil is Lipidol, a seed-oil from the opium poppy, in which iodine atoms are bound to the polyunsaturated fatty acids (Ingenbleek *et al.*,

1997). A portion of the iodized fatty acids is stored in adipose tissue (Wei & Li, 1985), permitting a slow release of iodine and thus providing long-lasting supplies. One year of iodine needs can be achieved with 200 to 480 mg in the form of oral Lipidol (Benmiloud *et al.*, 1994; Elnagar *et al.*, 1995). The advantage of oral iodized oil is that it can be selectively applied to circumscribed regions or geographical pockets of severe iodine deficiency, and within such a region, it can be restricted to certain target populations to reduce costs (Bürgi & Helbling, 1996).

Universal salt iodization

In nearly all countries where iodine deficiency occurs, it is now well recognized that the most effective way to eliminate IDD is through USI (WHO *et al.*, 2001a). Salt is an ideal vehicle for fortification due to the following reasons: 1) It is consumed by everyone, 2) the consumption is rather constant throughout the year, 3) its production is usually limited to a few centers which facilitates its quality control, 4) salt iodization is easy to implement and is cost-effective, 5) the addition of iodine to the salt does not change color and flavor. The recommended amounts for the daily intake of iodine is 150 µg/day for adults and adolescents, 200 µg/day for pregnant and lactating women and less for children (WHO *et al.*, 2001a). In order to achieve an optimal iodine intake through salt iodization the following factors have to be considered: 1) the consumption of salt per person, 2) the degree of iodine deficiency, 3) the iodine losses during storage and transport. Consequently, the optimal level of salt iodization vary from country to country (Delange *et al.*, 2001). However, WHO/UNICEF/ICCIDD recommend that iodine concentration should be 20-40 mg/kg salt in typical circumstances, where the average daily salt intake is 10 g per person, 20% of iodine from salt is estimated to be lost during transport from production to household and 20% during cooking (WHO *et al.*, 1996).

While there is much data on the effects of the health benefits of iodized oil, there is a lack of such data on iodized salt. However, long-term effects of USI are well known. Bürgi *et al.* (1990) has reviewed the effects of iodized salt in Switzerland, which has first started the introduction of iodized salt in 1922. After 1930 no new born endemic cretins have been identified, and goiter disappeared rapidly in newborns and school children, more slowly in army recruits, and incompletely in elderly adults. In Finland iodized salt was introduced in the 1940's. Consequently the goiter prevalence among school children decreased generally to 1-4%, having been 15-30% in most parts in the early 1950's (Lamberg *et al.*, 1981). However, results on the elimination of endemic cretinism, the prevention of blunting of intellectual and socio economic

potential and reduction in perinatal morbidity and mortality through salt iodization are needed (Delange *et al.*, 2001).

Monitoring universal salt iodization

There has been a remarkable progress in USI worldwide. In 1999, of the 130 countries with IDD, 98 had legislation on salt iodization in place (WHO *et al.*, 1999). However, the past has shown that once a national IDD control program is successfully implemented, monitoring is very important to maintain sustainability. If iodine content in the salt is too low, iodine deficiency will relapse soon (Delange *et al.*, 2001). At the same time, it is crucial to avoid iodine excess as this can lead to adverse effects. The principle adverse effect is iodine-induced hyperthyroidism which occurs essentially in older people with autonomous nodular goiters, especially when iodine intake is suddenly too much increased (Delange *et al.*, 1999). In this case, excess iodine can even have lethal consequences for some individuals. However, according to Delange & Lecomte (2000) the incidence of this disorder is usually low and reverts spontaneously to the background rate of hyperthyroidism or even below this rate after 1 to 10 years of iodine fortification. Iodine-induced hyperthyroidism can not be entirely avoided even when iodization programs use only physiological amounts of iodine (Delange & Lecomte, 2000). It is very important to introduce USI at the lowest iodine level to correct IDD and at the same time to minimize the risk of iodine-induced hyperthyroidism. Moreover, it is crucial to maintain the iodization level in the salt at the recommended level.

Iron

Iron deficiency, particularly and iron deficiency anemia (IDA), remains one of the most severe and important nutritional deficiencies in the world today, affecting millions of people mainly in the developing but also in the developed world (WHO *et al.*, 2001b). Iron is present in all cells and has several vital functions. Iron deficiency has therefore a wide range of adverse health effects. It is not the only cause of anemia, but where anemia is prevalent, iron deficiency is usually the most common cause (Stoltzfus & Dreyfuss, 1998).

Consequences of iron deficiency

Cognitive development

There is strong evidence that IDA during the first few years of life is associated with poor cognitive and motor development and behavioral problems. Longitudinal studies indicate consistently that children who were anemic in early childhood continue to have poor cognitive and motor development and school achievement into middle childhood (Pollitt, 1993; Walker, 1998; Grantham-McGregor & Ani, 2001). In a study in Costa Rica of 5 year-old children, those who had IDA in infancy were considered to be at risk of long-lasting developmental disadvantage as compared with their peers with better iron status (Lozoff *et al.*, 1991). Moreover, the effects of IDA in infancy and early childhood are not likely to be corrected by subsequent iron therapy (WHO *et al.*, 2001b). Grantham-McGregor & Ani (2001) have reviewed the effect of iron supplementation on cognition in children > 2 years and concluded that the beneficial effect of iron treatment on cognition in anemic older children is reasonably convincing. In four studies children benefited from iron treatment and in three other studies a benefit was highly likely, whereas two studies showed no effect. In children < 2 years causal relationship was inconsistent (Grantham-McGregor & Ani, 2001). However, according to the authors of this review, the results of many studies were difficult to interpret as only a few were randomized controlled trials and the sample size was often extremely small.

Studies in rats showed that at different stages of life different regions in the brain are high in iron content. These studies indicate that the effect of iron deficiency on brain iron content depends on the timing of the nutritional insult (Erikson *et al.*, 1997; Pinero *et al.*, 2000; Pinero *et al.*, 2001). It appears, that there may be one or more critical developmental periods during which insufficient iron availability can produce

deficits in neural functioning and behavior that may be impossible to remedy by iron replenishment (Beard *et al.*, 1993).

Immune function

Whereas most pathogens require iron and other micronutrients, and have evolved sophisticated strategies for acquiring these micronutrients, iron is also required by the host for mounting an effective immune response (Beard, 2001). Since both, iron deficiency and infectious diseases are common conditions, the effects of iron deficiency and iron supplementation, respectively, on the immune defense and on morbidity are of great interest. Experimental and clinical studies suggest that there is an increased risk of infection during iron deficiency, although a small number of reports indicate otherwise (Beard, 2001). On the other hand it is discussed, that iron deficiency may protect from infection in certain malaria-endemic situations, however evidence is weak, indirect and inconclusive (Oppenheimer, 2001). The difficulty in interpreting many studies is, that the confounding issues of poverty, generalized malnutrition and multiple micronutrient deficiencies are often present. Moreover, even laboratory measures of iron deficiency are confounded by the immediate presence of infection (Cook *et al.*, 1992). In a prospective randomized study of adult Somali nomads with IDA, 36 episodes of infection occurred in the iron treated group compared with 7 in the placebo group (Murray *et al.*, 1978). The most striking differences were in malaria, brucellosis and tuberculosis. However, these dramatic effects of oral iron treatment on tropical infections have not been confirmed by other randomized studies in similar populations (Hershko, 1993). Oppenheimer (2001) reviewed the effects of iron treatment and stated that oral iron supplementation has not been shown to cause an increased risk of infection in any age group in non-malarious countries, whereas in malarious regions, iron supplementation given in therapeutic doses may carry up to a 50% increased risk of clinical malaria at times of malaria transmission. However, based on a review of 13 randomized, controlled clinical trials, the International Nutritional Anemia Consultative Group (INACG, 2000) states that the known benefits of iron supplementation are likely to outweigh the risk of adverse effects in regions with endemic malaria. In a randomized study in Tanzania, infants were treated against malaria with sulphadoxine-pyrimethamine or placebo, whereas all received iron supplementation between 2 and 6 months of age. During the first year of life, the rate of clinical malaria was reduced by 59% and the rate of severe anemia was reduced by 50% in the treated group compared to the placebo group (Schellenberg *et al.*, 2001), indicating the significant impact malaria

has on anemia. As iron supplementation should not be withhold to the risk groups in malarious regions, it is recommended that iron treatment should be covered or preceded by effective antimalarial therapy to reduce risk (Oppenheimer, 2001).

Pregnancy

During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder pregnancy (Bothwell, 2000). Therefore, the risk of iron deficiency during pregnancy is high. A major concern about the adverse effects of IDA on pregnant women is the belief that the risk of maternal mortality and morbidity might be increased. Data indicate a strong association between severe anemia and maternal mortality but not for mild or moderate anemia (Rush, 2000; Brabin *et al.*, 2001). The iron deficiency component of this is unknown, but the more severe the anemia, the more likely it is to have multiple causes and not be related solely to iron deficiency (Brabin *et al.*, 2001). Data on the relative risk of low birth weight that results from moderate or severe anemia are inconsistent. Nonetheless it is generally higher than the also inconsistent relative risk of preterm birth that results from anemia (Rasmussen, 2001). An association between low maternal hemoglobin (Hb) and higher neonatal or perinatal mortality is likely, but the data remain insufficient (Rasmussen, 2001). Altogether evidence is insufficient to prove that iron deficiency plays a causal role in poor pregnancy outcome (Allen, 2001).

Work capacity and productivity

The overt physical manifestations of iron deficiency include the general symptoms of anemia, which are tiredness, lassitude and general feelings of lack of energy. From both the laboratory and field experiments, the evidence is strong and suggests that the potential magnitude of the effect of IDA on work productivity is substantial (Haas & Brownlie, 2001). A linear relationship between iron deficiency and work capacity for agricultural workers have been reported in different countries (WHO *et al.*, 2001b). The presumed mechanism for this effect is the reduced oxygen transport associated with anemia; tissue iron deficiency may also play a role through reduced cellular oxidative capacity (Haas & Brownlie, 2001). However, the social and economic effect of iron deficiency and IDA is difficult to assess and further field studies are necessary (Horton & Levin, 2001).

Altered metabolism

Iron deficiency is associated with alterations in many metabolic processes. The activity or concentration of multiple iron-containing enzymes in skeletal muscle and liver declines with IDA (Ackrell *et al.*, 1984; Cartier *et al.*, 1986; Chen *et al.*, 1997). The concentration of the key regulators of glucose production, the catecholamines epinephrine and norepinephrine, is abnormal in iron-deficient anemic humans and rats (Dillman *et al.*, 1980; Martinez-Torres *et al.*, 1984). In addition, a dose-response relationship among hyperglycemia, hyperinsulinemia and severity of IDA has been demonstrated (Henderson *et al.*, 1986; Brooks *et al.*, 1987; Borel *et al.*, 1991). Iron deficiency also influences the neurotransmitter system. However, Beard (2001) stated in a review that the dopaminergic system is the only neurotransmitter system in the central nervous system that has been consistently sensitive to experimental changes in iron status. One of the primary effects of chronic iron deficiency on the circulatory system is hypertrophy of the heart (Medeiros & Beard, 1998). It was suggested that the myocardial enlargement is a physiologic attempt to maintain oxygen delivery to peripheral tissues in anemic animals (Smith *et al.*, 1990). Other alterations on metabolism such as the effects on thyroid metabolism and thermoregulation will be discussed in a later section. Some of the adverse effects of iron deficiency can be attributed to the presence of anemia, whereas others are more clearly related to decreases in essential body iron and limitations in tissue oxidative capacity (Borel *et al.*, 1991). However, separating the effects of low oxygen transport and iron tissue deficits is difficult, as tissue iron deficits occur simultaneously with deficits in oxygen transport in naturally occurring IDA (Beard, 2001).

Physiological role of iron

In the human body, iron is present in all cells. It has several vital functions, including binding and transport of oxygen, electron transfer reactions, gene regulation and regulation of cell growth and differentiation (Bothwell *et al.*, 1979; Dallman, 1986; Beard, 2001). The iron-containing compounds in the body are grouped into two categories: those known to have metabolic or enzymatic functions and those associated with iron storage and transport (Bothwell *et al.*, 1979). The main iron-containing protein is Hb in erythrocytes. It accounts about two thirds of the body iron. Hb binds oxygen as the blood passes through the lungs, and distributes this oxygen to the body tissues. Similarly, myoglobin is the oxygen reserve in the muscle. It transports and stores oxygen for use during muscle contraction. Most other functional iron-containing proteins are enzymes. The so-called heme-enzymes, such as

cytochromes, catalase and peroxidase, depend on heme as a coenzyme (Dallman, 1986). They act as electron carriers within the cell. A very large number of other iron-containing enzymes have also been described. They play key roles not only in the oxygen and electron transport but also as signal-controlling substances in some neurotransmitter systems in the brain (Hallberg *et al.*, 1993).

Iron stores have no physiological function other than to serve as a buffer against increasing iron demands such as occur during pregnancy or with acute blood loss (Cook *et al.*, 1992). The major iron storage compounds are ferritin and hemosiderin. There are large quantities of ferritin in iron storage tissue such as the liver, spleen and bone marrow, but only small quantities are present in human serum, normally between 12 and 300 µg/L (Cook *et al.*, 1992). Hemosiderin is the major iron storage protein present when excessive iron accumulates in the tissues. The contribution of the two types of storage iron to total body iron can vary widely from less than 5% to more than 30% (Dallman, 1986).

The extracellular transport of iron within the body is accomplished by its binding to transferrin, a specific carrier protein. Transferrin accounts for only about 0.1% of the total body iron (Dallman, 1986). The movement of transferrin iron through the plasma compartment is controlled by the number of transferrin receptors on the surface of body cells. When a cell senses a need for iron, the synthesis of transferrin receptor is up regulated, allowing it to compete more effectively for circulating transferrin iron (Cook *et al.*, 1993).

Iron metabolism

While iron is an essential nutrient required by every human cell, quantitatively most of the iron in the body is found within the erythrocyte cycle and most of the daily movement of iron cycles through the erythron. Erythrocytes are constantly produced in the bone marrow and, after a live span of 120 days, are broken down in the spleen and the iron is released. Most of the iron required to produce new erythrocytes comes from the breakdown of old erythrocytes, and only a small portion comes from the absorption of food iron (Hallberg *et al.*, 1993). Movement of iron to and from the erythron accounts for about 80% of the iron flowing through the transferrin compartment each day. The remaining 20% of iron carried by transferrin includes: 1) iron exchange with hepatocytes, 2) movement between the plasma and extravascular transferrin compartments, 3) exchange between extravascular transferrin and parenchymal tissues, and 4) limited external exchange of iron through obligatory losses and absorption of iron from the gastrointestinal tract (Brittenham,

1994). The majority of iron is lost in sloughed cells via the gut, skin and urine. Additional losses occur through menstrual blood losses in women.

The metabolism for iron differs from other minerals and trace elements, as total amounts of iron in the body cannot be controlled by excretion of iron but only by a regulation of the absorption (Hallberg, 2001). Theoretically, when the body needs more iron, absorption is increased, and when the body is iron-sufficient, absorption is restricted (Hurrell, 1999). Dietary iron is absorbed mainly in the duodenum by an active process that transports iron from the gut lumen into the mucosal cell, from where it is transferred across the cell into the circulation when needed (Charlton & Bothwell, 1983). If iron is not required by the body, iron in the mucosal cell is stored as ferritin. Two factors have been considered to control the absorption of dietary iron: the amount of iron stores, and the erythropoietic activity (Bothwell *et al.*, 1979; Finch, 1994). The erythroid regulator mainly responds to acute marrow iron needs, whereas the store regulator would be mainly responsible for the maintenance of iron balance by influencing the absorption of dietary iron (Hallberg, 2001). According to Finch (1994), however, it is not possible to characterize these regulators in other than physiological terms.

Indicators of iron deficiency

Iron deficiency develops in three overlapping stages (Dallman, 1986). The three main iron compartments, storage, transport and erythroid iron, are affected sequentially with increasing deficits in body iron. When iron intake can not meet iron requirements, iron is mobilized from the iron stores in the body. In man, this stage is characterized by a decrease in the concentration of serum ferritin (SF). Storage depletion represents an increased risk of developing iron deficiency but by itself is not associated with any known liabilities (Cook *et al.*, 1992). The concentration of SF is considered the most practical indicator that correlates with total body iron stores. The cut-off level for SF suggested by WHO, below which iron stores are considered to be depleted, is < 15 µg/L (WHO *et al.*, 2001b). Once iron stores are depleted as defined by a SF concentration below the cut-off value, the measurement gives no indication of the severity of iron deficiency (Cook *et al.*, 1992). However, because ferritin is an acute-phase reactant, its concentration in the blood increases in the presence of subclinical and clinical inflammatory and infectious diseases. Thus it can not be used to accurately assess depleted iron stores in settings where poor health is common (Nestel & Davidsson, 2002).

The second stage of iron deficiency consists of a decrease in transport iron and is likely to be transient (Dallman, 1986). Serum iron concentrations decrease, transferrin (total iron-binding capacity) levels increase, and hence transferrin saturation (serum iron/total iron-binding capacity) is reduced. However, due to significant diurnal fluctuation and low specifity, these indicators are not very useful in assessing iron deficiency (WHO *et al.*, 2001b). A more sensitive measure for iron deficiency is serum transferrin receptor (TfR). Elevated TfR is the first laboratory sign of iron deficiency following depletion of body iron reserves and the rise continues in direct proportion to the severity of iron deficiency (Skikne *et al.*, 1990). The advantage of measuring TfR is that there are only two conditions that are associated with an elevation in TfR: 1) enhanced erythrocyte production and 2) tissue deficiency of iron (Cook, 1999). The concentration of circulating TfR have been found to be normal even in pregnancy, only being raised if iron deficiency is present (Carriaga *et al.*, 1991). TfR is also unaffected by inflammatory disorders as shown by several authors (Ferguson *et al.*, 1992; Kuvibidila *et al.*, 1995; Staubli Asobayire *et al.*, 2001). However, a number of studies found increased TfR concentrations in children with malaria, probably due to hemolysis during malaria resulting in increased erythropoiesis (Stoltzfus *et al.*, 2000; Verhoef *et al.*, 2001; Verhoef *et al.*, 2002).

At the second stage of iron deficiency, free protoporphyrin concentration in erythrocytes also increases (Nestel & Davidsson, 2002). Erythrocyte protoporphyrin is the precursor of heme. In iron deficiency, the incorporation of iron to form heme is reduced, which leads to a progressive accumulation of zinc protoporphyrin (ZPP) (Labbé & Rettmer, 1989). Besides iron deficiency, inflammation, lead poisoning and hemolytic anemia can significantly increase ZPP (WHO *et al.*, 2001b).

Anemia is the last and most severe stage of iron deficiency. It develops when the supply of transport iron decreases sufficiently to restrict the concentration of Hb and/or other iron compounds that fulfill known physiological function (Dallman, 1986). Any assessment of iron status must include the Hb concentration because it defines a more advanced stage of iron lack and it is the only laboratory assay that provides a quantitative measure of the severity of iron deficiency once anemia has developed (Cook *et al.*, 1992). The major limitation of Hb, however, is that many factors other than iron deficiency cause anemia. It can also result from infections such as malaria (Menendez *et al.*, 2000), from chronic inflammatory disorders (Yip & Dallman, 1988), or from other nutritional deficiencies of folate, vitamin A, B_{12} or riboflavin (Suharno *et al.*, 1993; Savage *et al.*, 1994; van den Broek & Letsky, 2000; Allen & Casterline-Sabel, 2001). Hb can, therefore, not be used in isolation as an indicator of iron status (Cook, 1999).

IDA should be considered as a subset of iron deficiency (WHO *et al.*, 2001b). It represents the extreme lower end of the distribution of iron deficiency. However, under conditions of poor health, anemia develops due to multiple factors of which iron deficiency is often the most frequent one.

Etiology of iron deficiency and anemia

Iron requirements

The prevalence of iron deficiency varies greatly according to host factors: age, gender, physiological, pathological, environmental, and socioeconomic conditions (WHO *et al.*, 2001b). However, it affects a large number of young children and pregnant women. Iron requirements on a body weight basis are proportional to growth velocity (WHO *et al.*, 2001b). In the weanling period, the iron requirements in relation to energy intake are the highest during the whole lifespan of man (Hallberg *et al.*, 1993). Although there are some differences in the iron dietary reference values given by the different organizations and nations, adolescent girls, women of childbearing age and adolescent boys have the highest recommended intake values (Hurrell, 1999). In particular, adolescent females have high iron requirements as they have to cover their growth needs and menstrual losses besides the obligatory losses (Hallberg & Rossander-Hulthén, 1991). Following menarche, adolescent females often do not consume sufficient iron to offset menstrual losses. As a result, a peak in the prevalence of iron deficiency frequently occurs among females during adolescence (WHO *et al.*, 2001b). Iron requirements increase significantly during the second half of pregnancy because of the expansion of the erythrocyte mass and the transfer of increasing amounts of iron to both the growing fetus and the placental structures (Bothwell, 2000). RDAs during pregnancy are 27 mg iron/day, whereas they are 18 mg/day for 19-50 year old non-pregnant women (Institute of Medicine, 2002b). After delivery, the iron used for the increased erythrocyte mass is returned to stores (Bothwell, 2000). During lactation, iron needs are equal or even lower than in the non-pregnant state because lactational amenorrhea more than compensates for iron lost through breast milk (WHO *et al.*, 2001b).

Factors influencing iron absorption

In many populations, the amount of iron absorbed from the diet is not sufficient to meet many individuals' requirements (Stoltzfus & Dreyfuss, 1998). Two main

physiological factors influence the amount of iron absorbed from the diet: 1) the iron status of the individual and 2) the composition of the diet (Rossander-Hulthén & Hallberg, 1996). There are two kinds of iron in the diet with respect to the mechanism for absorption, heme and non-heme iron. Heme iron is derived mainly from meat and usually forms only a small fraction of the dietary iron. Non-heme iron is the major part of dietary iron and derives mainly from plants. However, the iron content *per se* of individual foods has little meaning since iron bioavailability varies considerably (Fairweather-Tait & Hurrell, 1996). Heme iron is always relatively well absorbed (15-35%) and is little influenced by physiological and dietary factors (Monsen *et al.*, 1978). Non-heme iron bioavailability is strongly affected by dietary components mainly as a result of luminal interactions (Hallberg, 1981).

Phytic acid, phenolic compounds, calcium and certain milk or soy proteins are common dietary inhibitors of iron absorption (Hurrell, 2002). They can considerably reduce the absorption in both native food iron and fortification iron by forming unabsorbable complexes in the gastrointestinal tract. Phytic acid is found in cereal grains and legume seeds and is the major determinant of the low iron bioavailability in these foods. It strongly inhibits iron absorption in a dose-dependent relationship and even small amounts have a marked effect (Hallberg *et al.*, 1989). Different methods to reduce phytic acid during food preparation had a positive effect on iron absorption (Egli, 2001). Phenolic compounds are widespread in nature and are particularly high in beverages such as tea, coffee, herb teas, cocoa and red wine. The major inhibiting effect has been ascribed to the galloyl groups in different foods and spices (Brune *et al.*, 1989; Brune *et al.*, 1991). Ascorbic acid counteracts the inhibiting effects of phytic acid and phenolic compounds. It is the best known and most potent enhancer of iron absorption both in its natural form in fruits and vegetables (Ballot *et al.*, 1987) and when added as the free compound (Cook & Monsen, 1977). Due to poor overall dietary quality and a low intake of foods from animal sources in developing countries, dietary factors are likely to make a greater contribution to iron deficiency than in more developed countries (Yip & Ramakrishnan, 2002).

Other causes of anemia

The etiologic factors responsible for anemia are multiple and their relative contributions can be expected to vary by geographic area and season (van den Broek & Letsky, 2000). Besides iron and other nutrient deficiencies, general infections and chronic diseases including HIV/AIDS, may impair hematopoiesis and

consequently can cause anemia (Nestel & Davidsson, 2002). Some parasitic infections, e.g. hookworm, schistosomiasis, and trichuriasis cause blood loss directly (WHO *et al.*, 2001b). In developing countries, such as Côte d'Ivoire, where the prevalence of gastrointestinal infections is very high (Utzinger *et al.*, 1998; Keiser *et al.*, 2002), this blood loss can potentially aggravate iron deficiency and anemia.

Helminth infections and their contribution to anemia

There are two species of hookworm, *Anclystoma duodenale* and *Nectar americano*, which commonly parasitize and mature in humans. They contribute to anemia by several mechanisms: 1) by feeding on blood, 2) by causing blood loss into the gut at their attachment sites, and 3) hookworms live and feed in the duodenum and jejunum, the same site where most iron is absorbed (Hall *et al.*, 2001). The association between hookworm infection, intestinal blood loss and severity of anemia depend on the level of infection (Stoltzfus *et al.*, 1996; Dreyfuss *et al.*, 2000; Stoltzfus *et al.*, 2000). Hookworm transmission occurs by contact between skin and soil polluted by larvae from fecal material.

Adult worms of trematode flatworms *Schistosoma mansoni* and *Schistosoma haematobium* live and reproduce in human blood vessels that line the intestines and human urinary tract, respectively (Kloos, 1987). Both forms are highly prevalent in Sub-Saharan Africa and remain a significant health problem (WHO, 1998). Although the adults in the blood stream feed on blood and erythrocytes, the disease due to these worms and the blood loss they cause is largely due to the parasite's eggs. Because sharp spines of the eggs help to cut through tissue when penetrating into the gut or bladder lumen, the site of penetration bleeds (Hall *et al.*, 2001). Schistosomiasis transmission occurs through skin contact in contaminated streams and ponds.

Transmission with the nematode *Trichuris trichiura* occurs by consumption of food or water polluted with eggs, and larvae are liberated from eggs in the intestinal tract. *Trichuris trichiura* can cause anemia when the worm burden is heavy (Nestel & Davidsson, 2002). However, the intensity of a single helminth species and lowered Hb concentration is not consistent and it is typically found that more than one helminth contributes to anemia in a population (Hall *et al.*, 2001).

Malaria-related anemia

Malaria is another cause of anemia. The responsible factors are multiple, involving both the destruction and the decreased production of erythrocytes (Menendez *et al.*, 2000). However, malaria does not cause iron deficiency, because much of the iron in Hb released from the ruptured cells stays in the body (Nestel & Davidsson, 2002), but is not accessible for erythropoiesis. The greatest burden of malarial anemia is carried by young children and pregnant women in sub-Saharan Africa (Menendez *et al.*, 2000). A negative interaction between malaria and age on anemia has been documented, whereby the effect of malarial parasitemia on anemia decreases with age (Premji *et al.*, 1995; Schellenberg *et al.*, 1999). This might be explained either by: 1) the fall in parasite density with age leading to a decreased number of parasitized erythrocytes, and 2) the early and quick development of clinical immunity, as suggested be the parallel decrease in the incidence of severe disease and death (Menendez *et al.*, 2000). Malaria and nutritional deficiencies are more frequently responsible for the anemia in infants and young children (Staubli Asobayire *et al.*, 2001), whereas in school children, hookworm infestation, malnutrition and the anemia of acute and chronic infections are more important contributors to anemia (Stoltzfus *et al.*, 1997). Among pregnant women, the risk for malaria-induced anemia is greater in primigravidae than in mutligravidae (Brabin, 1983; Isah *et al.*, 1985; Matteelli *et al.*, 1994). However, Brabin *et al.* (2001) has calculated the risk factor of anemia for maternal mortality using cross-sectional, longitudinal and case-control studies and found that nutritional deficiency is a major component of severe anemia deaths even in malarious areas. Because factors contributing to IDA are multiple, iron treatment should be integrated into broader public health programs, which are directed to the same population groups. Iron treatment should be combined with malarial prophylaxis, hookworm control, immunization, environmental health and community-based primary health care (WHO *et al.*, 2001b).

Other nutritional anemias

Vitamin A

Several micronutrient deficiencies in addition to iron can cause anemia. The evidence that vitamin A deficiency causes anemia through modulation of iron metabolism is strong and supported by observations from both experimental animal models and human studies (Semba & Bloem, 2002). Studies indicate that the lack of vitamin A may lead to mild anemia characterized by low serum iron and elevated

levels of iron in storage depots, particularly in the liver (Mejia & Chew, 1988). Mechanisms which might underlie the effects of vitamin A deficiency on anemia are 1) impaired mobilization of iron stores, 2) impaired erythropoiesis, and 3) increased susceptibility to infection (Fishman *et al.*, 2000). This is of public health significance as iron deficiency and vitamin A deficiency often occur simultaneously, as they are prevalent in similar geographical areas and the most vulnerable groups to both deficiencies are children and pregnant women of reproductive age (WHO *et al.*, 2001b). Positive hematological responses to vitamin A, most consistently reflected in increased Hb and SF concentrations, have been observed among children and pregnant women, whether the vitamin A was delivered as a regular supplement, a single dose or a fortified food item (Fishman *et al.*, 2000). In a randomized, double-blind, placebo-controlled study in pregnant women in Indonesia, Hb concentration significantly increased after 8 weeks of vitamin A supplementation (Suharno *et al.*, 1993), indicating that improvement in vitamin A status may contribute to the control of anemia in pregnancy. A study among anemic school children in Tanzania showed that daily vitamin A supplementation was associated with an increase in Hb of 13.5 g/L after 3 months and a larger increase of 22.1 g/L was observed in children who received both vitamin A and iron (Mwanri *et al.*, 2000). It appears that interventions targeted at vitamin A deficiency alone may only eliminate a proportion of the anemia (Suharno *et al.*, 1993; Albalak *et al.*, 2000; Mwanri *et al.*, 2000). According to Semba & Bloem (2002), using available data, it is not possible to accurately estimate the impact of the elimination of vitamin A deficiency on the prevalence of anemia in developing countries.

Riboflavin

Riboflavin deficiency also tends to co-exist and interact with iron deficiency (Allen & Casterline-Sabel, 2001). Although riboflavin is ubiquitous in foods, riboflavin deficiency may be one of the most common vitamin deficiencies in developing countries, particularly in those regions where diets are predominantly rice-based and contain insufficient milk, meat, fish, fresh fruit and vegetables (Bates, 1987). Riboflavin deficiency impairs iron absorption and increases the gastrointestinal loss of endogenous iron (Powers *et al.*, 1991; Powers, 1995). It is also suggested that it reduces the efficiency of iron utilization for heme synthesis (Powers, 1995). Results from riboflavin supplementation trials that have assessed the effects on anemia are inconsistent. However, it is suggested that supplementation with riboflavin enhances the response of Hb, hematocrit and erythrocyte count to iron supplementation in

pregnant women and improves the hematological status of anemic children and men (Fishman *et al.*, 2000).

Folate

Folate is a central component of human erythropoiesis besides iron and vitamin B_{12}. One of the first clinical manifestations of folate deficiency is the production of megaloblastic marrow cells, macrocytic erythrocytes, and ultimately macrocytic anemia (Scholl & Johnson, 2000). However, the current global impact of folate on anemia is not clear (Allen & Casterline-Sabel, 2001). Folate deficiency was found in 21% of pregnant women in Malawi and was frequently associated with other micronutrient deficiencies (van den Broek & Letsky, 2000). Whether folate deficiency in this population was primarily the result of dietary insufficiency, problems with absorption, or the results of malaria could not be established. In a cross-sectional study in pregnant women in Tanzania, odds ratio for anemia was increased with low serum folate concentration (Hinderaker *et al.*, 2002), whereas it was decreased in a study in Nepal (Bondevik *et al.*, 2000). Folate trials have focused primarily on pregnancy and several studies indicate that folate supplementation fails to raise Hb concentration or lower the risk of anemia, while it can prevent the development of megaloblastosis (Fishman *et al.*, 2000).

Vitamin B_{12}

A second nutritional cause of megaloblastic anemia is vitamin B_{12} deficiency. There are few data on global prevalence of vitamin B_{12} deficiency and even less is known about its global contribution to anemia (Allen & Casterline-Sabel, 2001). In studies in Mexico and Guatemala, vitamin B_{12} deficiency was found in 19 - 47 % of young children, school children, adults, and pregnant and lactating women (Allen *et al.*, 1995; Casterline *et al.*, 1997). A study in anemic preschoolers in Mexico showed that children with higher initial vitamin B_{12} concentrations were more likely to respond to iron supplements with improved Hb concentrations than children with low vitamin B_{12} status (Allen *et al.*, 2000). However, Fishman *et al.* (2000) reviewed the effects of vitamin B_{12} supplementation on pregnant women and found no effects on Hb concentration. In premature and low birth weight infants, vitamin B_{12} supplementation may improve Hb status and reduce the severity on the anemia (Fishman *et al.*, 2000).

Strategies to combat iron deficiency

To combat iron deficiency and IDA several strategies are available. To ensure a sustainable prevention, a combination of different approaches is most likely to successfully reduce iron deficiency in a population (WHO *et al.*, 2001b). Besides the improvement of food diversity to increase iron bioavailability, the promotion of better care and feeding practices and the improvement of health services and sanitation are necessary. Ideally, all countries where IDA exists would have a comprehensive anemia control program that includes an appropriate mix of interventions adapted to the local conditions (Stoltzfus & Dreyfuss, 1998).

Iron supplementation

In iron supplementation, pharmaceutical iron preparations are given daily or several times a week during at least 3 months to improve iron status of the most vulnerable population groups, such as infants, children, adolescents and women of childbearing age and during pregnancy (WHO *et al.*, 2001b). Iron supplementation is most often used to treat existing IDA, but is also considered as a preventive public health strategy to control iron deficiency in populations at high risk. However, the major difficulty of iron supplementation is to reach the most vulnerable population groups as they are often difficult to contact through the health services. An other important reason for the relative lack of success of iron supplementation programs has been the perceived need for these supplements to be taken daily, over relatively long periods of time (Allen, 2002). Studies have therefore evaluated whether the frequency of iron supplements could be reduced from daily to once per week (Viteri *et al.*, 1999; Ekström *et al.*, 2002; Sungthong *et al.*, 2002). The efficacy of weekly supplementation in school children, adolescents, and non-pregnant women is promising, but the current recommendation remains daily supplementation for young children and pregnant women (Stoltzfus & Dreyfuss, 1998).

Iron fortification

A more long-term approach is food fortification with iron, as it does not require any active participation of the target group (Cook & Reusser, 1983). Iron fortification can cover the whole population, by use of fortified foods such as staple foods and condiments, which are widely consumed by all population groups, or it can focus on one target groups by fortifying a product mainly consumed by this target group. The food vehicle has to be carefully chosen, as in some areas of the developing world,

food derives mainly from subsistence farming, and not many processed foods are purchased. Special efforts are also needed, to enrich foods that young children and infants consume, as their consumption of staple foods, such as wheat flour, is low (Trowbridge & Martorell, 2002). Moreover, an important issue of fortification in developing countries is how to make the fortified products affordable to the poorest and most vulnerable populations (Yip & Ramakrishnan, 2002).

Iron is the most difficult mineral to add to foods and ensure adequate absorption (Hurrell, 1999). The main technical barriers to successful iron fortification are: 1) to select an iron compound that does not cause unwanted sensory changes in the food vehicle and is still adequately absorbed, and 2) to overcome inhibitory effects of phytic acid and other food components on iron absorption (Hurrell, 2002). Whereas successful practical solutions were found for some food vehicles, other foods still need further investigation. Major problems remain in fortifying cereal flours and salt, two foods with great potential for iron fortification in developing countries (Hurrell, 2002).

Bio-fortification

A newer strategy in combating micronutrient deficiencies is the enrichment of staple foods either by plant breeding or by genetic engineering (Bouis, 1996; Trowbridge & Martorell, 2002). Both strategies aim for increasing the iron content and for improving bioavailability by reducing the amount of inhibitors and/or by promoting enhancers. However, the difficulty in reducing inhibitors, such as phytic acid, is that it plays an important role in viability and vigor of the seedling. This can have an unacceptable impact on production and is therefore not recommended (Bouis, 1996). Lucca *et al.* (2002) has successfully produced transgenic rice grains with higher iron content, rich in phytase and cystein-protein, a potential enhancer of iron absorption. The impact of these changes on bioavailability remains to be evaluated. Despite the need of further research, these approaches show great promise as sustainable ways to reduce micronutrient deficiencies (Zimmermann & Hurrell, 2002).

Interactions between iodine and thyroid metabolism and other micronutrients

In many developing countries, the risk of multiple micronutrient deficiencies is high. Because of increased needs during growth and pregnancy, vulnerable groups are infants, children and pregnant women. As poor dietary quality and increased losses due to infections often occur in similar geographical areas, they are risk factors for several micronutrient deficiencies. Table 2 shows the etiology and vulnerable groups for iodine, iron, vitamin A and selenium deficiencies. Because micronutrient deficiencies co-exist and interact in different ways, it is now recommended to consider supplementation and fortification using multiple micronutrients (WHO *et al.*, 2001b). Besides preventing more than one deficiency at a time, multiple supplementation strategies could have additional benefits due to interactions between two or more micronutrient deficiencies.

Table 2: Etiology and vulnerable groups for iodine, iron, vitamin A and selenium deficiencies (Institute of Medicine, 2000; WHO *et al.*, 2001b)

	Iodine deficiency	**Iron deficiency**	**Vitamin A deficiency**	**Selenium deficiency**
Etiology	Geographic	Dietary Increased losses	Dietary Increased losses	Geographic
Vulnerable groups	Entire population	Pregnant and lactating women Infants Preschool children Women of childbearing age	Pregnant and lactating women Infants of less than 6 months old Preschool children	Entire population

Besides iodine, other micronutrients are essential for normal thyroid metabolism, e.g. iron, selenium, vitamin A and zinc. Deficiencies of iron and selenium can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine (Arthur *et al.*, 1999; Zimmermann & Köhrle, 2002). The effects of iron and selenium status on iodine and thyroid metabolism share certain parallels

(Zimmermann & Köhrle, 2002). In the following section, potential interactions between iodine and other micronutrients will be reviewed.

Iron and iodine metabolism

Deficiencies of iron and iodine are major public health problems in Africa, and many children are at high risk of both goiter and IDA. In regions of West and North Africa, 23 to 26% of school-aged children suffer from both goiter and IDA (Zimmermann *et al.*, 2000c; Zimmermann *et al.*, 2000d). Iron deficiency with or without anemia can have adverse effects on thyroid metabolism. If IDA is a nutritional factor that influences the pathogenesis of IDD, it may have a greater impact on IDD than previously described goitrogens because of its high prevalence in vulnerable groups (Zimmermann, 2002).

Evidence from rat studies

The initial data on thyroid hormone metabolism in iron-deficient anemic rats resulted from investigations concerning poor thermoregulation. Iron deficiency decreases plasma T_3 and T_4 concentrations compared with those of control rats, and the normal increase in plasma levels of T_3 and T_4 observed in control rats after cold exposure (4°C) was not seen in iron-deficient rats (Dillman *et al.*, 1980; Beard *et al.*, 1982; Tang *et al.*, 1988). Additionally, the TSH response to cold in iron-deficient anemic rats was lower than in the controls (Tang *et al.*, 1988). Overall, although iron-deficient rats were able to increase thyroid hormone production and utilization when challenged with a cool environment, iron deficiency limited their ability to fully up regulate thyroid hormone metabolism to the degree observed in iron-replete rats (Beard *et al.*, 1998). Normal animals whose hematocrits were lowered by exchange transfusion showed the same responses as iron-deficient animals who were chronically anemic, whereas transfusion of iron-deficient rats to normal hematocrits improved the defect (Beard *et al.*, 1984). Injecting iron-deficient anemic rats with T_3 improved the ability of rats with IDA to maintain body temperature at 4°C, but injections of T_4 had no such beneficial effects (Beard *et al.*, 1982). These findings led the authors to conclude that IDA blunts the TSH response to cold temperature, impairs the conversion of T_4 to T_3 and that anemia, rather than tissue iron deficiency, is the critical factor in causing an impaired thyroid response to low temperature (Brigham & Beard, 1996).

Severely iron-deficient anemic animals (16 % hematocrit) showed a blunted TSH response despite lower T_3 and T_4 concentrations (Beard *et al.*, 1989), whereas no effect was found in less severely iron-deficient rats (31% hematocrit) (Tang *et al.*, 1988). Beard *et al.* (1989) have also shown that T_3 turnover rate from the plasma pool and its irreversible loss from the system is significantly lower in iron deficiency. These results were confirmed in a second kinetic study, which showed lower T_4 and T_3 disposal rates in iron-deficient rats than in control rats (by 48% and 28%, respectively) at 15°C temperature (Beard *et al.*, 1998). Smith *et al.* (1994) demonstrated a decreased nuclear T_3 binding in iron deficiency. In addition, IDA leads to a decreased hepatic 5'-deiodinase activity, which catalyzes the conversion of T_4 to T_3 (Beard *et al.*, 1989; Smith *et al.*, 1992; Brigham & Beard, 1995). The depression of 5'-deiodinase activity is greater in more severely iron-deficient anemic rats (72%) than in the less severely anemic rats (25%) (Brigham & Beard, 1995). Although the lowered hepatic 5'-deiodinase activity observed in iron deficiency may be at least partially attributed to low plasma T_4 concentrations, normalizing plasma T_4 did not normalize hepatic 5'-deiodinase activity. These observations suggest that the mechanisms that control hepatic 5'-deiodinase activity (e.g. enzyme synthesis, allosteric regulation of enzyme activity) are directly affected by iron deficiency, regardless of thyroid hormone status (Brigham & Beard, 1995).

Evidence from human studies

IDA also reduces thyroid hormone concentration in humans. Although, Lukaski *et al.* (1990) observed no differences in thyroid hormone and TSH concentrations between iron depleted and iron repleted women at room temperature, the relative increases in TSH, T_4, and T_3 after cold exposure were smaller (18, 16, and 18% respectively) when iron balance was negative than when it was positive (23, 23, and 25%, respectively) . Martinez-Torres *et al.* (1984) reported 10% lower T_3 concentrations in both moderate to severe IDA (Hb 75 g/L) and iron deficiency without anemia compared to control subjects, also this difference was not significant. In contrast, Beard *et al.* (1990) found a highly significant difference in T_3 concentrations between anemic (Hb 110 g/L) and control women. This discrepancy might be due to a smaller within-group variance in the latter study because only women with a certain body fatness and during particular days of their menstruation cycle were included (Beard *et al.*, 1990). In the same study, plasma TSH concentrations of anemic women were within the normal range at baseline and were unaffected by iron status. The

subsequent iron supplementation corrected anemia, but only partially normalized thyroid hormone concentrations (Beard *et al.*, 1990).

Studies have also demonstrated a relationship between anemia and hypothyroidism; anemia was found in 25-50% of hypothyroid patients (Das *et al.*, 1975; Horton *et al.*, 1976). Hematological findings were diverse and anemia was due to iron deficiency only in a few cases. However, a recent study found significant differences in SF concentration and total iron binding capacity between 57 hypothyroid patients and 61 euthyroid controls (Duntas *et al.*, 1999). Moreover, in a group of hypothyroid patients with low serum iron levels, the Hb concentrations increased in response to T_4, but the increase was greater in response to T_4 and iron (Horton *et al.*, 1976). In thyroidectomized rats, gastrointestinal iron absorption was decreased compared to intact control rats and it increased in response to thyroid hormone therapy (Donati *et al.*, 1973). Poor iron absorption in hypothyroidism could be at least in part attributed to achlorhydria, a deficiency of hypochloric acid in gastric justice found in hypothyroidism (Seino *et al.*, 1978; Marqusee & Mandel, 2000). The reduced erythrocyte mass in the hypothyroid state may be an adaptive process, a result of reduced need for delivery of oxygen to peripheral tissues, one consequence being a decrease in serum erythropoietin concentration (Marqusee & Mandel, 2000).

Public health significance

Evidence from cross-sectional studies

Data from the few available cross-sectional studies which have investigated the correlation between IDD and IDA are equivocal. A survey in Ethiopian children found no correlation in goiter rate or thyroid hormone levels and iron status (Wolde-Gebriel *et al.*, 1993b). Also no significant difference was found in the prevalence of anemia between goitrous and non-goitrous subjects in the Philippines (Florentino *et al.*, 1996). However, in severely vitamin A-deficient Ethiopian children, low levels of T_3 were associated with serum iron and low transferrin saturation (Wolde-Gebriel *et al.*, 1993a). A national screening in 2917 children in Iran has reported a highly significant difference in goiter rates by palpation between children with low and normal SF levels (Azizi *et al.*, 2002). Goiter was 3.8 times more prevalent in school children with low SF levels than in children with normal SF concentrations. Moreover, Zimmermann *et al.* (2000c) assessed in 1997 iron status and goiter rate by palpation in 419 children aged 6-15 years in two villages in western Côte d'Ivoire and found a relative risk of 1.9 (confidence interval 1.5-2.3) for goiter for children with IDA. However, the

inconsistent data on the relationship between IDD and IDA are probably due to the fact that public health problems in developing countries are multiple. Several factors such as malaria, parasitic infections and other nutritional deficiencies also interfere with iodine and iron metabolism, as well as anemia, and therefore may obscure present interactions between two micronutrients.

Evidence from intervention studies

In a first study in 1997, Zimmermann *et al.* (2000c) investigated the effect of a 200 mg oral dose of iodine as iodized oil in non-anemic (n=51) and anemic (n=53) children with goiter in western Côte d'Ivoire. At 15 and 30 weeks Tvol was significantly reduced in the non-anemic group compared to the anemic group ($p<0.001$). A clear difference in goiter prevalence was apparent at 15 and 30 weeks, when goiter rates were 62% and 64% in the anemic group and only 31% and 12% in the non-anemic group, respectively. After 30 weeks, TSH and T_4 concentrations improved significantly in the non-anemic group compared to the anemic group. Beginning at 30 weeks, the anemic children were given 60 mg oral iron as ferrous sulfate four times/week for 12 weeks (Zimmermann *et al.*, 2000b). This resulted in an increase in Hb (±SD) from 97 (±8) g/L at 30 weeks to 122 (±8) g/L at 50 weeks. Change in Tvol, which had reached a plateau at weeks 10 through 30 in the iron-deficient anemic children, began to fall again after iron supplementation. Consequently, goiter prevalence in the anemic group, which had remained at 62% to 64% from weeks 10 through 30, was reduced after iron supplementation to 31% and 20% at 50 and 65 weeks. The findings in these studies suggest 1) that IDA in children may limit the thyroid response to an iodine prophylaxis and 2) that iron supplementation improves the efficacy of oral iodized oil in goitrous children with IDA. However, further investigation is needed as this study was not a randomized controlled trial.

Potential mechanisms of the iodine and iron interaction

As described above, certain aspects of thyroid metabolism in iron deficiency overlap with those observed in hypothyroid states. The plasma concentrations of T_4 and T_3 are lower and thyroid response to several different input stimuli is blunted in IDA. However, it is not clear how iron deficiency exerts its effects on thyroid and iodine metabolism.

Beard *et al.* (1998) suggest that IDA induces alterations in central nervous system control. Also the lowered [^{125}I]T_3 binding to hepatic nuclei shown in rats, could be a contributory mechanism (Smith *et al.*, 1993). Normalization of plasma T_4 kinetic parameters in iron-deficient anemic rats provided with exogenous T_4 suggests that low plasma T_4 concentrations contribute to the altered thyroid hormone kinetics associated with iron deficiency (Beard *et al.*, 1998). Presumably, in iron-deficient anemic rats, a smaller portion of T_4 is converted to T_3 and a larger portion is converted to reverse T_3, a physiologically inactive metabolite. This is in agreement with a study by Smith *et al.* (1994) who concluded that iron-deficient rats are functionally hypothyroid, with a tendency toward thyroid hormone inactivation versus activation. According to Beard *et al.* (1998) the effect of iron deficiency on either the hepatic 5'-deiodinase or the brown fat deiodinase II observed in rats is rather minimal. Moreover, using an in vitro method, outer ring deiodinase activity is not affected by either ferric or ferrous iron (Kaplan & Utiger, 1978).

Thyroid metabolism could also be impaired by iron deficiency through anemia and lowered oxygen transport, similar to the thyroid impairment of hypoxia found in animals (Surks, 1969; Galton, 1972). Thyroid impairment was also found in chronically hypoxic children, who had not only increased levels of reverse T_3, but also decreased concentrations of T_4 and T_3, whereas in acutely hypoxic children, mean serum T_4 and T_3 concentrations were not altered, but mean serum reverse T_3 concentration was significantly elevated (Moshang *et al.*, 1980). However, in healthy subjects hypoxic stress led to marked elevations in plasma T_4 and T_3 within 4 hours and the increased levels were maintained during the entire period of exposure (Sawhney & Malhotra, 1991; Basu *et al.*, 1995). This indicates that in healthy subject, hypoxia can not entirely explain hypothyroidism associated with IDA.

The association between anemia and hypothyroidism may be physiologic to some extent, that is, a result of reduced need for delivery of oxygen to peripheral tissues in hypothyroidism (Marqusee & Mandel, 2000). On the other hand, a widely recognized effect of thyroid hormones is their influence over energy metabolism (Lanni *et al.*, 2001). As food intake is reduced in anemia, lowering thyroid hormone concentration may be in part a physiologic adaptation. This has been confirmed by reduced thyroid hormone concentrations in modified fasting of rats (Schröder-van der Elst & van der Heide, 1992; Janssen *et al.*, 1994).

An additional mechanism, which could induce increased Tvol in IDA is the interaction of nitric oxide with Hb. Nitric oxide is a potent vasodilator that is produced in endothelial cells and has been assumed to act exclusively at its site of synthesis (Lane & Gross, 2002). McMahon *et al.* (2002) recently showed that binding of nitric

oxide to hemes and thiols of Hb varies as a function of HbO_2 saturation, suggesting that Hb is involved in the systematic transport and delivery of nitric oxide to tissues. Moreover, red blood cell/thiol-mediated vasodilator activity was inversely proportional to HbO_2 saturation (McMahon *et al.*, 2002). Theoretically, this inverse relationship could cause enlarged Tvol in IDA due to vasodilatation and explain the blunted Tvol response to iodine prophylaxis in IDA. According to Lane & Gross (2002) it is widely appreciated that nitric oxide bioactivity is scavenged by heme-iron in Hb (Jia *et al.*, 1996; Stamler *et al.*, 1997), and that animal and human blood contains low micromolar concentrations of nitric oxide-Hb. However, the possibility that Hb actually delivers nitric oxide bioactivity is highly controversial and further investigation is needed (Hobbs *et al.*, 2002; Lane & Gross, 2002).

Thyroid peroxidase activity in iron deficiency anemia

Another potential mechanism for reduced thyroid hormone concentration in IDA is impairment of TPO activity. TPO is a glycosylated, heme-enzyme bound to the apical membrane of the thyrocytes (Taurog, 2000). It plays a key role in thyroid hormone synthesis as it catalyzes the two initial steps, iodination of the Tg and coupling of the iodotyrosine residues (Dunn & Dunn, 2001). Whereas the thyroid hormone synthesis occurs at the apical membrane of the thyrocytes, TPO is localized in the endoplasmic reticulum and in the perinuclear membrane (Ekholm, 1981; Kuliawat *et al.*, 1995; Penel *et al.*, 1998). Only about 30% of the synthesized TPO is able to fold correctly and to reach the apical cell surface (Kuliawat *et al.*, 1995; Fayadat *et al.*, 1998). Fayadat *et al.* (1999) investigated if heme had to be inserted into TPO for its exit from the endoplasmic reticulum and found that hemin, a chemical derivative of Hb, increased the quantity of human TPO at the apical cell surface level by 20% and increased TPO activity at the cell surface by 120%. The authors concluded that some of the human TPO molecules at the cell surface of the thyrocytes are inactive because they lack heme which agrees with the conclusions of earlier studies (Fan *et al.*, 1996; Guo *et al.*, 1998). It has been shown in the case of lactoperoxidase, a mammalian peroxidase similar to TPO, that no other enzyme system is required to modify heme before incorporation into the enzyme (DePillis *et al.*, 1997). After preincubation of lactoperoxidase with H_2O_2, decreased the proportions of the free and the polar heme product, while the amount of protein-bound heme increased, as a function of H_2O_2 concentration. These results indicated that the formation of the covalently bound heme-protein complex was the result of an autocatalytic process (DePillis *et al.*, 1997). Fayadat *et al.* (1999) has demonstrated that H_2O_2 also had this

additional role at the apical membrane of thyrocytes. In the presence of H_2O_2, heme was autocatalytically modified and then covalently bound to TPO. Using a monolayer technique with an apical pole oriented toward the culture medium, Fayadat *et al.* (1999) showed that adding hemin had an increasing effect on cell surface TPO activity of 30%. Considering the crucial role of heme in TPO activity, IDA could lower TPO activity and thereby interfere with iodine and thyroid metabolism.

Selenium and iodine metabolism

Selenium

Selenium functions largely through an association with proteins, known as selenoproteins. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defense systems, and immune function (Rayman, 2002). Its role in thyroid hormone metabolism is crucial, and therefore selenium has the potential to play a major part in the outcome of IDD. These effects of selenium derive from two aspects of its biological function: 1) three selenium containing deiodinases regulate the synthesis and degradation of the biologically active thyroid hormone T_3, and 2) selenoperoxidases and possibly thioredoxin reductase protect the thyroid gland from H_2O_2 produced during the synthesis of thyroid hormones (Arthur *et al.*, 1999).

Evidence from rat studies

Studies with [^{75}]Se showed that, after severe selenium depletion, the brain and endocrine glands have priority on supplies of this element (Behne *et al.*, 1988). This was confirmed in rats, where 5'-deiodinase I activity in the thyroid is highly resistant to selenium depletion (Larsen & Berry, 1995). In contrast to the maintained thyroidal activity, 5'-deiodinase I in peripheral tissues like liver and kidney is strongly decreased by selenium deficiency in both short-term (Beech *et al.*, 1995; Hotz *et al.*, 1997) and long-term rat studies (Meinhold *et al.*, 1992).

In rat studies, it has been shown that selenium deficiency can further compound the adverse effects of iodine deficiency (Arthur *et al.*, 1992; Beckett *et al.*, 1993). Male weanling rats fed on diets deficient in selenium and iodine for 7 weeks showed lower concentrations of T_4, T_3 and depleted amounts of iodine in thyroid than in selenium or iodine deficiency alone (Beckett *et al.*, 1993). Furthermore, rats deficient in both trace

elements had larger thyroid glands, higher plasma TSH concentrations and higher cerebral deiodinase II activities than rats deficient in iodine alone. However, a rat study by Hotz *et al.* (1997) found that low dietary iodine alone produced lower serum T_4 and low dietary selenium alone produced higher serum T_4 concentrations compared to controls. Whereas in rats deficient in selenium and iodine, serum T_4 concentrations were not significantly different to those in controls (Hotz *et al.*, 1997). The lower synthesis level of T_4 of iodine deficiency was probably masked by the lower deiodination of T_4 seen as a result of concurrent selenium deficiency. Other feeding trials, however, found no significantly different plasma T_4 or TSH concentrations between rats with combined selenium and iodine deficiency and those with iodine deficiency alone (Meinhold *et al.*, 1992; Beckett *et al.*, 1993; Wu *et al.*, 1997). These discrepancies might be due to the severity of the selenium deficiency. Whether concurrent selenium deficiency aggravates iodine deficiency may depend on the acuteness and the severity of the selenium deficiency (Meinhold *et al.*, 1993; Hotz *et al.*, 1997).

Another important selenium containing enzyme is the glutathione peroxidase found in different cell fractions and tissues of the body (Arthur, 2000). Tissue glutathione peroxidase activity of rats is typically lower in selenium deficiency. Lower activities in liver, kidney and erythrocytes of rats were found, but not in the thyroid (Hotz *et al.*, 1997). The need for maintenance of thyroid glutathione peroxidase activity may be indicative of its important function in the thyroid to neutralize H_2O_2 and prevent cytotoxicity, as large amounts of H_2O_2 are generated during the biosynthesis of thyroid hormones. However, the lack of, or in cases a very small, difference in plasma T_3 concentrations between iodine-deficient, and selenium- and iodine-deficient rats suggests that the thyroid gland is able to retain sufficient selenium to produce T_3 either by the *de novo* synthesis or by deiodination of T_4 by 5'-deiodinase I (Arthur *et al.*, 1999). This may be at the expense of increased peroxidative damage to the gland. It has been shown that thyroid cells from severely selenium-deficient rats were more necrotic on iodine re-feeding than were those from selenium-adequate rats (Contempré *et al.*, 1993). Furthermore, the thyroid gland morphology was restored to normal within 15 days of iodide administration in selenium-sufficient, but not in selenium-deficient rats (Contempré *et al.*, 1995).

Evidence from human studies

Selenium deficiency and cretinism

Severe deficiencies of selenium and iodine coexist in China, Southeast Asia, Russia, Egypt and Central Africa (Utiger, 1998). Interactions between those two trace elements have been associated with different diseases, such as Kashin-Beck disease in Tibet (Moreno-Reyes *et al.*, 1998), and myxedematous cretinism in Central Africa (Goyens *et al.*, 1987; Vanderpas *et al.*, 1990; Corvilain *et al.*, 1993).

In iodine deficiency, TSH concentration is increased and consequently the production of H_2O_2 in thyroid cells is elevated. Selenium deficiency results in reduced levels of glutathione peroxidase leading to an accumulation of H_2O_2. This excess H_2O_2 could induce thyroid cell damage, resulting in myxedematous cretinism which is caused by severe thyroid insufficiency (Contempré *et al.*, 1994). Although this hypothesis has not been proven, it is clear that myxedematous cretinism and its consequences are due to a failure of the thyroid in the cretin, while neurological cretinism is principally due to the failure of the mother to provide enough T_4 to the fetus resulting in extreme mental retardation (Dumont *et al.*, 1994a).

Another hypothesis describes the mechanism why selenium deficiency could protect the fetus from brain damage despite severe iodine deficiency in the mother. Besides the increased risk of oxidative thyroid damage in selenium deficiency, a beneficial effect regarding the T_4 transfer from the mother to the fetus was suggested. In selenium deficiency, T_4 is preserved from deiodination due to decreased activity of peripheral 5'-deiodinase, a selenium-containing enzyme, and thus T_4 remains available for the fetus. As brain deiodinase II is not a selenium enzyme (Safran *et al.*, 1991), the conversion of T_4 to T_3 in the brain remains intact even in selenium deficiency. This could protect fetal brain from the deleterious consequences of T_4 deficiency in the mother (Dumont *et al.*, 1994a).

However, some studies have failed to provide convincing support for the hypothesis that selenium deficiency is the only compounding factor responsible for endemic cretinism seen in some iodine-deficient areas (Arthur *et al.*, 1999). Ngo *et al.* (1997) found in an area in Zaire, where cretinism had not been reported, the same degree of combined selenium and iodine deficiency as in northern Zaire, an area of endemic cretinism. Similarly the distribution of myxedematous cretinism is not related to selenium deficiency in China (Ma *et al.*, 1993). According to Arthur *et al.* (1999) other additional factors in endemic cretinism, besides selenium deficiency, must be considered.

Evidence from cross-sectional studies

Only a few studies investigated the association between selenium and iodine status in humans. Two studies have investigated the association of selenium deficiency and goiter prevalence in school children in Turkey. One found an association between low enzymatic antioxidants (glutathione peroxidase, catalase, and superoxide dismutase) and low selenium status and goiter (Giray *et al.*, 2001), whereas the other study has found that low serum selenium had little or no impact on goiter endemics (Erdogan *et al.*, 2001). In a study in Poland, no association was found between selenium status and free T_4 and TSH concentrations in goitrous and non-goitrous children (Zagrodzki *et al.*, 2000). Further studies are needed to evaluate the public health significance of the effect of selenium deficiency on iodine and thyroid metabolism.

Supplementation trials

After 2 months of selenium supplementation in 52 school children deficient in selenium and iodine, mean serum T_4 and reverse T_3 fell significantly, whereas T_3 and TSH remained stable (Contempré *et al.*, 1992). The authors assumed that deiodinase I could account for the changes seen in thyroid hormones concentrations. In cretins, 2 months of selenium supplementation further worsened thyroid failure with decreased serum T_4 and T_3 and increased TSH (Contempré *et al.*, 1991; Vanderpas *et al.*, 1993). This led to the conclusion, that selenium deficiency may protect from some of the effects of iodine deficiency and that it is important to correct iodine deficiency first before supplementing with selenium. However, low dose selenium administration did not show any effect on thyroid hormone synthesis in subjects with mild iodine deficiency and sufficient selenium (Roti *et al.*, 1993). Because of the multiplicity of roles of selenoproteins in thyroid hormone metabolism and elsewhere, selenium may have both beneficial and adverse effects on man and animals with iodine deficiency (Arthur *et al.*, 1999).

In a supplementation trial with oral iodized oil in western Côte d'Ivoire, the response to oral iodized oil among goitrous, selenium-deficient children was impaired by increasing severity of selenium deficiency (Zimmermann *et al.*, 2000e). These results suggest that more severe selenium deficiency may partially blunt the thyroid response to iodine supplementation.

Vitamin A and thyroid metabolism

Vitamin A

Vitamin A (retinol) in the body comes from two sources, preformed vitamin A in animal foods and from β-carotene and other provitamin A carotenoids in plant sources (McLaren & Frigg, 2001). For transport in the body, retinol is attached to the retinol-binding protein, which is almost entirely associated with another protein called transthyretin (Ingenbleek & Young, 1994). Besides its essential function in vision, retinol plays an important role in activating nuclear receptors in virtually all cell types via acidic forms of retinol, such as retinoic acid (McLaren & Frigg, 2001).

Evidence from animal studies

There are a number of animal studies suggesting a linkage between vitamin A metabolism and thyroid function. Histologic alterations of the thyroid have been described in vitamin A deficiency (Drill, 1943; Strum, 1979). Increased thyroid weight in vitamin A-deficient animals was found by some investigators (Drill, 1943; Nockels *et al.*, 1984), but not by others (Ingenbleek, 1983). Also results on the effects of vitamin A deficiency on thyroid hormone concentration are not consistent. One study in rats (Oba & Kimura, 1980) and one in chickens (Nockels *et al.*, 1984) found lower thyroid hormone levels in vitamin A-deficient animals than in controls. All other studies, however, found hyperthyroidism in rats with vitamin A deficiency (Morley *et al.*, 1978; Garcin & Higueret, 1983; Ingenbleek, 1983) with a negative correlation between the amount of free T_3 and serum vitamin A (Garcin & Higueret, 1980).

The interaction between thyroid metabolism and vitamin A deficiency is partially attributed to the retinol-binding protein, of which lower concentrations were found in vitamin A-deficient rats (Muto *et al.*, 1972). The retinol-binding protein is important for the transport of retinol as well as T_4 and T_3, as it forms a complex with T_4-binding prealbumin, called transthyretin. Under physiologic condition, transthyretin binds only about 10-15% of the thyroid hormones but is responsible for much of the immediate delivery of T_4 and T_3 to cells because its affinity for the hormones is lower and therefore they dissociate from it more rapidly (Robbins, 2000). In vitamin A-deficient rats, this was found to be changed as radioactive T_4 and T_3 bound to the prealbumin zone was significantly decreased compared to controls (Higueret & Garcin, 1979; Garcin & Higueret, 1980). Moreover, a reduced transport of T_3 into the target cells was indicated, when smaller radioactivity was counted in kidney and liver of vitamin A-deficient rats after injection of ^{125}I-T_3 compared to controls (Higueret & Garcin,

1984). These studies suggest that vitamin A deficiency causes a change in plasma transport and consequently a change in plasma concentration of thyroid hormones.
Morley *et al.* (1978) found an increase in hypothalamic TRH and pituitary TSH content in vitamin A deficiency, implying an abnormality in thyroid hormone feedback on the hypothalamic-pituitary axis. In normal thyroid hormone feedback, the inhibition of TSH formation occurs through T_3 binding to the thyroid receptor, a nuclear protein belonging to the family of nuclear receptors that include the retinoid X receptor activated by retinoic acid (Evans, 1988). The increase in thyroid hormone concentration and TSH observed when rats were made vitamin A-deficient, let to the hypothesis, that retinoic acid is involved in the suppression of TSH expression (Wolf, 2002). Breen *et al.* (1995) showed that vitamin A-deficient rats expressed increased levels of the TSHβ subunit of TSH. Following studies showed that the regulation of the anterior pituitary hormone TSH depends on two factors: the binding of the thyroid receptor, which is activated by T_3 and T_4, to the TSHβ gene, and the binding of the retinoid X receptor, which is activated by retinoic acid, to the same gene (Haugen *et al.*, 1997; Brown *et al.*, 2000). Each interaction alone and both interactions simultaneously inhibit expression of TSHβ mRNA and TSH hormone production. In this way, there occurs not only the feedback inhibition of serum TSH by serum T_3 and T_4, but also inhibition by vitamin A (Wolf, 2002). It was recently found that the association between vitamin A and the pituitary-thyroid axis also applied to humans when, patients with T-cell lymphoma developed hypothyroidism after treatment with a synthetic retinoid, that specifically binds to the retinoid X receptor (Sherman *et al.*, 1999). However, the physiological significance of the inhibitory action by vitamin A on a hormone remains unclear (Wolf, 2002).

Evidence from human studies

Also some human studies showed some association between thyroid function and vitamin A metabolism. Ingenbleek & de Visscher (1979) found a negative correlation between palpable goiter and serum retinol concentration in Senegal. In an area of severe vitamin A deficiency, total T_3 was significantly correlated with retinol, transthyretin, and albumin in children, while T_4 was associated with none of these biochemical parameters (Wolde-Gebriel *et al.*, 1993a). In a total of 14740 school children in Ethiopia, those children with visible goiters had significantly lower serum retinol levels than children without or only small palpable goiters (Wolde-Gebriel *et al.*, 1993b). In India, hypothyroid women had increased retinol concentrations and

hyperthyroid women had decreased retinol levels compared to controls (Goswami & Choudhury, 1999).
Further research is necessary to evaluate the association between thyroid and vitamin A metabolism and its public health significance.

Zinc and thyroid metabolism

Zinc

Zinc is the most abundant intracellular trace element and is involved in a multitude of diverse catalytic, structural, and regulatory functions (King & Keen, 1999). Zinc is a constituent of many enzymes, including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Vallee & Falchuk, 1993). Therefore, zinc participates in protein, nucleic acid, carbohydrate, and lipid metabolism, as well as in control of gene transcription and other fundamental biological process.

Evidence from rat studies

Several studies investigating the influence of zinc deficiency on thyroid metabolism in rats found no effect (Root *et al.*, 1979; Oliver *et al.*, 1987; Pekary *et al.*, 1991; Freake *et al.*, 2001), whereas other studies found reduced serum T_3 concentrations in zinc-deficient rats compared to pair-fed controls (Morley *et al.*, 1980; Kralik *et al.*, 1996). Studies investigating the effect of concurrent zinc and iodine deficiency in rats did not find further impairment of thyroid hormone status compared to iodine deficiency alone (Smit *et al.*, 1993; Ruz *et al.*, 1999). Results on the effect of zinc deficiency on hepatic 5'-deiodinase activity are also inconsistent: decreased (Kralik *et al.*, 1996); unchanged (Fujimoto *et al.*, 1986); as well as increased 5'-deiodinase activity (Oliver *et al.*, 1987) has been found. Similar to iron deficiency, zinc-deficient rats showed blunted thermoregulation (Lukaski *et al.*, 1992). A study in guinea pigs found that the thyroid gland weights of zinc-deficient animals were significantly decreased and glands looked paler than compared to ad libitum controls (Gupta *et al.*, 1997). Moreover, the authors reported that the glands showed changes of atrophy and degeneration in the follicles. These histopathological changes could be due to the role of zinc in the structure and function of biomembranes. A loss of zinc from the membrane could result in increased susceptibility to oxidative damage, structural

changes, and alterations in specific receptor sites and transport systems (King & Keen, 1999).

The relationship between zinc and thyroid metabolism is based on the assumption that T_3 receptors, in common with other member of the nuclear receptor family, are thought to be included among nuclear zinc-binding proteins (Freake *et al.*, 2001). Miyamato *et al.* (1991) showed that the removal of zinc from bacterially expressed T_3 receptors impaired their ability to bind DNA. Although results from in vitro experiments have not been consistent probably due to interfering substances within the assay, this finding agrees with the generally accepted model for zinc-nuclear receptor interactions (Freake *et al.*, 2001).

Evidence from human studies

In a study of zinc-deficient males in Egypt, no evidence of hypothyroidism was found (Sandstead *et al.*, 1967). Moreover, normal zinc concentrations in patients with hyperthyroidism (Nishi *et al.*, 1980) and hypothyroidism (Bremner & Fell, 1977) have been reported. Wada & King (1986) showed that marginal zinc deficiency in humans was associated with a reduction in basal metabolic rate. In addition, there was a trend toward decreased thyroid hormone concentration when a low zinc diet was fed for 54 days, although only free T_4 was significantly decreased at the midpoint of the low zinc feeding period (Wada & King, 1986). Although no significant difference in thyroid function was found in 6 healthy male subjects with low serum zinc levels compared to 8 subjects with high serum zinc levels, serum T_4 concentration increased in the low zinc group following zinc administration (Hartoma *et al.*, 1979). A decrease in the T_3 concentration and the free T_3 index were observed in alcoholic cirrhotic patients with low serum zinc levels (Morley *et al.*, 1981). However, zinc supplementation did not normalize T_3 levels. In a cross-sectional study in Turkey, plasma zinc concentration in goitrous male adults was significantly lower than in the control group (Ozata *et al.*, 1999). A limitation to these studies is the fact that plasma zinc level is not a very useful indicator of zinc status, as it is influenced by stress, infection, food intake and hormonal state (King & Keen, 1999).

In conclusion, although there are potential interactions between zinc and thyroid metabolism, evidence is inconclusive. According to Freake *et al.* (2001) it remains possible that interactions will be found as other indices of thyroid hormone action are examined.

Study site

Three of the present studies were carried out in primary schools of nine remote villages of the Danané Health District in western Côte d'Ivoire. The region is mountainous and covered with dense tropical forest. All villages are within a radius of about 10 km and are approximately 50 km distance on unpaved roads from Danané, the nearest small town with electricity and medical facilities. The villages are similar ethnically and socioeconomically. There is no access to running water or electricity. The staple foods are cassava, rice and plantain.

Figure 3: Map of Côte d'Ivoire in West Africa

The mountainous region of western Côte d'Ivoire has been shown to be an area of endemic goiter. In 1980 goiter prevalence by palpation in the region of Man was 54% (Latapie *et al.*, 1981). In the same area, goiter prevalence by ultrasound was 62% in 6-15 year old children in 1996 (Franke *et al.*, 1999) using the reference values recommended by Gutekunst & Treichert (1993). In an effort to combat IDD in Côte d'Ivoire, the Ivorian government began USI in 1997, at a level of 30 – 50 ppm. However, the introduction of iodized salt in western Côte d'Ivoire was slow. In late 1997, iodized salt was not yet available in the Danané Health District, as UI was still only 28 µg/L indicating moderate to severe iodine deficiency (Zimmermann *et al.*, 2000c). Moreover, due to the cassava consumption, the median UI/thiocyanate ratio was low (3 µg/mg), indicating increased risk for exacerbation of goiter by thiocyanate (Zimmermann *et al.*, 2000e).

As in most developing countries, many children in Côte d'Ivoire are also at high risk of IDA. In a survey in 4 different regions of Côte d'Ivoire in 1996, the prevalence of iron deficiency in school-age children was 47% and that of IDA was 25%, respectively (Staubli Asobayire *et al.*, 2001). About half of the children were anemic (Staubli Asobayire *et al.*, 2001). Similar results were found in two villages in the Danané Health District in 1997. The prevalence of iron deficiency and IDA in school-age children was 50% and 27%, respectively (Zimmermann *et al.*, 2000c). Moreover, 18% of the school-age children suffered from both goiter and IDA.

As in many other developing countries, anemia can also result from infections such as malaria (Menendez *et al.*, 2000), from chronic inflammatory disorders (Yip & Dallman, 1988), or from other nutritional deficiencies (Nestel & Davidsson, 2002). Staubli Asobayire *et al.* (2001) found a prevalence of at least mild malaria infection in Ivorian school-age children of 54%. The prevalence of inflammation of infection, indicated by elevated C-reactive protein, was 21% in school-age children (Staubli Asobayire *et al.*, 2001). In a survey in 1997, polyparasitism was very common in 260 community members in the region of Man. *Schistosomiasis mansoni*, *Entamoeba coli*, and hookworm were the predominant species with prevalences of 72, 65 and 52%, respectively (Keiser *et al.*, 2002). Only 8 individuals displayed no infection, whereas two-thirds of the population harbored 3 or more parasites concurrently. This observation is in agreement with earlier work carried out in nearby villages, where polyparasitism is also a common feature among school children (Utzinger *et al.*, 1999). The impact on anemia prevalence was not evaluated, but could be substantial.

Besides iron and iodine deficiency, other micronutrient deficiencies are frequent in the region of Danané and Man. In 1997, of 51 children studied, all were selenium-deficient and mean selenium concentration was only 14.8 $\pm$ 10.7 µg/L (Zimmermann *et al.*, 2000e). Serum retinol was 0.65 $\pm$ 0.39 µmol/L and about a quarter were vitamin A-deficient (Zimmermann *et al.*, 2000c). Similar results were found in 1997 near Man, 23 out of 50 subjects studied, had plasma selenium concentration below 0.35 µmol/L, indicating severe selenium deficiency (Arnaud *et al.*, 2001). In the same group, riboflavin deficiency was 74% assessed by erythrocyte glutathione reductase activation coefficient (Arnaud *et al.*, 2001).

It can be concluded that overall health situation is poor in the studied region mainly due to a high risk of parasitic infections (Keiser *et al.*, 2002), malaria (Staubli Asobayire *et al.*, 2001) and monotonous and poor quality diets (Staubli Asobayire, 2000).

References

Abrams GM & Larsen PR (1973) Triiodothyronine and thyroxine in the serum and thyroid glands of iodine-deficient rats. *Journal of Clinical Investigation* **52**, 2522-2531.

Ackrell BA, Maguire JJ, Dallman PR & Kearney EB (1984) Effect of iron deficiency on succinate- and NADH-ubiquinone oxidoreductases in skeletal muscle mitochondria. *Journal of Biological Chemistry* **259**, 10053-10059.

Albalak R, Ramakrishnan U, Stein AD, Van der Haar F, Haber MJ, Schroeder D & Martorell R (2000) Co-occurrence of nutrition problems in Honduran children. *Journal of Nutrition* **130**, 2271-2273.

Allen L & Casterline-Sabel J (2001) Prevalence and causes of nutritional anemias. In *Nutritional anemias*, pp. 7-21 [U Ramakrishnan, editor]. London: CRC Press.

Allen LH, Rosado JL, Casterline JE, Martinez H, Lopez P, Munoz E & Black AK (1995) Vitamin B-12 deficiency and malabsorption are highly prevalent in rural Mexican communities. *American Journal of Clinical Nutrition* **62**, 1013-1019.

Allen LH, Rosado JL, Casterline JE, Lopez P, Munoz E, Garcia OP & Martinez H (2000) Lack of hemoglobin response to iron supplementation in anemic mexican preschoolers with multiple micronutrient deficiencies. *American Journal of Clinical Nutrition* **71**, 1485-1494.

Allen LH (2001) Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. *Journal of Nutrition* **131**, 581S-589S.

Allen LH (2002) Iron supplements: scientific issues concerning efficacy and implications for research and programs. *Journal of Nutrition* **132**, 813S-819S.

Arnaud J, Malvy D, Richard MJ, Faure H & Chaventre A (2001) Selenium status in an iodine deficient population of the West Ivory Coast. *J Physiol Anthropol Appl Human Sci* **20**, 81-84.

Arthur JR, Nicol F & Beckett GJ (1992) The role of selenium in thyroid hormone metabolism and effects of selenium deficiency on thyroid hormone and iodine metabolism. *Biological Trace Element Research* **34**, 321-325.

Arthur JR, Beckett GJ & Mitchell JH (1999) The interactions between selenium and iodine deficiencies in man and animals. *Nutrition Research Reviews* **12**, 55-73.

Arthur JR (2000) The glutathione peroxidases. *Cellular and Molecular Life Sciences* **57**, 1825-1835.

Azizi F, Mirmiran P, Sheikholeslam R, Hedayati M & Rastmanesh R (2002) The relation between serum ferritin and goiter, urinary iodine and thyroid hormone concentration. *International Journal for Vitamin and Nutrition Research* **72**, 296-299.

Ballot D, Baynes RD, Bothwell TH, Gillooly M, MacFarlane BJ, MacPhail AP, Lyons G, Derman DP, Bezwoda WR, Torrance JD & et al. (1987) The effects of fruit juices and fruits on the absorption of iron from a rice meal. *British Journal of Nutrition* **57**, 331-343.

Basu M, Pal K, Malhotra AS, Prasad R & Sawhney RC (1995) Free and total thyroid hormones in humans at extreme altitude. *International Journal of Biometeorology* **39**, 17-21.

Bates CJ (1987) Human riboflavin requirements, and metabolic consequences of deficiency in man and animals. *World Review of Nutrition and Dietetics* **50**, 215-265.

Bautista A, Barker PA, Dunn JT, Sanchez M & Kaiser DL (1982) The effects of oral iodized oil on intelligence, thyroid status, and somatic growth in school-age children from an area of endemic goiter. *American Journal of Clinical Nutrition* **35**, 127-134.

Beard J, Finch CA & Green WL (1982) Interactions of iron deficiency, anemia, and thyroid hormone levels in response of rats to cold exposure. *Life Sciences* **30**, 691-697.

Beard J, Green W, Miller L & Finch C (1984) Effect of iron-deficiency anemia on hormone levels and thermoregulation during cold exposure. *American Journal of Physiology* **247**, R114-119.

Beard J, Tobin B & Green W (1989) Evidence for thyroid hormone deficiency in iron-deficient anemic rats. *Journal of Nutrition* **119**, 772-778.

Beard JL, Borel MJ & Derr J (1990) Impaired thermoregulation and thyroid function in iron-deficiency anemia. *American Journal of Clinical Nutrition* **52**, 813-819.

Beard JL, Connor JR & Jones BC (1993) Iron in the brain. *Nutrition Reviews* **51**, 157-170.

Beard JL, Brigham DE, Kelley SK & Green MH (1998) Plasma thyroid hormone kinetics are altered in iron-deficient rats. *Journal of Nutrition* **128**, 1401-1408.

Beard JL (2001) Iron biology in immune function, muscle metabolism and neuronal functioning. *Journal of Nutrition* **131**, 568S-579S; discussion 580S.

Beckett GJ, Nicol F, Rae PW, Beech S, Guo Y & Arthur JR (1993) Effects of combined iodine and selenium deficiency on thyroid hormone metabolism in rats. *American Journal of Clinical Nutrition* **57**, 240S-243S.

Beech SG, Walker SW, Beckett GJ, Arthur JR, Nicol F & Lee D (1995) Effect of selenium depletion on thyroidal type-I iodothyronine deiodinase activity in isolated human thyrocytes and rat thyroid and liver. *Analyst* **120**, 827-831.

Behne D, Hilmert H, Scheid S, Gessner H & Elger W (1988) Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochimica et Biophysica Acta* **966**, 12-21.

Benmiloud M, Chaouki ML, Gutekunst R, Teichert HM, Wood WG & Dunn JT (1994) Oral iodized oil for correcting iodine deficiency: optimal dosing and outcome indicator selection. *Journal of Clinical Endocrinology and Metabolism* **79**, 20-24.

Bleichrodt N & Born MP (1994) A metaanalysis of research on iodine and its relationship to cognitive development. In *The damaged brain of iodine deficiency*, pp. 195-200 [JB Stanbury, editor]. New York, NY: Cognizant Communication Corporation.

Bondevik GT, Eskeland B, Ulvik RJ, Ulstein M, Lie RT, Schneede J & Kvale G (2000) Anaemia in pregnancy: possible causes and risk factors in Nepali women. *European Journal of Clinical Nutrition* **54**, 3-8.

Borel MJ, Smith SH, Brigham DE & Beard JL (1991) The impact of varying degrees of iron nutriture on several functional consequences of iron deficiency in rats. *Journal of Nutrition* **121**, 729-736.

Bothwell T, Charlton R, Cook JD & Finch C (1979) *Iron metabolism in man*. London: Blackwell Scientific Publications.

Bothwell TH (2000) Iron requirements in pregnancy and strategies to meet them. *American Journal of Clinical Nutrition* **72**, 257S-264S.

Bouis H (1996) Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition Reviews* **54**, 131-137.

Boyages SC (1993) Iodine deficiency disorders. *Journal of Clinical Endocrinology and Metabolism* **77**, 587-591.

Brabant G, Bergmann P, Kirsch CM, Kohrle J, Hesch RD & von zur Muhlen A (1992) Early adaptation of thyrotropin and thyroglobulin secretion to experimentally decreased iodine supply in man. *Metabolism: Clinical and Experimental* **41**, 1093-1096.

Brabin BJ (1983) An analysis of malaria in pregnancy in Africa. *Bulletin of the World Health Organization* **61**, 1005-1016.

Brabin BJ, Hakimi M & Pelletier D (2001) An analysis of anemia and pregnancy-related maternal mortality. *Journal of Nutrition* **131**, 604S-614S; discussion 614S-615S.

Bray GA (1968) Increased sensitivity of the thyroid in iodine-depleted rats to the goitrogenic effects of thyrotropin. *Journal of Clinical Investigation* **47**, 1640-1647.

Breen JJ, Matsuura T, Ross AC & Gurr JA (1995) Regulation of thyroid-stimulating hormone beta-subunit and growth hormone messenger ribonucleic acid levels in the rat: effect of vitamin A status. *Endocrinology* **136**, 543-549.

Bremner WF & Fell GS (1977) Zinc metabolism and thyroid status. *Postgraduate Medical Journal* **53**, 143-145.

Brigham D & Beard J (1996) Iron and thermoregulation: a review. *Critical Reviews in Food Science and Nutrition* **36**, 747-763.

Brigham DE & Beard JL (1995) Effect of thyroid hormone replacement in iron-deficient rats. *American Journal of Physiology* **269**, R1140-1147.

Brittenham GM (1994) The red cell cycle. In *Iron metabolism in health and disease*, pp. 31-62 [JH Brock, JW Halliday, MJ Pippard and LW Powell, editors]. London: W. B. Saunders Company Ltd.

Brooks GA, Henderson SA & Dallman PR (1987) Increased glucose dependence in resting, iron-deficient rats. *American Journal of Physiology* **253**, E461-466.

Brown NS, Smart A, Sharma V, Brinkmeier ML, Greenlee L, Camper SA, Jensen DR, Eckel RH, Krezel W, Chambon P & Haugen BR (2000) Thyroid hormone resistance and increased metabolic rate in the RXR-gamma-deficient mouse. *Journal of Clinical Investigation* **106**, 73-79.

Brune M, Rossander L & Hallberg L (1989) Iron absorption and phenolic compounds: importance of different phenolic structures. *European Journal of Clinical Nutrition* **43**, 547-557.

Brune M, Hallberg L & Skanberg AB (1991) Determination of iron-binding phenolic groups in foods. *Journal of Food Science* **56**, 128-131.

Brunn J, Block U, Ruf G, Bos I, Kunze WP & Scriba PC (1981) Volumetrie der Schilddrüsenlappen mittels Real-time-Sonographie. *Deutsche Medizinische Wochenschrift* **106**, 1338-1340.

Bürgi H, Supersaxo Z & Selz B (1990) Iodine deficiency diseases in Switzerland one hundred years after Theodor Kocher's survey: a historical review with some new goitre prevalence data. *Acta Endocrinologica* **123**, 577-590.

Bürgi H & Helbling B (1996) Methods of iodine supplementation. What is best where? In *The thyroid and iodine*, pp. 51-61 [J Nauman, D Glinoer, LE Braverman and U Hostales, editors]. Stuttgart: Schattauer.

Cao XY, Jiang XM, Dou ZH, Rakeman MA, Zhang ML, O'Donnell K, Ma T, Amette K, DeLong N & DeLong GR (1994) Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. *New England Journal of Medicine* **331**, 1739-1744.

Capen CC (2000) Comparative anatomy and physiology. In *The thyroid. A fundamental and clinical text*, pp. 20-43 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Carrasco N (2000) Thyroid hormone synthesis. In *The thyroid. A fundamental and clinical text*, pp. 52-61 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Carriaga MT, Skikne BS, Finley B, Cutler B & Cook JD (1991) Serum transferrin receptor for the detection of iron deficiency in pregnancy. *American Journal of Clinical Nutrition* **54**, 1077-1081.

Cartier LJ, Ohira Y, Chen M, Cuddihee RW & Holloszy JO (1986) Perturbation of mitochondrial composition in muscle by iron deficiency. Implications regarding regulation of mitochondrial assembly. *Journal of Biological Chemistry* **261**, 13827-13832.

Casterline JE, Allen LH & Ruel MT (1997) Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. *Journal of Nutrition* **127**, 1966-1972.

Chaouki ML & Benmiloud M (1994) Prevention of iodine deficiency disorders by oral administration of lipiodol during pregnancy. *European Journal of Endocrinology* **130**, 547-551.

Charlton RW & Bothwell TH (1983) Iron absorption. *Annual Review of Medicine* **34**, 55-68.

Chen OS, Schalinske KL & Eisenstein RS (1997) Dietary iron intake modulates the activity of iron regulatory proteins and the abundance of ferritin and mitochondrial aconitase in rat liver. *Journal of Nutrition* **127**, 238-248.

Chopra IJ, Hershman JM & Hornabrook RW (1975) Serum thyroid hormone and thyrotropin levels in subjects from endemic goiter regions of New Guinea. *Journal of Clinical Endocrinology and Metabolism* **40**, 326-333.

Chopra IJ & Sabatino L (2000) Nature and source of circulating thyroid hormones. In *The thyroid. A fundamental and clinical text*, pp. 121-135 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Cobra C, Muhilal, Rusmil K, Rustama D, Djatnika, Suwardi SS, Permaesih D, Muherdiyantiningsih, Martuti S & Semba RD (1997) Infant survival is improved by oral iodine supplementation. *Journal of Nutrition* **127**, 574-578.

Cohen RN, Weintraub BD & Wondisford FE (2000) Thyrotropin. In *The thyroid. A fundamental and clinical text*, pp. 202-219 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Contempré B, Dumont JE, Ngo B, Thilly CH, Diplock AT & Vanderpas J (1991) Effect of selenium supplementation in hypothyroid subjects of an iodine and selenium deficient area: the possible danger of indiscriminate supplementation of iodine-deficient subjects with selenium. *Journal of Clinical Endocrinology and Metabolism* **73**, 213-215.

Contempré B, Duale NL, Dumont JE, Ngo B, Diplock AT & Vanderpas J (1992) Effect of selenium supplementation on thyroid hormone metabolism in an iodine and selenium deficient population. *Clinical Endocrinology* **36**, 579-583.

Contempré B, Denef JF, Dumont JE & Many MC (1993) Selenium deficiency aggravates the necrotizing effects of a high iodide dose in iodine deficient rats. *Endocrinology* **132**, 1866-1868.

Contempré B, Many MC, Vanderpas J & Dumont JE (1994) Interactions between two trace elements: selenium and iodine. Implications of both deficiencies. In *The damaged brain of iodine deficiency*, pp. 133-138 [JD Stanbury, editor]. New York: Cognizant Communication.

Contempré B, Dumont JE, Denef JF & Many MC (1995) Effects of selenium deficiency on thyroid necrosis, fibrosis and proliferation: a possible role in myxoedematous cretinism. *European Journal of Endocrinology* **133**, 99-109.

Cook JD & Monsen ER (1977) Vitamin C, the common cold, and iron absorption. *American Journal of Clinical Nutrition* **30**, 235-241.

Cook JD & Reusser ME (1983) Iron fortification: an update. *American Journal of Clinical Nutrition* **38**, 648-659.

Cook JD, Baynes RD & Skikne BS (1992) Iron deficiency and the measurement of iron status. *Nutrition Research Reviews* **5**, 189-202.
Cook JD, Skikne BS & Baynes RD (1993) Serum transferrin receptor. *Annual Review of Medicine* **44**, 63-74.
Cook JD (1999) Defining optimal body iron. *Proceedings of the Nutrition Society* **58**, 489-495.
Corvilain B, van Sande J, Laurent E & Dumont JE (1991) The H2O2-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* **128**, 779-785.
Corvilain B, Contempré B, Longombe AO, Goyens P, Gervy-Decoster C, Lamy F, Vanderpas JB & Dumont JE (1993) Selenium and the thyroid: how the relationship was established. *American Journal of Clinical Nutrition* **57**, 244S-248S.
Dallman PR (1986) Biochemical basis for the manifestations of iron deficiency. *Annual Review of Nutrition* **6**, 13-40.
Das KC, Mukherjee M, Sarkar TK, Dash RJ & Rastogi GK (1975) Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism* **40**, 211-220.
Delange F, Hershman JM & Ermans AM (1971) Relationship between the serum thyrotropin level, the prevalence of goiter and the pattern of iodine metabolism in Idjwi Island. *Journal of Clinical Endocrinology and Metabolism* **33**, 261-268.
Delange F, Benker G, Caron P, Eber O, Ott W, Peter F, Podoba J, Simescu M, Szybinsky Z, Vertongen F, Vitti P, Wiersinga W & Zamrazil V (1997) Thyroid volume and urinary iodine in European schoolchildren: standardization of values for assessment of iodine deficiency. *European Journal of Endocrinology* **136**, 180-187.
Delange F, de Benoist B & Alnwick D (1999) Risks of iodine-induced hyperthyroidism after correction of iodine deficiency by iodized salt. *Thyroid* **9**, 545-556.
Delange F (2000a) Endemic cretinism. In *The thyroid. A fundamental and clinical text*, pp. 743-754 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.
Delange F (2000b) Iodine deficiency. In *The thyroid. A fundamental and clinical text*, pp. 295-316 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.
Delange F & Lecomte P (2000) Iodine supplementation: benefits outweigh risks. *Drug Safety* **22**, 89-95.
Delange F, de Benoist B, Pretell E & Dunn JT (2001) Iodine deficiency in the world: where do we stand at the turn of the century? *Thyroid* **11**, 437-447.
Delange F, de Benoist B, Bürgi H & ICCIDD Working Group (2002) Determining median urinary iodine concentration that indicates adequate iodine intake at population level. *Bulletin of the World Health Organization* **80**, 633-636.
DeLong GR, Leslie PW, Wang SH, Jiang XM, Zhang ML, Rakeman M, Jiang JY, Ma T & Cao XY (1997) Effect on infant mortality of iodination of irrigation water in a severely iodine-deficient area of China. *Lancet* **350**, 771-773.
DePillis GD, Ozaki S, Kuo JM, Maltby DA & Ortiz de Montellano PR (1997) Autocatalytic processing of heme by lactoperoxidase produces the native protein-bound prosthetic group. *Journal of Biological Chemistry* **272**, 8857-8860.
Dillman E, Gale C, Green W, Johnson DG, Mackler B & Finch C (1980) Hypothermia in iron deficiency due to altered triiodothyronine metabolism. *American Journal of Physiology* **239**, R377-381.
Dillon JC & Milliez J (2000) Reproductive failure in women living in iodine deficient areas of West Africa. *British Journal of Obstetrics and Gynaecology* **107**, 631-636.
Djokomoeljanto R, Setyawan H, Dramaix M, Hadisaputro S, Soehartono T & Delange F (2001) The ThyroMobil model for standardized evaluation of iodine deficiency disorder control in Indonesia. *Thyroid* **11**, 365-372.
Donati RM, Fletcher JW, Warnecke MA & Gallagher NI (1973) Erythropoiesis in hypothyroidism. *Proceedings of the Society for Experimental Biology and Medicine* **144**, 78-82.
Dreyfuss ML, Stoltzfus RJ, Shrestha JB, Pradhan EK, LeClerq SC, Khatry SK, Shrestha SR, Katz J, Albonico M & West KP, Jr. (2000) Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. *Journal of Nutrition* **130**, 2527-2536.
Drill VA (1943) Interrelations between thyroid function and vitamin metabolism. *Physiological Reviews* **23**, 355-379.
DuBois D & DuBois EF (1916) Clinical calorimetry. X. A formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine* **17**.

Dumont JE, Lamy F, Roger P & Maenhaut C (1992) Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiological Reviews* **72**, 667-697.
Dumont JE, Corvilain B & Contempré B (1994a) The biochemistry of endemic cretinism: roles of iodine and selenium deficiency and goitrogens. *Molecular and Cellular Endocrinology* **100**, 163-166.
Dumont JE, Corvilain B & Contempré B (1994b) Endemic cretinism: the myxedematous and neurological forms of a disease caused by severe iodine deficiency. In *The damaged brain of iodine deficiency*, pp. 259-263 [JD Stanbury, editor]. New York: Cognizant Communication.
Dumont JE, Ermans AM, Maenhaut C, Coppee F & Stanbury JB (1995) Large goitre as a maladaptation to iodine deficiency. *Clinical Endocrinology* **43**, 1-10.
Dunn JT (1994) Societal implications of iodine deficiency and the value of its prevention. In *The damaged brain of iodine deficiency*, pp. 309-315 [JD Stanbury, editor]. New York: Cognizant Communication.
Dunn JT (1998) What's happening to our iodine? *Journal of Clinical Endocrinology and Metabolism* **83**, 3398-3400.
Dunn JT & Dunn AD (2000) Thyroglobulin: chemistry, biosynthesis, and proteolysis. In *The thyroid. A fundamental and clinical text*, pp. 91-104 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.
Dunn JT & Delange F (2001) Damaged reproduction: the most important consequence of iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* **86**, 2360-2363.
Dunn JT & Dunn AD (2001) Update on intrathyroidal iodine metabolism. *Thyroid* **11**, 407-414.
Duntas LH, Papanastasiou L, Mantzou E & Koutras DA (1999) Incidence of sideropenia and effects of iron repletion treatment in women with subclinical hypothyroidism. *Experimental and Clinical Endocrinology and Diabetes* **107**, 356-360.
Durkin MS, Khan NZ, Davidson LL, Huq S, Munir S, Rasul E & Zaman SS (2000) Prenatal and postnatal risk factors for mental retardation among children in Bangladesh. *American Journal of Epidemiology* **152**, 1024-1033.
Egli I (2001) Traditional food processing methods to increase mineral bioavailability from cereal and legume based weaning foods. Thesis/Dissertation, Swiss Federal Institute of Technology Zürich, Switzerland. Diss ETH No. 13980.
Ekholm R (1981) Iodination of thyroglobulin. An intracellular or extracellular process? *Molecular and Cellular Endocrinology* **24**, 141-163.
Ekström EC, Hyder SM, Chowdhury AM, Chowdhury SA, Lonnerdal B, Habicht JP & Persson LA (2002) Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues. *American Journal of Clinical Nutrition* **76**, 1392-1400.
Elnagar B, Eltom M, Karlsson FA, Ermans AM, Gebre-Medhin M & Bourdoux PP (1995) The effects of different doses of oral iodized oil on goiter size, urinary iodine, and thyroid-related hormones. *Journal of Clinical Endocrinology and Metabolism* **80**, 891-897.
Erdogan MF, Erdogan G, Sav H, Gullu S & Kamel N (2001) Endemic goiter, thiocyanate overload, and selenium status in school-age children. *Biological Trace Element Research* **79**, 121-130.
Erikson KM, Pinero DJ, Connor JR & Beard JL (1997) Regional brain iron, ferritin and transferrin concentrations during iron deficiency and iron repletion in developing rats. *Journal of Nutrition* **127**, 2030-2038.
Ermans AM, Dumont JE & Bastenie PA (1963a) Thyroid function in a goiter endemic: I. Impairment of hormone synthesis and secretion in the goitrous gland. *Journal of Clinical Endocrinology and Metabolism* **23**, 539-549.
Ermans AM, Dumont JE & Bastenie PA (1963b) Thyroid function in a goitrous endemic: II. Nonhormonal iodine escape from the goitrous gland. *Journal of Clinical Endocrinology and Metabolism* **23**, 550-560.
Evans RM (1988) The steroid and thyroid hormone receptor superfamily. *Science* **240**, 889-895.
Fairweather-Tait SJ & Hurrell RF (1996) Bioavailability of minerals and trace elements. *Nutrition Research Reviews* **9**, 295-324.
Fan JL, Patibandla SA, Kimura S, Rao TN, Desai RK, Seetharamaiah GS, Kurosky A & Prabhakar BS (1996) Purification and characterization of a recombinant human thyroid peroxidase expressed in insect cells. *Journal of Autoimmunity* **9**, 529-536.
Fayadat L, Niccoli-Sire P, Lanet J & Franc JL (1998) Human thyroperoxidase is largely retained and rapidly degraded in the endoplasmic reticulum. Its N-glycans are required for folding and intracellular trafficking. *Endocrinology* **139**, 4277-4285.

Fayadat L, Niccoli-Sire P, Lanet J & Franc JL (1999) Role of heme in intracellular trafficking of thyroperoxidase and involvement of H2O2 generated at the apical surface of thyroid cells in autocatalytic covalent heme binding. *Journal of Biological Chemistry* **274**, 10533-10538.
Fenwick GR & Heaney RK (1983) Glucosinolates and their breakdown products in cruciferous crops, foods and feedingstuffs. *Food Chemistry* **11**, 249-271.
Ferguson BJ, Skikne BS, Simpson KM, Baynes RD & Cook JD (1992) Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *Journal of Laboratory and Clinical Medicine* **119**, 385-390.
Fierro-Benitez R, Cazar R, Stanbury JB, Rodriguez P, Garces F, Fierro-Renoy F & Estrella E (1988) Effects on school children of prophylaxis of mothers with iodized oil in an area of iodine deficiency. *Journal of Endocrinological Investigation* **11**, 327-335.
Finch C (1994) Regulators of iron balance in humans. *Blood* **84**, 1697-1702.
Fishman SM, Christian P & West KP (2000) The role of vitamins in the prevention and control of anaemia. *Public Health Nutr* **3**, 125-150.
Florentino RF, Tanchoco CC, Rodriguez MP, Cruz AJ & Molano WL (1996) Interactions among micronutrient deficiencies and undernutrition in the Philippines. *Biomedical and Environmental Sciences* **9**, 348-357.
Foo LC, Zulfiqar A, Nafikudin M, Fadzil MT & Asmah AS (1999) Local versus WHO/International Council for Control of Iodine Deficiency Disorders-recommended thyroid volume reference in the assessment of iodine deficiency disorders. *European Journal of Endocrinology* **140**, 491-497.
Franke D, Filler G, Zivicnjak M, Kouame P, Ohde I, Eckhardt L, Doehring E & Ehrich JH (1999) Sonomorphologic evaluation of goiter in an iodine deficiency area in the Ivory Coast. *American Journal of Public Health* **89**, 1857-1861.
Freake HC, Govoni KE, Guda K, Huang C & Zinn SA (2001) Actions and interactions of thyroid hormone and zinc status in growing rats. *Journal of Nutrition* **131**, 1135-1141.
Fujimoto S, Yasuhiro I, Higashi A, Matsuda I, Kashiwabara N & Nahashima I (1986) Conversion of thyroxin into tri-iodothyronine in zinc-deficient rat liver. *Journal of Pediatric Gastroenterology and Nutrition* **5**, 799-805.
Gaitan E, Lindsay RH, Reichert RD, Ingbar SH, Cooksey RC, Legan J, Meydrech EF, Hill J & Kubota K (1989) Antithyroid and goitrogenic effects of millet: role of C-glycosylflavones. *Journal of Clinical Endocrinology and Metabolism* **68**, 707-714.
Gaitan E (1990) Goitrogens in food and water. *Annual Review of Nutrition* **10**, 21-39.
Gaitan E, Cooksey RC, Legan J, Lindsay RH, Ingbar SH & Medeiros-Neto G (1994) Antithyroid effects in vivo and in vitro of babassu and mandioca: a staple food in goiter areas of Brazil. *European Journal of Endocrinology* **131**, 138-144.
Gaitan JE, Mayoral LG & Gaitan E (1983) Defective thyroidal iodine concentration in protein-calorie malnutrition. *Journal of Clinical Endocrinology and Metabolism* **57**, 327-333.
Galton VA (1972) Some effects of altitude on thyroid function. *Endocrinology* **91**, 1393-1403.
Garcin H & Higueret P (1980) Free and protein-bound tri-iodothyronine in the serum of vitamin A-deficient rats. *Journal of Endocrinology* **84**, 135-140.
Garcin H & Higueret P (1983) Thyroid hormones in vitamin A-deficient rats: effect of retinoic acid supplementation. *Annals of Nutrition and Metabolism* **27**, 495-500.
Giray B, Hincal F, Tezic T, Okten A & Gedik Y (2001) Status of selenium and antioxidant enzymes of goitrous children is lower than healthy controls and nongoitrous children with high iodine deficiency. *Biological Trace Element Research* **82**, 35-52.
Glinoer D & Delange F (2000) The potential repercussions of maternal, fetal, and neonatal hypothyroxinemia on the progeny. *Thyroid* **10**, 871-887.
Glinoer D (2001) Pregnancy and iodine. *Thyroid* **11**, 471-481.
Goswami UC & Choudhury S (1999) The status of retinoids in women suffering from hyper- and hypothyroidism: interrelationship between vitamin A, beta-carotene and thyroid hormones. *International Journal for Vitamin and Nutrition Research* **69**, 132-135.
Goyens P, Golstein J, Nsombola B, Vis H & Dumont JE (1987) Selenium deficiency as a possible factor in the pathogenesis of myxoedematous endemic cretinism. *Acta Endocrinologica* **114**, 497-502.
Grantham-McGregor S & Ani C (2001) A review of studies on the effect of iron deficiency on cognitive development in children. *Journal of Nutrition* **131**, 649S-666S; discussion 666S-668S.
Greer MA, Grimm Y & Studer H (1968) Qualitative changes in the secretion of thyroid hormones induced by iodine deficiency. *Endocrinology* **83**, 1193-1198.

Guo J, McLachlan SM, Hutchison S & Rapoport B (1998) The greater glycan content of recombinant human thyroid peroxidase of mammalian than of insect cell origin facilitates purification to homogeneity of enzymatically protein remaining soluble at high concentration. *Endocrinology* **139**, 999-1005.

Gupta RP, Verma PC & Garg SL (1997) Effect of experimental zinc deficiency on thyroid gland in guinea-pigs. *Annals of Nutrition and Metabolism* **41**, 376-381.

Gutekunst R & Teichert HM (1993) Requirements for goiter surveys and the determination of thyroid size. In *Iodine deficiency in Europe: a continuing concern*, pp. 109-118 [F Delange, JT Dunn and D Glinoer, editors]. New York: Plenum Press.

Haas JD & Brownlie Tt (2001) Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *Journal of Nutrition* **131**, 676S-688S; discussion 688S-690S.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD & Klein RZ (1999) Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New England Journal of Medicine* **341**, 549-555.

Hall A, Drake L & Bundy D (2001) Public health measures to control helminth infections. In *Nutritional anemias*, pp. 215-239 [U Ramakrishnan, editor]. London: CRC Press.

Hallberg L (1981) Bioavailability of dietary iron in man. *Annual Review of Nutrition* **1**, 123-147.

Hallberg L, Brune M & Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition* **49**, 140-144.

Hallberg L & Rossander-Hulthén L (1991) Iron requirements in menstruating women. *American Journal of Clinical Nutrition* **54**, 1047-1058.

Hallberg L, Sandström B & Aggett PJ (1993) Iron, zinc and other trace elements. In *Human nutrition and dietetics*, pp. 174-207 [JS Garow and WPT James, editors]. Edinburgh: Churchill Livingstone.

Hallberg L (2001) Perspectives on nutritional iron deficiency. *Annual Review of Nutrition* **21**, 1-21.

Hartoma RT, Sotaniemi EA & Maattanen J (1979) Effect of zinc on some biochemical indices of metabolism. *Nutrition and Metabolism* **23**, 294-300.

Haugen BR, Brown NS, Wood WM, Gordon DF & Ridgway EC (1997) The thyrotrope-restricted isoform of the retinoid-X receptor-gamma1 mediates 9-cis-retinoic acid suppression of thyrotropin-beta promoter activity. *Molecular Endocrinology* **11**, 481-489.

Henderson SA, Dallman PR & Brooks GA (1986) Glucose turnover and oxidation are increased in the iron-deficient anemic rat. *American Journal of Physiology* **250**, E414-421.

Hershko C (1993) Iron, infection and immune function. *Proceedings of the Nutrition Society* **52**, 165-174.

Hess SY & Zimmermann MB (2000) Thyroid volumes in a national sample of iodine-sufficient swiss school children: comparison with the World Health Organization/International Council for the control of iodine deficiency disorders normative thyroid volume criteria. *European Journal of Endocrinology* **142**, 599-603.

Hetzel BS (1983) Iodine deficiency disorders (IDD) and their eradication. *Lancet* **2**, 1126-1129.

Hetzel BS (1993) Iodine deficiency disorders. In *Human nutrition and dietetics*, pp. 534-555 [JS Garow and WPT James, editors]. Edinburgh: Churchill Livingstone.

Hetzel BS (1994) Historical development of the concepts of the brain - thyroid relationships. In *The damaged brain of iodine deficiency*, pp. 1-7 [JD Stanbury, editor]. New York: Cognizant Communication.

Higueret P & Garcin H (1979) Transport of thyroxine in the serum of vitamin A-deficient rats. *Journal of Endocrinology* **80**, 223-228.

Higueret P & Garcin H (1984) Triiodothyronine and vitamin A-deficiency in the rat. *Journal de Physiologie* **79**, 373-377.

Hinderaker SG, Olsen BE, Lie RT, Bergsjo PB, Gasheka P, Bondevik GT, Ulvik R & Kvale G (2002) Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections. *European Journal of Clinical Nutrition* **56**, 192-199.

Hobbs AJ, Gladwin MT, Patel RP, Williams DL & Butler AR (2002) Haemoglobin: NO transporter, NO inactivator or NOne of the above? *Trends in Pharmacological Sciences* **23**, 406-411.

Horton L, Coburn RJ, England JM & Himsworth RL (1976) The haematology of hypothyroidism. *Quarterly Journal of Medicine* **45**, 101-123.

Horton S & Levin C (2001) Commentary on "evidence that iron deficiency anemia causes reduced work capacity". *Journal of Nutrition* **131**, 691S-696S.

Hosoya T, Matsukawa S & Nagai Y (1971) Localization of peroxidase and other microsomal enzymes in thyroid cells. *Biochemistry* **10**, 3086-3093.
Hotz CS, Fitzpatrick DW, Trick KD & L'Abbe MR (1997) Dietary Iodine and selenium interact to affect thyroid hormone metabolism of rats. *Journal of Nutrition* **127**, 1214-1218.
Hurrell R (1999) Iron. In *The mineral fortification of foods*, pp. 54-93 [R Hurrell, editor]. Surrey: Leatherhead Food RA.
Hurrell RF (2002) Fortification: overcoming technical and practical barriers. *Journal of Nutrition* **132**, 806S-812S.
Ingenbleek Y & De Visscher M (1979) Hormonal and nutritional status: critical conditions for endemic goiter epidemiology? *Metabolism: Clinical and Experimental* **28**, 9-19.
Ingenbleek Y (1983) Vitamin A deficiency impairs the normal mannosylation, conformation and iodination of the thyroglobulin: a new etiological approach to endemic goiter. *Experientia. Supplementum* **44**, 264-297.
Ingenbleek Y & Young V (1994) Transthyretin (prealbumin) in health and disease: nutritional implications. *Annual Review of Nutrition* **14**, 495-533.
Ingenbleek Y, Jung L, Ferard G, Bordet F, Goncalves AM & Dechoux L (1997) Iodised rapeseed oil for eradication of severe endemic goitre. *Lancet* **350**, 1542-1545.
Institute of Medicine (2000) Selenium. In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, pp. 284-324. Washington, D.C.: National Academy Press.
Institute of Medicine (2002a) Iodine. In *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, pp. 258-289. Washington, D.C.: National Academy Press.
Institute of Medicine (2002b) Iron. In *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, D.C.: National Academy Press.
International Nutritional Anemia Consultive Group (2000) *Safety of iron supplementation programs in malaria-endemic regions*. Washington, DC: ILSI press.
Isah HS, Fleming AF, Ujah IA & Ekwempu CC (1985) Anaemia and iron status of pregnant and non-pregnant women in the guinea savanna of Nigeria. *Annals of Tropical Medicine and Parasitology* **79**, 485-493.
Janssen KP, Van Der Heide D, Visser TJ, Kaptein E & Beynen AC (1994) Thyroid function and deiodinase activities in rats with marginal iodine deficiency. *Biological Trace Element Research* **40**, 237-246.
Jia L, Bonaventura C, Bonaventura J & Stamler JS (1996) S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* **380**, 221-226.
Jooste PL, Weight MJ & Lombard CJ (2000) Short-term effectiveness of mandatory iodization of table salt, at an elevated iodine concentration, on the iodine and goiter status of schoolchildren with endemic goiter. *American Journal of Clinical Nutrition* **71**, 75-80.
Kaplan MM & Utiger RD (1978) Iodothyronine metabolism in rat liver homogenates. *Journal of Clinical Investigation* **61**, 459-471.
Keiser J, N'Goran EK, Traore M, Lohourignon KL, Singer BH, Lengeler C, Tanner M & Utzinger J (2002) Polyparasitism with Schistosoma mansoni, geohelminths, and intestinal protozoa in rural Côte d'Ivoire. *Journal of Parasitology* **88**, 461-466.
King JC & Keen CL (1999) Zinc. In *Modern nutrition in health and disease*, pp. 223-239 [ME Sihls, JA Olson, M Shike and AC Ross, editors]. Baltimore: Williams & Wilkins.
Kloos H (1987) Schistosomiasis Ecology: Water resources development in the Awash Valley o Ethopia. In *Health and Disease in Tropical Africa*, pp. 305-333 [R Akhtar, editor]. Chur: Harwood Academic Press.
Knudsen N, Bulow I, Jorgensen T, Perrild H, Ovesen L & Laurberg P (2001) Serum Tg--a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. *Journal of Clinical Endocrinology and Metabolism* **86**, 3599-3603.
Kralik A, Eder K & Kirchgessner M (1996) Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. *Hormone and Metabolic Research* **28**, 223-226.
Kuliawat R, Lisanti MP & Arvan P (1995) Polarized distribution and delivery of plasma membrane proteins in thyroid follicular epithelial cells. *Journal of Biological Chemistry* **270**, 2478-2482.
Kuvibidila S, Mark JA, Warrier RP, Yu L, Ode D & Tshefu KA (1995) Soluble transferrin receptor as an index of iron status in Zairian children with malaria. *Journal of Tropical Medicine and Hygiene* **98**, 373-378.

Labbé RF & Rettmer RL (1989) Zinc protoporphyrin: a product of iron-deficient erythropoiesis. *Seminars in Hematology* **26**, 40-46.
Lamberg BA, Haikonen M, Makela M, Jukkara A, Axelson E & Welin MG (1981) Further decrease in thyroidal uptake and disappearance of endemic goitre in children after 30 years of iodine prophylaxis in the east of Finland. *Acta Endocrinologica* **98**, 205-209.
Lane P & Gross S (2002) Hemoglobin as a chariot for NO bioactivity. *Nature Medicine* **8**, 657-658.
Lanni A, Moreno M, Lombardi A, de Lange P & Goglia F (2001) Control of energy metabolism by iodothyronines. *Journal of Endocrinological Investigation* **24**, 897-913.
Larsen PR & Berry MJ (1995) Nutritional and hormonal regulation of thyroid hormone deiodinases. *Annual Review of Nutrition* **15**, 323-352.
Latapie JL, Clerc M, Beda B, Delmas M, Le Bras P, Lanet MJ & Ducassou D (1981) Aspect clinique et biologique du goitre endémique dans la région de Man (Côte d'Ivoire). *Annales d Endocrinologie* **42**, 517-530.
Lazarus JH & Kokandi A (2000) Thyroid disease in relation to pregnancy: a decade of change. *Clinical Endocrinology* **53**, 265-278.
Lozoff B, Jimenez E & Wolf AW (1991) Long-term developmental outcome of infants with iron deficiency. *New England Journal of Medicine* **325**, 687-694.
Lucca P, Hurrell R & Potrykus I (2002) Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition* **21**, 184S-190S.
Lukaski HC, Hall CB & Nielsen FH (1990) Thermogenesis and thermoregulatory function of iron-deficient women without anemia. *Aviation Space and Environmental Medicine* **61**, 913-920.
Lukaski HC, Hall CB & Marchello MJ (1992) Impaired thyroid hormone status and thermoregulation during cold exposure of zinc-deficient rats. *Hormone and Metabolic Research* **24**, 363-366.
Ma T, Guo J & Wang F (1993) The epidemiology of iodine-deficiency diseases in China. *American Journal of Clinical Nutrition* **57**, 264S-266S.
Marqusee E & Mandel SJ (2000) The blood in hypothyroidism. In *The thyroid. A fundamental and clinical text*, pp. 800-802 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.
Martinez-Torres C, Cubeddu L, Dillmann E, Brengelmann GL, Leets I, Layrisse M, Johnson DG & Finch C (1984) Effect of exposure to low temperature on normal and iron-deficient subjects. *American Journal of Physiology* **246**, R380-383.
Matteelli A, Donato F, Shein A, Muchi JA, Leopardi O, Astori L & Carosi G (1994) Malaria and anaemia in pregnant women in urban Zanzibar, Tanzania. *Annals of Tropical Medicine and Parasitology* **88**, 475-483.
McLaren DS & Frigg M (2001) *Sight and life guidebook on vitamin A in health and disease*. Basel: Task Force Sight and Life.
McMahon TJ, Moon RE, Luschinger BP, Carraway MS, Stone AE, Stolp BW, Gow AJ, Pawloski JR, Watke P, Singel DJ, Piantadosi CA & Stamler JS (2002) Nitric oxide in the human respiratory cycle. *Nature Medicine* **8**, 711-717.
Medeiros DM & Beard JL (1998) Dietary iron deficiency results in cardiac eccentric hypertrophy in rats. *Proceedings of the Society for Experimental Biology and Medicine* **218**, 370-375.
Meinhold H, Campos-Barros A & Behne D (1992) Effects of selenium and iodine deficiency on iodothyronine deiodinases in brain, thyroid and peripheral tissue. *Acta Medica Austriaca* **19 Suppl 1**, 8-12.
Meinhold H, Campos-Barros A, Walzog B, Kohler R, Muller F & Behne D (1993) Effects of selenium and iodine deficiency on type I, type II and type III iodothyronine deiodinases and circulating thyroid hormones in the rat. *Experimental and Clinical Endocrinology* **101**, 87-93.
Mejia LA & Chew F (1988) Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *American Journal of Clinical Nutrition* **48**, 595-600.
Menendez C, Fleming AF & Alonso PL (2000) Malaria-related anaemia. *Parasitol Today* **16**, 469-476.
Michot JL, Osty J & Nunez J (1980) Regulatory effects of iodide and thiocyanate on tyrosine oxidation catalyzed by thyroid peroxidase. *European Journal of Biochemistry* **107**, 297-301.
Missler U, Gutekunst R & Wood WG (1994) Thyroglobulin is a more sensitive indicator of iodine deficiency than thyrotropin: development and evaluation of dry blood spot assays for thyrotropin and thyroglobulin in iodine-deficient geographical areas. *European Journal of Clinical Chemistry and Clinical Biochemistry* **32**, 137-143.
Miyamoto T, Sakurai A & DeGroot LJ (1991) Effects of zinc and other divalent metals on deoxyribonucleic acid binding and hormone-binding activity of human alpha 1 thyroid hormone receptor expressed in Escherichia coli. *Endocrinology* **129**, 3027-3033.

Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W & Finch CA (1978) Estimation of available dietary iron. *American Journal of Clinical Nutrition* **31**, 134-141.
Moreno-Reyes R, Suetens C, Mathieu F, Begaux F, Zhu D, Rivera MT, Boelaert M, Neve J, Perlmutter N & Vanderpas J (1998) Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. *New England Journal of Medicine* **339**, 1112-1120.
Morley JE, Damassa DA, Gordon J, Pekary AE & Hershman JM (1978) Thyroid function and vitamin A deficiency. *Life Sciences* **22**, 1901-1905.
Morley JE, Gordon J & Hershman JM (1980) Zinc deficiency, chronic starvation, and hypothalamic-pituitary-thyroid function. *American Journal of Clinical Nutrition* **33**, 1767-1770.
Morley JE, Russell RM, Reed A, Carney EA & Hershman JM (1981) The interrelationship of thyroid hormones with vitamin A and zinc nutritional status in patients with chronic hepatic and gastrointestinal disorders. *American Journal of Clinical Nutrition* **34**, 1489-1495.
Moshang T, Jr., Chance KH, Kaplan MM, Utiger RD & Takahashi O (1980) Effects of hypoxia on thyroid function tests. *Journal of Pediatrics* **97**, 602-604.
Murray MJ, Murray AB, Murray MB & Murray CJ (1978) The adverse effect of iron repletion on the course of certain infections. *British Medical Journal* **2**, 1113-1115.
Muto Y, Smith JE, Milch PO & Goodman DS (1972) Regulation of retinol-binding protein metabolism by vitamin A status in the rat. *Journal of Biological Chemistry* **247**, 2542-2550.
Mwanri L, Worsley A, Ryan P & Masika J (2000) Supplemental vitamin A improves anemia and growth in anemic school children in Tanzania. *Journal of Nutrition* **130**, 2691-2696.
Nath SK, Moinier B, Thuillier F, Rongier M & Desjeux JF (1992) Urinary excretion of iodide and fluoride from supplemented food grade salt. *International Journal for Vitamin and Nutrition Research* **62**, 66-72.
National Research Council (1989) *Recommended dietary allowances*, 10th ed. Washington, DC: National Academy Press.
Nestel P & Davidsson L (2002) *Anemia, iron deficiency, and iron deficiecy anemia*. Washington, DC: International Nutritional Anemia Consultative Group.
Ngo DB, Dikassa L, Okitolonda W, Kashala TD, Gervy C, Dumont J, Vanovervelt N, Contempré B, Diplock AT, Peach S & Vanderpas J (1997) Selenium status in pregnant women of a rural population (Zaire) in relationship to iodine deficiency. *Tropical Medicine and International Health* **2**, 572-581.
Nishi Y, Kawate R & Usui T (1980) Zinc metabolism in thyroid disease. *Postgraduate Medical Journal* **56**, 833-837.
Nockels CF, Ewing DL, Phetteplace H, Ritacco KA & Mero KN (1984) Hypothyroidism: an early sign of vitamin A deficiency in chickens. *Journal of Nutrition* **114**, 1733-1736.
Oba K & Kimura S (1980) Effects of vitamin A deficiency on thyroid function and serum thyroxine levels in the rat. *Journal of Nutritional Science and Vitaminology* **26**, 327-334.
Okamura K, Taurog A & Krulich L (1981a) Strain differences among rats in response to Remington iodine-deficient diets. *Endocrinology* **109**, 458-463.
Okamura K, Taurog A & Krulich L (1981b) Elevation of serum 3,5,3'-triiodothyronine and thyroxine levels in rats fed Remington diets; opposing effects of nutritional deficiency and iodine deficiency. *Endocrinology* **108**, 1247-1256.
Oliver JW, Sachan DS, Su P & Applehans FM (1987) Effects of zinc deficiency on thyroid function. *Drug-Nutrient Interactions* **5**, 113-124.
Oppenheimer SJ (2001) Iron and its relation to immunity and infectious disease. *Journal of Nutrition* **131**, 616S-633S; discussion 633S-635S.
Ozata M, Salk M, Aydin A, Sayin S, Oktenli C, Beyhan Z, Isimer A & Ozdemir IC (1999) Iodine and zinc, but not selenium and copper, deficiency exists in a male Turkish population with endemic goiter. *Biological Trace Element Research* **69**, 211-216.
Patel YC, Pharoah PO, Hornabrook RW & Hetzel BS (1973) Serum triiodothyronine, thyroxine and thyroid-stimulating hormone in endemic goiter: a comparison of goitrous and nongoitrous subjects in New Guinea. *Journal of Clinical Endocrinology and Metabolism* **37**, 783-789.
Pazos-Moura CC, Moura EG, Dorris ML, Rehnmark S, Melendez L, Silva JE & Taurog A (1991) Effect of iodine deficiency and cold exposure on thyroxine 5'-deiodinase activity in various rat tissues. *American Journal of Physiology* **260**, E175-182.
Pekary AE, Lukaski HC, Mena I & Hershman JM (1991) Processing of TRH precursor peptides in rat brain and pituitary is zinc dependent. *Peptides* **12**, 1025-1032.

Penel C, Gruffat D, Alquier C, Benoliel AM & Chabaud O (1998) Thyrotropin chronically regulates the pool of thyroperoxidase and its intracellular distribution: a quantitative confocal microscopic study. *Journal of Cellular Physiology* **174**, 160-169.

Pinero D, Jones B & Beard J (2001) Variations in dietary iron alter behavior in developing rats. *Journal of Nutrition* **131**, 311-318.

Pinero DJ, Li NQ, Connor JR & Beard JL (2000) Variations in dietary iron alter brain iron metabolism in developing rats. *Journal of Nutrition* **130**, 254-263.

Pintar JE (2000) Normal development of the hypothalamic-pituitary-thyroid axis. In *The thyroid. A fundamental and clinical text*, pp. 7-19 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Pisarev MA & Gärtner R (2000) Autoregulatory actions of iodine. In *The thyroid. A fundamental and clinical text*, pp. 85-90 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Pollitt E (1993) Iron deficiency and cognitive function. *Annual Review of Nutrition* **13**, 521-537.

Potter JD (1980) Hypothyroidism and reproductive failure. *Surgery, Gynecology and Obstetrics* **150**, 251-255.

Powers HJ, Weaver LT, Austin S, Wright AJ & Fairweather-Tait SJ (1991) Riboflavin deficiency in the rat: effects on iron utilization and loss. *British Journal of Nutrition* **65**, 487-496.

Powers HJ (1995) Riboflavin-iron interactions with particular emphasis on the gastrointestinal tract. *Proceedings of the Nutrition Society* **54**, 509-517.

Premji Z, Hamisi Y, Shiff C, Minjas J, Lubega P & Makwaya C (1995) Anaemia and Plasmodium falciparum infections among young children in an holoendemic area, Bagamoyo, Tanzania. *Acta Tropica* **59**, 55-64.

Rasmussen K (2001) Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? *Journal of Nutrition* **131**, 590S-601S; discussion 601S-603S.

Rayman MP (2002) The argument for increasing selenium intake. *Proceedings of the Nutrition Society* **61**, 203-215.

Robbins J (2000) Thyroid hormone transport proteins and the physiology of hormone binding. In *The thyroid. A fundamental and clinical text*, pp. 105-120 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Root AW, Duckett G, Sweetland M & Reiter EO (1979) Effects of zinc deficiency upon pituitary function in sexually mature and immature male rats. *Journal of Nutrition* **109**, 958-964.

Rossander-Hulthén L & Hallberg L (1996) Dietary factors influencing iron absorption - an overview. In *Iron nutrition in health and disease*, pp. 105-115 [L Hallberg and NG Asp, editors]: John Libbey & Company Ltd.

Roti E, Minelli R, Gardini E, Bianconi L, Ronchi A, Gatti A & Minoia C (1993) Selenium administration does not cause thyroid insufficiency in subjects with mild iodine deficiency and sufficient selenium intake. *Journal of Endocrinological Investigation* **16**, 481-484.

Roti E & Vagenakis AG (2000) Effects of excess iodide: clinical aspects. In *The thyroid. A fundamental and clinical text*, pp. 316-329 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Rush D (2000) Nutrition and maternal mortality in the developing world. *American Journal of Clinical Nutrition* **72**, 212S-240S.

Ruz M, Codoceo J, Galgani J, Munoz L, Gras N, Muzzo S, Leiva L & Bosco C (1999) Single and multiple selenium-zinc-iodine deficiencies affect rat thyroid metabolism and ultrastructure. *Journal of Nutrition* **129**, 174-180.

Safran M, Farwell AP & Leonard JL (1991) Evidence that type II 5'-deiodinase is not a selenoprotein. *Journal of Biological Chemistry* **266**, 13477-13480.

Sandstead HH, Prasad AS, Schulert AR, Farid Z, Miale A, Jr., Bassilly S & Darby WJ (1967) Human zinc deficiency, endocrine manifestations and response to treatment. *American Journal of Clinical Nutrition* **20**, 422-442.

Sartelet H, Serghat S, Lobstein A, Ingenbleek Y, Anton R, Petitfrere E, Aguie-Aguie G, Martiny L & Haye B (1996) Flavonoids extracted from fonio millet (Digitaria exilis) reveal potent antithyroid properties. *Nutrition* **12**, 100-106.

Sava L, Delange F, Belfiore A, Purrello F & Vigneri R (1984) Transient impairment of thyroid function in newborn from an area of endemic goiter. *Journal of Clinical Endocrinology and Metabolism* **59**, 90-95.

Savage D, Gangaidzo I, Lindenbaum J, Kiire C, Mukiibi JM, Moyo A, Gwanzura C, Mudenge B, Bennie A, Sitima J & et al. (1994) Vitamin B12 deficiency is the primary cause of megaloblastic anaemia in Zimbabwe. *British Journal of Haematology* **86**, 844-850.

Sawhney RC & Malhotra AS (1991) Thyroid function in sojourners and acclimatised low landers at high altitude in man. *Hormone and Metabolic Research* **23**, 81-84.

Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, Acosta C, Schellenberg JA, Aponte JJ, Kimario J, Urassa H, Mshinda H, Tanner M & Alonso P (1999) African children with malaria in an area of intense Plasmodium falciparum transmission: features on admission to the hospital and risk factors for death. *American Journal of Tropical Medicine and Hygiene* **61**, 431-438.

Schellenberg D, Menendez C, Kahigwa E, Aponte J, Vidal J, Tanner M, Mshinda H & Alonso P (2001) Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet* **357**, 1471-1477.

Scholl TO & Johnson WG (2000) Folic acid: influence on the outcome of pregnancy. *American Journal of Clinical Nutrition* **71**, 1295S-1303S.

Schröder-van der Elst JP & van der Heide D (1992) Effects of streptozocin-induced diabetes and food restriction on quantities and source of T4 and T3 in rat tissues. *Diabetes* **41**, 147-152.

Seino Y, Matsukura S, Inoue Y, Kadowaki S, Mori K & Imura H (1978) Hypogastrinemia in hypothyroidism. *American Journal of Digestive Diseases* **23**, 189-191.

Semba RD & Bloem MW (2002) The anemia of vitamin A deficiency: epidemiology and pathogenesis. *European Journal of Clinical Nutrition* **56**, 271-281.

Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P & Duvic M (1999) Central hypothyroidism associated with retinoid X receptor-selective ligands. *New England Journal of Medicine* **340**, 1075-1079.

Shrestha R (1994) Effect of iodine and iron supplementation on physical, psychomotor and mental development in primary school children in Malawi. Thesis/Dissertation, Wageningen Agriculture University, Netherlands.

Skikne BS, Flowers CH & Cook JD (1990) Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* **75**, 1870-1876.

Smit JG, van der Heide D, van Tintelen G & Beynen AC (1993) Thyroid function in rats with iodine deficiency is not further impaired by concurrent, marginal zinc deficiency. *British Journal of Nutrition* **70**, 585-592.

Smith SM, Smith SH & Beard JL (1990) Heart norepinephrine content in iron deficiency anemia. *Proceedings of the Society for Experimental Biology and Medicine* **193**, 306-312.

Smith SM, Deaver DR & Beard JL (1992) Metabolic rate and thyroxine monodeiodinase activity in iron-deficient female Sprague-Dawley rats: Effects of the ovarian steroids. *Journal of Nutritional Biochemistry* **3**, 461-466.

Smith SM, Johnson PE & Lukaski HC (1993) In vitro hepatic thyroid hormone deiodination in iron-deficient rats: effect of dietary fat. *Life Sciences* **53**, 603-609.

Smith SM, Finley J, Johnson LK & Lukaski HC (1994) Indices of in vivo and in vitro thyroid hormone metabolism in iron-deficient rats. *Nutrition Research* **14**, 729-739.

Spencer CA (2000) Thyroglobulin. In *The thyroid. A fundamental and clinical text*, pp. 403-413 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.

Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K & Piantadosi CA (1997) Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. *Science* **276**, 2034-2037.

Staubli Asobayire F (2000) Development of a food fortification strategy to combat iron deficiency in the Ivory Coast. Thesis/Dissertation, Swiss Federal Institute of Technology Zürich, Switzerland. Diss ETH No. 13730.

Staubli Asobayire F, Adou P, Davidsson L, Cook JD & Hurrell RF (2001) Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. *American Journal of Clinical Nutrition* **74**, 776-782.

Stoltzfus R & Dreyfuss ML (1998) *Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia*. Washington DC: ILSI Press.

Stoltzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch J, Schulze K & Yip R (1996) Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *American Journal of Tropical Medicine and Hygiene* **55**, 399-404.

Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M & Savioli L (1997) Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *American Journal of Clinical Nutrition* **65**, 153-159.

Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L & Tielsch JM (2000) Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old

Zanzibari children and these relationships change with age. *Journal of Nutrition* **130**, 1724-1733.
Strum JM (1979) Alterations within the rat thyroid gland during vitamin A deficiency. *American Journal of Anatomy* **156**, 169-182.
Stübner D, Gartner R, Greil W, Gropper K, Brabant G, Permanetter W, Horn K & Pickardt CR (1987) Hypertrophy and hyperplasia during goitre growth and involution in rats--separate bioeffects of TSH and iodine. *Acta Endocrinologica* **116**, 537-548.
Suharno D, West CE, Muhilal, Karyadi D & Hautvast JG (1993) Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* **342**, 1325-1328.
Sullivan K & May S (1999) *Urinary iodine assessment: A manual on survey and laboratory methods*: United Nations Children's Fund / Program Against Micronutrient Malnutrition.
Sungthong R, Mo-Suwan L, Chongsuvivatwong V & Geater AF (2002) Once weekly is superior to daily iron supplementation on height gain but not on hematological improvement among schoolchildren in Thailand. *Journal of Nutrition* **132**, 418-422.
Surks MI (1969) Effect of thyrotropin on thyroidal iodine metabolism during hypoxia. *American Journal of Physiology* **216**, 436-439.
Tang F, Wong TM & Loh TT (1988) Effects of cold exposure or TRH on the serum TSH levels in the iron-deficient rat. *Hormone and Metabolic Research* **20**, 616-619.
Taurog A (1970) Thyroid peroxidase and thyroxine biosynthesis. *Recent Progress in Hormone Research* **26**, 189-247.
Taurog AM (2000) Hormone synthesis: thyroid iodine metabolism. In *The thyroid. A fundamental and clinical text*, pp. 61-85 [LE Braverman and RD Utiger, editors]. Philadelphia: Lippincott.
Thilly CH, Swennen B, Moreno-Reyes R, Hindlet JY, Bourdoux P & Vanderpas JB (1994) Maternal, fetal and juvenile hypothyroidism, birth weight and infant mortality in the etiopathogenesis of the IDD spectra in Zaïre and Malawi. In *The damaged brain of iodine deficiency*, pp. 241-257 [JD Stanbury, editor]. New York: Cognizant Communication.
Todd CH & Dunn JT (1998) Intermittent oral administration of potassium iodide solution for the correction of iodine deficiency. *American Journal of Clinical Nutrition* **67**, 1279-1283.
Torrens JI & Burch HB (2001) Serum thyroglobulin measurement. Utility in clinical practice. *Endocrinology and Metabolism Clinics of North America* **30**, 429-467.
Trowbridge F & Martorell R (2002) Summary and recommendations. *Journal of Nutrition* **132**, 875S-879S.
Utiger RD (1998) Kashin-Beck disease - expanding the spectrum of iodine-deficiency disorders. *New England Journal of Medicine* **339**, 1156-1158.
Utzinger J, N'Goran EK, Esse Aya CM, Acka Adjoua C, Lohourignon KL, Tanner M & Lengeler C (1998) Schistosoma mansoni, intestinal parasites and perceived morbidity indicators in schoolchildren in a rural endemic area of western Cote d'Ivoire. *Tropical Medicine and International Health* **3**, 711-720.
Utzinger J, N'Goran EK, Marti HP, Tanner M & Lengeler C (1999) Intestinal amoebiasis, giardiasis and geohelminthiases: their association with other intestinal parasites and reported intestinal symptoms. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 137-141.
Vallee BL & Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiological Reviews* **73**, 79-118.
van den Briel T, West CE, Hautvast JG, Vulsma T, de Vijlder JJ & Ategbo EA (2001) Serum thyroglobulin and urinary iodine concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply in schoolchildren in Benin. *Journal of Nutrition* **131**, 2701-2706.
van den Broek NR & Letsky EA (2000) Etiology of anemia in pregnancy in south Malawi. *American Journal of Clinical Nutrition* **72**, 247S-256S.
Vanderpas JB, Contempré B, Duale NL, Goossens W, Bebe N, Thorpe R, Ntambue K, Dumont J, Thilly CH & Diplock AT (1990) Iodine and selenium deficiency associated with cretinism in northern Zaire. *American Journal of Clinical Nutrition* **52**, 1087-1093.
Vanderpas JB, Contempré B, Duale NL, Deckx H, Bebe N, Longombe AO, Thilly CH, Diplock AT & Dumont JE (1993) Selenium deficiency mitigates hypothyroxinemia in iodine-deficient subjects. *American Journal of Clinical Nutrition* **57**, 271S-275S.
Verhoef H, West CE, Ndeto P, Burema J, Beguin Y & Kok FJ (2001) Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. *American Journal of Clinical Nutrition* **74**, 767-775.

Verhoef H, West CE, Kraaijenhagen R, Nzyuko SM, King R, Mbandi MM, Van Laatum S, Hogervorst R, Schep C & Kok FJ (2002) Malarial anemia leads to adequately increased erythropoiesis in asymptomatic Kenyan children. *Blood* **100**, 3489-3494.

Virion A, Deme D, Pommier J & Nunez J (1980) Opposite effects of thiocyanate on tyrosine iodination and thyroid hormone synthesis. *European Journal of Biochemistry* **112**, 1-7.

Viteri FE, Ali F & Tujague J (1999) Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. *Journal of Nutrition* **129**, 2013-2020.

Vitti P, Martino E, Aghini-Lombardi F, Rago T, Antonangeli L, Maccherini D, Nanni P, Loviselli A, Balestrieri A, Araneo G & et al. (1994) Thyroid volume measurement by ultrasound in children as a tool for the assessment of mild iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* **79**, 600-603.

Vought RL & London WT (1967) Iodine intake, excretion and thyroidal accumulation in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* **27**, 913-919.

Wada L & King JC (1986) Effect of low zinc intakes on basal metabolic rate, thyroid hormones and protein utilization in adult men. *Journal of Nutrition* **116**, 1045-1053.

Walker ARP (1998) The remedying of iron deficiency: what priority should it have? *British Journal of Nutrition* **79**, 227-235.

Wei J & Li J (1985) Metabolism of iodized oil after oral administration in guinea pigs. *Nutrition Reports International* **31**, 1085-1092.

Wolde-Gebriel Z, Gebru H, Fisseha T & West CE (1993a) Severe vitamin A deficiency in a rural village in the Hararge region of Ethiopia. *European Journal of Clinical Nutrition* **47**, 104-114.

Wolde-Gebriel Z, West CE, Gebru H, Tadesse AS, Fisseha T, Gabre P, Aboye C, Ayana G & Hautvast JG (1993b) Interrelationship between vitamin A, iodine and iron status in schoolchildren in Shoa Region, central Ethiopia. *British Journal of Nutrition* **70**, 593-607.

Wolf G (2002) The regulation of the thyroid-stimulating hormone of the anterior pituitary gland by thyroid hormone and by 9-cis-retinoic acid. *Nutrition Reviews* **60**, 374-377.

Wollman SH (1962) Inhibition by thiocyanate of accumulation of radioiodine by thyroid gland. *American Journal of Physiology* **203**, 517-524.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (1994) Indicators for assessing iodine deficiency disorders and their control through salt iodization. WHO, Geneva; WHO/NUT/94.6.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (1996) Recommended iodine levels in salt and guidelines for monitoring their adequacy and effectiveness. WHO, Geneva; WHO/NUT/96.13.

World Health Organization & International Council for Control of Iodine Deficiency Disorders (1997) Recommended normative values for thyroid volume in children aged 6-15 years. World Health Organization & International Council for Control of Iodine Deficiency Disorders. *Bulletin of the World Health Organization* **75**, 95-97.

World Health Organization (1998) Report of the WHO informal consultation on schistosomiasis control. WHO, Geneva; WHO/CDS/CPC/SIP/99.2.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (1999) Progress towards the elimination of iodine deficiency disorders (IDD). WHO, Geneva; WHO/NHD/99.4.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (2001a) Assessment of iodine deficiency disorders and monitoring their elimination. WHO, Geneva; WHO/NHD/01.1.

World Health Organization, United Nations Children's Fund & United Nations University (2001b) Iron deficiency anemia: Assessment, prevention, and control. WHO, Geneva; WHO/NHD/01.3.

Wu HY, Xia YM, Ha PC & Chen XS (1997) Changes in myocardial thyroid hormone metabolism and alpha-glycerophosphate dehydrogenase activity in rats deficient in iodine and selenium. *British Journal of Nutrition* **78**, 671-676.

Xu F, Sullivan K, Houston R, Zhao J, May W & Maberly G (1999) Thyroid volumes in US and Bangladeshi schoolchildren: comparison with European schoolchildren. *European Journal of Endocrinology* **140**, 498-504.

Yip R & Dallman PR (1988) The roles of inflammation and iron deficiency as causes of anemia. *American Journal of Clinical Nutrition* **48**, 1295-1300.

Yip R & Ramakrishnan U (2002) Experiences and challenges in developing countries. *Journal of Nutrition* **132**, 827S-830S.

Zagrodzki P, Szmigiel H, Ratajczak R, Szybinski Z & Zachwieja Z (2000) The role of selenium in iodine metabolism in children with goiter. *Environmental Health Perspectives* **108**, 67-71.

Zhao J, Xu F, Zhang Q, Shang L, Xu A, Gao Y, Chen Z, Sullivan KM & Maberly GF (1999) Randomized clinical trial comparing different iodine interventions in school children. *Public Health Nutr* **2**, 173-178.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000a) Low dose oral iodized oil for control of iodine deficiency in children. *British Journal of Nutrition* **84**, 139-141.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000b) Iron supplementation in goitrous, iron-deficient children improves their response to oral iodized oil. *European Journal of Endocrinology* **142**, 217-223.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000c) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. *American Journal of Clinical Nutrition* **71**, 88-93.

Zimmermann M, Saad A, Hess S, Torresani T & Chaouki N (2000d) Thyroid ultrasound compared with World Health Organization 1960 and 1994 palpation criteria for determination of goiter prevalence in regions of mild and severe iodine deficiency. *European Journal of Endocrinology* **143**, 727-731.

Zimmermann M, Moretti D, Chaouki N & Torresani T (in press) Development of dried whole blood spot thyroglobulin assay and its evaluation as an indicator of thyroid status in goitrous children receiving iodized salt. *American Journal of Clinical Nutrition*.

Zimmermann MB, Adou P, Torresani T, Zeder C & Hurrell RF (2000e) Effect of oral iodized oil on thyroid size and thyroid hormone metabolism in children with concurrent selenium and iodine deficiency. *European Journal of Clinical Nutrition* **54**, 209-213.

Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess S & Delange F (2001a) Toward a consensus on reference values for thyroid volume in iodine-replete schoolchildren: results of a workshop on inter-observer and inter-equipment variation in sonographic measurement of thyroid volume. *European Journal of Endocrinology* **144**, 213-220.

Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess S & Delange F (2001b) Updated provisional WHO/ICCIDD reference values for sonographic thyroid volume in iodine-replete school-age children. *IDD Newsletter* **17**, 12.

Zimmermann MB (2002) Iron status influences the efficacy of iodine prophylaxis in goitrous children in Côte d'Ivoire. *International Journal for Vitamin and Nutrition Research* **72**, 19-25.

Zimmermann MB & Hurrell RF (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Current Opinion in Biotechnology* **13**, 142-145.

Zimmermann MB & Köhrle J (2002) The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. *Thyroid* **12**, 867-878.

Treatment of iron deficiency in goitrous children improves the efficacy of iodized salt in Côte d'Ivoire

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***American Journal of Clinical Nutrition* 75, 743-748, 2002.**

This study was supported by the Thrasher Research Fund (Salt Lake City, USA), the Swiss Federal Institute of Technology (Zürich) and the Foundation for Micronutrients in Medicine (Rapperswil, Switzerland).

Abstract

Background: In many developing countries, children are at high risk for both goiter and iron-deficiency anemia. Iron deficiency adversely effects thyroid metabolism and may reduce the efficacy of iodine prophylaxis in areas of endemic goiter.

Objective: The aim of this study was to determine if iron supplementation in goitrous, iron-deficient children would improve their response to iodized salt.

Design: We conducted a prospective randomized double-blind placebo-controlled trial in 5-14 yr-old children in western Côte d'Ivoire. Goitrous, iron-deficient children (n=166) consuming iodized salt (10-30 mg iodine/kg salt at household level) received either iron supplementation (60 mg iron/day, 4 days/week for 16 weeks) or placebo. To ensure a constant supply of iodine during the study, half of the children in both groups were given an additional single oral dose of 200 mg iodine as iodized oil. At 0, 1, 6, 12 and 20 weeks, hemoglobin, serum ferritin and transferrin receptor, whole blood zinc protoporphyrin, thyrotropin, thyroxine, urinary iodine and ultrasonographic thyroid gland volume were measured.

Results: Hemoglobin and iron status at 20 weeks were significantly better after iron treatment than after placebo (P<0.05). At 20 weeks, the mean reduction in thyroid size in the iodine+iron group was nearly twice that of the iodine+placebo group [mean (SD) percentage change in thyroid volume from baseline= –22.8 (10.7)% vs. –12.7 (10.1)%] (P<0.001). At 20 weeks, goiter prevalence was 43% in the iodine+iron group vs. 62% in the iodine+placebo group (p<0.05). There were no significant differences in TSH and T_4 at baseline or during intervention.

Conclusions: Iron supplementation improves the efficacy of iodized salt in goitrous children with iron deficiency. A high prevalence of iron deficiency among children in areas of endemic goiter may reduce the effectiveness of iodine prophylaxis.

Introduction

Iodine deficiency produces a spectrum of disorders - endemic goiter, hypothyroidism, cretinism, and congenital anomalies - that are termed the iodine deficiency disorders (IDD) (1). In western and central Africa, it is estimated 250 million people are at risk for IDD and 50 million have goiter (2). Universal salt iodization is the preferred strategy for IDD control (1). In iodine-deficient areas, multiple nutritional factors, including goitrogenic foods, protein-calorie malnutrition, and selenium deficiency may influence the prevalence and severity of IDD and modify response to iodine prophylaxis (3-5).

Iron status also has an impact on thyroid metabolism and IDD. The two initial steps of thyroid hormone synthesis are catalyzed by thyroid peroxidase and are dependent on iron. In addition, iron deficiency may alter central nervous system control of thyroid metabolism (6) and modify nuclear triiodothyronine (T_3) binding (7). Iron-deficiency anemia (IDA) decreases plasma thyroxine (T_4) and T_3 levels, reduces peripheral conversion of T_4 to T_3, and increases circulating thyroid stimulating hormone (TSH) (6,8,9). In goitrous children, the therapeutic response to oral iodized oil is impaired in children with iron-deficiency anemia (IDA), compared to iron-sufficient children (10). In addition, in an open, uncontrolled trial, iron treatment of goitrous children with IDA improved their response to oral iodized oil (11).

Deficiencies of iron and iodine are major overlapping public health problems in the developing world, and many children are at high risk for both goiter and iron-deficiency anemia. In western Côte d'Ivoire, 30-50% of school-aged children are goitrous and 37-47% are iron deficient (11). Therefore, the aim of this study was to determine if iron treatment could increase the efficacy of iodized salt and oral iodized oil in children with both goiter and iron deficiency.

Subjects and Methods

The study was done in nine primary schools of the Danané Health District, an area of endemic goiter in the mountains of western Côte d'Ivoire (11). The University Children's Hospital in Zürich and the Ministry of Research of Côte d'Ivoire gave ethical approval for the study. Informed oral consent was obtained from the village chiefs and the childrens' teachers and families. In 1997, the median urinary iodine concentration (UI) and the goiter rate by palpation in school-aged children in this region were 28 μg/L and 45%, respectively (11), indicating moderate-severe IDD (1).

Côte d'Ivoire began a universal salt iodization program in 1997 at a production level 30-50 ppm. In late 1998, iodized salt was introduced into the Danané region. Access to iodized salt in this region had steadily increased so that by November 1999, it was estimated that >80% of households were using iodized salt at a household level of 20-30 ppm (unpublished data, P. Adou, National Institute of Public Health of Côte d'Ivoire). This study was done from November 1999 through June 2000.

Screening study

All children in the nine schools were screened (n=1014). Weight and height were measured, and spot urine samples for collected for measurement of UI. Thyroid gland volume (Tvol) was measured using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5 MHz linear transducer (12). Measurements were performed on subjects sitting upright with the neck extended. Blood was collected by venipuncture for determination of hemoglobin (Hb), whole-blood zinc protoporphyrin (ZPP), serum ferritin (SF) and serum transferrin receptor (TfR). Blood was spotted onto filter paper for measurement of thyrotropin (TSH) and thyroxine (T_4). Random salt samples (n=213) from households of children in the screening were collected for determination of iodine concentration.

Intervention study

From the screening, all children who were both goitrous and iron-deficient (using criteria described below) were invited to join a double-blind intervention study. Children with Hb <80 g/L were excluded and treated with oral iron. The remaining children (n=169) were randomized to two groups. One group received oral ferrous sulfate (60 mg elemental iron) 4 tablets/week for 16 weeks; the second group received identical-appearing placebo tablets. The teachers gave the tablets to the children at school at mid-morning with water. Pill counts were done at 6, 12 and 20 weeks to determine compliance. At baseline, half of the children in each group were randomly selected to also receive a single oral dose of 0.4 ml iodized poppyseed oil (Lipiodol, Guerbet, France) containing 200 mg of iodine (13). All children enrolled in the study received a single 400 mg oral dose of albendazole (Zentel, SmithKline Beecham) at baseline.

At baseline, 1, 6, 12 and 20 weeks, spot urines were collected for measurement of UI, and dried blood spots for determination of TSH and T_4. At baseline, 6, 12, and 20 weeks, weight, height and ultrasonographic Tvol were measured, and at baseline, 12 and 20 weeks, Hb, SF, TfR and ZPP were measured. Salt samples were collected

from random households (n=45) of both groups of children at 1, 12 and 20 weeks. On completion of the study, the children who had received placebo and remained anemic were treated with iron.

Laboratory analyses

Serum and urine samples were aliquoted and frozen at –20^{o} C until analysis. UI was measured using a modification of the Sandell-Kolthoff reaction (14). Salt iodine content was measured by titration with sodium thiosulphate (15). Dried blood spots on filter paper were analysed for whole blood TSH and serum T_4 using immunoassay (16). Hb was measured using a AcT8 Counter (Beckman Coulter, Krefeld, Germany). ZPP was measured on washed red blood cells using a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA). SF and TfR were measured using enzyme-linked immunosorbent assay (17,18). Iron deficiency was defined using multiple criteria (19): SF < 15 µg/L; or TfR > 8.5 mg/L + ZPP > 40 µmol/mole heme; or TfR/SF ratio >500 (18). Anemia was defined as Hb < 120 g/L in children aged ≥12 yrs, and Hb < 115 g/L in children aged 5-12 yrs (20). Tvol was calculated using the method of Brunn et al (21). S.H. or M.Z. performed the ultrasound measurements during the screening, and S.H. performed all ultrasound measurements during the intervention. To estimate intra- and interobserver variability, S.H. measured 20 children twice and M.Z. measured the same children once. The mean (SD) intra- and interobserver errors were 4.9 (4.0)% and 3.7 (3.5)%, respectively. Because current World Health Organization/ International Council for the Control of Iodine Deficiency Disorders (WHO/ICCIDD) normative values for Tvol (12) are being revised, we used previous ICCIDD references for school-age children according to sex and age to define the presence or absence of goiter (22).

Statistical analyses

Data processing and statistics were done using SPLUS 2000 (Mathsoft, Seattle, USA), Prism3 (GraphPad, San Diego, USA) and Excel (Microsoft, Seattle, WA, USA). Normally distributed data were expressed as means (SD) and were compared by Student‘s *t* test. Parameters not normally distributed were expressed as medians and ranges, and were compared by Wilcoxon and Mann-Whitney tests. A 2-factor repeated measures ANOVA was done to compare effects of time and group and time by group for Hb, indices of iron status, UI, TSH, T_4, Tvol, and percentage change in Tvol (%ΔTvol). Logistic regression was done to compare effects of time and group and time by group for binary variables of goiter, anemia, and iron deficiency. Multiple regression was used to test for associations.

Results

The results of the screening are shown in Table 1. The median UI (range) was 162 (16-1017) µg/L. Only 1% and 3% of the children had a UI <20 µg/L and <50µg/L, respectively. Mean salt iodine content (SD) was 25.2 (18) ppm. Despite adequate UI and salt iodine levels, the prevalence of goiter by ultrasound was 58.6%. The median TSH and the mean serum T_4 were within the normal reference range; only 3% of the children had an elevated TSH and 1% had a low serum T_4. The prevalence of iron deficiency was 38% and 224 (23%) children were both goitrous and iron deficient.

Table 1. Characteristics of children (n=1017) at screening.

Characteristic	Value
Age (yr)	8.9 ± 2.5 (4 – 16)
Sex (M / F)	698 / 316
BMI (kg/m^2)	15.2 ± 1.5
No. subjects with goiter	594 [59]
Urinary iodine (µg/L)[1]	162 (16 – 1017)
Whole blood thyrotropin (mU/L)[2]	0.7 (0.2 – 4.4)
Serum thyroxine (nmol/L)[2]	126 ± 29
Hemoglobin (g/L)	120 ± 13
No. of subjects with iron deficiency	364 [38]
No. of subjects with iron-deficiency anemia	178 [19]
No. of subjects with iron-deficiency + goiter	224 [23]

As means ±SD or medians (range). Percentages in brackets.
[1]Measured in 400 randomly-selected children. [2]Measured in 160 randomly-selected children.

Characteristics of the iron-treated and placebo groups at basline are compared in Table 2. There were no significant differences in measured baseline characteristics between groups. Of the 169 children who began the study, 166 completed it. Three children moved away and could not be found (all in the placebo group). Pill counts

estimated compliance to be >90% in both the iron-treated and control groups. The median iodine concentration (range) in the salt samples from random households in the iron-treated and placebo groups at 1, 12 and 20 weeks was 20.1 (4.3 - 86.6), 16.1 (9.5 - 40.2), and 12.8 (7.6 - 67.3) ppm, respectively. Within both the iron-treated and placebo groups, comparing the subgroups of children who received the additional iodized oil to those who consumed only iodized salt, we found no significant differences in Hb, iron status indicators, TSH, T_4, percentage change in thyroid volume from baseline (%ΔTvol) or goiter prevalence at 6, 12 or 20 weeks (data not shown). Therefore, we combined the subgroups and compared iron+iodine to placebo+iodine in the final data analyses.

Table 2. Baseline characteristics of the iron-treated and control children

Characteristic	**Iron-treated (n=85)**	**Placebo (n=81)**
Age (yrs)	8.5 ± 1.9	8.5 ± 2.2
Sex (M / F)	60 / 25	57 / 24
BMI (kg/m^2)	15.7 ± 1.2	15.4 ± 1.6
Hemoglobin (g/L)	110 ± 10	110 ± 11
Serum ferritin (μg/L)	52.0 ± 34.2	46.7 ± 25.7
Serum transferrin receptor (mg/L)	14.0 ± 5.2	13.3 ± 4.8
Serum transferrin receptor/ferritin	513 ± 883	519 ± 861
Whole-blood zinc protoporphyrin (μmol/mol heme)	59 ± 26	70 ± 52
Urinary iodine (μg/L)	143 (24-814)	156 (22-788)
Whole-blood thyrotropin (mU/L)	0.5 (0.3-6.0)	0.5 (0.2-2.0)
Serum thyroxine (nmol/L)	109 ± 30	121 ± 39
Thyroid volume (ml)	5.6 (3.5-16.4)	5.8 (3.4-24.7)

There were no significant differences in baseline characteristics between groups.
As means ±SD or medians (range).

Table 3. Change in hemoglobin and prevalence of anemia and iron deficiency in iron-treated (n=85) and placebo (n=81) groups

	Hemoglobin (g/L)[1]		No. of anemic children[2]		No. of iron-deficient children[2]	
Time	Iron	Placebo	Iron	Placebo	Iron	Placebo
0 wk baseline	110 ± 10	109 ± 11	71 [83]	70 [87]	85 [100]	81 [100]
12 wk	118 ± 10	114 ± 11	50 [59]	55 [67]	51 [60][6]	53 [66][6]
20 wk	124 ± 9[3,6]	115 ± 10	28 [33][4,6]	51 [63][5]	33 [39][3,6]	42 [52][6]

[1] Means±SD. [2] Percentages in brackets.
[3] p <0.05 vs. placebo; [4] p <0.01 vs. placebo
[5] p <0.05 vs. baseline; [6] p <0.01 vs. baseline

Iron treatment significantly increased mean Hb and significantly decreased the prevalence of iron deficiency ($p<0.01$ vs. baseline, $p<0.05$ vs. placebo at 20 weeks) (Table 3). At 20 weeks, comparing the iron-treated group to placebo, mean ZPP (SD) was 59 (26) vs. 70 (52) μmol/mol heme ($p<0.05$); mean SF (SD) was 80.2 (39.6) vs. 67.1 (38.3) μg/L ($p<0.05$); mean TfR (SD) was 10.4 (5.3) vs. 10.7 (3.5) mg/L (N.S.) and mean TfR/SF (SD) was 183 (172) vs. 452 (527) ($p<0.05$). Iron treatment had no measured effect on growth; there was no significant difference in weight, height or BMI between the iron and placebo groups at 0, 6, 12 or 20 weeks.

Changes in Tvol and goiter prevalence in the iron-treated and placebo groups are shown in Table 4. At 12 and 20 weeks, Tvol and goiter rate were significantly reduced in the iron-treated group compared to placebo. At 20 weeks the mean %ΔTvol in the iron-treated and placebo groups was –22.8 (10.7)% and –12.7 (10.1)%, respectively ($p<0.01$ between groups). This difference was reflected in the goiter rate at 20 weeks, which was 43% in the iron-treated group and 62% in the placebo group. As modeled by logistic regression, the probability of goiter was significantly lower in the iron-treated group, and the group difference increased with time ($p<0.05$ comparing time and group model relative to time only model).

Table 4. Changes in thyroid volume and goiter prevalence in schoolchildren treated with iron or placebo at 6, 12, and 20 wk after baseline

Thyroid volume	Iron-treated (n=85)	Placebo (n=81)
Baseline (mL)	5.6 (3.5-16.4)	5.8 (3.4-24.7)
6 wk (mL)	5.6 (2.9-15.4)	5.8 (2.9-22.5)
%change from baseline	-0.9 ± 13.4	3.4 ± 13.5
No. subjects with goiter	58 [68]	64 [78]
12 wk (mL)	4.9 (2.5-16.0)[1]	5.2 (2.4-22.7)
%change from baseline	-13.2 ± 11.6	-7.9 ± 11.1
No. subjects with goiter	46 [54]	51 [63]
20 wk (mL)	4.3 (2.1-12.9)[2,4]	5.1 (2.1-21.4)[1]
%change from baseline	-22.8 ± 10.7[5]	-12.7 ± 10.1
No. subjects with goiter	37 [43]	50 [62]

As means ±SD or medians (range). Percentages in brackets. To reduce the effects of variability among individuals, % change from baseline was calculated for each child before deriving means.
[1] p <0.05 vs. baseline; [2] p < 0.01 vs. baseline.
[3] p <0.05 vs. placebo; [4] p < 0.01 vs. placebo; [5] p < 0.001 vs. placebo

Table 5 shows the TSH, T_4, and UI of the iron-treated and control groups over the 20 weeks of follow-up. Median TSH and mean serum T_4 were within the normal range in both groups at baseline and throughout the study. The median UI throughout the study in the children consuming iodized salt alone was well above the WHO/ICCIDD cut-off value (100 µg/L) for risk of iodine deficiency (1). In the groups that received the additional dose of oral iodized oil, UI was significantly increased at 1 week ($p<0.01$), and at 6, 12, and 20 weeks ($p<0.05$).

Multiple regression of %ΔTvol at 20 weeks on group, baseline characteristics (in Table 2), and change in Hb from baseline to 20 weeks (ΔHb) was done. The regression of %ΔTvol at 20 weeks on group was significant ($p<0.0001$). There was a significant effect (beyond group) of height, weight, and BMI, as well as baseline Hb, ΔHb and baseline Tvol. Regression applied to bootstrapped data consistently selected group, baseline height, baseline Tvol, baseline Hb, and ΔHb as significant predictors for % ΔTvol (multiple $R^2 = 0.27$, $p < 0.0001$).

Table 5. Changes in whole blood thyrotropin, serum thyroxine and urinary iodine in iron-treated and placebo children after baseline

	Thyrotropin (mU/L)		**Thyroxine (nmol/L)**		**Urinary iodine (μg/L)**			
Time	Iron	Placebo	Iron	Placebo	Iron + oral iodine	Iron + iodized salt	Placebo+ oral iodine	Placebo+ iodized salt
0 wk (baseline)	0.5 (0.3-6.0)	0.5 (0.2-2.0)	109 ± 30	121 ± 39	131 (24-819)	155 (35-449)	161 (38-788)	151 (22-652)
1 wk	0.6 (0.2-3.8)	0.6 (0.3-2.4)	99 ± 29	105 ± 25	690[2,4] (62-2889)	178 (15-1013)	336[2,3] (82-2899)	140 (33-676)
6 wk	0.6 (0.3-2.0)	0.6 (0.2-1.9)	102 ± 24	106 ± 30	208[3] (33-1032)	176 (33-1129)	274[1,4] (28-1019)	179 (26-898)
12 wk	0.7 (0.1-2.3)	0.7 (0.2-2.4)	121 ± 25	120 ± 32	163[1,3] (10-664)	128 (13-505)	193[1] (27-963)	135 (25-373)
20 wk	0.7 (0.7-4.2)	0.8 (0.2-4.2)	105 ± 25	104 ± 29	164[1,3] (25-625)	110 (17-271)	179[1] (42-484)	125 (23-445)

As means±SD or medians (range).
[1] p <0.05 vs. iodized salt; [2] p <0.01 vs. iodized salt
[3] p <0.05 vs. baseline; [4] p <0.01 vs. baseline

Children with larger thyroids at baseline tended to have a greater %ΔTvol, while taller children, more severely anemic children and those with a poorer response to iron treatment tended to have a smaller %ΔTvol.

Discussion

In this study, iron supplementation improved the efficacy of iodized salt and oral iodized oil in goitrous children with iron deficiency. In our previous studies in goitrous children in Côte d'Ivoire, the therapeutic response to oral iodized oil was impaired in children with iron-deficiency anemia (IDA), compared to iron-sufficient, non-anemic children (10). In addition, in an open, uncontrolled trial, iron treatment of goitrous children with IDA improved their response to oral iodized oil (11). However, the mechanism by which iron deficiency influences response to iodine in children with IDD is unclear. Iron deficiency impairs thyroid metabolism in animal and human studies (6-9). In rats, iron-deficiency anemia lowers plasma thyroid hormone levels, reduces activity of hepatic thyroxine-5-deiodinase, impairs peripheral conversion of T_4 to triiodothyronine (T_3), and blunts the TSH response to thyrotropin-releasing hormone (6,7). Compared to healthy controls, iron-deficient adults have lower circulating T_4 and T_3 levels (8,9) and higher TSH concentrations (8). Iron deficiency may influence IDD through alterations of central nervous system control of thyroid metabolism (6) or through modification of nuclear T_3 binding (7). Also, the initial steps of thyroid hormone synthesis —iodide incorporation into tyrosine residues of thyroglobulin and covalent bridging of the residues— are catalyzed by heme-containing thyroid peroxidases. Other iron-containing enzymes (e.g. cytochrome oxidase, myeloperoxidase and succinate-ubiquinone oxidoreductase) are sensitive to depletion of iron deficiency (23). Theoretically, severe iron deficiency could lower thyroperoxidase activity in the thyroid and interefere with thyroid hormone synthesis.

We gave half the children in both the iron-treated and control groups a single 200 mg dose of oral iodine (13) in addition to their daily iodine intake from salt. We were concerned about potential fluctuations in iodine intake in the subjects from iodized salt alone, in a region where transportation, food supply and infrastructure are precarious. The iodized oil was given to ensure that at least half the children would have an ample and steady supply of iodine during the study period. In hindsight, this was unnecessary. Median UI remained adequate (<100 μg/L) (1) throughout the study in the children consuming only iodized salt. The additional iodine as iodized oil increased UI significantly but otherwise had no discernible effect. Within both the iron-treated and

placebo groups, comparing children who received iodized oil to those who consumed only iodized salt, we found no significant differences in TSH, T4, %ΔTvol or goiter rate at 6, 12 and 20 weeks.

The high prevalence of malaria and gastrointestinal infections in children in rural Côte d'Ivoire both contributes to and complicates the diagnosis of iron deficiency in this population (24). Therefore, we used multiple iron status indicators (SF, TfR, ZPP) to confirm iron deficiency at baseline and monitor response to iron supplementation (19). Because we wished to investigate the influence of iron status and not anemia *per se* on response to iodine, we included both iron-deficient and iron-deficient anemic children in the study. By regression, baseline Hb negatively correlated with %ΔTvol in both the iron-treated and control groups, while improvement in Hb from baseline to 20 weeks was positively associated with %ΔTvol. This suggests that iodine was less efficacious in children with more severe anemia at baseline and in those with a poorer response to iron. In a previous study, we also found a strong correlation between severity of IDA and %ΔTvol after oral iodized oil (10).

The high prevalence of malaria and other infections also blunts the response to iron repletion in anemic African children (25). In the present study, response to iron was only clearly evident after 16 weeks of supplementation. Moreover, reductions in thyroid size lag behind improvements in thyroid function during introduction of iodized salt in an area of endemic goiter (26). For these reasons, the impact of iron treatment on thyroid size may have been greater if follow-up had been longer. We did not extend the study past 20 weeks because we wanted to limit the delay in iron treatment of the iron-deficient children in the placebo group (27).

The significant improvement in iron status in the placebo group compared to baseline (Table 3) was likely due to several factors. First, we explained to the parents that the children were enrolled in the study because they were sick due to poor nutrition. This may have precipitated a change in feeding patterns at home; for example, the children may have received a greater share of the small amounts of meat available at mealtimes. Second, the availability of mango and pineapple increases during spring months in rural Côte d'Ivoire, so it is possible that intakes of ascorbic acid (a potent enhancer of iron absorption (28)) increased over the course of the study. Third, all of the children were dewormed at the beginning of the study. This is likely to have reduced iron losses from hookworm and other parasitic infections endemic in this region and may have contributed to the improvement in iron status (25).

Our findings suggest a high prevalence of iron deficiency among children in areas of endemic goiter may reduce the effectiveness of iodized salt programs. In developing countries, it is estimated that 40-45% of school-age children are anemic (29), of which approximately 50% is due to iron deficiency. Children are also highly vulnerable to iodine deficiency and are one of the main target groups of iodized salt programs (1). These deficiencies often coexist— in regions of West and North Africa, 20-25% of school-age children suffer from both goiter and iron-deficiency anemia (10,30). Our findings argue strongly for improving iron status in areas of overlapping deficiency, not only to combat anemia but also to increase the efficacy of iodine prophylaxis.

Acknowledgements

We would like to thank the participating children and the teachers; Mr. JB Gbato (Public Health, Côte d'Ivoire); Dr L Molinari (Children's Hospital, Zürich, Switzerland) for statistical assistance; and Carol Flowers (University of Kansas Medical Center, Kansas City, USA) for assistance with the laboratory analyses.

References

1. World Health Organization, United Nations Children's Fund & International Council for the Control of Iodine Deficiency Disorders. Indicators for Assessing Iodine Deficiency Disorders and their Control through Salt Iodization. Geneva: WHO; WHO/NUT 94.6,1994.
2. Bailey KV, Clugston GA. Iodine deficiency disorders. In: Murray CJL, Lopez AD, eds. The Global Burden of Disease and Risk Factors in 1990. WHO/World Bank. Geneva, World Health Organization.
3. Boyages SC. Iodine deficiency disorders. J Clin Endocrinol Metab 1993;77:587.
4. Thilly CH, Vanderpas JB, Bebe N, et al. Iodine deficiency, other trace elements and goitrogenic factors in the etiopathology of iodine deficiency disorders. Biol Trace Elem Res 1992;32:229-43.
5. Zimmermann MB, Torresani T, Adou P, Zeder C, Hurrell RF. Effect of oral iodized oil on thyroid size and thyroid hormone metabolism in children with concurrent selenium and iodine deficiency. Eur J Clin Nutr 2000;54:209-13.
6. Beard JL, Brigham DE, Kelley SK, Green MH. Plasma thyroid hormone kinetics are altered in iron-deficient rats. J Nutr 1998;128:1401-8.
7. Smith SM, Finley J, Johnson LK, Lukaski HC. Indices of in vivo and in vitro thyroid hormone metabolism in iron-deficient rats. Nutr Res 1994;14:729-39.
8. Beard JL, Borel MJ, Derr J. Impaired thermoregulation and thyroid function in iron-deficiency anemia. Am J Clin Nutr 1990;52:813-9.
9. Dillman E, Gale C, Green W, Johnson DG, Mackler B, Finch C. Hypothermia in iron deficiency due to altered triiodothyronine metabolism. Am J Physiol 1980;239:R377-81.
10. Zimmermann MB, Adou P, Zeder C, Torresani T, Hurrell RF. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. Am J Clin Nutr 2000;71:88-93.
11. Zimmermann MB, Adou P, Torresani T, Zeder C, Hurrell RF. Iron supplementation in goitrous, iron-deficient children improves their response to oral iodized oil. Eur J Endocrinol 2000;142:217-23.

12. World Health Organization & International Council for the Control of Iodine Deficiency Disorders. Recommended normative values for thyroid volume in children aged 6-15 years. Bull World Health Organ 1997;75:95-97.
13. Zimmermann MB, Adou P, Torresani T, Zeder C, Hurrell RF. Low dose oral iodized oil for control of iodine deficiency in children. Br J Nutr 2000;84:139-41.
14. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem 1996;42:239-43.
15. Sullivan KH, Houston R, Gorstein J, Cervinskas J. Monitoring Universal Salt Iodization Programmes. 1995; PAMM/MI/ICCIDD, Atlanta, USA.
16. Torresani T, Scherz R. Thyroid screening of neonates without use of radioactivity: evaluation of time-resolved fluoroimmunoassay of thyrotropin. Clin Chem 1986;32:1013-16.
17. Flowers CH, Kuizon M, Beard J, Skikne B, Covell A, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141-51.
18. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990;75:1870-76.
19. Cook JD, Baynes RD, Skikne BS. Iron deficiency and the measurement of iron status. Nutr Res Rev 1992;5:189-202.
20. United Nations Children's Fund/World Health Organization/United Nations University /Micronutrient Initiative Technical Workshop (1998). Preventing iron deficiency in women and children: background and consensus on key technical issues and resources for advocacy, planning and implementing national programmes. 1999, International Nutrition Foundation, Canada.
21. Brunn J, Block U, Ruf G, Bos I, Kunze WP, Scriba PC. Volumetrie der Schilddrüsenlappen mittels Real-time-Sonographie. Deutsch Med Wochenschrift 1981;106:1338-40.
22. Gutekunst R, Martin-Teichert H. Requirements for goiter surveys and the determination of thyroid size. In: Delange F, Dunn JT, Glinoer D (Eds.) Iodine Deficiency in Europe: A Continuing Concern. 1993, New York: Plenum Press.
23. Murakawa H, Bland CE, Willis WT, Dallman PR. Iron deficiency and neutrophil function: different rates of correction of the depression in oxidative burst and myeloperoxidase activity after iron treatment. Blood 1987;69:1464-8.
24. Staubli-Asobayire F, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency, with or without concurrent anemia, in population groups with a high prevalence of malaria and other infections: a study in Côte d'Ivoire. Am J Clin Nutr (in submission)
25. International Nutritional Anemia Consultative Group/World Health Organization/United Nations Children's Fund. Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia. 1998, INACG, Washington, DC.
26. Zhao J, Xu F, Zhang Q et al. Randomized clinical trial comparing different iodine interventions in children. Publ Health Nutr 1999;2:173-8.
27. Walter T. Effect of iron deficiency anaemia on cognitive skills in infancy and childhood. In: Hallberg L, Asp N-G. Iron Nutrition in Health and Disease, 1996, John Livvey & Co., London.
28. Ballot D, Baynes RD, Bothwell TH et al. The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr 1987;57:331-43.
29. World Health Organization/United Nations Children's Fund/United Nations University. IDA. Prevention, assessment and control. Report of a joint WHO/UNICEF/UNU consultation. 1998; Geneva, World Health Organization.
30. Zimmermann M, Saad A, Hess S, Torresani T, Chaouki N. Thyroid ultrasound compared to WHO 1960 and 1994 palpation criteria for determination of goiter prevalence in regions of mild and severe iodine deficiency. Eur J Endocrinol 2000;143:727-31.

Iron deficiency anemia reduces thyroid peroxidase activity in rats

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Journal of Nutrition **132, 1951-1955, 2002**

Supported by the Swiss Foundation for Nutrition Research and the Swiss Federal Institute of Technology in Zürich, Switzerland.

Abstract

Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism. However, the mechanism is not yet clear. The objective of this study was to investigate if iron (Fe) deficiency lowers thyroid peroxidase (TPO) activity. TPO is a heme-containing enzyme catalyzing the two initial steps in thyroid hormone synthesis. Male weanling Sprague-Dawley rats (n=84) were assigned to seven groups. Three groups (ID-3, ID-7, ID-11) were fed a Fe-deficient diet containing 3, 7 and 11 μg Fe/g, respectively. Because IDA reduces food intake, three groups were pair-fed to each of the ID groups and one control group was fed ad libitum, all with Fe-sufficient diets (35 μg Fe/g). After 4 weeks, mean hemoglobin, triiodothyronine (T_3) and thyroxine (T_4) were significantly lower in the Fe-deficient groups than in the control group ($p<0.001$). Food restriction had a highly significant, independent effect on T_4 ($p<0.0001$), but not on T_3. TPO activity (by both guaiacol and iodine assays) was markedly reduced by food restriction ($p<0.05$). IDA also independently and significantly reduced TPO activity ($p< 0.05$). Compared to ad libitum fed controls, mean TPO activity in GU/ total thyroid in the ID-3, ID-7, and ID-11 groups was decreased by 56, 45 and 33%, respectively. These data indicate Fe deficiency sharply reduces TPO activity, and suggest that decreased TPO activity contributes to the adverse effects of IDA on thyroid metabolism.

Introduction

Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism. IDA decreases plasma total thyroxine (T_4) and triiodothyronine (T_3) concentrations, reduces peripheral conversion of T_4 to T_3, and may increase circulating thyrotropin (TSH) (1-8). In regions of endemic goiter, the thyroid response to iodized oil is impaired in children with IDA, compared to Fe-sufficient children (9). In addition, Fe supplementation of goitrous children with IDA improves the efficacy of iodized oil and iodized salt (10-11).

The mechanism by which Fe status influences thyroid and iodine metabolism is unclear. IDA could impair thyroid metabolism through anemia and lowered oxygen transport (12-13). IDA may also alter central nervous system control of thyroid metabolism (14) and nuclear T_3 binding (15). Another potential mechanism is impairment of thyroid peroxidase (TPO) activity. TPO is a 103-kd Fe-dependent enzyme located at the apical membrane of the thyrocyte (16). TPO catalyzes the first two steps of thyroid hormone synthesis, iodination of thyroglobulin and coupling of the iodotyrosine residues (17). TPO activity requires a heme protein attached to ferriprotoporphyrin IX or a closely-related porphyrin (18-19). IDA lowers activity of other heme-containing enzymes: cytochrome oxidase, myeloperoxidase and succinate-ubiquinone oxidoreductases are all sensitive to depletion during Fe deficiency (20). Similarly, IDA could lower TPO activity and thereby interfere with iodine metabolism in the thyroid. Therefore, the aim of this study was to investigate if TPO activity is decreased in Fe-deficient anemic rats.

Materials and Methods

Animals and Diets

The Veterinary Department of the Canton of Zurich gave ethical approval for the study. Male weanling Sprague-Dawley rats (ZUR:SD, Institut für Labortierkunde, University Zurich) were randomly assigned to 7 groups (n=12 in each group) at 21 days of age. Three groups were assigned to receive low Fe diets of 3, 7 and 11 μg Fe/g, respectively, and three groups were pair-fed to each of these Fe-deficient groups. The pair-fed groups and one ad libitum group were fed a normal Fe diet containing 35 μg Fe/g. The low Fe and normal Fe diets were prepared by Dyets Inc. (Bethlehem, PA, USA). Other than their Fe content, the diets were equivalent and

conformed to the recommendations for AIN-93 purified diets of the American Institute of Nutrition (21). Fe content of all diets at baseline was confirmed by atomic absorption spectroscopy (SpecrAA-300/400 with GTA-96 Graphite Tube Atomizer, Varian Techtron Pty. Ltd. Mulgrave, Victoria, Australia). Each pair of anemic and pair-fed rats was matched by body weight. Animals were individually housed in plastic cages with grated stainless steel floors in random order. The rats were kept under controlled conditions at 21°C temperature and 55% humidity with a daily 12 hour light : dark cycle. Millipore water (Milli-Q UF Plus, Millipore, Bedford, USA) was provided for all animals ad libitum. To prepare the animals and reduce stress response at the time of sacrifice, all rats were picked up and handled daily. After a feeding period of 29 days, pentobarbital anesthesia (0.16 mg / g body weight) was induced intraperitoneally by injection. Blood was collected by cardiac puncture into EDTA-coated tubes and the rats killed by exsanguination. Thyroids were immediately dissected and removed, wrapped in aluminum foil, shock frozen in liquid nitrogen and stored at –60 °C.

Laboratory Analysis

TPO preparation and analysis was done using a modified mini-assay method of Hosoya et al. (22). The thyroids were thawed, washed three times with cold saline, blotted on filter paper and weighed. They were then repeatedly manually homogenized with a glass pestle in 30 µg/L buffer A (0.25 M sucrose, 20nM Tris-HCl, pH 7.4, 100 mM KCl, 40mM NaCl, 10 mM $MgCl_2$) / mg original tissue and cooled in ice between each repetition. After centrifugation, the pellet was again homogenized in buffer A and centrifuged. The combined supernatants were ultracentrifuged at 25.900 x g and 4°C for 4 hours. The pellet was suspended in 30 µg/L buffer A / mg original tissue and solubilized in an ice-cold ultrasound bath. This procedure resulted in a higher enzyme activity than the recommended treatment with desoxycholate and/or trypsin (22-24). Therefore, the reaction mixture was not treated with desoxycholate. TPO activity was measured by the method using guaiacol and iodide as the second substrate (22). After ultracentrifugation, supernatants of ID groups and CN group (n=2) had no measurable TPO activity by the guaiacol and iodine assay. For the guaiacol assay, the reaction mixture had a total volume of 450 µL and contained 33 mM guaiacol, 0.27 mM H_2O_2, 33 mM sodium phosphate buffer (pH 7.4) and a total of 50 µL of enzyme mixture. The reaction was started by the addition of 10 µL H_2O_2 and followed spectrophotometrically at 470 nm and 25 °C. For the iodide assay, the reaction mixture contained 0.135 mM H_2O_2, 4.95 mM potassium iodide, 33 mM sodium phosphate buffer (pH 7.0), 50 µL of enzyme mixture and had a total

volume of 400 µL. After starting the reaction with 10 µL of H_2O_2, the reaction was followed spectrophotometrically at 350 nm and 25 °C. For both assays, the total of 50 µL of enzyme mixture contained three different volumes of sample solution (20, 30, 40 µL). One guaiacol unit (GU) and iodide unit (IU), respectively, represent the amount of enzyme that produced a change of 1.0 optical density unit per second. Protein concentration was determined using the advanced protein assay (Cytoskeleton, Inc., Denver, CO, USA). Hemoglobin (Hb) concentration was measured in triplicate in whole blood using the cyanmethemoglobin method (Sigma Diagnostics, St. Louis, MO, USA). Whole blood samples were centrifuged and plasma samples were stored at –20°C. Total T_4 and total T_3 plasma concentrations were determined by radioimmunoassay kits for veterinary use (Immunotech S.A., Marseille, France).

Statistical Analysis

Data processing and statistics were done using SPSS 10.0 (SPSS Inc., Chicago, USA). Data were analyzed by a one-way ANOVA across diets. Post-hoc comparisons were performed by Tukey's test to detect significant differences among means. Linear regression was done to compare effects of IDA and food restriction using Hb and mean daily food intake, respectively, as an indicator.

Results

One rat in the PF-3 group died of a nonspecific illness on day 27. Three thyroids (ID-3, ID-7, PF-11) were discarded because of incomplete dissection. There were no significant differences in body weight between groups at baseline (mean body weight ± SD = 52.0 ± 9.5g). Mean Fe content (± SD) of the diets was 2.6 ± 0.4 µg/g (ID-3), 7.0 ± 0.3 (ID-7), 10.5 ± 1.5 (ID-11) and 31.1 ± 1.4 µg/g (pair-fed and control). As shown in Table 1, mean food intake was significantly lower in ID-3, ID-7 and ID-11 groups compared to the ad libitum control group (CN) ($p<0.05$). Mean body weight was significantly reduced in ID-3 and ID-7 groups compared to CN group ($p<0.05$). In the food-restricted, pair-fed rats (PF-3, PF-7, PF-11), mean final body weight was also lower than in the CN group, although this reduction was only significant in the PF-3 group ($p<0.01$). However, there were no significant differences between groups in absolute (Table 1) or relative thyroid weights (4.8 ± 0.9/100 g body). Mean protein content / thyroid was significantly lower in the ID-3 group compared to the CN group(Table 1). By multiple regression, decreased food intake in both PF and ID groups significantly reduced thyroid protein concentration ($P < 0.05$; data not shown).

TABLE 1: Final body weight, daily food intake, thyroid weight, and thyroid protein content in weanling rats fed iron-deficient diets containing 3, 7 and 11 μg Fe/g for 29 days (ID-3, ID-7, ID-11), their pair-fed controls (PF-3, PF-7, PF-11) and control rats fed ad libitum (CN)[1].

Group	n	Final body weight (g)	Food intake (g/d)	Thyroid weight (mg)	Thyroid protein (μg)
ID-3	12	164 ± 32^{b}	11.0 ± 1.6^{b}	8.0 ± 1.8	152 ± 40^{b}
PF-3	11	174 ± 31^{b}	11.1 ± 1.6^{b}	8.7 ± 2.3	183 ± 65^{ab}
ID-7	12	187 ± 38^{b}	12.4 ± 2.1^{b}	8.8 ± 1.7	178 ± 64^{ab}
PF-7	12	198 ± 39^{ab}	12.4 ± 2.0^{b}	9.9 ± 2.2	225 ± 54^{ab}
ID-11	12	201 ± 28^{ab}	13.1 ± 1.6^{b}	9.4 ± 2.2	200 ± 40^{ab}
PF-11	12	199 ± 33^{ab}	12.9 ± 1.6^{b}	9.7 ± 2.6	212 ± 68^{ab}
CN	12	232 ± 36^{a}	15.5 ± 1.7^{a}	10.7 ± 2.7	243 ± 51^{a}

[1]Results are given as mean ± SD. Means in a column without a common letter differ, $P < 0.05$.
[2]Final body weight is that on the day before killing.

Table 2 shows the Hb, T_3 and T_4 concentrations in the seven groups. Mean Hb in all three ID-groups was significantly reduced compared to that of their pair-fed controls and the CN group ($p<0.001$). By multiple regression, IDA was an independent and significant predictor of both reduced T_3 ($p<0.001$) and decreased T_4 ($p<0.0005$). Food restriction had a highly significant, independent effect on T_4 ($p<0.0001$), but not on T_3.

TABLE 2: Hemoglobin and plasma T_4 and T_3 concentrations in weanling rats fed iron-deficient diets for 29 days (ID-3, ID-7, ID-11), their pair-fed controls (PF-3, PF-7, PF-11) and control rats fed ad libitum (CN) [1].

Group	n	Hemoglobin (g/L)	Total T_3 (ng/100 mL)	Total T_4 (µg/100 mL)
ID-3	12	40.3 ± 5.2^{d}	32.4 ± 9.3^{b}	2.1 ± 0.5^{c}
PF-3	11	146.2 ± 11.6^{a}	42.3 ± 3.7^{ab}	2.6 ± 0.6^{bc}
ID-7	12	58.4 ± 5.9^{c}	33.5 ± 5.5^{b}	2.4 ± 0.3^{bc}
PF-7	12	137.9 ± 6.7^{a}	47.6 ± 6.0^{a}	3.0 ± 0.5^{ab}
ID-11	12	72.4 ± 6.1^{b}	31.2 ± 5.4^{b}	2.7 ± 0.5^{b}
PF-11	12	135.4 ± 5.1^{a}	51.1 ± 13.9^{a}	2.9 ± 0.9^{ab}
CN	12	136.3 ± 10.9^{a}	48.5 ± 11.4^{a}	3.9 ± 1.1^{a}

[1]Results are given as mean ± SD. Means in a column without a common letter differ, $P < 0.05$.

Table 3 shows mean TPO activity determined by the guaiacol and iodide assays. Linear regression showed a highly significant independent effect of IDA on TPO activity expressed as GU / thyroid ($p<0.001$). Compared to mean TPO activity in GU / thyroid in the CN group, mean TPO activity in the ID-3, ID-7, and ID-11 groups was reduced 56, 45 and 33%, respectively (Figure 1). IDA also reduced TPO activity in GU / mg thyroid tissue ($p<0.01$), IU / total thyroid ($p<0.005$) and IU / mg thyroid tissue ($p<0.05$). IDA had no significant effect on TPO activity per mg protein (data not shown). Food restriction also had a highly significant impact on TPO activity. As modeled by multiple regression, TPO activity was significantly decreased by food restriction whether expressed as GU / total thyroid ($p<0.0005$), GU / mg thyroid tissue ($p<0.05$), IU / total thyroid ($p<0.0005$), IU / mg thyroid tissue ($p<0.0005$) or IU / mg protein ($p<0.05$). There was a strong correlation between Hb, daily food intake and TPO activity in GU / thyroid ($R^2=0.40$) and in IU / thyroid ($R^2=0.55$) (Table 4).

TABLE 3: TPO activity determined by guaiacol and iodide assay per total thyroid and per mg thyroid tissue in iron-deficient rats (ID-3, ID-7, ID-11), their pair-fed controls (PF-3, PF-7, PF-11) and control rats fed ad libitum (CN)[1]

		Thyroid Peroxidase			
		Guaiacol Assay		Iodide Assay	
Group	n	GU / thyroid	GU / mg thyroid tissue	IU / thyroid	IU / mg thyroid tissue
ID-3	11	3.9 ± 1.6^{b}	0.46 ± 0.15^{b}	48.4 ± 16.5^{b}	5.8 ± 1.4^{b}
PF-3	11	6.0 ± 2.4^{b}	0.69 ± 0.20^{ab}	68.1 ± 23.2^{ab}	7.3 ± 1.3^{ab}
ID-7	11	4.9 ± 1.7^{b}	0.56 ± 0.17^{b}	60.5 ± 17.4^{b}	6.8 ± 1.3^{ab}
PF-7	12	6.1 ± 2.1^{b}	0.61 ± 0.17^{ab}	67.2 ± 24.2^{ab}	6.7 ± 1.6^{b}
ID-11	12	6.0 ± 2.3^{b}	0.65 ± 0.27^{ab}	63.3 ± 19.2^{b}	6.8 ± 1.3^{ab}
PF-11	11	6.5 ± 2.7^{ab}	0.69 ± 0.25^{ab}	70.4 ± 25.8^{ab}	7.0 ± 1.9^{ab}
CN	12	8.9 ± 2.9^{a}	0.86 ± 0.26^{a}	90.7 ± 23.0^{a}	8.5 ± 1.0^{a}

[1]Results are given as mean ± SD. Means in a column without a common letter differ, $P < 0.05$.
By multiple regression, there was a significant independent effect of both IDA and food restriction on TPO activity.
GU, guaiacol unit; IU, iodine unit.

TABLE 4: Multiple regression of daily food intake, hemoglobin, and TPO activity by guaiacol and iodide assay per total thyroid and per mg thyroid tissue in iron-deficient rats (ID-3, ID-7, ID-11), their pair-fed controls (PF-3, PF-7, PF-11) and control rats fed ad libitum (CN)[1]

TPO activity	Hemoglobin (g/L)		Food intake (g/d)		Adjusted R^2
	Coefficient	*P*	Coefficient	*P*	
GU/thyroid	0.02 ± 0.01	<0.001	0.61 ± 0.11	<0.001	0.39
GU/mg thyroid tissue	0.002 ± 0.001	0.007	0.02 ± 0.01	0.049	0.16
IU/thyroid	0.16 ± 0.05	0.001	7.36 ± 0.94	<0.001	0.53
IU/mg thyroid tissue	0.009 ± 0.004	0.02	0.35 ± 0.08	<0.001	0.30

[1]Results are given as mean ± SEM. n = 80.
GU, guaiacol unit; IU, iodine unit.

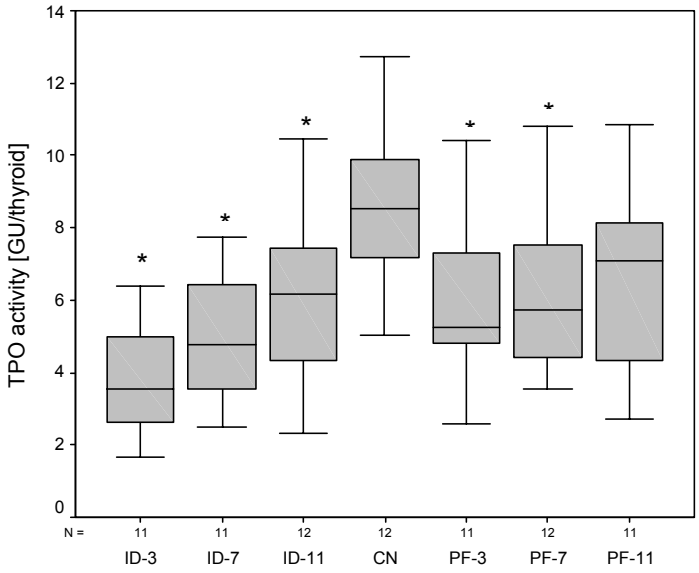

Figure 1: Thyroid peroxidase activity (TPO) expressed in guaiacol units (GU) per thyroid in Fe-deficient rats (ID-3, ID-7, ID-11), their pair-fed controls (PF-3, PF-7, PF-11) and control rats that consumed food ad libitum (CN). The plots show the median, 75th and 25th percentiles as boxes, and the ranges as whiskers, n = 11-12. One Gu represents the amount of enzyme that produced a change of 1.0 optical density unit/s. The Fe-deficient diets contained 3, 7 and 11 mg Fe/kg.
* Different from CN, $P < 0.05$.

Discussion

In humans, Martinez-Torres et al. (25) reported 10% lower T_3 levels in both moderate-to-severe IDA (mean Hb 75 g/L) and Fe deficiency without anemia. Beard et al. (7) compared women with mild IDA (mean Hb 110 g/L) to Fe-sufficient controls, and reported that the anemic group had significantly decreased serum T_3 and T_4 and significantly increased TSH. In Fe-deficient women without anemia, serum T_3 was significantly decreased and TSH significantly increased, compared to Fe-sufficient controls. Fe treatment of the IDA women significantly increased serum T_3 concentrations (7). Plasma T_4 and T_3 concentrations were also lower in Fe-deficient rats (2-4). Rats with Fe deficiency and moderate IDA (mean Hb 85 g/L) have reduced conversion of T_4 to T_3 (1), and lower serum T_4 and T_3 concentrations compared to controls (5). Fe-deficient rats have significantly lower hepatic thyroxine-5´-deiodinase activity, with hepatic production of T_3 only 46% of controls (7). Weanling rats fed Fe-

deficient diets had significantly blunted TSH responses to exogenous thyrotropin-releasing hormone (TRH), reduced turnover of serum T_3 (ca. 50% lower than controls), and lower hepatic thyroxine-5´-deiodinase activity (6). [^{125}I]T_3 binding in hepatic nuclei was lower in Fe-deficient compared to control rats (15).

In the present study, T_3 and T_4 levels were significantly decreased in IDA rats, in agreement with previous authors (1-2). Mean T_3 levels in IDA rats were only 65-68% of levels in the CN group. However, T_3 did not decrease in a dose-response fashion with increasing severity of IDA, in agreement with data from Brigham & Beard (8). Increasing severity of IDA did produce a significant step-wise decrease in mean T_4 (Table 2). Fe deficiency may impair activity of hepatic 5'-deiodinase that catalyzes conversion of T_4 to T_3 (8,26-27). The decreased T_3 levels in IDA rats in the present study may be related to decreased deiodinase activity and reduced peripheral formation of T_3 (6).

IDA may effect thyroid metabolism through several mechanisms. Using an *in vitro* incubation method, Kaplan and Utiger (28) found that outer ring deiodinase activity is not affected by either ferric or ferrous Fe. Thyroid metabolism could be impaired by Fe deficiency through anemia and lowered oxygen transport, similar to the thyroid impairment of hypoxia (12-13). Fe deficiency may influence iodine deficiency disorders through alterations of central nervous system control of thyroid metabolism (14) or through modification of nuclear T_3 binding (15). Our findings are the first to suggest an alternate contributing mechanism. TPO activity in the thyroid, measured by both the guaiacol and the iodide assays, was clearly sensitive to body Fe depletion. These data suggest that impairment of TPO activity contributes to the adverse effect of IDA on thyroid and iodine metabolism.

Of interest is the discrepancy between TPO activity expressed per total thyroid and per mg thyroid compared with TPO activity expressed per mg protein. There was no independent effect of IDA on TPO activity/mg protein. Because there was no independent reduction in protein concentration/mg thyroid by IDA, this cannot explain the discrepancy. The precision of the protein assay (CV = 4.7%) was adequate and is unlikely to have obscured a potential difference. We are therefore unable to explain the lack of an independent effect of IDA on TPO activity expressed per mg protein.

We included pair-fed controls to distinguish the effects of reduced food intake associated with IDA from IDA *per se* as a cause of lowered thyroid hormone levels

and TPO activity. This is important as lower food intake in rats predictably lowers serum concentrations of thyroid hormones (29-33). During the final ten days of the present study, PF-3, PF-7 and PF-11 rats were food restricted by 41%, 26% and 23%, respectively, compared to the CN group. Consistent with previous reports, food restriction in our PF groups significantly reduced mean T_4, compared to the CN group. In addition, food restriction was an independent predictor of reduced TPO activity. However, comparing the IDA rats to their PF controls, IDA *per se* had a clear impact on T_4 and T_3 levels and TPO activity (Tables 2 and 3).

These data provide a possible explanation for the observed impairment in thyroid response to iodine repletion in goitrous children with IDA (9). By reducing TPO activity, Fe deficiency may decrease iodine incorporation into thyroglobulin and subsequent coupling of iodotyrosines to form thyroid hormone. These data also provide a potential mechanism for our previous studies showing that Fe supplementation in goitrous children with IDA improves the thyroid response to both iodized oil and iodized salt (10-11).

Acknowledgements

We would like to thank Claudia von Meyenburg and Christophe Zeder (Swiss Federal Institute of Technology, Zürich) for technical assistance and Dr. Luciano Molinari (Children's Hospital, Zürich, Switzerland) for statistical advice.

Literature Cited

1. Dillman, E., Gale, C., Green, W., Johnson, D.G., Mackler, B. & Finch, C. (1980) Hypothermia in Iron Deficiency Due to Altered Triiodothyronine Metabolism. Am. J. Physiol. 239: R377-R381.
2. Beard, J.L., Green, W. & Finch, C.A. (1982) Interactions of Iron Deficiency, Anemia and Thyroid Hormone Levels in the Response of Rats to Cold Exposure. Life Sci. 30: 691-697.
3. Chen, S.C.H., Shirazi, M.R.S. & Orr, R.A. (1983) Triiodothyronine and thyroxine levels in iron-deficient, hypertriglyceridemic rats. Nutr. Res. 3: 91-106.
4. Beard, J., Green, W., Miller, L. & Finch, C. (1984) Effect of iron-deficiency anemia on hormone levels and thermoregulation during cold exposure. Am. J. Physiol. 247: R114-R119.
5. Tang, F., Wong, T.M. & Loh, T.T. (1988) Effects of Cold Exposure of TRH on the Serum TSH Levels in the Iron-Deficient Rat. Horm. Metabol. Res. 20: 616-619.
6. Beard, J., Tobin, B. & Green, W. (1989a) Evidence of Thyroid Hormone Deficiency in Iron-Deficient Anemic Rats. J. Nutr. 119: 772-778.
7. Beard, J.L., Borel, M.J. & Derr, J. (1990) Impaired Thermoregulation and Thyroid Function in Iron-Deficiency Anemia. Am. J. Clin. Nutr. 52: 813-819.
8. Brigham, D.E. & Beard, J.L. (1995) Effect of Thyroid Hormone Replacement in Iron-Deficient Rats. Am. J. Physiol. 38: R1140-R1147.
9. Zimmermann, M.B., Adou, P., Zeder, C., Torresani, T. & Hurrell, R.F. (2000a) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. Am. J. Clin. Nutr. 71: 88-93.

10. Zimmermann, M.B., Adou, P., Torresani, T., Zeder, C. & Hurrell R.F. (2000b) Iron supplementation in goitrous, iron-deficient children improves their response to oral iodized oil. Eur. J. Endocrinol. 142: 217-223.
11. Hess, S.Y., Zimmermann, M.B., Adou, P., Torresani, T. & Hurrell, R.F. (2001) Treatment of Iron Deficiency in Goitrous Children Improves the Efficacy of Iodized Salt in Côte d'Ivoire. Am J Clin Nutr (in press).
12. Surks, M.I. (1969) Effects of thyrotropin and thyroidal iodine metabolism during hypoxia. Am. J. Physiol. 216: 426-429.
13. Galton, V.A. (1972) Some effects of altitude on thyroid function. Endocrinology 91: 1393-1403.
14. Beard, J.L., Brigham, D.E., Kelley, S.K. & Green, M.H. (1998) Plasma Thyroid Hormone Kinetics Are Altered in Iron-Deficient Rats. J. Nutr. 128: 1401-1408.
15. Smith, S.M., Finley, J., Johnson, L.K. & Lukaski, H.C. (1994) Indices of In Vivo and In Vitro Thyroid Hormone Metabolism in Iron-Deficient Rats. Nutr. Res. 14: 729-739.
16. Kimura, S., Kotani, T., McBride, O.W., Umeki, K., Hirai, K., Nakayama, T. & Ohtaki, S. (1987) Human thyroid peroxidase: Complete CDNA and protein sequence, chromosome mapping, and identification of two alternately spiced mRNAs. Proc. Natl. Acad. Sci. USA 84: 5555-5559.
17. Dunn, J.T. & Dunn, A.D. (2001) Update on intrathyroidal iodine metabolism. Thyroid 11: 407-414.
18. Ohtaki, S., Nakagawa, H., Nakamura, M. & Yamazaki, I. (1982a) Reactions of purified hog thyroid peroxidase with H2O2, tyrosine, and methylmercaptoimidazole in comparison with bovine lactoperoxidase. J. Biol. Chem. 257: 761-766.
19. Ohtaki, S., Nakagawa, H., Nakamura, M. & Yamazaki, I. (1982b) One- and two-electron oxidations of tyrosine, monoiodotyrosine, and diiodotyrosine catalyzed by hog thyroid peroxidase. J. Biol. Chem. 257: 13398-13403.
20. Ackrell, B.A., Maguire, J.J., Dallman, P.R. & Kearney, E.B. (1984) Effect of iron deficiency on succinate- and NADH-ubiquinone oxidoreductases in skeletal muscle mitochondria. J. Biol. Chem. 259: 10053 – 10059.
21. Reeves, P.G., Forrest, H.N. & Fahey, G.C. (1992) AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hod Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J. Nutr.123: 1939-1951.
22. Hosoya, T., Sato, I., Hiyama, Y., Yoshimura, H., Niimi, H. & Tarutani, O. (1985) An improved Assay Method for Thyroid Peroxidase Applicable for a Few Milligram of Abnormal Human Thyroid Tissue. J. Biochem. 98: 637-647.
23. Hosoya, T. & Morrison, M. (1967) The isolation and purification of thyroid peroxidase. J. Biol. Chem. 242: 2828-2836.
24. Nakashima, T. & Taurog, A. (1978). Improved assay procedure for thyroid peroxidase; application to normal and adenomatous human thyroid tissue. Clin. Chim. Acta 83: 129-140.
25. Martinez-Torres, C., Cebeddu, L, Dillmann, E., Brengelmann, G.L., Leets, I., Layrisse, M., Johnson, D.G. & Finch, C. (1984) Effect of exposure to low temperature on normal and iron-deficient subjects. Am. J. Physiol. 246: R380-R383.
26. Beard, J.L., Tobin, B.W. & Smith, S.M. (1989b) Effects of Iron Repletion and Correction of Anemia on Norepinephrine Turnover and Thyroid Metabolism in Iron Deficiency. Proc. Soc. Exp. Biol. Med. 193: 306-312.
27. Smith, S.M., Deaver, D.R. & Beard, J.L. (1992) Metabolic Rate and Thyroxine Monodeiodinase Activity in Iron-Deficient Female Sprague-Dawley Rats: Effects of the Ovarian Steroids. J. Nutr. Biochem. 3: 461-466.
28. Kaplan, M.M. & Utiger, R.D. (1978) Iodothyronine metabolism in liver and kidney homogenates from hyperthyroid and hypothyroid rats. Endocrinology 103: 156-161.
29. Morley, J.E., Gordon, J. & Hershman, J.M. (1980) Zinc deficiency, chronic starvation and hypothalamic-pituitary-thyroid function. Am. J. Clin. Nutr. 33: 1767-1770.
30. Herlihy, J.T., Stacy, C. & Bertrand, H.A. (1990) Long-term food restriction depresses serum thyroid hormone concentrations in the rat. Mech. Ageing. Dev. 31: 9-16.
31. Schröder-van der Elst, J.P. & van der Heide, D. (1992) Effects of Streptozocin-Induced Diabetes and Food Restriction on Quantities and Source of T_4 and T_3 in Rat Tissue. Diabetes 41: 147-152.
32. Darras, V.M., Cokelaere, M., Dewil, E., Arnouts, S., Decuypere, E. & Kuhn, E.R. (1995) Partial food restriction increases hepatic inner ring deiodinating activity in the chicken and the rat. Gen. Comp. Endocrinol. 100: 334-338.
33. Cokelaere, M., Decuypere, E., Flo, G., Darras, V.M. & Kuhn, E.R. (1996) Influence of feeding pattern on thyroid hormones in long-term food-restricted rats. Horm. Metab. Res. 28: 315-318.

Low iron stores predict persisting goiter in Côte d'Ivoire after salt iodization

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Manuscript in preparation

Abstract

Although salt iodization is the recommended long-term strategy to control iodine deficiency and reduce goiter, a high goiter rate may persist for months to years after iodine repletion. Deficiencies of iron, vitamin A and selenium may impair thyroid metabolism and blunt the thyroid response to iodine. The aim of the study was to determine if deficiencies of iron, selenium or vitamin A, as well as high consumption of cassava, could account for persisting goiter after successful salt iodization. In a cross-sectional study of primary school children (n=1013) in western Côte d'Ivoire, hemoglobin, plasma ferritin and transferrin receptor, erythrocyte zinc protoporphyrin, plasma selenium, plasma retinol, urinary iodine and thiocyanate, serum thyroxine and thyrotropin were measured and compared to thyroid volume by ultrasound. Two years after salt iodization, goiter prevalence was 74%, despite a normal median urinary iodine concentration of 162 µg/L. The prevalence of iron deficiency was 35% and of anemia 38%, respectively. Forty-five percent of children were vitamin A deficient and 92% selenium deficient. Thiocyanate excretion in urine was elevated due to daily consumption of cassava. However, despite their high prevalence and severity, neither vitamin A nor selenium deficiencies nor urinary thiocyanate predicted goiter. Persisting goiter in children in Côte d'Ivoire two years after beginning salt iodization was associated with low plasma ferritin concentrations.

Besides low iron stores, other factors, not identified in this study, probably contributed to the persisting high goiter rate despite two years of succesful salt iodization.

Introduction

Universal salt iodization is the most effective long-term strategy to eliminate iodine deficiency disorders (IDD) in most regions of the world (WHO *et al.*, 2001a). Careful monitoring of salt iodine content, urinary iodine concentration (UI) and goiter rate is important to ensure success of universal salt iodization. While UI responds rapidly to increasing iodine intake, several authors have reported persisting high goiter rates despite normalization of iodine intake with salt iodization (Pardede *et al.*, 1998; Jooste *et al.*, 1999; Azizi *et al.*, 2002b). The reasons for the persisting high goiter rate after the introduction of salt iodization are unclear. The goiter rate should normalize after iodine repletion; for example, in goitrous, iron-sufficient children, the goiter rate was reduced from 100 to 12% measured 30 weeks after 200 mg oral iodized oil (Zimmermann *et al.*, 2000a). Deficiencies of key micronutrients and/or high consumption of dietary goitrogens could potentially play a role. Along with iodine, adequate intakes of iron, selenium and vitamin A are essential for normal thyroid status.

Iron deficiency can act in concert with iodine deficiency and impair thyroid metabolism and modify the response to prophylactic iodine (Zimmermann & Köhrle, 2002). Iron deficiency anemia (IDA) decreases plasma total thyroxine (T_4) and triiodothyronine (T_3) concentrations, reduces peripheral conversion of T_4 to T_3, and may increase circulating thyrotropin (TSH) (Dillman *et al.*, 1980; Beard *et al.*, 1989; Brigham & Beard, 1995; Beard *et al.*, 1998). In regions of endemic goiter, the thyroid response to iodized oil is impaired in children with IDA, compared to iron-sufficient children (Zimmermann *et al.*, 2000b) and is improved by iron supplementation as well as iron fortification (Zimmermann *et al.*, 2000a; Hess *et al.*, 2002; Zimmermann *et al.*, 2002).

Selenium is an essential component of three deiodinases and of glutathione peroxidase, enzymes that play central roles in thyroid metabolism (Arthur *et al.*, 1999). In humans, several diseases have been attributed to combined deficiencies in iodine and selenium; myxedematous cretinism in Zaire (Goyens *et al.*, 1987; Vanderpas *et al.*, 1990) and Kashin-Beck disease in Tibet (Moreno-Reyes *et al.*, 1998). Cross-sectional studies investigating the impact of selenium status on thyroid size and goiter rate are equivocal (Zagrodzki *et al.*, 2000; Erdogan *et al.*, 2001; Giray *et al.*, 2001). Studies in rats have reported that vitamin A deficiency impairs transport of thyroid hormones and increases their plasma concentrations (Higueret & Garcin,

1979; Ingenbleek, 1983; Higueret *et al.*, 1989). In humans, studies have found lower serum retinol levels in subjects with palpable goiter compared to non-goitrous subjects (Ingenbleek & De Visscher, 1979; Wolde-Gebriel *et al.*, 1993).

Côte d'Ivoire began universal salt iodization in early 1998 at a production level 30-50 µg iodine/g salt. We recently reported that thyroid volume and the goiter rate in rural Ivorian children remains sharply elevated 4 years after normalization of iodine intake (Zimmermann *et al.*, in press). Poor vitamin A, selenium and iron nutrition adversely affect thyroid function and many children in western Côte d'Ivoire suffer from these deficiencies, which are often overlapping (Zimmermann *et al.*, 2000b; Arnaud *et al.*, 2001). Cassava, a known goitrogen, is a staple food eaten daily by children in this region. Therefore, we investigated whether micronutrient deficiencies, together with high consumption of cassava, might contribute to the persisting high goiter rate in children in this region.

Subjects, methods and materials

Subjects

The study was done in primary schools in the Danané Health District, an area of endemic goiter in the mountains of western Côte d'Ivoire. The University Children's Hospital in Zürich and the Ministry of Research of Côte d'Ivoire gave ethical approval for the study. Informed oral consent was obtained from the village chiefs and the children's teachers and families. The screening was done in November 1999. All school children in the nine villages were screened (n=1013). The ages of the children were recorded from dates of birth in the school register. Weight and height were measured and spot urine samples were collected for measurement of UI. Thyroid gland volume (Tvol) was measured using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5 MHz linear transducer (Zimmermann *et al.*, 2001). Measurements were performed on subjects sitting upright with the neck extended. Blood was collected by venipuncture for determination of hemoglobin (Hb), erythrocyte zinc protoporphyrin (ZPP), plasma ferritin (SF), plasma transferrin receptor (TfR), plasma retinol and plasma selenium. Blood was spotted onto filter paper for measurement of TSH and T_4. Random salt samples (n=213) from households of children were collected for determination of iodine concentration measurements.

Laboratory analyses

Urine and blood samples were transported on ice to the regional hospital laboratory. Plasma and urine samples were aliquoted and frozen at –20 °C until analysis. Randomly selected sub-samples were analyzed for UI and SCN (n=399), for T_4 and TSH (n=158), for Hb, SF, TfR and ZPP (n=978), for plasma selenium (n=50) and for plasma retinol (n=352).

UI was measured using a modification of the Sandell-Kolthoff reaction (Pino *et al.*, 1996). At UI concentrations of 47 µg/L and 79 µg/L, the CV of this assay in our laboratory is 10.3 and 12.7%. Iodine concentration in salt was measured by titration with thiosulfate (Sullivan *et al.*, 1995). Dried blood spots on filter paper were analyzed for whole blood TSH and serum T_4 using immunoassay (Torresani & Scherz, 1986). To convert whole blood TSH values to serum values, whole blood TSH values were multiplied by 2 (Torresani & Scherz, 1986). Normal reference values are TSH, <3.5 mU/L; T_4, 65-165 nmol/L. Hb was measured using a AcT8 Counter (Beckman Coulter, Krefeld, Germany). Anemia was defined as Hb < 120 g/L in children aged ≥12 years (y), and Hb < 115 g/L in children aged 5-12 y (WHO *et al.*, 2001b). ZPP was measured on washed red blood cells using a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA). SF and TfR were measured using an enzyme-linked immunosorbent assay (Flowers *et al.*, 1986; Skikne *et al.*, 1990). Normal reference values are: SF, 12-300 µg/L; TfR, 2.9-8.5 mg/L; ZPP, <40 µmol/mole heme. Iron deficiency was defined using multiple criteria: SF < 15 µg/L; or TfR > 8.5 mg/L + ZPP > 40 µmol/mole heme. Urinary thiocyanate (SCN) was analyzed by a colorimetric method (Lundquist *et al.*, 1979). Plasma selenium was measured by hydride generation atomic absorption spectrometry (SpecrAA-400 with a hydride generator VGA-77, Varian Techtron Pty. Ltd. Mulgrave, Victoria, Australia) (Borella *et al.*, 1998) with a limit of detection of 0.11 µmol/L; undetectable concentrations were assigned a value of 0.11 µmol selenium/L. Plasma retinol was measured by HPLC (Merck-Hitachi, Tokyo, Japan) on a reversed phase column (Hypersil ODS RP-18 200 x 4.6 mm, 3 µm, Crom, Herrenberg-Kayh, Germany) according to Tanumihardjo *et al.* (1994). A commercial reference material for the retinol measurement (National Institute of Standards and Technology, Gaithersburg, MD) and for the selenium measurement (Sero A/S, Billingstad, Norway) was analyzed together with the plasma samples, respectively. Normal reference values are: UI/SCN, >3µg/mg (Delange *et al.*, 1983); plasma retinol >0.70 µmol/L (WHO *et al.*, 1996a); plasma selenium, >0.5 µmol/L (FAO/WHO expert consultation, 2002). Tvol was calculated using the method of Brunn *et al.* (1981). In countries with a high prevalence of child growth retardation, Tvol is considered to be more directly a function of body surface area (BSA) than of

age (WHO et al., 2001a). Therefore, BSA was calculated from weight and height measurements taken with each ultrasonography measurement (DuBois & DuBois, 1916). Updated WHO/ICCIDD normative values for Tvol in school-age children according to sex and body surface area (BSA) were used to define goiter (Zimmermann *et al.*, 2001).

Statistical analyses

Data processing and statistics were done using SPSS 10.0 (SPSS Inc., Chicago, USA). Z-scores for height for age (HAZ), weight for age (WAZ) and weight for height (WHZ) were calculated by using EPI-Info 6.02 (Centers for Disease Control and Prevention, Atlanta, USA). Stunting was defined as HAZ <-2 SD and wasting WHZ <-2SD. Normally distributed data were expressed as mean and standard deviation and compared by student's t-test. Data not normally distributed were expressed as medians with ranges. Associations between age, gender, height, weight, Hb, SF, TfR, ZPP, ID, IDA, plasma retinol, plasma selenium, and UI/SCN ratio with goiter were analyzed by binary logistic regression using a backward deletion procedure. *P* values <0.05 were considered significant.

Results

The mean age of sample (n=1013) was 8.8 ± 2.4 y. As boys more often attend primary school in rural Côte d'Ivoire, 68% of the subjects were male. Mean height and weight was 1.29 ± 0.13 m and 25.7 ± 7.2 kg, respectively. Based on the growth reference recommended by the WHO (Dibley *et al.*, 1987), HAZ, WAZ and WHZ was -0.4 ± 1.4, -0.7 ± 1.0 and -0.6 ± 0.8, respectively. The prevalence of stunting and wasting was 12% and 9 %, respectively.

Mean (±SD) salt iodine content at the household level was 25 ± 18 µg/g (n=213). Based on salt intakes from weighed food records of 5 g/day in 7-12 y old children in rural Côte d'Ivoire (Hess *et al.*, 1999), daily iodine intake was estimated to be 125-200 µg. Median UI and TSH, and mean T_4 were well within normal ranges and only 2% of children had either a UI <50 µg/L or an abnormal TSH or T_4 (Table 1) (WHO *et al.*, 2001a). Despite adequate iodine nutrition and normal thyroid hormone levels, goiter prevalence was 74%.

Table 1: Urinary iodine, TSH and T_4, thyroid volume and goiter prevalence in school children Côte d'Ivoire[1]

Characteristic		n
Urinary iodine (µg/L) No. of subjects with < 100 µg/L No. of subjects with < 50 µg/L	162 (16-1017) 45 [21] 5 [2]	399
TSH (mU/L) No. of subjects with >3.5 mU/L	1.4 (0.4-8.8) 1 [2]	158
Thyroxine (nmol/L) No. of subjects with <65 nmol/L	126 ± 29 1 [2]	158
Thyroid volume (mL) Thyroid volume in 5-9 yr-olds Thyroid volume in 10-14 yr-olds	5.9 ± 2.9 5.0 ± 1.4 7.6 ± 3.5	1013 662 351
Goiter prevalence Goiter prevalence in 5-9 yr-olds Goiter prevalence in 10-14 yr-olds Goiter prevalence in boys Goiter prevalence in girls	745 [74] 472 [71] 273 [78] 537 [77] 208 [65]	1013

[1]Values are given as: means ± SD; medians (range); or numbers [percentage]

The prevalence of iron deficiency was 35% and the prevalence of anemia was 38%, with nearly half of the anemia due to iron deficiency (17%). Deficiencies of selenium and vitamin A were common, with 92 and 45% of children affected (Table 2). We detected no clinical eye signs of vitamin A deficiency in the study population.

Table 2: Indicators of micronutrient status of school children in Côte d'Ivoire[1]

Characteristics		n
No. of subjects with iron deficiency No. of subjects with iron deficiency anemia	341 [35] 166 [17]	
Hemoglobin (g/L) No. of anemic subjects[2]	120.1 ± 13.5 378 [38]	1005
Serum ferritin (µg/L) No. of subjects with < 15 µg/L	61.5 ± 33.4 26 [3]	978
Transferrin receptor (mg/L) No. of subjects with > 8.5 mg/L	11.7 ± 3.9 817 [84]	978
Erythrocyte zinc protoporphyrin (µmol/mol heme) No. of subjects with > 40 µmol/mol heme	41 ± 29 391 [39]	994

Serum retinol (µmol/L) No. of subjects with < 0.7 µmol/L	0.74 ± 0.22 159 [45]	352
Serum selenium (µmol/L) No. of subjects with < 0.5 µmol/L	0.27 ± 0.17 46 [92]	50
Urinary iodine /thiocyanate (µg/mg) No. of subjects with < 3 µg/mg	26 (1-1688) 18 [5]	399

[1] Values are given as: means ± SD; medians (range); or numbers [percentage]
[2] Anemia was defined as Hb < 120 g/L in children aged ≥12 yrs, and Hb <115 g/L in children aged 5-12 yrs
[3] Iron deficiency was defined using multiple criteria: SF <15 µg/L; or TfR >8.5 mg/L + ZPP >40 µmol/mol heme

The goiter prevalence, using the provisional WHO/ICCIDD reference cut-offs based on BSA, was higher in boys than in girls (*P*<0.001) (Zimmermann *et al.*, 2001). By regression, village (*P*<0.001) and greater height (*P*=0.013) significantly predicted goiter, whereas lower weight showed a trend (*P*=0.057). Of the iron status indicators, only SF significantly predicted goiter (*P*=0.046); that is, the probability of goiter was increased in children with lower SF concentrations. Neither ID nor IDA significantly predicted goiter. Despite the high prevalence of low serum retinol and selenium, neither deficiency was associated with goiter. Cassava consumption, as reflected by the UI/SCN ratio in urine, also did not significantly predict goiter.

Table 3: Binary logistic regression of the association between goiter and gender, village, plasma ferritin, height and weight in school children in western Côte d'Ivoire (n=955)

Variables	β	**S.E.**	**df**	***P***
Gender	.559	.160	1	< .001
Village			8	< .001
Height	.042	.017	1	.013
Plasma Ferritin	- .005	.002	1	.046
Weight	-.058	.031	1	.057
Constant	-2.86	1.46	1	.051

Discussion

Compared to measurements made in school children in this region 2 years earlier (Zimmermann *et al.*, 2000b), median UI had increased from 28 to 162 µg/L, and the prevalence of elevated TSH values was reduced from 8% to 2%. Despite normalization of these indicators for >1 year, 3 out 4 school children remained

goitrous. As previously reported (Pardede *et al.*, 1998; Jooste *et al.*, 1999; Azizi *et al.*, 2002b), there is a lag period between increasing iodine intake and normalization of Tvol after salt iodization. The length of this lag period is unclear, with reports suggesting Tvol may remain elevated for several years after iodine repletion (Zimmermann *et al.*, in press) or may not completely normalize (Aghini-Lombardi *et al.*, 1997; Sullivan & May, 1999).

Many children in western Côte d'Ivoire suffer from vitamin A, selenium and iron deficiencies. Because they all adversely affect thyroid function, we investigated whether these deficiencies, together with high consumption of cassava, might at least partially explain the persisting high goiter rate. These factors may adversely affect Tvol through impaired iodine metabolism and altered thyroid hormone concentrations, or through other mechanisms directly acting on the thyroid gland. The strengths of this cross-sectional study were the large sample (>1000 children), consideration of the 3 main micronutrient deficiencies recognized to affect thyroid function, rigorous definition of micronutrient status, and inclusion of cassava, the major dietary goitrogen in this region.

In the present study, low SF concentrations significantly predicted goiter 2 years after salt iodization. Similar results were found in Iran, where goiter was more prevalent in children with low SF concentrations compared to children with normal SF values (Azizi *et al.*, 2002a). Moreover, our findings are consistent with previous intervention studies, which have clearly shown that iron deficiency blunts the thyroid response to iodine prophylaxis (Zimmermann *et al.*, 2000a; Hess *et al.*, 2002; Zimmermann *et al.*, 2002). The lack of association of goiter with iron deficiency or IDA (defined by multiple criteria) in this study may have been due to the relatively mild anemia present in the sample (mean Hb was 120 g/L with only 3% of children <100 g/L) or to difficulties in defining iron deficiency in tropical regions where infections are common. The prevalence of malaria in rural Côte d'Ivoire is high, with 54% of the school-age children showing at least mild infection (Staubli Asobayire *et al.*, 2001). Also gastrointestinal infections are very frequent, as indicated by a prevalence of polyparasitism (> 2 species of parasites) of 77% in rural Côte d'Ivoire (Keiser *et al.*, 2002). Hemolysis due to malaria and blood loss from helminthic infections can increase the risk of anemia (Nestel & Davidsson, 2002). The presence of inflammation and/or infection can spuriously increase SF and ZPP concentrations and reduce their sensitivity as predictors of iron deficiency (Lipschitz *et al.*, 1974; Hastka *et al.*, 1993). Staubli Asobayire *et al.* (2001) found that TfR, compared to SF

and ZPP, was the iron status indicator least affected by inflammatory disorders in Côte d'Ivoire. Therefore, we expected that TfR rather then SF might have significantly predicted goiter. However, 84% of the children had elevated TfR concentrations in the present study. This could possibly be due to hemolysis during malaria, as reported by Stoltzfus *et al.* (2000) in 0- to 5 y-old children in Zanzibar. The same study found the relation between malaria and increased SF values disappearing in older children (2.5 to 5 y-olds). This finding supported data from an earlier study in primary school children (mean age 10.5 y), in which malarial infection and elevated SF concentration were not associated (Stoltzfus *et al.*, 1997). These studies suggested that SF can provide accurate information on iron status, particularly in older children, despite its role as an acute phase reactant (Stoltzfus *et al.*, 2000).

Although previous studies reported low serum retinol concentrations in subjects with palpable goiter (Ingenbleek & De Visscher, 1979; Wolde-Gebriel *et al.*, 1993), we found no association between vitamin A status and goiter. We measured plasma retinol concentrations using high-performance liquid chromatography, the recommended method to ensure high precision (de Pee & Dary, 2002). However, we may have failed to detect an association because diagnosis of vitamin A deficiency may be confounded by infection and/or inflammation. Serum retinol concentrations may decrease during the acute phase response to infection (Filteau *et al.*, 1993; Mitra *et al.*, 1998) or chronic inflammation (Stephensen & Gildengorin, 2000). This can lead to an overestimation of the prevalence of vitamin A deficiency. We did not measure an indicator of inflammation or infection, such as C-reactive protein (Fleck & Myers, 1985), and this may have limited our ability to distinguish the effects of inflammation/infection from vitamin A deficiency.

Selenium concentration in plasma is a useful indicator of status and is well-correlated with glutathione peroxidase activity in erythrocytes (Diplock, 1993). Data from cross-sectional studies investigating the association between plasma selenium and Tvol are equivocal. Giray *et al.* (2001) reported that goitrous children had significantly lower serum selenium levels than controls, and Zimmermann *et al.* (2000c) found that shrinkage in thyroid size after iodized oil was reduced with increasing severity of selenium deficiency. However, other investigators have found no association between selenium status and Tvol (Zagrodzki *et al.*, 2000; Erdogan *et al.*, 2001). Depending on the reference range applied, the prevalence of selenium deficiency in a randomly-selected subgroup of our sample was 92-100% (WHO, 1996b; FAO/WHO expert consultation, 2002). The narrow distribution of plasma selenium

concentrations in the sample, with many near the detection limit of our method, likely decreased our statistical power to detect an association between poor selenium status and goiter.

Logistic regression showed that gender was a significant predictor of goiter. Although more boys were defined goitrous than girls, there was no significant gender difference found for Tvol (*P*=0.86), similar to other reports (Vitti *et al.*, 1994; Xu *et al.*, 1999; Hess & Zimmermann, 2000). The significant impact of gender on goiter prevalence might be due to the difference in the provisional ICCIDD reference criteria for boys and girls (Zimmermann *et al.*, 2001). Village also had a significant impact on goiter. Although the 9 villages participating in this study were ethnically and economically equal, general health seemed to vary as indicated by significant differences between the different nutritional status indicators measured in this study (data not shown). However, we were unable to explain the significant impact the village had on goiter.

In this study, Tvol did not return to normal size despite 2 years of successful salt iodization in Côte d'Ivoire. Although low SF concentration significantly predicted goiter in school children two years after iodization of salt, other factors not identified in this study probably act in concert with iron depletion to blunt the thyroid response to iodized salt. Future research could focus on other micronutrient deficiencies (e.g. zinc) and/or other environmental factors (dietary goitrogens in food and water) which may affect thyroid metabolism.

Acknowledgments

We thank participating children and teachers, JB Gbato (Public Health, Côte d'Ivoire), Carol Flowers (University of Kansas Medical Center, Kansas City, KS, USA), Toni Torresani (Children's Hospital, Zürich) and Karin Lüscher, Marie-Helene Balsat, Sabine Renggli and Christophe Zeder (Swiss Federal Institute of Technology, Zürich, Switzerland) for assistance with the laboratory analyses. Financial support by The Thrasher Research Fund (Salt Lake City, UT, USA) and The Swiss Federal Institute of Technology (Zürich, Switzerland) is gratefully acknowledged.

References

Aghini-Lombardi F, Antonangeli L, Pinchera A, Leoli F, Rago T, Bartolomei AM & Vitti P (1997) Effect of iodized salt on thyroid volume of children living in an area previously characterized by moderate iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* **82**, 1136-1139.

Arnaud J, Malvy D, Richard MJ, Faure H & Chaventre A (2001) Selenium status in an iodine deficient population of the West Ivory Coast. *J Physiol Anthropol Appl Human Sci* **20**, 81-84.

Arthur JR, Beckett GJ & Mitchell JH (1999) The interactions between selenium and iodine deficiencies in man and animals. *Nutrition Research Reviews* **12**, 55-73.

Azizi F, Mirmiran P, Sheikholeslam R, Hedayati M & Rastmanesh R (2002a) The relation between serum ferritin and goiter, urinary iodine and thyroid hormone concentration. *International Journal for Vitamin and Nutrition Research* **72**, 296-299.

Azizi F, Navai L & Fattahi F (2002b) Goiter prevalence, urinary iodine excretion, thyroid function and anti-thyroid antibodies after 12 years of salt iodization in Shahriar, Iran. *International Journal for Vitamin and Nutrition Research* **75**, 291-295.

Beard J, Tobin B & Green W (1989) Evidence for thyroid hormone deficiency in iron-deficient anemic rats. *Journal of Nutrition* **119**, 772-778.

Beard JL, Brigham DE, Kelley SK & Green MH (1998) Plasma thyroid hormone kinetics are altered in iron-deficient rats. *Journal of Nutrition* **128**, 1401-1408.

Borella P, Bargellini A, Caselgrandi E, Menditto A, Patriarca M, Taylor A & Vivoli G (1998) Selenium determination in biological matrices. *Microchemical Journal* **58**, 325-336.

Brigham DE & Beard JL (1995) Effect of thyroid hormone replacement in iron-deficient rats. *American Journal of Physiology* **269**, R1140-1147.

Brunn J, Block U, Ruf G, Bos I, Kunze WP & Scriba PC (1981) Volumetrie der Schilddrüsenlappen mittels Real-time-Sonographie. *Deutsche Medizinische Wochenschrift* **106**, 1338-1340.

de Pee S & Dary O (2002) Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. *Journal of Nutrition* **132**, 2895S-2901S.

Delange F, Bourdoux P & Colinet P (1983) Nutritional factors involved in the goitrogenic action of cassava. In *Cassava toxicity and thyroid: research and public health issues*, pp. 17-27 [F Delange and R Ahluwahlia, editors]. Ottawa: IDRC.

Dibley MJ, Staehling N, Nieburg P & Trowbridge FL (1987) Interpretation of Z-score anthropometric indicators derived from the international growth reference. *American Journal of Clinical Nutrition* **46**, 749-762.

Dillman E, Gale C, Green W, Johnson DG, Mackler B & Finch C (1980) Hypothermia in iron deficiency due to altered triiodothyronine metabolism. *American Journal of Physiology* **239**, R377-381.

Diplock AT (1993) Indexes of selenium status in human populations. *American Journal of Clinical Nutrition* **57**, 256S-258S.

DuBois D & DuBois EF (1916) Clinical calorimetry. X. A formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine* **17**.

Erdogan MF, Erdogan G, Sav H, Gullu S & Kamel N (2001) Endemic goiter, thiocyanate overload, and selenium status in school-age children. *Biological Trace Element Research* **79**, 121-130.

FAO/WHO expert consultation (2002) Selenium. In *Human vitamin and mineral requirements*, pp. 235-255 [G Nantel and K Tontisirin, editors]. Rome: FAO.

Filteau SM, Morris SS, Abbott RA, Tomkins AM, Kirkwood BR, Arthur P, Ross DA, Gyapong JO & Raynes JG (1993) Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. *American Journal of Clinical Nutrition* **58**, 192-197.

Fleck A & Myers MA (1985) Diagnosistic and prognostic significance of the acute-phase proteins. In *The acute-phase response to injury and infection*, pp. 249-271 [AH Gordon and A Koj, editors]. Amsterdam: Elsevier Science Publisher B.V.

Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM & Cook JD (1986) A serum ferritin assay for prevalence studies of iron deficiency. *American Journal of Hematology* **23**, 141-151.

Giray B, Hincal F, Tezic T, Okten A & Gedik Y (2001) Status of selenium and antioxidant enzymes of goitrous children is lower than healthy controls and nongoitrous children with high iodine deficiency. *Biological Trace Element Research* **82**, 35-52.

Goyens P, Golstein J, Nsombola B, Vis H & Dumont JE (1987) Selenium deficiency as a possible factor in the pathogenesis of myxoedematous endemic cretinism. *Acta Endocrinologica* **114**, 497-502.

Hastka J, Lasserre JJ, Schwarzbeck A, Strauch M & Hehlmann R (1993) Zinc protoporphyrin in anemia of chronic disorders. *Blood* **81**, 1200-1204.

Hess SY, Zimmermann MB, Staubli-Asobayire F, Tebi A & Hurrell RF (1999) An evaluation of salt intake and iodine nutrition in a rural and urban area of the Côte d'Ivoire. *European Journal of Clinical Nutrition* **53**, 680-686.

Hess SY & Zimmermann MB (2000) Thyroid volumes in a national sample of iodine-sufficient swiss school children: comparison with the World Health Organization/International Council for the control of iodine deficiency disorders normative thyroid volume criteria. *European Journal of Endocrinology* **142**, 599-603.

Hess SY, Zimmermann MB, Adou P, Torresani T & Hurrell RF (2002) Treatment of iron deficiency in goitrous children improves the efficacy of iodized salt in Côte d'Ivoire. *American Journal of Clinical Nutrition* **75**, 743-748.

Higueret P & Garcin H (1979) Transport of thyroxine in the serum of vitamin A-deficient rats. *Journal of Endocrinology* **80**, 223-228.

Higueret P, Pailler I & Garcin H (1989) Vitamin A deficiency and tri-iodothyronine action at the cellular level in the rat. *Journal of Endocrinology* **121**, 75-79.

Ingenbleek Y & De Visscher M (1979) Hormonal and nutritional status: critical conditions for endemic goiter epidemiology? *Metabolism: Clinical and Experimental* **28**, 9-19.

Ingenbleek Y (1983) Vitamin A deficiency impairs the normal mannosylation, conformation and iodination of the thyroglobulin: a new etiological approach to endemic goiter. *Experientia. Supplementum* **44**, 264-297.

Jooste PL, Weight MJ, Locatelli-Rossi L & Lombard CJ (1999) Impact after 1 year of compulsory iodisation on the iodine content of table salt at retailer level in South Africa. *International Journal of Food Sciences and Nutrition* **50**, 7-12.

Keiser J, N'Goran EK, Traore M, Lohourignon KL, Singer BH, Lengeler C, Tanner M & Utzinger J (2002) Polyparasitism with Schistosoma mansoni, geohelminths, and intestinal protozoa in rural Côte d'Ivoire. *Journal of Parasitology* **88**, 461-466.

Lipschitz DA, Cook JD & Finch CA (1974) A clinical evaluation of serum ferritin as an index of iron stores. *New England Journal of Medicine* **290**, 1213-1216.

Lundquist P, Martensson J, Sorbo B & Ohman S (1979) Method for determining thiocyanate in serum and urine. *Clinical Chemistry* **25**, 678-681.

Mitra AK, Alvarez JO, Wahed MA, Fuchs GJ & Stephensen CB (1998) Predictors of serum retinol in children with shigellosis. *American Journal of Clinical Nutrition* **68**, 1088-1094.

Moreno-Reyes R, Suetens C, Mathieu F, Begaux F, Zhu D, Rivera MT, Boelaert M, Neve J, Perlmutter N & Vanderpas J (1998) Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. *New England Journal of Medicine* **339**, 1112-1120.

Nestel P & Davidsson L (2002) *Anemia, iron deficiency, and iron deficiecy anemia*. Washington, DC: International Nutritional Anemia Consultative Group.

Pardede LV, Hardjowasito W, Gross R, Dillon DH, Totoprajogo OS, Yosoprawoto M, Waskito L & Untoro J (1998) Urinary iodine excretion is the most appropriate outcome indicator for iodine deficiency at field conditions at district level. *Journal of Nutrition* **128**, 1122-1126.

Pino S, Fang SL & Braverman LE (1996) Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. *Clinical Chemistry* **42**, 239-243.

Skikne BS, Flowers CH & Cook JD (1990) Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* **75**, 1870-1876.

Staubli Asobayire F, Adou P, Davidsson L, Cook JD & Hurrell RF (2001) Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. *American Journal of Clinical Nutrition* **74**, 776-782.

Stephensen CB & Gildengorin G (2000) Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. *American Journal of Clinical Nutrition* **72**, 1170-1178.

Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L & Tielsch JM (1997) Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. *Journal of Nutrition* **127**, 293-298.

Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L & Tielsch JM (2000) Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. *Journal of Nutrition* **130**, 1724-1733.

Sullivan K, Houston R, Gorstein J & Cervinskas J (1995) *Monitoring universal salt iodization programmes*. Atlanta: Program Against Micronutrient Malnutrition / Micronutrient Initiative / International Council for Control of Iodine Deficiency Disorders.

Sullivan K & May S (1999) *Urinary iodine assessment: A manual on survey and laboratory methods*: United Nations Children's Fund / Program Against Micronutrient Malnutrition.

Tanumihardjo SA, Muherdiyantiningsih, Permaesih D, Dahro AM, Muhilal, Karyadi D & Olson JA (1994) Assessment of the vitamin A status in lactating and nonlactating, nonpregnant Indonesian women by use of the modified-relative-dose-response (MRDR) test. *American Journal of Clinical Nutrition* **60**, 142-147.

Torresani TE & Scherz R (1986) Thyroid screening of neonates without use of radioactivity: evaluation of time-resolved fluoroimmunoassay of thyrotropin. *Clinical Chemistry* **32**, 1013-1016.

Vanderpas JB, Contempré B, Duale NL, Goossens W, Bebe N, Thorpe R, Ntambue K, Dumont J, Thilly CH & Diplock AT (1990) Iodine and selenium deficiency associated with cretinism in northern Zaire. *American Journal of Clinical Nutrition* **52**, 1087-1093.

Vitti P, Martino E, Aghini-Lombardi F, Rago T, Antonangeli L, Maccherini D, Nanni P, Loviselli A, Balestrieri A, Araneo G & et al. (1994) Thyroid volume measurement by ultrasound in children as a tool for the assessment of mild iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* **79**, 600-603.

Wolde-Gebriel Z, West CE, Gebru H, Tadesse AS, Fisseha T, Gabre P, Aboye C, Ayana G & Hautvast JG (1993) Interrelationship between vitamin A, iodine and iron status in schoolchildren in Shoa Region, central Ethiopia. *British Journal of Nutrition* **70**, 593-607.

World Health Organization (1996a) Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. WHO, Geneva; WHO/NUT/96.10.

World Health Organization (1996b) *Trace elements in human nutrition and health*. Geneva: WHO.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (2001a) Assessment of iodine deficiency disorders and monitoring their elimination. WHO, Geneva; WHO/NHD/01.1.

World Health Organization, United Nations Children's Fund & United Nations University (2001b) Iron deficiency anemia: Assessment, prevention, and control. WHO, Geneva; WHO/NHD/01.3.

Xu F, Sullivan K, Houston R, Zhao J, May W & Maberly G (1999) Thyroid volumes in US and Bangladeshi schoolchildren: comparison with European schoolchildren. *European Journal of Endocrinology* **140**, 498-504.

Zagrodzki P, Szmigiel H, Ratajczak R, Szybinski Z & Zachwieja Z (2000) The role of selenium in iodine metabolism in children with goiter. *Environmental Health Perspectives* **108**, 67-71.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000a) Iron supplementation in goitrous, iron-deficient children improves their response to oral iodized oil. *European Journal of Endocrinology* **142**, 217-223.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000b) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. *American Journal of Clinical Nutrition* **71**, 88-93.

Zimmermann MB, Adou P, Torresani T, Zeder C & Hurrell RF (2000c) Effect of oral iodized oil on thyroid size and thyroid hormone metabolism in children with concurrent selenium and iodine deficiency. *European Journal of Clinical Nutrition* **54**, 209-213.

Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess S & Delange F (2001) Updated provisional WHO/ICCIDD reference values for sonographic thyroid volume in iodine-replete school-age children. *IDD Newsletter* **17**, 12.

Zimmermann MB & Köhrle J (2002) The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. *Thyroid* **12**, 867-878.

Zimmermann MB, Zeder C, Chaouki N, Torresani T, Saad A & Hurrell RF (2002) Addition of microencapsulated iron to iodized salt improves the efficacy of iodine in goitrous, iron-deficient children: a randomized, double-blind, controlled trial. *European Journal of Endocrinology* **147**, 747-753.

Zimmermann MB, Hess SY, Adou P, Torresani T, Wegmüller R & Hurrell RF (in press) Thyroid size and goiter prevalence after introduction of iodized salt: a 5-year prospective study using ultrasonography in schoolchildren in Côte d'Ivoire. *American Journal of Clinical Nutrition*.

Thyroid size and goiter prevalence after introduction of iodized salt: a 5-year prospective study using ultrasonography in school children in Côte d'Ivoire

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***American Journal of Clinical Nutrition* 77, 663-667, 2003.**

This study was supported by The Thrasher Research Fund (Salt Lake City, UT, USA) and The Swiss Federal Institute of Technology (Zürich, Switzerland).

Abstract

Background A long-term goal of salt iodization programs is reduction of the goiter rate to <5% in school-aged children. Normalization of this impact indicator likely signifies disappearance of the iodine deficiency disorders as a public health problem. However, thyroid size may not return to normal for months or years after correction of iodine deficiency.

Objective The study aim was to describe the time course and pattern of changes in thyroid size and goiter rate in response to introduction of iodized salt in an area of long-standing, severe endemic goiter.

Design A 5-year prospective study with measurements of thyroid size by ultrasonography, urinary iodine and thyroid hormones in 5-14 yr-old school children 6 months before introduction of iodized salt and annually for 4 years thereafter.

Results Four years after introduction of iodized salt and normalization of median urinary iodine concentration, mean thyroid size had decreased –56% (P<0.0001). However, 29% of children remained goitrous. There was a significant age shift in the distribution of goiter in the sample. At baseline, the goiter rate was significantly higher in younger (5-9 yr olds) than in older children (10-14 yr-olds) (P<0.0001). At 2, 3 and 4 years after salt iodization, the goiter rate was significantly higher in the older compared to the younger children (at 4 yrs: 52% vs. 19%), and the difference was increasing with time (P<0.0001).

Conclusion The goiter rate in school-aged children may remain sharply elevated for up to 4 yrs after successful introduction of iodized salt, primarily due to persisting goiter in older children.

Introduction

The success of universal salt iodization (USI) for control of the iodine deficiency disorders (IDD) requires impact monitoring at a population level. The principal indicator of impact is the median urinary iodine concentration (UI), because it is highly sensitive to recent changes in iodine intake (1). A second indicator is thyroid size as reflected by the goiter rate. Although thyroid size changes inversely in response to alterations in iodine intake, there is a lag before goiter rate normalizes after iodine repletion. The duration of this lag period is unclear, with experts suggesting it may last from months to years (2). During this period, goiter rate is a poor impact indicator because it reflects a population's history of iodine nutrition but not its present iodine status. Cross-sectional studies have reported a discrepancy between UI and goiter rate in the immediate post-USI introduction period (3,4).

Despite this, goiter rate, when accurately assessed, remains an important and sensitive long-term indicator of the success of an iodine program. The ultimate goal of USI is not only to increase access to iodized salt and increase UI, but to normalize thyroid function in individuals affected by IDD. Because goiter represents maladaptation of the thyroid to iodine deficiency (5,6), the reduction of goiter rate to <5% in school-aged children likely indicates disappearance of IDD as a significant public health problem (1).

Although large doses of iodine as iodized oil, either intramuscularly or orally, rapidly reduce goiter rate (7,8), many of these studies used thyroid palpation to grade goiter. Palpation is subjective and its sensitivity and specificity are low (1). Particularly in areas of mild-to-moderate IDD and for monitoring the impact of USI, measurement of thyroid size by ultrasonography is preferable to palpation (9). Although estimating goiter rate in children based on thyroid size has been hampered by the difficulty in establishing references for thyroid volume in school-aged children, updated WHO/ICCIDD reference criteria have recently been published (10).

In Chinese school children affected by mild IDD, goiter rate by ultrasonography was reduced from 18% to 5-9% after 18 months of salt iodization (11). We are aware of no other long-term, prospective studies using ultrasonography to measure changes in thyroid size and goiter rate after introduction of iodized salt in IDD-affected children. Populations in western Côte d'Ivoire were severely affected by IDD until 1998 (12), when USI was successfully introduced. We therefore conducted a 5-year

study of school-aged children in this region, measuring thyroid size, UI, and thyroid hormones, before and after introduction of USI.

Subjects and Methods

The study was done in six remote villages in the Danané Health District, a mountainous region of western Côte d'Ivoire. The villages are located within a □10 km radius in dense forest and have no electricity or running water. Most families are engaged in small-scale subsistence farming. The staple foods are rice and cassava. The villages are similar ethnically and socioeconomically. Before introduction of USI, this region was affected by severe IDD and goiter was endemic (12). The study was approved by the Ethical Review Board of the Children's Hospital of the University of Zürich, the National Institute of Public Health and the Ministry of Research of the Côte d'Ivoire. Informed oral consent was given by the village chiefs, teachers and parents. In late 1997, Côte d'Ivoire legislated mandatory USI at a production level 30-50 ppm. In February-March of 1998, iodized salt was introduced into the Danané region. By 1999, it was estimated that >80% of Ivorian households had access to iodized salt at a market level of 20-30 ppm (unpublished data, 2000, P. Adou, National Institute of Public Health of Côte d'Ivoire). The present study was done from 1997 through 2001.

The subjects were school children recruited from six primary schools. The study visits were done in the same month (November) in the midst of the dry season for 5 consecutive years. All children 5-14 yrs-old attending school on days when the fieldwork was done were measured. School attendance is only sporadic in this region so samples from the 5 years varied in size. Children were recruited from two schools in 1997 and 1998, and all six schools in 1999-2001. Age and sex were recorded, and weight was measured using a calibrated and leveled digital scale to the nearest 100 g. Height was measured to the nearest mm using a pull-down metal measuring tape. Spot urine samples were collected for measurement of UI. Whole-blood was spotted onto filter paper for measurement of thyroxine (T_4) in 1997-99 and thyrotropin (TSH) in 1997-2001. In 1999, T_4 and TSH were measured on 51 children randomly selected from the sample; in other years, all children were measured. In 1997, goiter was graded by either palpation using WHO criteria (n=291) or thyroid ultrasonography (n=128) (1). In 1998-2001, thyroid size was measured using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5 MHz linear transducer, with subjects sitting and the neck slightly extended. S.H. or M.Z. performed all

ultrasonography measurements over the 5 years. Each year, salt samples were collected from random households of participating children. In addition, to evaluate potential goitrogenic factors, in 1997 and in 1999 whole blood was collected by venipuncture for determination of hemoglobin (Hb), serum ferritin (SF), whole-blood zinc protoporphyrin (ZPP), serum transferrin receptor (TfR), serum selenium and serum retinol, and a spot urine sample was collected for measurement of urinary thiocyanate.

Laboratory analyses

Urine and blood samples were transported on ice to the regional hospital laboratory. Serum and urine samples were aliquoted and frozen at –20° C until analysis. UI was measured using a modification of the Sandell-Kolthoff reaction (13). At UI concentrations of 47 μg/L and 79 μg/L, the CV of this assay in our laboratory is 10.3 and 12.7%. Iodine concentration in salt was measured by titration with thiosulfate (14). The CV of this measurement in our laboratory is 0.64 at 10 μg/g. Dried blood spots on filter paper were analysed for whole blood TSH and serum T_4 using immunoassay (15). To convert whole blood TSH values to serum values, whole blood TSH values were multiplied by 2. Normal reference values are TSH, <3.5 mU/L; T4, 65-165 nmol/L. Hb was measured using a AcT8 Counter (Beckman Coulter, Krefeld, Germany). ZPP was measured on washed red blood cells using a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA). SF and TfR were measured using an enzyme-linked immunosorbent assay (16,17). Normal reference values are: SF, 12-300 μg/L; TfR, 2.9-8.5 mg/L; ZPP, <40 μmol/mole heme. Iron deficiency was defined using multiple criteria: SF < 15 μg/L; or TfR > 8.5 mg/L + ZPP > 40 μmol/mole heme. Thyroid volume was calculated using the method of Brunn *et al.* (19). In countries with a high prevalence of child growth retardation, thyroid volume is considered to be more directly a function of body surface area (BSA) than of age (1). Therefore, BSA was calculated from weight and height measurements taken with each ultrasonography measurement. Updated World Health Organization /International Council for the Control of Iodine Deficiency Disorders (WHO/ICCIDD) normative values for thyroid volume in school-age children according to sex and BSA were used to define goiter (10). Urinary thiocyanate (SCN) was analyzed by a colorimetric method (20). Serum selenium was measured by atomic absorption spectrometry with the Zeeman background correction (Perkin-Elmer Model 4100 ZL, Norwalk, CT, USA9 (21) with a limit of sensitivity of 6.5 μg/L; undetectable concentrations were assigned a value of 6.5 μg Se/L. Serum retinol was measured

by HPLC (22). Normal reference values are: UI/SCN, >3µg/mg (23); serum retinol >0.70 µmol/L; serum selenium, 65-105 µg/L.

Statistical analyses

Data processing and statistics were done using GraphPad Prism3 (GraphPad, San Diego, USA) and Excel 97 (Microsoft, Seattle, WA, USA). Although follow-up data were not obtained for individual children, the same schools were sampled at yearly visits, so overlap between the samples was considerable. For the data analysis, a conservative approach was taken and the samples were considered independent. Age, height, weight, salt iodine concentration, UI, TSH, T_4, and thyroid volume were compared using one-way ANOVA across years and Tukey's test for post-hoc comparisons. Parameters not normally distributed (UI, TSH, thyroid volume) were logarithmically transformed before analysis. Proportions were compared using the chi-square test. Logistic regression was done to compare effects of time and group (older vs. younger children) on percentage change in thyroid volume from baseline and goiter rate. Significance was set at $P < 0.05$.

Results

There were no significant differences in mean age, weight, or height of the children sampled at each visit (Table 1). Reflecting the local preference for sending boys to school, 63% of the total sample was male. In 1997 (baseline), before introduction of USI, there was no measurable iodine in salt. UI and goiter rate were 28 µg/L and 45%, respectively, indicating moderate-to-severe IDD (1). Significantly more young children (5-9 yr-olds) were goitrous than older children (10-14 yr-olds) (P <0.0001) (Table 2). In early 1998, the USI program was introduced. By November of 1998, the mean (SD) iodine concentration in household salt had increased to 11(9) µg/g, and UI had increased to 86 µg/L (P<0.0001). There was a small, non-significant reduction in thyroid size compared to baseline, and goiter rate remained high, with 84% of children affected (Table 2). While either palpation or ultrasonography were used to measure goiter rate in 1997, only ultrasonography was used in 1998. The increase in goiter rate between 1997 and 1998 is likely an artifact reflecting the increased sensitivity of ultrasonography to detect mildly enlarged thyroids (4,9). In 1999, mean iodine concentration in household salt was 25 µg/g, and UI was 161 µg/L, indicating adequate iodine intake (Table 1). This was associated with a significant -35% reduction in mean thyroid size compared to baseline (P<0.0001), but only a 8%

Table 1. Age, sex ratio, height, weight, salt iodine concentration, serum TSH and T_4, and median urinary iodine concentration in 5-14 year-old children in Côte d'Ivoire before and after introduction of iodized salt.

	Preiodization	***Postiodization***			
	1997 (n=419)	***1998 (n=204)***	***1999 (n=641)***	***2000 (n=507)***	***2001 n=526)***
Age (years)[1]	8.8±2.7[a]	8.5±2.5[a]	8.9±2.3[a]	8.8±2.3[a]	8.5±2.3[a]
Sex (M / F)	231 / 186	115 / 89	441 / 200	333 / 170	337 / 188
Height (m)[1]	1.27±0.17[a]	1.24±0.19[a]	1.28±0.13[a]	1.25±0.14[a]	1.25±0.16[a]
Weight (kg)[1]	24.9±7.1[a]	25.1±6.7[a]	25.8±7.3[a]	24.8±7.4[a]	24.4±6.9[a]
Serum TSH (mU/L)[2]	2.2 (0.4-76.4)[a]	1.2 (0.6-24.6)[b]	1.4 (0.4-8.8)[b]	1.4 (0.4-8.2)[b]	1.4 (0.4-16.2)[b]
No. of subjects with >3.5 mU/L[3]	35 [8][a]	12 [6][a]	1 [2][b]	16 [3][b]	13 [2][b]
Serum thyroxine (nmol/L)[1]	137±36[a]	122±25[a]	126±29[a]	Not measured	Not measured
No. of subjects with <65 nmol/L[3]	12 [2][a]	4 [2][a]	1 [2][a]		
Urinary iodine (μg/L)[2]	28 (5-176)[a]	86 (12-541)[b]	161 (16-936)[c]	133 (4-1339)[c]	94 (8-785)[b]
No. of subjects < 20	117 [28]	16 [8]	3 [1]	14 [3]	12 [2]
No. of subjects < 50	337 [80]	31 [15]	5 [2]	66 [13]	59 [11]
No. of subjects < 100	392 [94]	106 [52]	45 [21]	188 [37]	276 [52]
Salt iodine (μg/g)[4]	<2 (n=52)[a]	11±9 (n=23)[b]	25±18 (n=213)[c]	16±5 (n=58)[d]	11±5 (n= 94)[b]

Values are given as: means±SD; medians (range); or numbers [percentage].
[1] Compared using one-way ANOVA across years (N.S.).
[2] Compared using one-way ANOVA on logarithmically transformed data across years (P<0.0001). Tukey's test for post-hoc comparisons.
[3]Compared using Chi square test.
[4] Compared using one-way ANOVA across years (P<0.0001). Tukey's test for post-hoc comparisons.
Across rows, values without a common letter are significantly different. Significance of post-hoc comparisons are given in the text.

reduction in goiter rate (Table 2). In 2000, goiter rate had decreased significantly to 42%, half of the prevalence in 1998 (*P*<0.0001). In 2001, 4 years after USI, although mean thyroid size had decreased 57% compared to baseline (*P*<0.0001), 29% of children remained goitrous.

Over the course of the study, there was an age shift in the distribution of goiter (Table 2). Preiodization, goiter rate was significantly higher in younger (5-9 yr olds) than in older children (10-14 yr-olds) (p<0.0001). At 2, 3 and 4 yrs after USI, although goiter rate had decreased significantly compared to baseline in both younger and older children, the decrease was greater in the younger children (*P*<0.0001). As modeled by logistic regression, at 2, 3 and 4 yrs post iodization, the goiter rate was significantly greater in the older children compared to the younger children (*P*<0.0001), and the group difference increased with time (*P*<0.0001 comparing time and group model relative to time only model). After 4 yrs, the goiter rate in the younger children had fallen to 19%, compared to 52% in the older children (*P*<0.0001). The percentage decrease in mean thyroid size after 4 yrs was significantly greater in the younger (-63%) compared to the older children (-41%) (p<0.0001), and the group difference increased with time (*P*<0.0001 comparing time and group model relative to time only model).

Mean serum T_4 and median serum TSH were within the normal reference ranges both pre- and post-USI, and there was no significant change in mean serum T_4 over the course of the study (Table 1). However, in response to salt iodization, there was a significant decrease in median serum TSH and in the number of children with an elevated TSH concentrations (*P*<0.0001). Measured in 1997 and again in 1999, there was a high prevalence of potential goitrogenic factors in the children. The prevalence of iron-deficiency anemia (IDA) in 1997 and 1999 was 27% and 19%, respectively. In 1997, mean serum selenium (SD) was only 15.4 (8.4) μg/L, and 92% of children had low serum selenium concentrations. Deficiencies of vitamin A were common, with 64% and 45% of children having low concentrations of serum retinol in 1997 and 1999. In 1997, the median urinary iodine/urinary thiocyanate (UI/SCN) ratio was only 1.8 μg/mg, indicating risk for exacerbation of goiter (23).

Table 2. Thyroid volume and prevalence of goiter in 5-14 yr-old children in Côte d'Ivoire before and after introduction of iodized salt.

	Preiodization	***Postiodization***			
	1997 (n=419)	***1998 (n=204)***	***1999 (n=641)***	***2000 (n=507)***	***2001 (n=526)***
Thyroid volume (ml)[1]	8.3 (3.1-20.1)[a]	7.1 (2.1-21.4)[a]	5.4 (1.4-39.7)[b]	3.9 (1.2-14.5)[c]	3.4 (1.1-22.5)[d]
Thyroid volume (ml) in 5-9 yr-olds	7.2 (4.9-17.9)[a]	6.7 (2.1-19.3)[a]	4.7 (1.4-16.0)[b]	3.1 (1.2-10.9)[c]	2.7 (1.1-10.4)[d]
Thyroid volume (ml) in 10-14 yr-olds	9.3 (7.0-20.1)[a]	8.3 (3.5-21.4)[a]	7.1 (1.9-39.7)[b]	5.6 (1.7-14.5)[c]	5.4 (1.7-22.5)[c]
Number of subjects with goiter[2]	188 [45][a]	172 [84][b]	486 [76][b]	207 [42][a]	152 [29][c]
No. of females with goiter	72 [40][a]	67 [76][b]	132 [65][b]	57 [34][a]	46 [24][c]
No. of males with goiter	116 [49][a]	105 [91][b]	354 [80][b]	150 [46][a]	106 [31][c]
No. of 5-9 yr-olds with goiter	112 [52][a]	119 [84][b]	295 [71][c]	120 [37][d]	71 [19][e]
No. of 10-14 yr-olds with goiter	76 [38][a]	53 [84][b]	291 [83][b]	87 [54][c]	81 [52][c]

Values are given as medians (range) or numbers [percentage].

[1] Compared using one-way ANOVA on logarithmically transformed data across years (P<0.0001). Tukey's test for post-hoc comparisons

[2] Compared using Chi-square test.

Across rows, values without a common letter are significantly different. Significance of post-hoc comparisons are given in the text.

Discussion

In this study, USI rapidly normalized UI, decreased mean TSH and reduced the proportion of children with an elevated TSH. These impact indicators are highly sensitive to recent changes in iodine intake (1). In contrast, goiter rate was 29% four years after USI, and using WHO/ICCIDD/UNICEF criteria for assessing severity of IDD using goiter rate in children, should indicate moderate-severe IDD (1). A discrepancy between a normal UI and an elevated goiter rate in the immediate post-USI period has been reported in several cross-sectional studies (3,4). There are several potential reasons for the long delay in goiter rate response. Endemic goiter is due to thyroid overstimulation by TSH in an effort to maximize the utilization of available iodine. In this study, mean TSH concentration decreased significantly in the first year and remained in the low-normal range thereafter. Only 2-3% of children exhibited elevated TSH levels after the 1st year. Thus, persisting TSH overstimulation does not appear to explain the high goiter rate. Although it has been suggested that long-standing goiters may become autoimmune (24), we have measured antithyroid antibodies in these children and found no evidence of increased thyroid autoimmunity (M. Zimmermann, unpublished data). Multiple goitrogens present in the children may have blunted the effect of USI. Deficiencies of selenium, iron and vitamin A were common and may impair the thyroid response to iodine repletion (12,25). Also, cassava is one of the staple foods of this region and median UI/SCN ratios were low (<3 µg/mg) indicating increased risk for exacerbation of goiter by thiocyanate (23).

A potential limitation of goiter rate in children as a USI impact indicator is the possibility that enlarged thyroids in children who are iodine deficient during the first years of life may not regress completely after introduction of iodized salt (26). If true, this suggests that to achieve a goiter rate < 5% in children aged 6-12 yrs may require that the children grow up under conditions of iodine sufficiency. This implies that the lag time to normalization of thyroid size and goiter rate in children aged 10-12 yrs could be a decade or more. In support of the premise that enlarged thyroids in children growing up in IDD-affected areas may not regress completely, our data indicate a clear age shift in the goiter rate in the present study (Table 2). Before iodization, significantly more younger children than older children were goitrous. In response to 4 years of adequate iodine supply, mean percentage decrease in thyroid size from baseline was significantly greater in the younger than in the older children. This was reflected in a significantly higher goiter rate in the older children at 2, 3 and

4 yrs post introduction of USI. After 4 yrs, the goiter rate in the younger children was nearly 1/3rd that of the older children (19% vs. 52%).

Several authors have reported trials of iodized oil in children and used ultrasonography to measure thyroid response. In Algerian schoolchildren, iodized oil providing iodine doses of 960 mg (orally) or 480 mg (intramuscularly) decreased mean thyroid volume 23-29% after 1 year (27). In Côte d'Ivoire, 200 mg iodine as oral iodized oil given to school-aged children was associated with a –41% reduction in mean thyroid volume after 1 year (28). Other studies have evaluated the impact on goiter rate of smaller doses of oral iodine given as potassium iodide solution or in iodized salt. Oral administration of potassium iodide solution providing 30 mg of iodine monthly or 8 mg biweekly to school-aged children in Zimbabwe significantly reduced thyroid volumes measured by ultrasonography over 13 months (29). In South African children, after 1 year of mandatory USI, UI was normalized but goiter rate by palpation was unchanged (3). In a small study in Chinese schoolchildren comparing iodized oil to iodized salt, provision of iodized salt normalized goiter rate after 18 months (11). However, the children were only mildly iodine deficient at baseline and the UI throughout the intervention was maintained >200 μg/L. In contrast, our subjects were severely iodine-deficient at baseline and USI maintained UI at a lower level of 86-161 μg/L.

The strengths of this study are its prospective design and long follow-up, as well as the use of ultrasonography to measure thyroid size and updated WHO/ICCIDD references to classify goiter. Our data emphasize that goiter rate is a poor IDD indicator up to 4 years after introduction of USI because it reflects chronic, rather than immediate, iodine deficiency. Compared to the strong and rapid reduction in thyroid size induced by large doses of iodine as iodized oil, shrinkage and remodeling of the goitrous thyroid in response to the modest iodine doses associated with USI appears to be much more gradual. Despite this, goiter rate is a sensitive long-term indicator of the success of an iodine program, and normalization of goiter rate in schoolchildren previously affected by IDD have been reported by sustained USI programs (2,30). Governments and program managers monitoring USI impact, encouraged by rapid improvements in salt iodine levels and UI, may expect a parallel improvement in goiter rate. It is important to recognize the limitation of goiter rate in judging the short-term efficacy of salt iodization programs.

References

1. WHO/UNICEF/ICCIDD. Assessment of Iodine Deficiency Disorders and Monitoring their Elimination. WHO/NHD/01.1. Geneva: World Health Organization, 2001.
2. Delange F, de Benoist B, Pretell E, Dunn JT. Iodine deficiency in the world: where do we stand at the turn of the century? 2001 Thyroid 11:437-447.
3. Jooste PL, Weight MJ, Lombard CJ. Short-term effectiveness of mandatory iodization of table salt, at an elevated iodine concentration, on the iodine and goiter status of schoolchildren with endemic goiter. Am J Clin Nutr 2000;71:75-80.
4. Pardede LVH, Hardjowasito W, Gross R, Dillon DHS, Totoprajogo OS, Yosoprawoto M et al. Urinary iodine excretion id the most appropriate outcome indicator for iodine deficiency at field conditions at district level. J Nutr 1998;128:122-1126.
5. Dumont JE, Ermans AM, Maenhaut G, Coppée F, Stanbury JB. Large goiter as a maladaptation to iodine deficiency. Clin Endocrinol 1995; 43:1-10.
6. Delange F. Adaptation to iodine deficiency during growth: etiopathogenesis of endemic goiter and cretinism. In: Delange F, Fisher D, Malvaux P, eds. Pediatric thyroidology. Basel: Karger,1985: 295-396.
7. Dunn JT. Iodized oil in the treatment and prophylaxis of IDD. In: Hetzel BS, Dunn JT, Stanbury J, eds. The prevention and control of iodine deficiency disorders. Amsterdam: Elsevier, 1987: 127-139.
8. Furnée CA, West CE, van der Haar, F, Hautvast JGAJ. Efficacy of oral iodized oil is associated with anthropometric status in severely iodine-deficient schoolchildren in rural Malawi. Br J Nutr 2000;84:343-352.
9. Zimmermann MB, Saad A, Hess SY et al. Thyroid ultrasound compared to WHO 1960 and 1994 palpation criteria for determination of goiter prevalence in regions of mild and severe iodine deficiency. Eur J Endocrinol 2000;143:727-31
10. Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess SY, Delange F. Updated provisional WHO/ICCIDD reference values for sonographic thyroid volume in iodine-replete school-age children. ICCIDD Newsletter 2001;1:12.
11. Zhao J, Xu F, Zhang Q, Shang L, Xu A, Ghao Y et al. Randomized clinical trial comparing different iodine interventions in school children. Publ Health Nutr 1999;2:173-178.
12. Zimmermann MB, Adou P, Zeder C, Torresani T, Hurrell RF. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. Am J Clin Nutr 2000;71:88-93.
13. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem 1996;42:239-43.
14. Sullivan KH, Houston R, Gorstein J, Cervinskas J. Monitoring Universal Salt Iodization Programmes. 1995; PAMM/MI/ICCIDD, Atlanta, USA.
15. Torresani T, Scherz R. Thyroid screening of neonates without use of radioactivity: evaluation of time-resolved fluoroimmunoassay of thyrotropin. Clin Chem 1986;2:1013-16
16. Flowers CH, Kuizon M, Beard J, Skikne B, Covell A, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141-51.
17. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990;75:1870-76
18. Perry GS, Byers T, Yip R, et al. Iron nutrition does not account for the hemoglobin differences between blacks and whites. J Nutr 1992; 122: 1417-24.
19. Brunn J, Block U, Ruf G, Bos I, Kunze WP, Scriba PC. Volume measurement of the thyroid gland using real-time sonography. Deutsch Med Wochenschrift 1981;106:1338-40 (In German).
20. Bowler RG. The determination of thiocyanate in blood serum. Biochem J 1944;38:385-89.
21. Van Dael P, Van Cauwenbergh R, Robberecht H, et al. Determination of Se in human serum by AAS using electrothermal atomization with longitudinal Zeeman-effect background correction or flow injection hydride generation. Atomic Spectroscopy 1995;16:251-255.
22. Catignani GL, Bieri JG. Simultaneous determination of retinol and tocopherols in serum or plasma by liquid chromatography. Clin Chem 1983;29:708-712.
23. Delange F, Bourdoux P, Colinet P. Nutritional factors involved in the goitrogenic action of cassava. In: Delange F, Ahluwahlia R, eds. Cassava toxicity and thyroid: research and public health issues. Ottawa: IDRC, 1983: 17-27.

24. Dremier S, Coppee F, Delange F, Vassart G, Dumont JE, Van Sande J.J Clinical review 84: Thyroid autonomy: mechanism and clinical effects.Clin Endocrinol Metab 1996 Dec;81(12):4187-93.
25. Zimmermann MB, Torresani T, Adou P, Zeder C, Hurrell RF. Effect of oral iodized oil on thyroid size and thyroid hormone metabolism in children with concurrent selenium and iodine deficiency. Eur J Clin Nutr 2000; 54:209-213.
26. Aghini-Lombardi F, Antonangeli L, Pinchera A, Leoli F, Rago T, Bartolomei AM, et al. Effect of iodized salt on thyroid volume of children living in an area previously characterized by moderate iodine deficiency. J Clin Endocrinol Metab 1997;82:1136-39.
27. Benmiloud M, Lamine Chaouki M, Gutekunst R, Teichert HM, Graham Wood W, Dunn JT. Oral iodized oil for correcting iodine deficiency: optimal dosing and outcome indicator selection. J Clin Endocrinol Metab 1994;79:20-24.
28. Zimmermann MB, Adou P, Torresani T, Zeder C, Hurrell RF. Low dose oral iodized oil for control of iodine deficiency in children. Br J Nutr 2000; 84: 139-141.
29. Todd CH, Dunn JT. Intermittent oral administration of potassium iodide solution for the correction of iodine deficiency.Am J Clin Nutr. 1998;67:1279-83.
30. Delange F, Lecomte P. Iodine supplementation: benefits outweigh risks. Drug Safety 2000;22:89-95.

Thyroid volumes in a national sample of iodine-sufficient Swiss school children: comparison to the WHO / ICCIDD normative thyroid volume criteria

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European Journal of Endocrinology **142, 599-603, 2000.**

Abstract

Objective: The determination of goiter prevalence in children by thyroid ultrasound is an important tool for assessing iodine deficiency disorders (IDD). The current WHO / ICCIDD normative values, based on thyroid volume in iodine-sufficient European children, have recently been questioned, as thyroid volumes in iodine-sufficient children from the USA and Malaysia are smaller than the WHO / ICCIDD reference data. Our objective was to describe ultrasonographic thyroid volumes in a representative national sample of iodine-sufficient Swiss school children, and compare these with the current reference data for thyroid volume.

Design and Methods: A 3-stage, probability-proportionate-to-size cluster sampling method was used to obtain a representative national sample of 600 Swiss children aged 6-12 years. The following data were collected: thyroid size by ultrasound, urinary iodine concentration, weight, height, sex and age.

Results: The median urinary iodine concentration (range) of the children was 115 µg/l (5-413). Application of the WHO / ICCIDD thyroid volume references to the Swiss children resulted in a prevalence of 0%, using either age/sex-specific or body surface area (BSA)/sex-specific cut-off values. Upper limits of normal (97^{th} percentile) of thyroid volume from Swiss children calculated using BSA, sex and age were similar to those reported in iodine-sufficient children in the US, but 20-56% lower than the corresponding WHO / ICCIDD references.

Conclusions: Swiss children had smaller thyroids than the European children on which the WHO/ICCIDD references are based, perhaps due to a residual effect of a recent past history of iodine deficiency in many European regions. However, there were sharp differences between our data and a recent set of thyroid volume data in Swiss children produced by the operator and equipment that generated the WHO/ICCIDD reference data. This suggests that interobserver and/or interequipment variability may contribute to the current disagreement on normative values for thyroid size by ultrasound in iodine-sufficient children.

Introduction

The median urinary iodine concentration (UI) and goiter prevalence are the most widely-used indicators for assessing iodine deficiency disorders (IDD) in a population (1). In areas of mild endemicity, ultrasonographic measurement of thyroid volume is preferable to inspection and palpation for determination of goiter prevalence (2). However, interpretation of thyroid ultrasonography requires valid reference criteria from iodine-sufficient populations. Although school-age children are a useful target group for IDD surveillance (1), defining normal values for thyroid size in children has proven difficult. The original normative criteria for thyroid volume proposed by Gutekunst and Martin-Teichert (3) were criticized as too low because the 97th percentiles classified a high percentage of children as goitrous in areas where iodine supply was sufficient (4).

The World Health Organization / International Council for the Control of Iodine Deficiency Disorders (WHO/ICCIDD) adopted a new thyroid volume reference in 1997 (2). However, recent reports have suggested the WHO/ICCIDD reference criteria may be too high (5,6). Thyroid volumes in iodine-sufficient US children (5) and iodine-sufficient Malaysian children (6) are distinctly smaller than the European children from whom the WHO reference data are derived (4). The reason for this discrepancy is unclear: it may be due to interobserver/equipment variability in ultrasonography and/or may be a residual effect of iodine deficiency that existed in many European countries up to the early 1990s (5,7, 9).

In contrast to much of Europe, iodized table salt has been available nationwide in Switzerland for 50 years and has been iodized at 15 mg iodine/kg since 1980 (8,9). Swiss children who are 6-12 years old today very likely have had a steady and sufficient iodine intake since birth (10-12). It would therefore be valuable to evaluate the current WHO/ICCIDD reference data in Swiss children. Bürgi *et al.* reported that age-adjusted median thyroid volumes and 97th percentiles measured in early 1997 in children from 2 cities in Switzerland closely agreed with WHO/ICCIDD reference data (12). The study by Bürgi *et al.* (12) employed identical equipment and the same operator whose ultrasound measurements had been used to generate the WHO/ICCIDD reference data (4).

We recently completed a national survey of iodine nutrition in Switzerland, to assess iodine status 1 year after the iodine content in salt was increased from 15 to 20

mg/kg. One objective was to describe thyroid volumes by ultrasonography in a representative national sample of 6-12 yr old Swiss school children with an assumed lifetime of iodine sufficiency, and compare these with the WHO reference data. In addition, by comparing our data to that of Bürgi *et al.* (12), we wished to examine if interobserver/equipment variability in thyroid ultrasound may contribute to the current disagreement on normative values for thyroid size in iodine-sufficient children.

Subjects and Methods

Subjects

A 3-stage probability proportionate to size (PPS) cluster sampling method (1) was used to obtain a representative national sample of 600 Swiss children aged 6-12 years. The design used current census data to provide a systematic sampling of communities based on the cumulative population. Stage 1 of the sampling involved a stratified random selection and recruitment of 30 schools for participation in the study. Written consent was then obtained from the community school boards. If a school declined participation another randomly selected school from the same stratum replaced it. In the second stage, 2 classrooms at the appropriate grade level were randomly selected from each school. Finally, the teachers of the classrooms randomly selected students to participate. An average of 20 students was sampled at each school, the number varying depending on the size of the classrooms. Data were collected from April through October 1999. Ethical approval for the study was obtained from the Human Subjects Committee of the Swiss Federal Institute of Technology in Zürich. Written consent was obtained from the community school boards, as well as the teachers and parents of the children involved.

Methods

Height and weight were measured using standard anthropometric techniques (13). For the measurements, subjects removed their shoes, emptied their pockets and wore light indoor summer clothing. Height was recorded to the nearest cm and weight to the nearest 100 g. Body surface area (BSA) was calculated from weight and height measurements using the formula: BSA = weight $(kg)^{0.425}$ x height $(cm)^{0.725}$ x 71,84 x 10^{-4} (2). Spot urine samples were collected from all children and stored at -20°C until analysis. The iodine concentration in the urine was measured using a modification of the Sandell-Kolthoff reaction as described by Pino *et al*. (14) with ammonium persulfate as the oxidizing reagent. Thyroid gland volume was measured using an Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a 7.5 MHz linear

transducer. Measurements were performed on subjects sitting upright with the neck slightly extended. Volume of each lobe was calculated according to the formula: width x length x thickness x 0.479 and the lobe volumes were summed (2). The volume of the isthmus was not included. S.H. or M.Z performed the ultrasound measurements. To estimate intra- and interobserver variability, S.H. measured 20 school children twice and M.Z. measured the same children once. The mean (SD) intra- and interobserver errors were 4.9 (4.0)% and 3.7 (3.5)%, respectively.

Statistical analysis

Data processing and statistics were done using SPLUS 4.5 (Mathsoft, Seattle, USA) and Excel (Microsoft, Seattle, WA, USA). The thyroid volume distributions for each age and BSA group for both sexes were skewed to the right. The distributions were logarithmically transformed and the Kolmogorov-Smirnov test was used to verify normality of the transformed data. Means and standard deviations of the logarithm of the thyroid volume were then used as parameters to fit a normal distribution, and 97th percentiles (P97) were calculated from the P97 of the standard normal distribution. Differences in thyroid volume between groups were tested using the Mann-Whitney test. Curves of the P97 thyroid volumes against age and BSA were constructed and smoothed using regression.

Results

A total of 612 students from 30 schools throughout Switzerland were studied. This represents approximately 1 in 1000 children in this age group in Switzerland (15). The sample included 310 females and 302 males aged 6-12 yrs. Mean age (SD) was 9.3 (1.9). The median UI (range) of the children was 115 µg/l (5-413). The goiter prevalence in our sample using the P97 of the original normative data of Gutekunst and the current WHO/ICCIDD-recommended cut-off values was 3.9% (n=23) and 0%, respectively. There were significant gender differences in median thyroid volume ($p<0.01$) only at age 12 years, when females had a median thyroid volume 17% greater than males. By BSA, significant differences ($P<0.05$) between females and males were found only when BSA was > 1.4 m^2. At a BSA of 1.5 and 1.6, females had a median thyroid volume 9% and 10% greater than males, respectively.

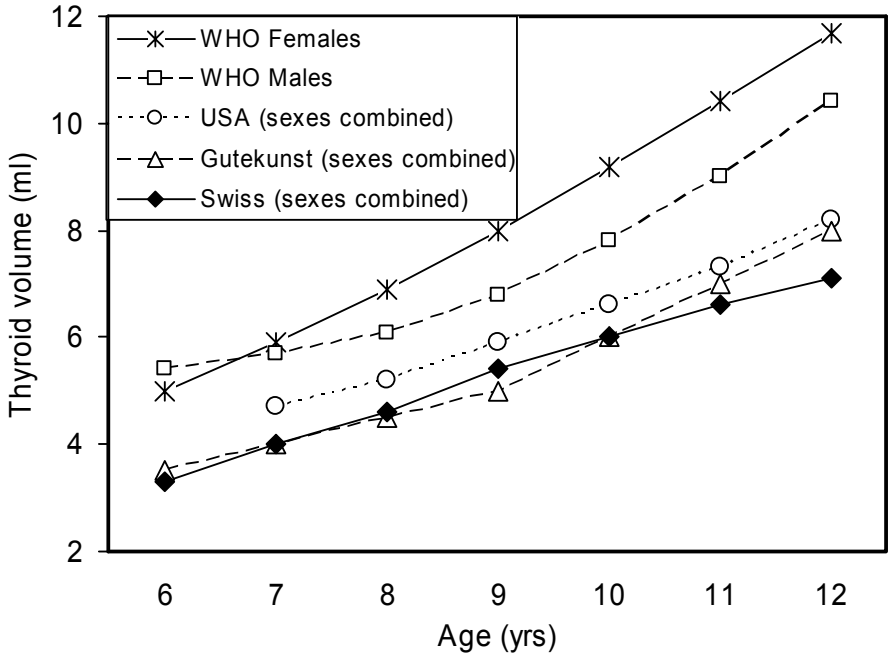

Figure 1: Comparison of the age/sex-specific WHO/ICCIDD upprt limits of normal thyroid volume (2) (females and males) with 97th percentiles of thyroid volume from Gutekunst & Martin-Teichert (3), Xu *et al.* in US children (5), and the Swiss children in this study (sexes combined)

Figure 1 compares our age/sex-specific P97 curve of thyroid volume with the WHO/ICCIDD-recommended reference curves (2), as well as the original normative data proposed by Gutekunst (3). Our age/sex-specific P97 volumes are similar to the values of Gutekunst (3) but are 20-42% smaller than the WHO/ICCIDD cut off values (2). Figure 2 compares our BSA/sex-specific P97 curve of thyroid volume with the WHO/ICCIDD-recommended reference curves (2) and recent data from Xu *et al.* on iodine-sufficient children in the US (5). Our BSA/sex-specific P97 volumes are similar to the values of Xu *et al.* (5), but are 30-56% smaller than the WHO/ICCIDD cut-off values (2).

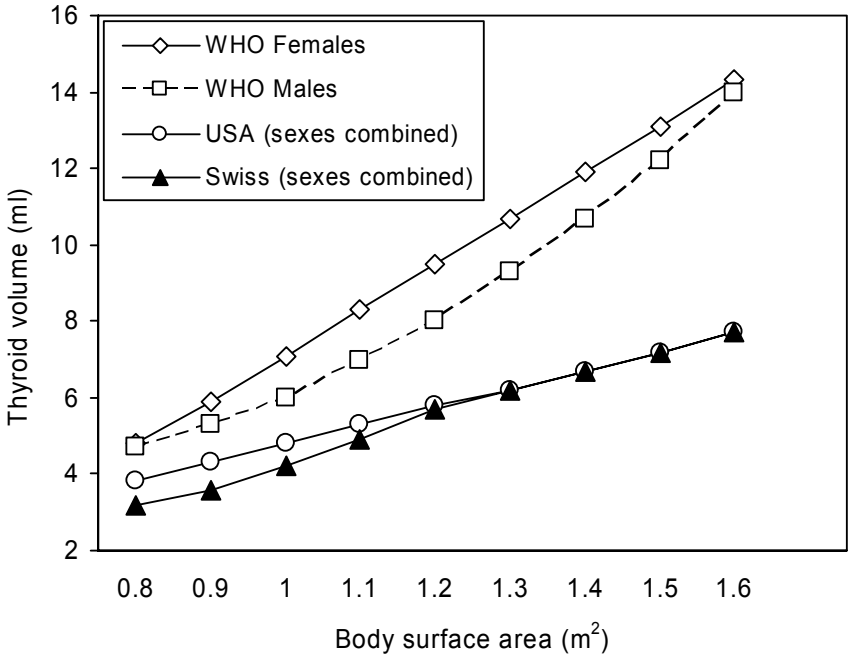

Figure 2: Comparison of the BSA/sex-specific WHO/ICCIDD upper limits of normal for thyroid volume (2) (females and males) with the 97th percentiles of thyroid volume from Xu *et al.* in US children (5) and the Swiss children in this study (sexes combined)

Discussion

Using age/sex-specific or BSA/sex-specific criteria, the Swiss children in this study had distinctly smaller thyroid volumes than the iodine-sufficient European children from which the WHO/ICCIDD reference data are derived (4). Because the WHO/ICCIDD reference cut-off points are the 97th percentiles of thyroid volume in iodine-sufficient European children, applying the WHO/ICCIDD references to this population of iodine-sufficient Swiss children should yield a goiter prevalence of approximately 3%. However, using either the age/sex-specific or the BSA/sex-specific WHO/ICCIDD cut-offs, there were no goitrous children in our sample.

The difference in thyroid size between the Swiss children in this study and the iodine-sufficient European children studied by Delange *et al.* (4) may be explained by the residual, long-term effects of a recent past history of iodine deficiency in Europe (5,7). Enlarged thyroids in children who are iodine deficient during the first years of

life may not regress completely after introduction of iodized salt (16). Iodine deficiency existed in many European countries up to the early 1990s (8). This may help explain why differences in thyroid volume increase sharply with age and/or BSA when the European children of Delange *et al.* (4) are compared to US children (5) and our Swiss children (with a lifetime of iodine-suficiency) (Figures 1 and 2).

Interobserver and/or interequipment variation in ultrasonographic thyroid measurement may also contribute to reported differences in thyroid volume from iodine-sufficient children (7). In 40 Malaysian children aged 7-10 years, Foo *et al.* found an interobserver error (SD) in ultrasound measurement of thyroid volume of 3.4 (3.7)% (6). In 20 Italian children aged 6-14 years, Vitti *et al.* reported an interobserver error of 4.2 –5.2% (17). These values are similar to the interobserver error (SD) of 3.7 (3.5)% reported in the present study. In contrast, Özgen *et al.* recently reported a mean interobserver error (SD) of 13.4 (8.3)% in the ultrasound measurement of thyroid volume in 30 healthy 7-16 yr-old Turkish children (18).

Table 1: Age specific thyroid volume (ml) by ultrasound in iodine-sufficient Swiss school children: 1994-1999.

Age (years)	**Troung *et al.* 1994 (11) (n=217)**	**Bürgi *et al.* 1997 (12) (n=280)**	**Present study (n=612)**
6	2.1	2.6	2.2
7	2.2	3.3	2.4
8		3.8	2.9
9	3.3	4.0	3.4
10	3.4	4.9	3.6
11		6.0	4.1
12		6.5	4.4

In attempting to distinguish between interobserver/equipment error vs. a past history of iodine deficiency to explain the current discrepancies in thyroid volume measurements from iodine-sufficient children, it is of particular interest to compare the results of our study to that of Bürgi *et al.* (12) and Truong *et al.* (11). These 3 studies report ultrasonographic thyroid volume measurements in iodine-sufficient Swiss school children between 1994 and 1999 (Table 1). Bürgi *et al.* employed

identical equipment and the same operator whose ultrasound measurements had been used to generate the 1997 WHO/ICCIDD reference data. They found that age/sex-specific median thyroid volumes and 97th percentiles measured in 1997 in 6-16 yr-old children from two cities in Switzerland closely agreed with the WHO/ICCIDD reference data (2, 12). In contrast, the age/sex-specific median thyroid volumes reported in this study—which used different investigators and equipment—are 10-56% smaller than the median volumes of Bürgi *et al*. (Table 1). Moreover, our age/sex-specific median thyroid volume are similar to those of Truong *et al.* (11), who measured thyroid volumes in iodine-sufficient Swiss children in 1994. Although 5 years separate the three data collections and the iodine level in Swiss table salt was increased from 15 to 20 mg/kg in the intervening period, it is unlikely that this could account for the sharp differences in thyroid volumes obtained by the different investigators. These data strongly suggest that interobserver and/or interequipment variability may contribute to the current disagreement on normative values for thyroid size by ultrasound in iodine-sufficient children. It also argues for the intercalibration of the methods used for ultrasonography in the measurement of thyroid volume in children.

Acknowledgements

We thank Dr Hans Bürgi for his helpful criticisms and advice, Dr Luigi Molinari of the Zürich Children‘s Hospital for assistance with the statistical analyses, and specially thank the teachers and students involved in the study. The study was supported by the Swiss Foundation for Nutrition Research and the Swiss Federal Institute of Technology in Zürich.

References

1. WHO, UNICEF and ICCIDD. *Indicators for assessing iodine deficiency disorders and their control through salt iodization.* WHO/NUT/94.6. Geneva: WHO, 1994.
2. WHO & ICCIDD. Recommended normative values for thyroid volume in children aged 6-15 years. *Bulletin WHO* 1997 **75** 95-97.
3. Gutekunst R, Martin-Teichert H. Requirements for goiter surveys and the determination of thyroid size. In: Delange F, Dunn JT, Glinoer D (Eds.) *Iodine Deficiency in Europe: A Continuing Concern*. New York: Plenum Press, 1993, pp-109-118.
4. Delange F, Benker G, Caron P, Eber O, Ott W, Peter F et al. Thyroid volume and urinary iodine in European schoolchildren: standardization of values for assessment of iodine deficiency. *European Journal of Endocrinology* 1997 **136** 180-187.
5. Xu F, Sullivan K, Houston R, Zhao J, May W, Maberly G. Thyroid volumes in US and Bangladeshi schoolchildren: comparison with European schoolchildren. *European Journal of Endocrinology* 1999 **140** 498-504.

6. Foo LC, Zulfiqar A, Nafikudin M, Fadzil MT, Asmah ASA. Local versus WHO/ICCIDD-recommended thyroid volume reference in the assessment of iodine deficiency disorders. *European Journal of Endocrinology* 1999 **140** 491-497.
7. Delange F. What do we call a goiter? *European Journal of Endocrinology* 1999 **140** 486-488.
8. Bürgi H, Supersaxo Z, Selz B. Iodine deficiency diseases in Switzerland one hundred years after Theodor Kocher's survey: a historical review with some new goiter prevalence data. *Acta Endocrinologica* 1990 **123** 577-590.
9. Delange F, Dunn JT, Glinoer D (Eds.) *Iodine Deficiency in Europe: A Continuing Concern*. New York: Plenum Press, 1993.
10. Bürgi H, Supersaxo Z, Dürig P. Status of iodine nutrition in Switzerland. In: Delange F, Dunn JT, Glinoer D (Eds.) *Iodine Deficiency in Europe: A Continuing Concern*. New York: Plenum Press, 1993.
11. Truong TH, Gerber H, Haenel A, Bürgi H. Jodversorgung in verschiedenen Lebensphasen und sonographische Schilddrüsenvolumina bei Schulkindern in einer Gegend der Schweiz. *Schweizerische Medizinische Wochenschrift* 1997 **127** 715-721.
12. Bürgi H, Portmann L, Podoba J, Vertongen F, Srbecky M. Thyroid volumes and urinary iodine in Swiss school children, 17 years after improved prophylaxis of iodine deficiency. *European Journal of Endocrinology* 1999 **140** 104-106.
13. World Health Organization. *Physical Status: The Use and Interpretation of Anthropometry.* 1995, WHO, Geneva, p.427-29.
14. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. *Clinical Chemistry* 1996; 42: 239-243.
15. Bundesamt für Statistik. *Statistisches Jahrbuch der Schweiz*. 1996, Verlag NZZ, Zürich.
16. Aghini-Lombardi F, Antonangeli L, Pinchera A, Leoli F, Rago T, Bartolomei AM, et al. Effect of iodized salt on thyroid volume of children living in an area previously characterized by moderate iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1136-39.
17. Vitti P, Martino E, Aghini-Lombardi F, Rago T, Antonangeli L, Maccherini D et al. Thyroid volume measurement by ultrasound in children as a tool for the assessment of mild iodine deficiency. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 600-603.
18. Özgen A, Erol C, Kaya A, Özmen MN, Akata D, Akhan O. Interobserver and intraobserver variations in sonographic measurement of thyroid volume in children. *European Journal of Endocrinology* 1990 **140** 328-331.

Conclusions and Perspectives

In the intervention trial, the decrease in thyroid volume (Tvol) and goiter prevalence were significantly greater in the iron-supplemented group compared to placebo. Because iron supplementation has now been shown to improve the efficacy of both iodized salt and oral iodized oil in goitrous iron-deficient children, our findings argue strongly that a high prevalence of iron deficiency in areas of endemic goiter may reduce the effectiveness of iodine prophylaxis. This finding indicates an important, not-previously-described benefit of iron supplementation strategies in areas where concomitant iron and iodine deficiencies occur. Iron supplementation provides not only a health benefit for the iron-deficient target group, but also improves the efficacy of a salt iodization program. In the past, research and public health intervention strategies were focused mainly on single micronutrients. However, as shown by the present study, these supplementation or fortification programs may have been less effective due to interactions between different micronutrients. Our findings strongly support the current recommendations by the World Health Organization (WHO *et al.*, 2001b) for combined supplementation and fortification using multiple micronutrients. Besides controlling more than one micronutrient deficiency, potential synergies (such as an improvement of iodine efficacy by iron) may occur. An example of a beneficial combined strategy would be dual fortification of salt with both iron and iodine in Côte d'Ivoire. Salt is an ideal vehicle, as it is universally consumed in rural villages of Côte d'Ivoire (Hess *et al.*, 1999), and its consumption is fairly constant throughout the year (WHO *et al.*, 2001a). In a recent efficacy trial in school children in Morocco, Zimmermann *et al.* (2002) compared iodized salt to dual-fortified salt with iron and iodine. In the group receiving the dual-fortified salt, Tvol and thyroid hormone concentrations were significantly improved compared to the group receiving iodized salt. Moreover, the prevalence of iron deficiency anemia (IDA) was reduced from 35% to 8% in the dual-fortified group. However, technical problems due to color changes and iodine loss caused by the addition of iron to the iodized salt remain to be solved.

Previous studies in human and animals found that IDA impairs thyroid metabolism, but the mechanism was not clear. We have shown that IDA reduces thyroid peroxidase (TPO) activity in rats. TPO is an essential heme-enzyme in thyroid metabolism that catalyzes the two initial steps of thyroid hormone synthesis. By reducing its activity, IDA may decrease the incorporation of iodine into thyroglobulin

and the subsequent coupling of the iodotyrosines to thyroid hormones. Further research could focus on the relative contribution of reduced TPO activity compared to other potential mechanisms of impaired thyroid metabolism in IDA, such as altered central nervous system control (Beard *et al.*, 1998), reduced nuclear binding of T_3 (Smith *et al.*, 1994), or the impairment of the thyrocyte because of reduced oxygen supply (Surks, 1969; Galton, 1972). Further investigation is also needed to elucidate the mechanism by which IDA reduces TPO activity on a cellular and/or molecular level, similar to the studies by Fayadat *et al.* (1999) but in an iron-deficient model. Moreover, it would be of interest to further investigate whether IDA could cause enlarged Tvol through reduced hemoglobin oxygenation, increased nitric oxide concentrations, and resulting vasodilatation (Jia *et al.*, 1996; Stamler *et al.*, 1997).

Two years after universal salt iodization (USI) had been introduced in Côte d'Ivoire, goiter rate was still 74%, although urinary iodine concentration and TSH values indicated a sufficient iodine intake in school children. The aim of the cross-sectional study was to investigate whether a high goiter rate persists despite successful salt iodization due to overlapping micronutrient deficiencies and/or a high consumption of cassava, a known goitrogen commonly consumed in this region. However, despite their high prevalence neither selenium, nor vitamin A deficiencies, nor urinary thiocyanate predicted goiter. Plasma ferritin was the only iron status indicator associated with goiter, that is the probability of goiter was increased in children with lower plasma ferritin concentrations. This confirms other studies of this thesis and the studies by Zimmermann *et al.* (2000; 2002), suggesting that iron depletion blunts thyroid response to iodine prophylaxis. However, although the participating 9 villages were ethnically and economically equal, village was a highly significant predicting factor of goiter. Although results indicated significant differences between villages in all nutritional status indicators measured in this study, we were unable to explain the significant impact village had on goiter prevalence. Therefore, other factors, not identified in this study, probably act in concert with iron depletion to blunt the thyroid response to iodized salt. In similar settings, future studies should control for infection as a confounding factor on iron and vitamin A status indicators. There is a need to further investigate potential factors affecting thyroid metabolism, such as other micronutrient deficiencies, e.g. zinc, and other goitrogenic dietary factors. In terms of the effect of selenium and vitamin A deficiency on thyroid metabolism, further research is needed to determine the public health significance as earlier results are equivocal.

The results from the prospective study investigating the thyroid response to salt iodization found that there was an age shift in goiter distribution. Before salt iodization more children were goitrous in the age group of the 5-9 year olds compared to 10-14 years old children, whereas 4 years after iodized salt was introduced goiter rate was higher in the older children (52%) compared to the younger children (19%). This indicates that Tvol may not normalize completely in children who have been born and/or have grown up iodine deficient. If true, this would potentially limit the use of Tvol as a monitoring indicator after USI, as it would take a decade or more to normalize goiter prevalence in 10-12 years old children born under iodine deficiency. This points to the urgent need to clarify longitudinal changes in Tvol after salt iodization to help governments and program managers chose appropriate monitoring indicators of the success of their USI program. It also remains to be investigated if normalization of Tvol is of physiological importance in iodine repletion. As the aim of USI is the prevention of adverse effects of iodine deficiency, normalization of the thyroid function is the major goal. When USI assures sufficient iodine intake, thyroid function improves (as indicated by adequate thyroid hormone concentrations), and complete normalization of Tvol might only be of secondary importance.

The study of iodine-replete Swiss children from a representative national sample of school children suggested, together with other studies (Foo *et al.*, 1999; Xu *et al.*, 1999), that reference criteria at that time (WHO & ICCIDD, 1997) were too high. This led to a workshop organized by WHO and ICCIDD where interobserver and interequipment variation in ultrasound Tvol measurements was evaluated. This workshop produced new updated, but provisional reference criteria (Zimmermann *et al.*, 2001). The next step will be the generation of a new, truly international reference criteria for Tvol by ultrasound in school children who grew up iodine-sufficient. At the same time, to ensure that ultrasonographic Tvol measurements are internationally comparable, standardization of the measurement technique will be vital.

References

Beard JL, Brigham DE, Kelley SK & Green MH (1998) Plasma thyroid hormone kinetics are altered in iron-deficient rats. *Journal of Nutrition* **128**, 1401-1408.

Fayadat L, Niccoli-Sire P, Lanet J & Franc JL (1999) Role of heme in intracellular trafficking of thyroperoxidase and involvement of H2O2 generated at the apical surface of thyroid cells in autocatalytic covalent heme binding. *Journal of Biological Chemistry* **274**, 10533-10538.

Foo LC, Zulfiqar A, Nafikudin M, Fadzil MT & Asmah AS (1999) Local versus WHO/International Council for Control of Iodine Deficiency Disorders-recommended thyroid volume reference in

the assessment of iodine deficiency disorders. *European Journal of Endocrinology* **140**, 491-497.

Galton VA (1972) Some effects of altitude on thyroid function. *Endocrinology* **91**, 1393-1403.

Hess SY, Zimmermann MB, Staubli-Asobayire F, Tebi A & Hurrell RF (1999) An evaluation of salt intake and iodine nutrition in a rural and urban area of the Côte d'Ivoire. *European Journal of Clinical Nutrition* **53**, 680-686.

Jia L, Bonaventura C, Bonaventura J & Stamler JS (1996) S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* **380**, 221-226.

Smith SM, Finley J, Johnson LK & Lukaski HC (1994) Indices of in vivo and in vitro thyroid hormone metabolism in iron-deficient rats. *Nutrition Research* **14**, 729-739.

Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K & Piantadosi CA (1997) Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. *Science* **276**, 2034-2037.

Surks MI (1969) Effect of thyrotropin on thyroidal iodine metabolism during hypoxia. *American Journal of Physiology* **216**, 436-439.

World Health Organization & International Council for Control of Iodine Deficiency Disorders (1997) Recommended normative values for thyroid volume in children aged 6-15 years. World Health Organization & International Council for Control of Iodine Deficiency Disorders. *Bulletin of the World Health Organization* **75**, 95-97.

World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency Disorders (2001a) Assessment of iodine deficiency disorders and monitoring their elimination. WHO, Geneva; WHO/NHD/01.1.

World Health Organization, United Nations Children's Fund & United Nations University (2001b) Iron deficiency anemia: Assessment, prevention, and control. WHO, Geneva; WHO/NHD/01.3.

Xu F, Sullivan K, Houston R, Zhao J, May W & Maberly G (1999) Thyroid volumes in US and Bangladeshi schoolchildren: comparison with European schoolchildren. *European Journal of Endocrinology* **140**, 498-504.

Zimmermann M, Adou P, Torresani T, Zeder C & Hurrell R (2000) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. *American Journal of Clinical Nutrition* **71**, 88-93.

Zimmermann MB, Molinari L, Spehl M, Weidinger-Toth J, Podoba J, Hess S & Delange F (2001) Updated provisional WHO/ICCIDD reference values for sonographic thyroid volume in iodine-replete school-age children. *IDD Newsletter* **17**, 12.

Zimmermann MB, Zeder C, Chaouki N, Torresani T, Saad A & Hurrell RF (2002) Addition of microencapsulated iron to iodized salt improves the efficacy of iodine in goitrous, iron-deficient children: a randomized, double-blind, controlled trial. *European Journal of Endocrinology* **147**, 747-753.

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