Iodine deficiency and functional performance of schoolchildren in Benin

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit, Prof. Dr. Ir. L. Speelman,
in het openbaar te verdedigen
op vrijdag 14 december 2001
des namiddags om 16.00 uur in de Aula

Van den Briel-van Ingen, Tina

Iodine deficiency and functional performance of schoolchildren in Benin
Thesis Wageningen University- With references- With summary in Dutch

ISBN 90-5808-536-8

To the children of Benin,
That they may have a brighter life…

Abstract

Iodine deficiency and functional performance of schoolchildren in Benin

PhD thesis by Tina van den Briel-van Ingen, Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands, 14 December 2001.

The notion that iodine deficiency not only leads to goiter and cretinism, but to a much wider range of disorders, from stillbirth and abortions, to hearing problems and mental and physical underdevelopment began to be accepted beyond the research community since the early 1980's. In 1990 it was estimated that these problems, collectively called iodine deficiency disorders (IDD), presented a public health problem in 118 countries and that over 40 million people were affected by some degree of mental impairment. Children who have been exposed to iodine deficiency in the pre- and early post-natal phases of life show varying degrees of mental and psychomotor retardation, depending on the duration and degree of the deficiency. Adequate maternal iodine intake before and during pregnancy prevents such disorders. The question however whether or not deficits in mental and psychomotor performance of iodine deficient children may be reversed by supplementation with iodine later in life has not yet been answered unequivocally.

The research described in this thesis was set up to address this question. A double-blind placebo-controlled intervention was carried out in an iodine deficient area of northern Benin in the period 1995-1996. A single oral dose of iodized oil or placebo was administrated to 2 groups of schoolchildren, aged 7-11 years. The observation period was 10-11 months. However, 3 to 4 months after supplementation the population started to have access to iodized salt, in addition to non-iodized salt. Because iodine became available to both groups, the main hypothesis, i.e. that iodine supplementation would improve mental performance had to be modified. It was decided to take children whose iodine status, as measured by different indicators, did not change during the observation period as the "control" group. In addition to mental and psychomotor performance, other aspects associated with iodine deficiency were studied, including behavioral change and hearing thresholds and their relation with mental performance. The suitability of several indicators for measuring iodine status and thyroid function was evaluated.

Results showed that an improvement in iodine status as measured by urinary iodine concentration, was reflected in a significantly improved performance on the combination of mental tests, 10 months after supplementation. Moreover, children with better iodine status could hear better than their peers with a poorer iodine status, while hearing thresholds were negatively correlated with performance on all mental tests, but one. The serum concentration of thyroglobulin and the urinary iodine concentration were found to be indicators most suitable for measuring change in iodine status in this age group. Although the influx of iodine into the area precludes the drawing of "hard" conclusions, the results presented suggest that iodine supplementation is likely to promote a "catch-up" process in functional performance of iodine deficient schoolchildren.

Stellingen

1 Jodiumsuppletie bevordert waarschijnlijk een inhaalslag in het mentaal functioneren van jodiumdeficiënte kinderen.

- Dit proefschrift.

2 Jodiumstatus kan niet omschreven worden aan de hand van één indicator. De concentratie van thyroglobuline in serum en die van jodium in urine vormen de beste combinatie voor het vaststellen van jodiumgebrek bij schoolkinderen.

- Dit proefschrift.

3 Investeringen in de strijd tegen een tekort aan micronutriënten in minder ontwikkelde landen, teneinde het cognitief functioneren van bepaalde bevolkingsgroepen te verbeteren, zouden meer rendement opleveren als het onderwijs verbeterde.

- Reactie op: Scrimshaw N. "Investments in education and community development would be more effective if the physical and cognitive capacity of underprivileged populations were not impaired by malnutrition" (*Malnutrition, brain development, learning and behavior*; Nutr Res 1998; 18: 351-379).

4 Het complete beeld van de functionele gevolgen van nutriëntensuppletie voor geestelijke en lichamelijke ontwikkeling kan niet vastgelegd worden in een enkel kiekje van geselecteerde aspecten van ontwikkeling, genomen voor en na behandeling.

- Gebaseerd op: Pollitt E. *The developmental and probabilistic nature of the functional consequences of iron-deficiency anemia in children.* J Nutr 2001; 131:669S-675S.

5 Om verzuiling in de wetenschap tegen te gaan en synergie te verbeteren zou het vak systeem-denken verplicht gesteld moeten worden in het wetenschappelijk onderwijs.

6 Vijftig jaar ontwikkelingssamenwerking heeft de kloof tussen rijk en arm niet verkleind: het wordt tijd voor een nieuw Marshall plan.

7 Het oplossen van (s)cryptogrammen is een probaat middel tegen het vastroesten van denkpatronen en, met name bij ouderen, tegen aftakeling van "fluid intelligence".

8 Het kunnen aanschouwen van de maanlanding op TV was unieker en dwingt tot meer reflectie over technologische ontwikkeling dan de maanlanding zelf.

- Vrij naar: Mulisch H. (1997) *De ontdekking van de hemel.*

Stellingen behorend bij het proefschrift "Iodine deficiency and functional performance of schoolchildren in Benin"

Tina van den Briel, Wageningen, 14 december 2001

Propositions

1. Supplementation with iodine is likely to promote a "catch-up" process in mental performance of iodine deficient schoolchildren.

    - This thesis.

2. The concept of iodine status cannot be captured in one indicator. The concentrations of thyroglobulin in serum and of iodine in urine form the best combination for assessing iodine deficiency in schoolchildren.

    - This thesis.

3. Investments in the fight against micronutrient malnutrition with the aim of improving cognitive performance of population groups would be more effective if the quality of education were improved.

    - In response to: Scrimshaw N, "Investments in education and community development would be more effective if the physical and cognitive capacity of underprivileged populations were not impaired by malnutrition" (*Malnutrition, brain development, learning and behavior*. Nutr Res 1998; 18: 351-379).

4. The full picture of the functional consequences of nutrient supplementation with respect to physical and mental development cannot be captured in a pre- and post-treatment snapshot of selected aspects of development.

    - Based on: Pollitt E. The developmental and probabilistic nature of the functional consequences of iron-deficiency anemia in children. J Nutr 2001; 131:669S-675S.

5. In order to prevent segregation of scientific disciplines, systems thinking should be a mandatory subject in all academic education programmes.

6. Fifty years of development cooperation have not reduced the gap between the poor and the rich: it is time to come up with a new Marshall plan.

7. Solving (s)cryptograms on a regular basis is a good remedy against patterns of thinking becoming fixed and, particularly in the elderly, against deterioration of fluid intelligence.

8. Being able to watch the landing on the moon on TV was more unique and necessitates more reflection on technological development than the landing itself.

    - Based on: Mulisch, H. (1997) *The discovery of heaven*

Propositions pertaining to the thesis "Iodine deficiency and functional performance of schoolchildren in Benin".

Tina van den Briel, Wageningen, 14 december 2001

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1

Introduction

Iodine deficiency: the path from scourge to success

In the past decade enormous progress has been made in combatting iodine deficiency, one of the world's leading causes of preventable mental underdevelopment. Goiter and cretinism were phenomena long known. However, only recently there has been an increase in awareness that iodine deficiency may lead to a broad spectrum of problems ranging from abortion and stillbirth to mental and physical retardation, deafness and goiter. Following a large number of surveys and intervention studies in the 1960's and 1970's, the collective term iodine deficiency disorders (IDD) was introduced in the early 1980's (1). With growing awareness of the range of problems, it was also realized that IDD might be much more widespread than originally thought. In Africa in particular, data were available for a few countries only, while many more were expected to have a high prevalence of IDD. The magnitude of the IDD problem did not become fully known until the early 1990's. Whereas in the mid-80's 800 million people were considered at risk of IDD and 200 million were estimated to have goiter (2), in 1990 almost 1.6 billion people were considered at risk of IDD and over 600 million people to have visible goiter (3). By then, IDD was regarded as a significant public health problem in 118 countries. Moreover, more than 40 million people were thought to be affected by some degree of mental impairment and it was estimated that iodine deficiency accounted for a difference of 10-15 IQ points between iodine-replete and iodine deficient population groups (4). Realizing that IDD not only posed a threat to healthy pregnancy outcome and adequate physical and mental development of young children, but, more importantly, to the overall development potential of their countries, 71 Heads of State signed a Plan of Action to eliminate IDD by the year 2000 at the World Summit for Children in 1990. Eventually about 160 countries committed themselves to this goal. Subsequently efforts were made in most countries to establish national programmes for the elimination of IDD through collaboration between government, international agencies, the private sector and non-governmental agencies (5). Data from 1999 show that 75 % of the countries with an IDD problem have legislation on salt iodization in place and about 10 % have draft legislation. Two-thirds of households living in IDD-affected countries now have access to iodized salt (6).

Iodine metabolism, growth and development

Iodine is a constituent of the thyroid hormones, triiodothyronine (T3) and tetraiodothyronine (T4, thyroxine), produced by the thyroid gland. These hormones play an essential role in growth and development of human beings and animals. In a healthy human being, the thyroid gland weighs only about 15-25 g and contains approximately 15-20 mg of iodine, or 70-80 % of the total amount of iodine in the body. The remainder is found in other tissues or in the circulation, either as free iodine or protein-bound iodine. The best known consequences of iodine deficiency are goiter and cretinism. Although these phenomena were well known in various parts of the world and were associated with mental retardation since the early Middle Ages (7), the role of iodine in the origin of these problems was not elucidated until the 19th century. The work done in Switerland and France in this context is well described in a historical review by Bürgi et al (8). When in Switzerland in the second half of the 19th century, approximately 10 % of 19-year old men were found to be unfit for military service owing to a large goiter, Bircher (9) compiled tables and maps of goiter prevalence for the whole country. The conclusion was that goiter prevalence varied enormously, which was ascribed to differences in soil geology and water supplies. This work was followed up in 1889 by Theodor Kocher who found a total goiter prevalence of 20 -100 %, and who confirmed the importance of drinking water but could not explain why. Meanwhile in France in 1831 Boussingault (10) was the first to suggest that iodine-containing salt should be made available in goitrous regions, while Chatin between 1850 and 1860 was the first to demonstrate that moderate iodine deficiency in soil, water and food caused goiter, whereas severe deficiency caused cretinism in addition (11). The Swiss did not become aware of this work until the end of the century when also some other important work was published, including that of Baumann (12) who demonstrated that the thyroid gland accumulates iodine and later that of Marine and Kimball (13) who suggested prophylaxis in schools. These developments paved the way to a recommendation made in 1922 to all cantons by the Swiss Goiter Commission to start the sale of salt containing 2.5 to 5 mg KI per kg on a voluntary basis.

The famous study of McCarrison in northern Pakistan at the beginning of the previous century was one of the first to look into the relationship between goiter, cretinism and transferability of these phenomena from mother to child (14). The exact function of the thyroid gland was not yet known and McCarrison was of the opinion that "the function of the thyroid mechanism is to neutralise toxins produced in the ordinary course of metabolism". The conclusions of his study are noteworthy: ...." Defective thyroid function in the mother is the essential factor in the production of cretinism" and: "Cretinism is due to the action of toxic agents, notably that of endemic goiter, on the developing thyroid of the unborn child".

By 1960 methods for goiter survey techniques were standardized. The following decade saw a large increase in the number of studies into the prevalence of endemic cretinism in developing countries (15-17). It took until 1966 for a controlled trial to be set up in the western highlands of Papua New Guinea to see whether endemic cretinism could be prevented by the administration of iodized oil (18). At about the same time, similar studies were set up in Ecuador (19,20). The work done in Papua New Guinea showed that an injection of iodized oil given prior to pregnancy could prevent the occurrence of endemic cretinism in the infant. The findings also suggested that the damage to the developing nervous system was likely to occur in the first trimester of pregnancy. Initially it was assumed that a deficient supply to the fetus of iodine per se might be the key factor in the impairment of brain development (18,21). Various later studies looked into the transfer of maternal thyroid hormones across the placenta and the role these play before the onset of fetal thyroid function, which occurs at mid-gestation (22,23). Studies in both animals and humans have confirmed that maternal T4 plays a very important role in fetal brain development before the fetus starts producing its own supply of thyroid hormones (24,25) and the earlier view that the placenta is relatively impermeable to the transfer of maternal thyroid hormone has been revised (26). In this context maternal serum concentrations of T4 but not of T3 has been shown to be correlated with outcome measures in children, such as motor and cognitive function (27,28). This corresponds with observations that in cells of the cerebral cortex, approximately 80 % of the requirement for T3 is obtained by de-iodination of T4 in situ by type II de-iodinase (29), whereas most other tissues utilise circulating T3, derived from de-iodination by type I de-iodinase in liver or kidney.

The question as to what is the most crucial period in fetal life for thyroid hormones to exert their influence has been a recurring issue for several decades (22,30). In an overview of the data available in 1980 Morreale de Escobar (31) concluded: " Somehow thyroid hormones convey a message to developing cells to stop dividing and start differentiating". This would be most crucial in the period of the brain "growth spurt", starting in the 2nd trimester and continuing into the 3rd year of life. By the end of the 1980's the available body of evidence from studies in animals indicated that iodine deficiency is associated with reduction in the weight and DNA content of brain, delayed maturation of the cerebellum and diminished arborization of the dendrites in the cerebral hemisphere (32,33). Parts of the nervous system considered to be most affected are the cerebral neocortex, the cochlea and the basal ganglia. These grow most rapidly during the second trimester (33). In the mid-90's however there was still discussion about the question whether endemic cretinism originates early in gestation, i.e. during the 1st trimester or early second trimester (26), the period of neuronal migration and arborisation of dendrites, or whether the most crucial period is the 3rd trimester (34), the period of neuronal differentiation, formation of neural processes, synapses and neurotransmitters, myelination and gene expression on which these functions are based. In a recent review Delange (22) refers to maternal hypothyroxinemia during early pregnancy as a key factor in the development of neurological damage in the cretin. Morreale de Escobar further expands on the timing of insults to the developing brain, as a result of maternal or fetal hypothyroxinemia or a combination of the two (23) (Fig. 1). Early maternal hypothyroxinemia in combination with impairment of the fetal thyroid leads to central nervous system damage that is considered irreversible at birth.

Apart from the role that iodine deficiency plays during the *prenatal* period in the origin of endemic cretinism, differences in the clinical presentation of endemic cretinism may also be related to the duration and severity of *postnatal* hypothyroidism (35). However, very few data exist on the evolution of thyroid function in areas of severe iodine deficiency, from the neonatal period to adulthood (36).

Following the studies in Papua New Guinea and Ecuador, a number of other intervention studies addressed the question to what extent different degrees and duration of maternal iodine deficiency are reflected in the mental and psychomotor performance of children at various stages in their lives (see also Chapter 7). These studies generally support the notion that overcoming iodine deficiency before or even during pregnancy enhances mental performance of children subsequently born. Studies focusing on the association between iodine status of children later in life or changes therein and their mental and motor performance include observational (ecological, case-control) and intervention studies, with mixed results. The question however whether iodine supplementation of a hitherto iodine deficient population group redresses the arrears in mental and psychomotor functioning has been dealt with in only a few studies. In an intervention study in Spain (37), no improvement of mental and psychomotor development was found 32 months after supplementation, whereas in the Andes a significant effect was seen only in girls (38). The double-blind placebo-controlled studies carried out in Bolivia (39), Malawi (40) and Bangladesh (41) also showed different results. In Bolivia, no differences were found on mental and psychomotor tests 22 months after supplementation, in Malawi supplemented children performed significantly better on a number of mental tests, 1 year after supplementation, while in Bangladesh no difference in cognitive or motor performance was found 4 months after supplementation. These studies are reviewed in Chapter 7.

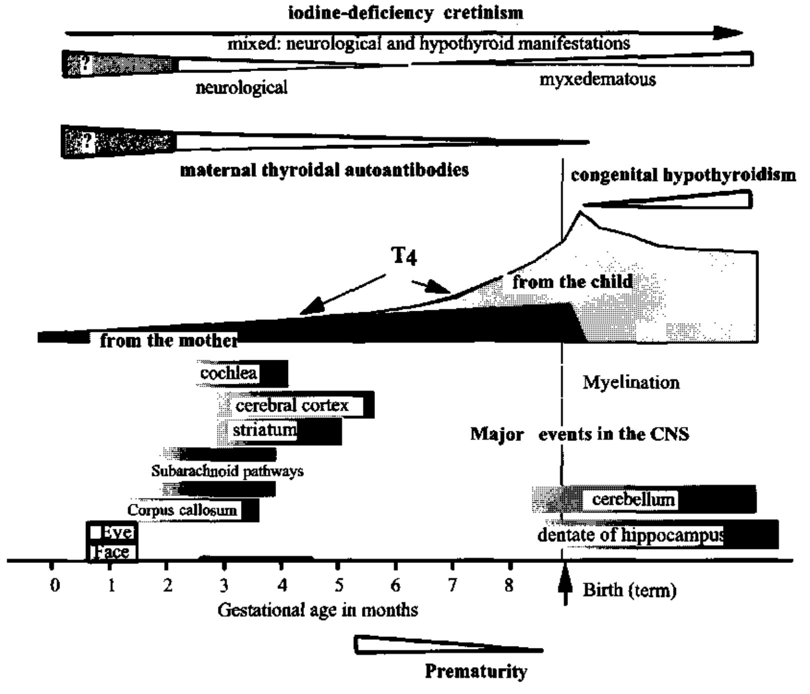


Figure 1. Approximate timing of major insults to the brain resulting from hypothyroxinemia, superimposed on major neurodevelopmental events. Conditions resulting in early maternal hypothyroxinemia, combined to later impairment of the fetal thyroid, are the most damaging, with central nervous system (CNS) damage that is irreversible at birth. The most frequent cause is maternal iodine deficiency (ID) and the presence of maternal autoimmune thyroid disorders. Unless ID is also present, the CNS damage in congenital hypothyroidism is preventable by early postnatal treatment because the normal maternal thyroxinemia has avoided damage to the brain until birth. If maternal hypothyroxinemia persists, normal maternal concentrations of T_3 do not protect the fetal brain because of its dependence on intracerebral regulation of local T_3 availability by deiodinating pathways using T_4 as a substrate. Interruption of the contribution of maternal T_4 in premature infants with an immature thyroid may also underlie their increased risk of neurodevelopmental problems, the more severe the earlier their birth. The *question mark* indicates that we do not know whether very early CNS development, corresponding to a period when the general morphogenesis of the pros encephalon (neurolation and segmentation) is being determined, is thyroid hormone sensitive or not.
Source: Morreale de Escobar, G. et al. (2000). J Clin Endocrin Metab 85(11): 3890. With permission of the Endocrine Society.

Measuring mental performance and personality traits in non-Western societies

Several conceptual and methodological issues are encountered when attempting to measure functional consequences of malnutrition in a particular target group, especially consequences related to mental performance or socio-emotional behavior. Cultural differences may further complicate the issues involved and have a bearing on the external validity of the findings.

Individual behavior is multi-dimensional and comprises sensory-perceptual, motor, cognitive and socio-emotional aspects. These aspects of behavior may be affected in different ways and to different degrees by malnutrition, depending on its nature, severity, duration and timing (42). Thus there is the question of selection of the dependent outcome measures and also of the nature and appropriateness of the methods to be used to assess these. Although these issues have been addressed in a number of studies (43), particularly in relation to protein-energy malnutrition (44-46), deficiencies of micronutrients such as iron (47,48), iodine (49,50), zinc (51), and various vitamins, or combinations of various deficiencies (52,53) there is still much to learn about which specific functions are affected by malnutrition as well as about the mechanisms involved.

Reliability and validity

Once outcome variables have been selected, requirements of reliability and validity need to be considered with respect to the tools used for and the procedures followed in measuring these variables (54,55). Reliability of measurements concerns the notion of consistency, i.e. there should be a reasonable degree of agreement in the short term between successive applications of a measurement procedure under "normal" circumstances (56). A test-retest correlation indicates the short term stability of the test measures in a particular setting. With respect to validity, several dimensions may be distinguished (57). **Internal** validity refers to the confidence with which one can infer a causal relationship between two variables within the context of the study population, whereas the **external** validity refers to the extent to which one can infer that the relationship found within the study population is true for other populations (58). As the ecological context and etiology of nutrition problems often differ between different populations, findings from studies on the effects of malnutrition on cognitive performance are not easily generalized. **Construct** validity refers to the degree of fitness between the underlying construct or conceptual attribute of the study population and the measurements used. As there is a substantial number of distinguishable mental abilities (56) and different mental and motor processes involved in tasks based on these abilities, aggregate scores of performance on IQ tests do not provide insight into the particular abilities that may be affected and may mask any changes in these. Performance on different subtests is much more informative. At the same time, further analysis of the mental and motor processes or other behavioral functions such as attention, motivation and arousal involved in completing such subtests, is required in order to be able to identify the mechanisms by which any changes in performance are realized.

Confounding variables

A poor nutritional status is generally found in environments that are poor in many other respects. In such an environment there may be not only insufficient access to food, water or adequate sanitation, but also a poor health care and education infrastructure. In addition, such conditions go hand in hand with socio-cultural deprivation. Thus many factors may, directly or indirectly, affect a child's physical, mental and social-emotional development (59-61).

In a school environment, optimal learning takes place when there is two-way interaction between teacher and child and when children are actively engaged in exploring stimuli, procesing information and exercising their creativity by applying what they have learned to novel situations. Hoever, poor health and nutrition, low levels of socio-economic status and parental stimulation and poor home and school environments all impinge on the child's active learning capacity, i.e. his or her propensity and ability to interact with, and to take optimal advantage of, resources offered (62). Therefore it is hard to distinguish the effects of poor nutrition from those of other deprivations on cognitive development, except by applying experimental or quasi-experimental study designs. Moreover, poor nutritional status is not easily defined in terms of deficiency of a single nutrient. In addition, different micronutrients may interact, with the status with respect to one micronutrient enhancing or diminishing the effects of a deficiency of another micronutrient on selected outcome variables, making it even more difficult to specify the consequences of specific nutrient deficiencies. Thus, in studies on the effect of a specific nutrient deficiency on mental development the possible effects of other relevant health or nutritional deficiencies must be taken into account.

Cultural bias

The most common use of psychometric tests in industrialised societies is in the context of predicting the development of certain competencies over time, including the ability to perform in a given work setting. The use of such tests across different cultures has been and still is subject to much debate. Even within one culture the use of psychometric tests has been said to be discriminatory, on the grounds that certain population groups, which are better off in socio-economic terms than others, are in a more advantageous position to do well on such tests. Tests as such however can never be discriminatory. It is only when differences in results (scores) are seen as differences in genetically determined intelligence potential, that discrimination comes in (56,63). Although psychologists have different opinions with respect to the relative importance of genetic and environmental factors (the "nature-nurture" issue) in the determination of cognitive abilities, it is clear that the socio-cultural environment of the persons being tested may play a role in test results, depending on the abilities that are involved in performing the tests (56). If ample language, or other schooling related skills are required in performing the test tasks, there may be a cultural bias if different cultural groups are being tested simultaneously. There are however no culture-free tests, although some tests are considered more "culture-fair" than others. Tests which require little or no vocabulary or other skills related to schooling or information-access are regarded as culture-fair. In this context it proves useful to position tests in the so-called fluid-crystallized spectrum, a spectrum of general abilities, ranging from subject-matter proficiency (crystallized achievements) to fluid ability (64). The term "crystallized" refers to an almost automatic response to a task, as it closely corresponds to past practices and experiences. On the other hand "fluid" ability refers to the adaptive process of apprehending an unfamiliar configuration and rearranging it to satisfy some requirement.

Therefore, when using psychometric tests in a society for which these have not been developed, one must be aware of the problems of translating the results into an Intelligence Quotient (IQ). Most of the mental abilities should be addressed, test items and procedures should be adapted and their use pretested. With children especially, tests should not require much explanation and subjects should feel at ease but not overly subdued. If such precautions are taken, it should be possible to develop an appropriate test battery for establishing local reference scores enabling the assessment of the IQ.

Measuring iodine deficiency

Various indicators can be used for measuring iodine deficiency. However, all of these indicators reflect different aspects of deficiency. There is no single indicator that fully represents iodine status. Indicators may be related to iodine intake, to iodine-dependent endocrinological processes in the human body ("outcome indicators") or to progress made with respect to provision of households with sources of iodine, such as iodized salt ("process indicators") (65). Thus the choice of indicators depends on the purposes that are to be served, such as assessing the prevalence of IDD, identifying high-risk populations, monitoring and evaluating IDD control programmes, or elucidating functional consequences of IDD. In addition, practical and economical considerations play a role in the selection of indicators.

Possible risk of a deficiency may be examined on the basis of data on food intake and water. If people live in an area where the soil is iodine deficient and where all food originates from such soil, then the people living in this area permanently would be at risk of developing IDD. There are however considerable individual differences in the propensity to develop IDD. Moreover, it is hard to know to what extent foods containing iodine come in from outside the study area. As food consumption studies are also very time-consuming if carried out on an individual basis and provide only a "snapshot" impression of actual intake, these are generally only carried out on a population basis, provided that dietary habits as well as iodine content of foods consumed are reasonably well known. In that case the average iodine intake of certain population groups may be estimated, data which would form the basis, together with clinical data, for decisions on policy measures in the field of salt iodization and the application of iodized salt in various processed foods (66).

Outcome indicators include clinical and biochemical indicators. In the public health context the most widely used clinical indicator originally was that of the size of the thyroid gland, measured by palpation. Classification has been simplified from a system with 5 grades to a system with only 3 grades (65):

Grade 0= no palpable or visible goiter;

Grade 1: a mass in the neck that is consistent with an enlarged thyroid gland that is palpable but not visible when the neck is in the normal position;

Grade 2: A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid gland when the neck is palpated.

This method is still widely used as it is a cheap and provides a quick way of assessing whether or not there is a problem of public health importance. However, the specificity and sensitivity of this method, especially for grades 0 and 1 are low due to a high inter-observer variation. Thus, the persons carrying out the survey must be well trained. The use of ultrasonography is a more accurate way of assessing the volume of the thyroid, but requires expensive equipment and an operator trained in its use. Even with this method however, inter-observer variation may be high (67).

Another clinical indicator is the presence of endemic cretinism, the most extreme form of IDD, that manifests itself by severe growth and mental retardation, often accompanied by deaf-mutism (see Chapter 7).

Biochemical outcome indicators include concentrations of hormones in serum or whole blood and concentration of iodine in urine. The serum or blood indicators most commonly used are T4, FT4, T3, thyroglobulin (Tg) and thyrotropin, or thyroid stimulating hormone (TSH). Assessment of the concentration of these indicators is however not readily done on a large scale as it is necessary to collect blood samples and the cost is high. Measurement of iodine in urine provides a good proxy for recent iodine intake, as most iodine is eventually excreted in urine following deiodination of the thyroid hormones. However, the amount of iodine excreted by an individual varies from day to day as well as during the day. Therefore it is recommended that the results obtained be used for making an estimate of iodine status of population groups rather than that of individuals. Iodine excretion used to be expressed per gram of creatinine. This is however no longer deemed necessary or not even reliable in areas where protein intake (and thus creatinine excretion) is very low (65,68). It is therefore also preferable to speak of urinary iodine concentration (expressed per volume unit of urine) rather than of urinary iodine excretion (expressed per gram of creatinine).

The choice of process indicators will depend on the specific interventions or programmes implemented to ensure improved availability and consumption of sources of iodine by those who suffer from or are at risk of IDD. Such indicators may include the measurements of iodine levels in the vehicle(s) chosen for iodine, usually salt, at various stages in the production and marketing chain as well as the availability of the iodized product in shops and households.

For some outcome indicators mentioned above (thyroid volume, urinary iodine concentration, TSH and Tg), criteria and cut-off points have been established that indicate whether or not iodine deficiency is a significant public health problem. However, not all indicators are equally suitable for use in different age groups. Blood TSH concentration for instance is widely used in neonatal screening programmes for congenital hypothyroidism, and compilation of such data may also be used for monitoring measures to correct iodine deficiency at the population level (69), but its usefulness in older groups is uncertain. Moreover, assay methods and reference materials are not universally standardized implying that criteria and cut-off points are also not universally applicable. This is for instance the case with Tg (70,71), which makes comparison between different studies difficult.

In studies into the functional consequences of IDD, criteria are required for classifying children with respect to the severity of iodine deficiency. Such criteria however do not exist. In clinical practice blood or serum concentrations of T4, FT4, T3 and TSH are used frequently and normal reference ranges have been established (72-74). However, these reference ranges were developed from the perspective of thyroid function and not endemic iodine deficiency. Their use in monitoring the extent and severity of IDD is limited. Moreover, in thyroid endocrinology, compensatory mechanisms play an important role. Thus, the significance of serum concentrations of different hormones may only be interpreted if the appropriate regulatory factors are taken into account. For instance, serum concentrations of TSH and T4 are closely interlinked: if serum T4 concentrations are increased, this will inhibit the secretion of TSH and block the action of thyroid releasing hormone (TRH). Similarly if serum T4 concentrations are decreased, TSH secretion increases. Low serum concentrations of T4 and high serum concentrations of TSH are indicative of hypothyroidism, which may be the result of iodine deficiency, but could also point to a different problem. On the other hand, in different field studies, both TSH and FT4 were shown to be in the "normal range" in schoolchildren who were considered iodine deficient on the basis of goiter rates and urinary iodine concentrations (75).

A further confounding factor is the interaction between micronutrients other than iodine, and thyroid metabolism. Examples are provided by zinc and selenium. Not much is known yet about the relative importance of zinc-deficiency in the context of thyroid hormone metabolism (76,77), but selenium does play an important role. It is an essential component of many selenoproteins that regulate thyroid hormone synthesis, preserve thyroid integrity in conditions of oxidative stress and control hormone metabolism in non-thyroidal tissues where T4 is converted to T3 (77). The seleno-enzymes include three iodothyronine de-iodinases (type I, active mostly in liver and kidneys, type II which is principally found in brain, central nervous system, brown adipose tissue and the pituitary and type III which is active at intracellular levels), and four Se-containing glutathione peroxidases, which protect against the potentially-injurious effects of lipid peroxides and hydrogen peroxide produced during normal metabolism (78-80). Selenium deficiency affects the supply of selenium to various tissues and also to different selenoenzymes within a tissue and thus may have both beneficial and adverse effects on man and animals (80). Under conditions of selenium deficiency, concentrations of seleno-enzymes are decreased in liver, kidney and muscle, whereas those in the brain and endocrine organs such as the thyroid gland are less affected. Based on studies in myxedematous cretins in endemic goiter areas of Zaire, which were also selenium-deficient, it was postulated that the thyroid atrophy found in the cretins was due to loss of protection from toxic levels of hydrogen peroxide and free radicals following diminished activity of glutathione peroxidase (81). Trials with selenium supplementation amongst school children and cretins in the same area led to a fall in serum T4 concentration in both groups and an increase in TSH concentration in the cretins. The fall in serum T4 concentration is ascribed to an increase in the expression of hepatic type I de-iodinase (82). These studies provided further support to the hypothesis that although selenium deficiency may exacerbate hypothyroidism associated with iodine deficiency and may be involved in the etiology of myxedematous cretinism, it may also protect the brain from some of the detrimental effects of iodine deficiency (78,81,83). Meanwhile, the etiology of myxedematous cretinism is not yet fully elucidated as this form of cretinism is also found for instance in China without concurrent selenium deficiency (84). Therefore other interacting factors, such as goitrogens, may be implicated as well (80).

Thus, for reasons mentioned above, one cannot define iodine deficiency on the basis of only one indicator. Moreover, it should be realized that other co-existing nutrient deficiencies may mask or aggravate the true severity of iodine deficiency.

IDD in Benin

When the goal to eliminate IDD by the year 2000 was set, data on the African continent were still scant. For Benin for example, the total goiter rate in the country was estimated at 23.7 %, on the basis of surveys dating from 1983 (3). Unpublished reports (85) and personal communication with nutrition experts in the country, further confirmed that goiter was (still) a public health problem in northern Benin by the mid-1990's. It was therefore decided to carry out this research in this country in collaboration with staff of the Département de Nutrition et des Sciences Agro-Alimentaires, Faculté de l' Agriculture, Université Nationale du Bénin.

Benin

Benin is a small country with an estimated population of 5.8 million people in West Africa, situated between Togo in the west, Burkina Faso and Niger in the north, Nigeria in the East and the Atlantic Ocean in the south (Figure 2). It is approximately 700 km long and between 125 and 325 km wide, with a total surface area of about 112000 km^2. The altitude ranges from sea level to about 400-600 m in the highest part of the country, the north-western part of the province of Atacora. In terms of geological origin, four large zones may be distinguished: the precambrian plateau, the volta basin, the sedimentary basin of Kandi and the sedimentary basin of lower Benin. The study area is situated in the precambrian zone, with very old rocks such as gneiss, micaschists and Dahomey quartzites as well as granites.

Benin became independent in 1960 and has been a democratic republic with a multiparty system since 1991. With an annual GNP per capita of US$ 380.- and one third of its population below the national poverty line, the country belongs to the least developed countries in the world (86), as may also be concluded from other demographic, health and education characteristics presented in Fig. 2. Sixty percent of the population is rural, the majority of whom is engaged in agriculture, which is the main source of income in the country. Agricultural practices, especially those in the north, are traditional and extensive and focused on the major food crops, such as maize, millet and sorghum as well as cassava and taro. In the north, cotton and tobacco are grown as cash crops. Towards the south agriculture is more diversified and more market-oriented. The country's most important commercial agricultural products include palm oil, cashew nuts, sugar cane, cotton and groundnuts. Tropical hardwood is also important. Large-scale industrial activities hardly exist, except for a few activities in the agrofood sector. The informal sector is another important source of income.

With an annual population growth rate of 2.7 %, almost half of the population is aged less than 15 years. There are about 20 different ethnic groups in the country, many of which have their own language. French is the official language of the country's administration and educational systems, but in rural areas French is only spoken by people who have had some formal education. Different tribes also have different socio-cultural and traditional religious practices. The two major religions which have been introduced from outside are Christianity and Islam, which prevail in the south and the north, respectively.

Less than 50 % of the rural population has access to safe drinking water and less than 10 % has access to adequate sanitation. Malaria is endemic and a major public health problem especially in the rainy season. Almost 30 % of the under-fives is underweight, one quarter is moderately or severely stunted, while 14 % is moderately or severely wasted (87).

The study area

The four villages which were selected for the study, Pénessoulou, Penelan, Kodowari and Nagayile, belong to the municipality of Pénessoulou, which is situated in the district of Basila, province of Atacora[1], northern Benin. Together with a few other villages, this municipality comprises about 10,000 inhabitants, of whom approximately 98

[1] Recently the province was split up in two new administrative units: Donga and Atacora. Basila now is situated in Donga.

% belong to the Anii, a minor tribe with its own language. The remaining 2 % come from other ethnic groups such as the Kotokoli, Peul and Lokpa. The majority of the people are Muslim. Polygamy is common and most people live in extended families

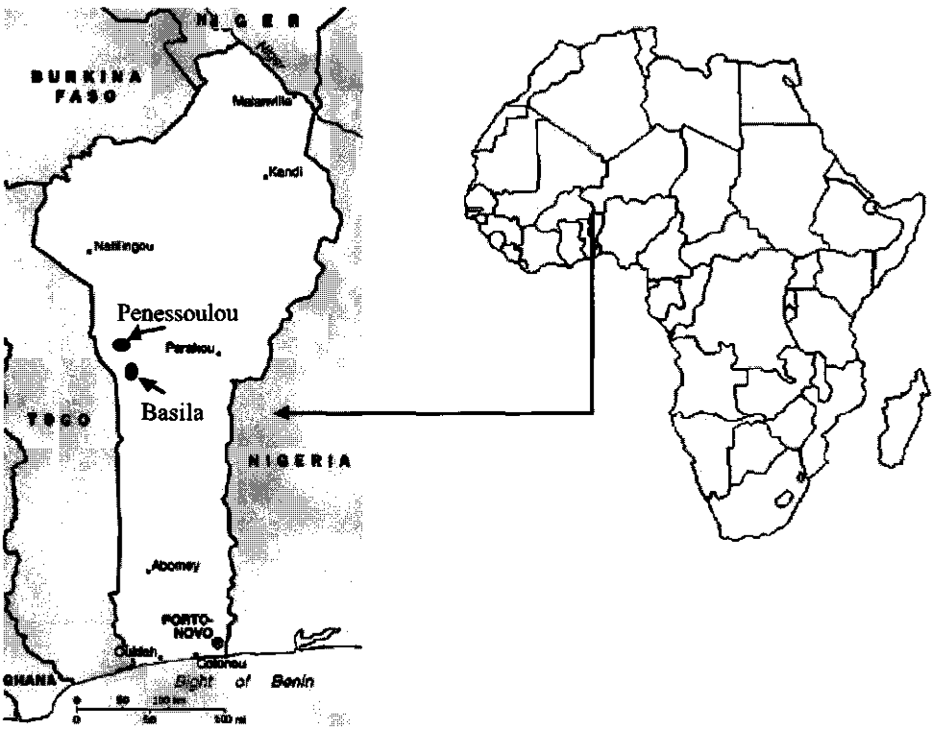
In terms of services, the area is underdeveloped. There is neither electricity nor potable water. The municipality has a small health centre, where a medical aide and a midwife provide basic health services and sell essential drugs. The nearest hospital is in Basila, about 35 km from Pénessoulou. Each of the four villages has a primary school, but the nearest secondary school is in Basila. Teaching is done in French, as most teachers come from the southern part of the country and generally do not speak the local language. Apart from the fact that not all children go to school, drop-out rates from school are also high. Over 80 children are found in the first grade, whereas about 20 children attend the sixth grade. The schools have very few written or visual teaching aids.

While rainfall in the south follows a bimodal pattern, in the north it is unimodal. The rainy season in this part of the country lasts from June to the beginning of October, followed by the dry season. Annual rainfall in the study area ranges from 1200 to 1300 mm. Subsistence farming is the major occupation, and maize, millet and sorghum, cassava and yam are the major food crops. Cotton and some tobacco are grown as cash crops. More than two-thirds of the people keep some animals, but these are mostly kept for special occasions or as a reserve for periods of hardship. Shortages of food may arise during the rainy season, when stocks are finished and food prices are high, and last to the harvesting period, early in the dry season. Besides being involved in agricultural activities and household chores, many women also engage in paid labour or petty trading in order to obtain a cash income with which household necessities such as salt, soap, oil and condiment cubes may be bought.

Food habits

Apart from rice virtually all the staple and other foods eaten by the study population are of local origin. In the morning, people eat a porridge made of maize, millet or a combination of the two, to which some sugar is added. At noon and dinner, a stiff porridge ("pâte") is prepared from mashed boiled yam, maize or cassava or a mixture of these. Akassa (fermented maize) and gari (fermented cassave) may also be used. The "pâte" is eaten with a sauce made of groundnut oil, red palm oil or karité butter with garlic, onions, red peppers, mustard from the locust bean seed, salt and condiment cubes (Maggi cubes, "cubes rouges" or a similar product). Usually tomatoes, okra or sesame seed sauce are added and, if the family can afford it, dried fish or meat.

Fig. 2. Benin



Benin: Basic data

Education
Adult literacy rate:
19/45 (females/males)
Net primary school enrollment ratio:
46/78 (females/males)
Net secondary school enrollment ratio:
10/23(females/males)

Demography

Total population:	5.8 million
Population density:	55 persons/km²
Annual growth rate:	2.7 %

Health and nutrition

Life expectancy:	53 yr
Infant Mortality Rate:	101 per 1000 live births
Under-5-Mortality Rate:	165 per 1000 live births

Sources
Data: UNICEF, The State of the world's children, New York, 2000.
Maps: courtesy of wwwtheodora.com/maps, used with permission.

Salt consumption

Salt is a precious commodity in this part of the world. Sold in small quantities, it is almost always available from most of the women traders along the main roads and from the markets. Iodized salt was not yet available in the north by the end of 1995 even though in November 1994 a resolution had been signed by 5 different ministers in the country (Health, Commerce and Tourism, Industry, Finance, Rural Development), banning the import of non-iodized salt and prescribing the levels of iodine in salt at all stages in the production and marketing chain. However, in the course of 1996 a project was started in the district's main town, Basila, distributing iodized salt through an extension worker to women traders. In a small market survey carried out in the study area by the end of 1996, it was shown that there were two types of salt on the market: iodized salt coming through the extension worker with a concentration of iodine of approximately 50 ppm, and non-iodized salt coming from different sources in the north and across the border with Togo. The sachets of iodized salt were 25-50 % more expensive than the non-iodized salt.

Objectives of the study and outline of the thesis

As described above, most studies on the relationship between iodine status and mental performance of children have concentrated on effects of iodine supply in utero and shortly after birth on mental and psychomotor development of children at various stages in their life. The most commonly held premise is that mental capacity of children may be affected by iodine deficiency in utero and shortly after birth and that, if iodine supply is not restored within this same period, mental performance is impaired permanently. A few intervention studies, with various designs, have been carried out to study whether mental and psychomotor performance of children who are growing up while iodine deficient may still benefit from iodine supplementation, with different results.

The two double-blind placebo-controlled studies involving oral supplementation of school children before 1995 produced different results. Bautista et al (39) found no difference in performance on the Stanford-Binet and Bender-gestalt tests between supplemented and non-supplemented children, aged 5.5-12 years, 22 months after supplementation. Shrestha et al (40) found that iodine supplementation had significantly improved performance of 6-8 year old Malawian children on a number of mental tests, fluency in particular, one year later.

The objectives of this study therefore were :

- To reconfirm the findings of the study done in Malawi in a different setting and to examine which specific aspects of mental performance are influenced most by supplementation;
- To assess the relationship between hearing thresholds and different variables representing iodine status and between hearing thresholds and mental performance;
- To determine whether iodine supplementation in iodine deficient children exerts an effect on their school behavior, including concentration;
- To assess the relationship between children's iodine status and their psychomotor performance;
- To evaluate the performance of different indicators of iodine status following iodine supplementation.

This research was set up as a double-blind placebo-controlled study, involving oral dosing with iodized oil in school children in an iodine deficient area in the northern part of Benin, with an observation period of 10 months. However, much faster progress than expected was made with the introduction of iodized salt in some parts of the country, including the study area. Approximately 3 to 4 months after supplementation the population started to have access to iodized salt, in addition to non-iodized salt. This resulted in an improvement of iodine status of the non-supplemented group. At the end of the intervention period both groups could be classified as mildly iodine deficient, as measured by urinary iodine concentration. Because iodine became available to both groups the main hypothesis, i.e. that iodine supplementation would improve mental performance had to be modified. It was decided to make a distinction between children whose iodine status, as measured by different indicators, improved during the observation period and children whose iodine status did not change. This latter group was taken as the "control" or "unchanged" group. The hypotheses subsequently formulated on a *post-hoc* basis are the following:

- *Children whose iodine status improves during the observation period show a greater improvement in scores on mental and psychomotor performance tests than those children whose iodine status shows little or no change;*
- *In an endemic iodine deficient area children with a better iodine status have lower hearing thresholds than those with a poorer iodine status;*
- *A change in iodine status of children during the observation period induces changes in their school performance and behavior as judged by their teachers;*
- *Serum concentration of thyroglobulin and urinary iodine excretion are the most suitable indicators for measuring iodine status under conditions of changing iodine supply.*

Outline of the thesis

The remainer of this thesis contains the following chapters:

Chapter 2 describes the effects of improving iodine status on the children's performance on a series of mental tests;

Chapter 3 describes the association between iodine status and hearing thresholds, as well as the relationship between hearing thresholds and mental performance.

Chapter 4 deals with the degree to which changes in iodine supply are reflected in indicators of iodine status and thyroid function.

Chapter 5 investigates the association between iodine deficiency and hyper-homocysteinemia.

Chapter 6 deals with the influence of a changing iodine status on changes in school performance and behavior, as judged by school teachers.

Chapter 7 presents a review of the main studies that have addressed the role of iodine in mental and psychomotor development.

Chapter 8 discusses the most important findings of the research described in this thesis, the issues that are still open to discussion and finally, implications for policies and programmes.

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Improved iodine status is associated with improved mental performance of schoolchildren in Benin

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American Journal of Clinical Nutrition 2000; 72:1179-85

ABSTRACT

Background: An adequate iodine supply in utero and shortly after birth is known to be crucial to an individual's physical and mental development. The question whether iodine supplementation later in life can exert a favorable influence on mental performance of iodine deficient populations has been addressed in various studies, but with contradictory results.

Objective: The aim of the study was to examine the effect of an improvement of iodine status on mental and psychomotor performance of schoolchildren (7-11 yr), who were moderately to severely iodine deficient.

Design: The study, which was originally planned as a double-blind randomized placebo-controlled intervention, was carried out in an iodine deficient population of school children (n=196) in northern Benin. As the population began to have access to iodized salt during the intervention period of 1 year, the study population was split, *post-hoc,* into a group with improved iodine status, based on increased urinary iodine concentration, and a group with unchanged iodine status. Changes in mental and psychomotor performance over the intervention period were compared.

Results: Children with increased urinary iodine concentration demonstrated a significantly greater increase in performance on the combination of mental tests than did the group where the concentration did not change.

Conclusions: Improvement in iodine status, rather than the status itself, determined mental performance in this population, which was initially iodine deficient. These findings suggest a "catch-up" effect in terms of mental performance.

INTRODUCTION

The public health importance of an adequate iodine supply for the physical and mental wellbeing of humankind has been well described (1,2). Most studies on the relationship between iodine status and mental performance of children have concentrated on effects of iodine supply in utero and shortly after birth on mental and psychomotor development (3-7). The most commonly held premise is that mental capacity of children, once affected by iodine deficiency in early life, is impaired permanently. Few intervention studies have been carried out to examine whether mental and psychomotor performance of children who are growing up while iodine deficient may still benefit from iodine supplementation. In a double blind, placebo-controlled study to examine the effect of iodine supplementation of children (5.5-12 yr) on mental performance, Bautista et al (8) found no difference between supplemented and non-supplemented children. In an unpublished double-blind placebo-controlled intervention study in Malawi, iodine supplementation significantly improved performance of 6-8 year old children on a number of mental tests (9).

The purpose of this study was to determine whether the findings in Malawi could be confirmed in a different setting and, if so, to examine which specific aspects of cognitive functioning are influenced most by supplementation. The research was planned as a double-blind, randomized, placebo-controlled study, involving oral dosing with iodized oil in school children in an iodine deficient area in northern Benin, West Africa. However, much earlier than expected, iodized salt was introduced into the study area. Halfway through the intervention period, the population began to have access to iodized salt. As iodine had become available to both groups, the hypothesis that iodine supplementation improves mental performance could no longer be tested. Therefore, we decided to test whether children whose iodine status improved over the intervention period showed a greater improvement in mental and psychomotor performance than those children whose iodine status changed to a limited extent or not at all.

SUBJECTS AND METHODS

Study area and subjects

The study was carried out in the district of Basila, province of Atacora, in northern Benin, where prevalence rates of goiter in schoolchildren aged 6-12 yr varied from 20 to 60 % (Doh, A. and Ategbo, EA. Prévalence de la carence en iode dans l'Atakora; unpublished report, 1994). The population, mostly Anii, was engaged in subsistence farming with cotton as the sole source of cash income. Food security was a seasonal concern. Polygamy was common and households were made up of extended families. The four study villages had neither electricity nor clean drinking water.

Children from standards (grades) 2 and 3, aged 7-11 years, in the four primary schools in the study area were considered for enrolment. Since in two out of four schools all female children had been given an iodized oil supplement in the previous year, only boys were selected from these two schools.

The study was approved by the health and education authorities of the province of Atacora, and by the Medical Ethics Committee of the Division of Human Nutrition and

Epidemiology of Wageningen University. The aim of the study was explained to local administrative and traditional authorities, parents and teachers. Having obtained verbal approval from local authorities, the parents and the parents-teachers association, all children selected were examined physically by a clinician. Several children with skin or respiratory infections, and malaria were treated. No children were excluded on health grounds.

Study design

Children were stratified by school, school class, and sex and subsequently matched on the basis of similar age and height-for-age. From each pair of children, one child was randomly allocated to one of two groups. The groups were then randomly allocated to receive iodine supplementation or a placebo. The study was double-blind, randomized and placebo controlled with the codes only being broken after the completion of the final test. Iodized oil (Lipiodol UF 7; 540 mg I/mL) and the placebo (poppyseed oil) were provided by Guerbet laboratories (Aulnay-sous-Bois, France). Iodized oil and poppyseed oil were dispensed as a single dose (1.0 mL), administered orally with a Swift 7 dispenser (English Glass Company, Leicester, England) in January 1996.

Baseline anthropometric measurements were made and urine and blood samples collected in October-November 1995. Baseline mental development tests were performed in the same period. All measurements and tests were repeated in October-November 1996. Additional urine samples were collected one week and 5 mo after supplementation.

Somatic and biochemical indicators

Anthropometric measurements were made in duplicate. Height was measured to the nearest mm, using a microtoise (Stanley ®). Weight was measured to the nearest 0.25 kg using a spring scale. Venous blood was drawn from the antecubical vein, immediately followed by the application of one drop of whole blood onto a filter paper card (Schleicher & Schuell, grade 903). These cards were air-dried for 1 to 2 h and packed in polyethylene bags, before being frozen. Hemoglobin was assessed using a Hemocue (Helsingborg, Sweden). Serum samples were prepared and frozen before being transported. Samples of casual urine (ca. 25 mL) were collected, to which some crystals of thymol were added. Blood spot cards, and frozen samples of urine and serum were transported to the Micronutrient Research Laboratory, University of Ghana at Legon, Accra for analysis of urinary iodine (chloric acid digestion followed by Sandell-Kolthoff reaction (10); thyroid stimulating hormone (TSH) in blood spots (Spectra Screen™ Dried Blood TSH EIA Kit) and serum ferritin (ELISA), within the next 6 to 9 mo. Frozen serum samples were also transported to the Laboratory for Endocrinology, Amsterdam Medical Centre, the Netherlands for assessment of thyroglobulin (RIA), free thyroxine (time-resolved fluoroimmunoassay; Delfia™, Wallac Oy, Turku, Finland) and thyroid stimulating hormone (immunoluminometric assay; Brahms Diagnostica Gmbh, Berlin, Germany).

Mental and psychomotor development tests

Since no comprehensive battery of mental tests has been developed for use in French-speaking West-Africa, a number of tests, consisting of nonverbal and abstract pictorial material of the French Kaufman ABC test battery (11) was pretested in a nearby village. Apart from the sequential memory test (hand movements), most tests contained images unknown to the children. Subsequently a battery was composed with mostly non-verbal tests which have been used under conditions comparable to those found in rural Benin, i.e. tests requiring very little or no vocabulary skills of the child being tested, thus avoiding confounding by education-related language skills. These tests covered as much as possible aspects of fluid intelligence, as opposed to crystallized intelligence (12-14). Fluid intelligence is regarded as one of the major constituents of intelligence; it refers to the ability to reason by analogy, to apprehend an unfamiliar configuration and to construct or extract a solution. Crystallized intelligence refers to subject-matter proficiency acquired in the past, which is reflected in results of tests measuring such aspects as vocabulary, arithmetic or factual knowledge. The mental test battery comprised the following 8 tests: block design (15), coloured progressive matrices (16), hand movements (11) as well as 5 tests from the African Child Intelligence Test (17): closure, mazes, exclusion, concentration, and fluency. Table 1 gives a description of the tests as well as their meaning, according to factors reported by Thurstone (18) and French (19). In addition 2 psychomotor tests, pegboard and ball throwing, were carried out. A psychologist (NB) trained two university graduates and two teachers from Benin to conduct the tests, which were subsequently pretested among school children from the study area who were not included in the study population. The testers worked in pairs. All four testers started with the same three tests in a fixed sequence. The remaining tests were administered either by one tester or the other, with the same tester always being responsible for the same set of tests. All children were given a snack prior to testing, which took place between 09.00 and 12.00 hrs. in a room which was quiet and free of distractions. The 8 mental tests plus the pegboard test took approximately 50-60 mins, after which the children were taken outside for the ball throwing test. Because of the diversity and short duration of tests, children did not become tired or bored. A simple reaction-time test and a choice reaction-time test, both measuring information processing time, as well as a tapping test measuring manual dexterity and accuracy, were administered at the end of the intervention period, but not on the same day as the mental tests.

Data analysis

Data analysis was related to the modified study design. Children were allocated to new groups, based on the magnitude of change over the intervention period in urinary iodine concentration. Differences in change in mental performance between these groups were assessed using Student's t-test. If not normally distributed, log transformations were made. Factor analysis, based on the intercorrelation of the tests, was carried out, using a principal component analysis with orthogonal Varimax rotation.. This enabled us to examine the construct validity of the test battery. All data were processed and analysed using SPSS-pc software (SPSS-Windows 6.1). Anthropometric indices were calculated using Epi-Info (version 6.02; CDC, Atlanta, GA, USA)

Table 1. Mental development tests used in the study

Test name	Test Description	Mental ability involved
Block design (WPPSI)	With a set of colored blocks (two colors; blocks having one or a combination of both colors), the tester arranges the blocks in a given design, after which the blocks are reshuffled and the child is asked to arrange the same design within a specified time period	Space [1)] Spatial Orientation [2)] Memory (partly)
Closure	A series of 35 test pages, each with one incomplete drawing of an everyday object is shown to the child; for each test page the child is asked to identify the incomplete figure within a specified time period, a task requiring mental reconstruction of what is missing	Perception [1)] Speed of closure [2)]
Concentration	A set of 2 pages is covered with small circles with an oblong dark "eye" in one of eight positions. The child is shown one example and is then asked to find and tick circles with the eye in the same position as the example as quickly as possible, working line by line, within a specified time period.	Perception [1)] Perceptual speed [2)]
Exclusion	A series of 30 test pages, each with 4 abstract figures, is shown to the child; from each test page the child is asked to choose one figure which lacks a common characteristic of the other three	Reasoning [1)] Induction [2)]
Fluency	In 2 fixed time periods the child is asked to mention first names of as many people as possible and subsequently, the names of as many animals as possible	Word fluency [1) 2)]
Mazes	The child is presented with a series of mazes en relief and asked to go with a small pointer through the maze as quickly as possible, while not being allowed to lift the pointer from the surface.	Space [1)] Spatial scanning [2)]
Coloured Progressive Matrices (sets A, Ab, B)	The child is presented with series of coloured figural rectangular pictures, out of which a small section has been cut. In addition the child is presented with 6 different pictures that all have the form, size and colours of the piece that was cut from the larger picture and is then asked which of the smaller pictures should be inserted into the larger one, in order to make it complete again.	General ability Reasoning by analogy
Hand Movements (Kaufman ABC)	The child is asked to imitate series of hand movements made by the instructor on a table; movements are specified and composed of three basic elements in varying sequence and number: the fist, the side of the hand and the palm of the hand	Sequential memory

[1)] Based on factors according to Thurstone
[2)] Based on factors according to French

RESULTS

Initially 211 children were enrolled in the study; 13 children had left school or moved out of the area by the end of the intervention period. Two children could not be located during urine collection. The socio-economic status of the families included in the studies was generally poor. Families were large, landholding size relatively small and levels of education among adults low (Table 2).

Nutritional status

One-third of the children were stunted (height-for-age <-2 SD of NCHS reference), 17 % had low weight-for-age (<-2 SD of NCHS reference) and 2 % were wasted (weight-for-height <-2 SD of NCHS reference) (Table 2). The proportion of children with anemia (hemoglobin <110 g/L) was one-third, while 11% had moderately to severely depleted iron stores (serum ferritin concentration < 18 μg/L). With an initial median urinary iodine concentration of 0.16 μmol/L (20.6 μg/L), the study population could be classified as moderately to severely iodine deficient. At the end of the intervention period both the original placebo and the iodine-supplemented group showed clearly improved iodine status. However, the total study population could still be classified as mildly iodine deficient based on median concentrations of urinary iodine. Initial urinary iodine concentration was positively correlated (Spearman) with the serum concentration of free thyroxin (r =0.19; P=0.007) and negatively correlated with serum concentrations of thyroglobulin (r = -0.51; P=0.000) and thyroid stimulating hormone (r = -0.34; P=0.000). At the end of the study urinary iodine concentration was related with serum thyroglobulin concentration only (r = -0.21; P=0.003).

Table 2. General characteristics of subjects and their families [1)]

	Improved group		*Unchanged group*	
Characteristics of subjects	Initial	Final	Initial	Final
n (males/females)		128 (109/19)		68 (58/10)
Suppl./non-suppl.		68/60		29/39
Age (y)	9.1 ± 1.2	10.1 ± 1.2	8.5 ± 1.2	9.5 ± 1.2
Height-for-age (Z-score)	-1.73 ± 0.91	-1.70 ± 0.87	-1.48 ± 1.04	-1.43 ± 1.01
Weight-for-age (Z-score)	-1.35 ± 0.70	-1.38 ± 0.70	-1.32 ± 0.77	-1.33 ± 0.75
Education (y)	2.8 ± 0.9		2.6 ± 0.7	
Characteristics of family				
Family size (n)	14.2 ± 7.2		14.8 ± 8.4	
Education of parents (y)				
- father	1.3 ± 0.8		1.4 ± 0.9	
- mother	1.2 ± 0.5		1.3 ± 0.8	
Size of landholding (ha)	3.1 ± 2.4		3.2 ± 2.7	

[1)] Results are expressed as mean ± SD, except for the number and proportion of supplemented/non-supplemented subjects

Mental performance

Correlations between the scores on the different tests at baseline were mostly positive (Table 3), in line with what is usually found (20). Factor analysis of the series of tests used followed by Varimax rotation produced three factors. Block design, closure, exclusion, maze, fluency and concentration tests loaded on one factor, the hand movements test on the second factor and the Coloured Progressive Matrices or Raven test loaded on the third factor (Table 4). This pattern is similar to the pattern found in a study on an intelligence test for Dutch, Spanish and Indian children, and comparison of this battery with the WISC-R battery (21-23). The first factor refers to spatial/perceptual reasoning skills, the second factor to sequential memory and the third factor to general intelligence, often referred to as *g* (20,24). The overall changes in performance over the intervention period were small but positive. Test-retest correlation of the full test battery in the unchanged group was 0.83 (*P*=0.000).

Table 3. Correlation between mental development tests in total group (n=196), at the beginning of the study

	Spearman correlation coefficients							
	1	2	3	4	5	6	7	8
1. Block design	1.00							
2. Closure	0.34***	1.00						
3. Concentration	0.51***	0.34***	1.00					
4. Exclusion	0.33***	0.35***	0.37***	1.00				
5. Fluency	0.23***	0.24***	0.39***	0.33***	1.00			
6. Mazes	0.44***	0.35***	0.47***	0.31***	0.29***	1.00		
7. Hand movements	0.09	- 0.03	0.22**	0.14*	0.19**	0.24***	1.00	
8. Raven test	0.05	0.08	0.18*	-0.01	0.14*	0.06	0.16*	1.00

Significance of correlation coefficients: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 4. Varimax-rotated factor matrix of results on mental tests at the beginning of the study

	VARIMAX-rotated Loadings [1]			
	Factor 1	*Factor 2*	*Factor 3*	Communality
Block design	0.76			0.58
Closure	0.65			0.64
Concentration	0.73			0.60
Exclusion	0.67			0.46
Fluency	0.50			0.42
Mazes	0.65			0.49
Hand movements		0.87		0.78
Raven test			0.96	0.94
				Total
Sum of squares (Eigenvalue)	2.84	1.12	0.96	4.92

[1] Only loadings ≥ 0.50 are included

Mental performance in relation to iodine status

Children were categorized (Table 5) both at the beginning and the end of the study with respect to their urinary iodine concentration as *normal/mild* (>0.40 μmol I/L urine), *moderate* (0.16 – 0.40 μmol I/L urine) *or severe* (<0.16 μmol I/L urine) based on criteria for establishing the severity of iodine deficiency as a public health problem (25). The categorization at the end of the study was based on the mean urinary iodine excretions at 5 months and 11 months after supplementation. Subsequently children were allocated to one of two groups, the criterion for allocation being whether or not they moved to a better category of iodine status. About two-thirds of the children, forming the "improved" group, showed a considerable increase in urinary iodine concentration (i.e. their status had moved from the severe to the moderate or mild/normal categories or, alternatively, from the moderate to the mild/normal category). The second or "unchanged" group comprised children whose urinary iodine concentration remained unchanged (i.e. they remained in the moderate or mild/normal categories or, in a few cases (n=7), they changed from mild/normal to moderate deficiency. None of the children in this group were severely deficient either at the beginning or at the end of the study). It should be noted that the unchanged group had, on average, better initial and end-of-study iodine status than their counterparts in the improved group (Table 5). Both groups comprised supplemented and non-supplemented children, but frequencies were not significantly different (Chi-Square test). Although the improved group was older than the unchanged group by 7 mo, groups were fully comparable in terms of blood hemoglobin concentrations, anthropometric and socio-economic indices as well as initial scores on the mental tests. No correlation was observed between age and change in mental performance in either one of the intervention groups or in the study population as a whole (data not shown).

Table 5. Indicators of iodine status [1] at beginning and end of the intervention in groups categorized as improved or unchanged on the basis of urinary iodine concentration (n=196)

	Improved		*Unchanged*	
	Initial	Final	Initial	Final
Urinary iodine concentration (µmol/L)	0.09 (0.03; 0.16) [4,5]	0.68 (0.30; 1.25)	0.62 (0.40; 1.43)	0.67 (0.27; 1.28)
Serum thyroglobulin concentration (pmol/L)	285.0(180.0; 460.0)[4,5]	95.0 (70.5; 142.5)	135.0 (85.0; 225.0) [4]	90.0 (64.0; 143.8)
Serum TSH concentration (mU/L) [2]	2.20 (1.70; 3.75) [4,5]	1.40 (1.10; 2.08)	1.80 (1.30; 2.45) [4]	1.20 (1.03; 1.80)
Blood TSH concentration (mU/L)	4.65 (2.70; 7.80)	n.a. [3]	3.75 (2.30; 6.88)	n.a. [3]
Serum free T4 concentration (pmol/L)	11.6 ± 2.4 [4,5]	13.9 ± 2.5 [6]	12.6 ± 2.2 [4]	14.8 ± 1.8
Blood hemoglobin concentration (g/L)	115.0 ± 1.2 [4]	118.5 ± 1.0	113.9 ± 1.3 [4]	117.0 ± 1.0
Serum ferritin concentration (µg/L)	47.2 (30.3; 72.4)	50.9 (26.8; 104.8)	48.2 (22.3; 70.4)	48.1 (22.8; 90.4)

[1] Values are expressed as means ± SD or as median (25th; 75th percentile)
[2] The initial serum concentration of TSH was measured in only 154 subjects (97 in the improved group, 57 in the unchanged group)
[3] Not available
[4] Initial/final values within same group are significantly different ($P<0.05$); paired samples t-test (non-normally distributed variables were log transformed)
[5] Initial values between groups are significantly different ($P< 0.05$); independent samples t-test (non-normally distributed variables were log transformed)
[6] Final values between groups are significantly different ($P< 0.05$); independent samples t-test (non-normally distributed variables were log transformed)

The performance at baseline and at the end of the study, as well as the changes in performance on the series of mental tests during the study period were expressed as Z-scores. Thus the Z-scores of the improved and unchanged groups together were zero, both at baseline and at the end of the study. The mean initial Z-score of the unchanged group (n=68) was -0.02 ± 0.58, while that of the improved group (n=128) was 0.01 ± 0.58. Mean Z-scores at the end of the study were -0.10 ± 0.59 in the unchanged group and 0.05 ± 0.57 in the improved group. The Z-scores for the change in performance (i.e. the differences between scores on each test at baseline and at the end of the study period) in the unchanged group were set at 0 with an SD at 1. Thus, the scores of the improved group represent the difference from the unchanged group. Comparison of the performance on the range of tests shows a consistent pattern in favor of the improved group (Table 6) and the overall results in the improved group were significantly better than those in the unchanged group. With respect to the reaction-time and tapping tests no effect of change in iodine status could be demonstrated.

Table 6. Change in mental performance during intervention in the group of children with improved urinary iodine concentration [1)]

	Z-scores
Block design	0.05 ± 1.03
Closure	0.04 ± 1.09
Concentration	0.06 ± 0.87
Exclusion	0.25 ± 1.00
Fluency	0.18 ± 1.21
Mazes	0.11 ± 0.88
Hand movements	0.05 ± 1.29
Colored progressive matrices (Raven test)	0.24 ± 1.14
Mean ± SE	0.12 ± 0.06 [2)]

[1)] Z-scores, mean ± SD. Mean performance for each test in the group of children with unchanged urinary iodine concentration was set at 0 ± 1 (mean ± SD)
[2)] Significantly different from unchanged group (P= 0.044; 2-tailed independent samples t-test)

DISCUSSION

This study demonstrates that in this population improvement of urinary iodine concentration is reflected in significantly improved mental performance on a combination of tests. Children whose urinary iodine concentration was basically unchanged during the intervention period showed less progress in performance, even though their iodine status as measured by several variables was, on average, better than that of their improved counterparts both initially and at the end of the study. These findings are indicative of "catch-up", that is, improvement towards their full potential, as a result of iodine supplementation.

The improvement found in the present study is most pronounced in tests on exclusion and the coloured progressive matrices or Raven test, suggesting an improvement in general abstract reasoning. Improvement was also seen on the test for verbal fluency. The latter finding concurs with a study in Malawi in which improved verbal fluency was one of the most pronounced effects of iodine supplementation (9). Since all tests, except for the coloured progressive matrices, have a time limit within which a response must be given, these findings may point to improvement in the level of task performance, improvement in speed of task performance, or a combination of the two.

Improved attention or concentration may facilitate improvement in mental function (20). According to Tiwari and colleagues (26) iodine deficient children are slow learners with a concurrent low “motivation to achieve”. They ascribe poor performance to both neurologic impairment and paucity of psychologic stimulation. In hypothyroidism, mood disorders including depression, social withdrawal, and paucity of speech are common phenomena (27). Thus it may well be that poor performance under conditions as found in our study reflects a general state of apathy, accounting not only for the lack of motivation but also for other factors important in cognitive functioning, such as attentiveness and concentration.

The marked improvement in mental and psychomotor performance as a result of iodine supplementation which was seen in a study in schoolchildren in Malawi (>10 IQ points; (9)), was not achieved in the present study. Although by the end of the intervention period the iodine status of the majority of children had improved substantially, the improvement in mental performance amounted to approximately 5 IQ points. The Beninese children were older than the children in Malawi (mean age 7.1 yr), but otherwise comparable in terms of initial iodine and iron status and in anthropometric indices. The improvement in iodine status, whether through iodized oil supplementation or through iodized salt, may have come too late in their life to enable comparable catch-up to take place.

The functional classification of children with respect to degrees of iodine deficiency remains problematic for several reasons. Firstly, there is as yet no universally accepted single indicator for "iodine status" in this age group and secondly, cut-off points enabling different degrees of iodine deficiency to be distinguished are based on populations rather than on individuals. The four iodine status variables which we measured could not be captured in one variable by factor analysis, which can be explained by the fact that each of these variables reflects different facets of iodine metabolism. While initial urinary iodine concentrations were indicative of a serious iodine deficiency in our study population, initial serum TSH and free T4 concentrations in our group were found to be within the normal range (Table 5). These findings concur with those of Benmiloud et al (28), Pardede et al (29) and Untoro et al (30) who, in studies in iodine deficient populations, also find values for TSH and free T4 in the normal range. These authors therefore maintain that urinary iodine excretion is the best outcome indicator for interventions involving iodine supplementation. As in our study TSH and free T4 concentrations showed significant improvement over the study period, it might also be argued that the normal range of TSH and free T4 values is too wide, at least for this age group. Although thyroglobulin concentration is considered to be very sensitive to changes in iodine metabolism, assay methods for thyroglobulin are not standardized among laboratories and therefore normal ranges and cut-off points for various degrees of iodine status cannot be established. For these reasons, our subjects were categorized on the basis of their urinary iodine concentrations. Although regarded as the best alternative for measuring iodine status at the population level, using urinary iodine concentration also has its limitations, especially when used at the individual level. This is because it primarily reflects the previous day's iodine intake, which may not be representative of long term intake. In addition, urinary iodine concentrations vary throughout the day. Thus, caution should be exercised in the interpretation of results.

With the current rate of progress being made with universal salt iodization, further research in this field is increasingly difficult to carry out. However, constraints in iodine supply and metabolism both in individuals and in population groups with insufficient access to sources of iodine will continue to call for a better insight into the relationships between iodine status and mental functioning. While our study has shown that restoration of impaired psycho-neurological processes is to some extent still possible in school children, a number of questions remain. Is there an age-threshold beyond which restoration is no longer possible? Are different aspects of cognitive functioning restored at different speeds? Which iodine variables are most closely associated with changes in cognitive functioning? This study was not set up to answer these questions. It does however indicate that mental performance of iodine deficient children is positively influenced by iodine supplementation, whether through the use of iodized salt or the administration of iodized oil.

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3

Mild iodine deficiency is associated with elevated hearing thresholds in children in Benin

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European Journal of Clinical Nutrition 2001; 55: 763-768

ABSTRACT

Objective: Elevated hearing thresholds have been demonstrated in populations afflicted by endemic cretinism as a result of severe iodine deficiency. However, data on the effects of less severe iodine deficiency on hearing thresholds in apparently normal children are scant. This study addresses the question whether there is a relationship among iodine variables, hearing and mental performance in a mildly iodine deficient population.

Design: A randomized, placebo-controlled intervention trial with an observation period of 11 months.

Setting: An iodine deficient area in northern Benin.

Subjects: 197 school children, aged 7-11 yr.

Interventions: 97 children received an oral dose of iodized oil, containing 540 mg I, while 100 children received a placebo. About 3-4 months after supplementation, the whole population began to have access to iodized salt. Non-verbal mental tests were administered and biochemical indicators (thyrotropin, free thyroxine, thyroglobulin and urinary iodine) were measured at the beginning and the end of the study. Hearing was measured at the end of the study in both ears by pure-tone audiometry at 7 frequencies.

Results: In this mildly iodine deficient child population children with higher serum thyroglobulin concentrations had significantly higher hearing thresholds in the higher frequency range (≥2000Hz) than children with lower serum thyroglobulin concentration. Moreover children with lower hearing thresholds performed significantly better on the mental tests used.

Conclusions: Even when iodine deficiency is "mild", promotion of adequate iodine intake through salt iodization programs and other means remains crucial.

INTRODUCTION

Hearing loss in thyroid dysfunction was first recognized more than one hundred years ago (1). Since then several types of thyroid abnormalities have been distinguished in relation to hearing problems. These include non-genetic sporadic and endemic hypothyroidism and genetic deviations such as Pendred's syndrome and resistance to thyroid hormone. However, the exact nature of the link between the endocrine and auditory systems has not been elucidated (2-5). Earlier studies on endemic hypothyroidism mostly focused on one of the most extreme consequences of iodine deficiency, endemic cretinism, and its relation to deafness and deaf-mutism. More recently ecological studies in China (6), Iran (7), Italy (8) and France (9) have shown that hearing thresholds in apparently normal persons living in iodine deficient areas are higher than those of persons living in iodine-replete areas.

Therefore, as part of a study on iodine status and mental performance of school children aged 7-11 yr in an iodine deficient area in northern Benin, hearing thresholds in the study population were measured with the aim of determining their relationship to mental performance and to different variables representing iodine status.

SUBJECTS AND METHODS

Study area and subjects

The study was carried out in 197 children from four villages in the province of Atacora, district of Basila, northern Benin, where iodine deficiency was a problem of public health importance. The population is mainly engaged in subsistence farming. The villages have neither electricity nor clean drinking water. Children from grades 2 and 3 in the four primary schools in the study area were considered for enrollment. From 2 of the 4 schools all girls were excluded as they had been supplemented orally with iodized oil in the previous year. The socio-economic conditions of the large, extended households to which the children belonged were poor: subsistence farming was the main occupation, while landholding size was small. Levels of parental education were low (Table 1).

The study was approved by the health and education authorities of the province of Atacora, Benin and by the Medical Ethics Committee of the Division of Human Nutrition and Epidemiology of Wageningen University. The aim of the study was explained to local administrative and traditional authorities, parents and teachers. Having obtained verbal approval from local authorities, the parents and the parents-teachers association, all children selected were examined by a physician. Several children with skin or respiratory infections, and malaria were treated, but no children were excluded on health grounds.

Study design

The study was set up as a double-blind placebo-controlled intervention. Children were stratified by school, school class and sex and subsequently matched on the basis of age and height-for-age. From each pair of children, one child was randomly allocated to one of two groups. The groups were then randomly allocated to receive iodine supplementation or a placebo. Iodized oil (Lipiodol UF 7; 540 mg I/mL) and the placebo (poppyseed oil) were provided by Guerbet Laboratories (Aulnay-sous-Bois, France). Iodized oil and poppyseed oil were dispensed as a single dose (1.0 mL), administered orally with a Swift 7 dispenser (English Glass Company, Leicester, England) in January 1996.

A baseline survey was carried out before supplementation, in October-November 1995, including the administration of mental development tests, anthropometric measurements and collection of urine and blood samples. All these measurements were repeated in the same period in 1996. Additional urine samples were collected one week and 5 mo after supplementation, i.e. in January and May 1996. Approximately 3-4 mo after the start of the intervention period of 11 mo the population began to have access to iodized salt. Audiometry was performed only at the end of the study in December 1996. Thus, at that point in time the study population consisted of one group of children who had received iodine supplementation 11 mo earlier (n=97) and one group who had received a placebo (n=100) while both groups had had access to iodized salt for a period of approximately 6-7 mo.

Table 1. General characteristics of the study population at baseline [1)]

Characteristics of subjects	
n (males/females)	197 (167/30)
Age (yr)	8.9 ± 1.2
Height-for-age (z-score)	-1.64 ± 0.96
Weight-for-age (z-score)	-1.34 ± 0.72
Education (yr)	2.8 ± 0.8
Characteristics of family	
Family size (n)	14.5 ± 7.6
Education of parents (yr)	
- father	1.4 ± 0.8
- mother	1.2 ± 0.6

[1)] Results are expressed as mean ± SD, except for the number of subjects

Somatic and biochemical indicators

Anthropometric measurements were made in duplicate. Height was measured to the nearest mm, using a microtoise (Stanley ®; Besançon, France). Weight was measured to the nearest 0.25 kg using a spring scale, which was calibrated with a 20 kg weight after every 25 children. Venous blood was drawn from the antecubical vein for assessment of free thyroxine, thyroid stimulating hormone, thyroglobulin and ferritin in serum and hemoglobin in whole blood. Casual urine samples were collected for determination of iodine concentrations. Procedures followed and assay methods used for assessment of these variables have been described elsewhere (10).

Hearing thresholds were measured using a simple battery-operated audiometer (model EB-60; Eckstein Bros., Inc.; Hawthorne, CA, USA) with 7 frequencies (250; 500; 1000; 2000; 3000; 4000; 6000 Hz) and an attenuator range of 0 to 80 decibels (dB). Both ears were tested separately at all frequencies for the lowest intensities (in 5 dB steps) that could still be heard. Measurements were carried out in rooms kept as quiet as possible, away from the main school activities.

Mental tests

A set was composed of mostly non-verbal mental tests, measuring different mental abilities (Ekstrom *et al*, 1979; Thurstone, 1969) and one psychomotor test (see van den Briel *et al*, 2000). These tests have been used under conditions comparable to those found in rural Benin, requiring very little or no vocabulary skills of the child being tested. After pretesting in a nearby village, the tests were administered by a team of 4 Beninese (2 teachers, 2 university graduates) who had been trained to conduct the tests by a psychologist.

Data analysis

To check the normality of data, the Kolmogorov-Smirnov test was used. Pearson correlation coefficients were calculated for the relationship between variables reflecting iodine status and hearing threshold, after log-transformation of non-normally distributed variables. Results on mental tests were expressed as z-scores. For determining the correlation between hearing threshold and results on mental tests a non-parametric (Spearman) test was used as 4 of the 9 variables were not normally distributed. All children were also allocated to one of three groups, based on tertile values for each of 4 iodine variables, as well as on tertile values for hearing thresholds. Differences between groups were assessed using Student's t-test or the Mann-Whitney test. Differences in hearing thresholds between the groups were assessed using analysis of variance tests (one-way ANOVA). All data were processed and analysed using SPSS-pc software (SPSS-Windows 6.1; SPSS Inc, Chicago, IL, USA). Anthropometric indices were calculated using Epi-Info (version 6.02; CDC, Atlanta, GA, USA)

RESULTS

Somatic and biochemical characteristics

Based on a reference population (11), the proportion of children who were stunted (height-for-age <-2 SD) at the beginning of the study was one-third, while 17% had low weight-for-age (<-2 SD) and 2% were wasted (weight-for-height <-2 SD). These proportions did not change significantly during the study.

Based on criteria established by WHO/Unicef/ICCIDD (1994) the study population was moderately to severely iodine deficient (median urinary iodine concentration, 0.16 µmol/L) at the start of this study. During the study the population began to have access to iodized salt. This resulted in improvement of the iodine status of the whole group, which was mildly iodine deficient 10 mo after supplementation, one year after the baseline survey (median urinary iodine concentration, 0.67 µmol/L; Table 2). Initially one-third of the children were anemic (hemoglobin <110 g/L), while at the end of the study 19% of the children were anemic.

Table 2. Biochemical characteristics [1] of study population at baseline and end of the study, 1 yr later

	Baseline	*End of study*
Serum thyrotropin concentration (mU/L) [2]	2.1 (1.5; 3.4)	1.4 (1.1; 1.9)
Serum thyroglobulin concentration (pmol/L)	215.0 (135.0; 362.0)	95.0 (66.0; 140.0)
Urinary iodine concentration (μmol/L)	0.16 (0.06; 0.42)	0.67 (0.30;1.25)
Serum free T4 concentration (pmol/L)	12.0 ± 2.4	14.2 ± 2.3
Blood hemoglobin concentration (g/L)	114.5 ± 12.0	117.9 ± 10.3
Serum ferritin concentration (μg/L)	46.9 (28.4; 71.8)	49.2 (25.4; 99.5)

[1] Results are expressed as median (25th, 75th percentiles) or as mean ± SD.
[2] The baseline serum concentration of thyrotropin was measured in only 154 subjects because of insufficient serum

Hearing thresholds

Based on the WHO (12) definition and classification, which recommends that the mean value from the better ear for 4 frequencies (500,1000,2000 and 4000 Hz) be used, 4 % of the children had slight hearing impairment (i.e. a threshold between 26 and 40 decibels). Based on the mean of values obtained from the left and right ears, 7% of the children had slight hearing impairment. The mean hearing threshold was 17.1 dB. Of the four iodine indicators measured, only serum thyroglobulin concentration showed a significant correlation with mean hearing threshold over the 7 frequencies used (Table 3).

There was a significant difference in the mean hearing threshold on 7 frequencies between the tertile with the lowest and that with the highest thyroglobulin concentration (one-way ANOVA, LSD; P=0.047). The pattern was very consistent, with the group with the higher thyroglobulin concentration having a higher hearing threshold at each of the frequencies applied, the differences becoming significant at the higher frequencies (Fig.1). The same consistent pattern was seen when children were divided into tertiles based on urinary iodine concentration, with the children with lower urinary iodine concentration hearing less well at each of the frequencies applied, but here the differences did not reach significance (data not shown).

Table 3. Correlations between hearing threshold and concentrations of iodine variables

	Correlation coefficient [1]	P [2]
Serum thyroglobulin	0.15	0.032
Serum thyrotropin	0.03	0.661
Serum free thyroxin	0.08	0.279
Urinary iodine	-0.08	0.254

[1] Pearson; non-normally distributed variables (serum concentrations of thyroglobulin and thyrotropin as well as urinary iodine concentration) were log-transformed
[2] Two-tailed significance level

Table 4. Correlations between performance on mental tests and hearing threshold

Mental tests	Correlation coefficient [1]	P [2]
Block design	-0.23	0.001
Closure	-0.32	<0.0001
Concentration	-0.17	0.016
Exclusion	-0.14	0.059
Fluency	-0.23	0.001
Mazes	-0.20	0.005
Raven test	0.02	0.749
Hand movements	-0.27	<0.0001
Pegboard	-0.19	0.007

[1] Spearman
[2] Two-tailed significance level

Hearing thresholds and mental performance

Hearing thresholds were negatively associated with performance on almost all the mental tests administered (Table 4). Similarly, if children were divided into groups on the basis of tertile values for overall hearing thresholds, children in the higher hearing threshold tertile performed significantly worse on the total mental test battery than did children in the lower tertile ($P < 0.001$; Table 5).

Table 5. Performance on mental tests of groups with high/low hearing threshold (tertiles)

	Hearing thresholds z-scores [2]		
Mental tests	Low	High	P [1]
Block design	0.34 ± 0.80	-0.24 ± 1.03	0.001
Closure	0.35 ± 1.00	-0.36 ± 1.02	<0.0001
Concentration	0.22 ± 1.03	-0.19 ± 0.91	0.017
Exclusion	0.07 ± 0.97	-0.22 ± 1.08	0.104
Fluency	0.28 ± 1.00	-0.19 ± 1.01	0.013
Mazes	0.33 ± 0.88	-0.25 ± 1.11	0.004
Raven test	0.02 ± 1.06	0.01 ± 1.00	0.970
Hand movements	0.43 ± 1.02	-0.28 ± 0.93	<0.0001
Pegboard	0.20 ± 1.10	-0.24 ± 0.90	0.007

[1] Two-tailed significance level; Mann-Whitney test
[2] Means±SD

Fig.1

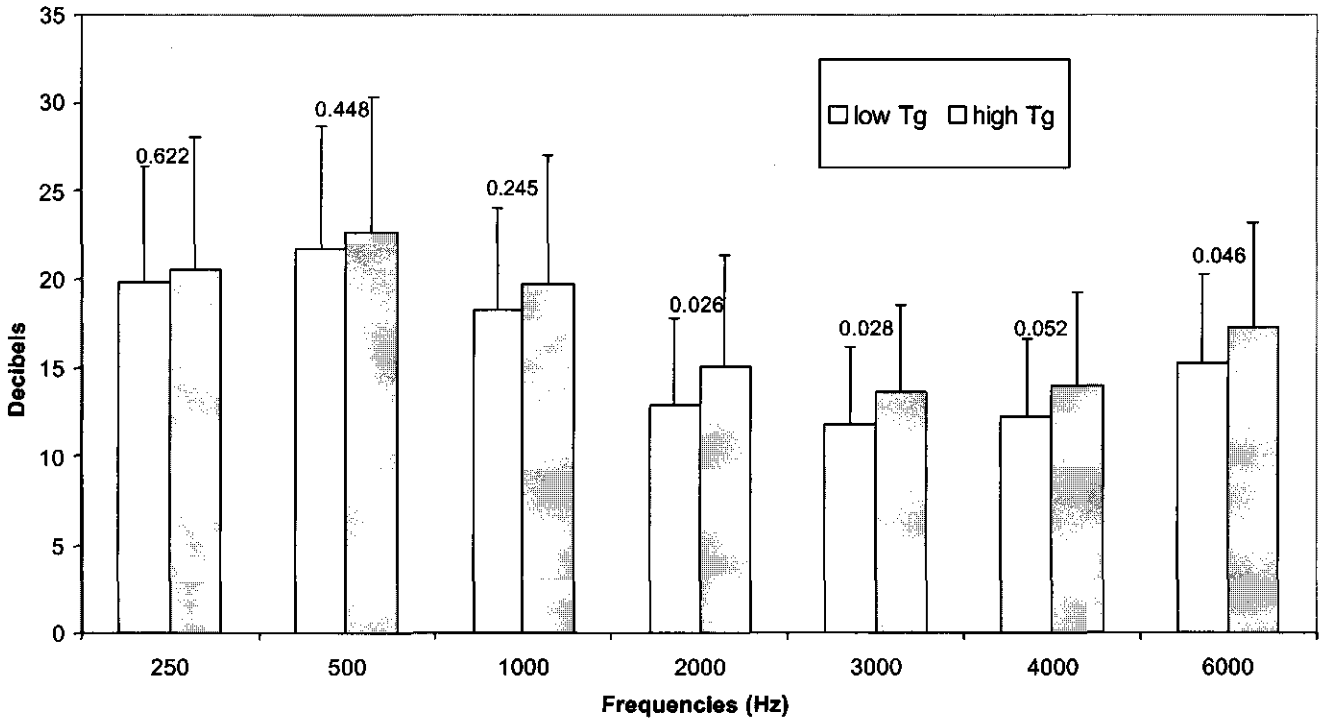

Fig. 1. Hearing thresholds in decibels (means + SD) in tertile groups with high or low concentrations of serum thyroglobulin. *P*-values representing significance of differences between groups are indicated above each set of bars (independent samples t-test; 2-tailed).

DISCUSSION

This study demonstrates that even in a mildly iodine deficient population, hearing threshold is associated with iodine status as measured by serum thyroglobulin concentration. Hearing thresholds in groups with higher concentrations of thyroglobulin in serum are higher than in groups with lower concentrations of thyroglobulin. Thus, children with poorer iodine status could hear less well. The differences are most marked in the higher frequency range, i.e. >2000 Hz. Goslings et al. (1975) and Delong (1987) in their studies on endemic cretinism have reported similar findings in Indonesia and Ecuador respectively.

Of the iodine variables studied, serum thyroglobulin concentration is the one most closely associated with hearing performance. The population studied was regarded as iodine deficient because of the low concentration of urinary iodine and elevated concentration of serum thyroglobulin, even though serum concentrations of thyrotropin and free thyroxine were within the normal range. Such discrepancies have been reported earlier in iodine deficient populations in Indonesia (13,14) and Algeria (15). Although opinions differ as to which indicator of iodine deficiency is the best for this age group, urinary iodine concentration or serum thyroglobulin concentration, serum thyroglobulin concentration is considered to be a more sensitive indicator of iodine deficiency in this age group than thyrotropin or free thyroxine (16,17).

The association we found between hearing and mental performance is in agreement with other studies, such as that in China by Boyages (1989). This finding raises the question whether there is interaction between the two. Do children perform less well on mental tests because of their hearing problems? As the tests addressed abilities not related to learning in school, and the children had only mild hearing impairment, it is unlikely that their higher hearing threshold results in impaired performance on the mental tests used. Data on the relationship between improvement in iodine status and mental performance reported earlier (10), showed that the group of children whose iodine status improved, as measured by an increase in urinary iodine concentration, demonstrated a greater improvement in mental performance than the group of children whose iodine status remained unchanged during this period. Although hearing was measured only at the end of the study period, urinary iodine concentration and serum Tg concentration at baseline did not differ between children with higher hearing thresholds and those with lower hearing thresholds. However, by the time hearing thresholds were measured, children with lower hearing thresholds had higher urinary iodine concentration and lower serum Tg concentration than those with higher hearing thresholds (data not shown). Conversely, children with better iodine status had lower hearing thresholds (see data with respect to serum thyroglobulin concentration in Figure 1). This would suggest that the differences in hearing result from the recent differentiation in iodine status.

In studies into neurologic manifestations of cretinism, hearing problems have been ascribed to poor development of the cochlea and of the cerebral neurons, induced by a critical degree of hypothyroidism during the early second trimester of pregnancy (18). That in-utero and neonatal hypothyroidism result in impaired development of both hearing and mental function has been demonstrated in studies in various animal species, rodents in particular (19-21). In congenital and acquired hypothyroidism in man, hearing deficits seem to be mostly of the sensorineural type, but conductive or mixed type deficits also occur (22,23). This would imply multiple lesion sites, including the middle ear, cochlear and retrocochlear sites. A number of recent studies have focused on the molecular genetic aspects of auditory defects in patients with resistance to thyroid hormone or with Pendred's syndrome (2,4,24). Although these defects and congenital hypothyroidism often have deafness and goiter in common, the genetic basis for these syndromes appears to be different (3).

Studies on changes in hearing in patients with sporadic, congenital or acquired hypothyroidism following treatment have not produced consistent results. Some studies have shown that hearing impairment may be reversed (23). Others do not allow conclusions about reversibility to be drawn as treatment had been going on for some time and baseline data were not available (25,26). However, hearing impairment was shown to be reversed in apparently normal children living in an iodine deficient area in China one year after the introduction of iodized salt and continued to improve for a further year (27). In the latter study the hearing impairment observed was ascribed to subclinical, acquired hypothyroidism as a result of prolonged severe iodine deficiency.

Hypothyroidism may affect various aspects of the auditory system differentially depending on its timing, severity and duration. The mechanisms by which a lesion may be reversed will depend on its site and nature. Some authors have claimed that an improvement in general wellbeing as a result of improved iodine status would in itself be sufficient to explain better performance on hearing tests (23). Our study was not aimed at elucidating types of auditory lesions, but at addressing the question whether, in a mildly iodine deficient child population, there is a relationship between iodine status, hearing and mental performance.

The findings in this study provide further justification for the large salt iodization programmes being undertaken worldwide. Although the causes of hearing impairment in relation to iodine deficiency are not fully understood, our results suggest that access to iodine affects hearing as well as mental performance. Public health officials should continue to promote adequate iodine intake through salt iodization programs and other means.

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4

Serum thyroglobulin and urinary iodine concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply

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Journal of Nutrition 2001; 131: 2701-2706

ABSTRACT

Objective: Iodine deficiency control programmes have greatly reduced iodine deficiency disorders (IDD) worldwide. For monitoring changes in iodine status, different indicators may be used. The aim of this study was to evaluate the suitability of indicators of iodine status and thyroid function (thyroglobulin, thyrotropin, and free thyroxine in serum, thyroid volume and urinary iodine concentration) in iodine deficient schoolchildren under conditions of increasing iodine supply.
Design: A randomized double-blind placebo-controlled intervention trial with an observation period of 11 months
Setting: An iodine deficient area in northern Benin
Subjects: 198 schoolchildren, aged 7-11 yr.
Intervention: 97 children received a single oral dose of iodized oil, containing 540 mg I, while 101 children received a placebo (poppyseed oil). However, 3-4 mo after supplementation, iodized salt became available in the area. The study population therefore comprised an iodized oil supplemented group and a non-supplemented group, both of which had variable, uncontrolled intakes of iodized salt during the last 6-7 mo of the study.
Results: Initial mean serum concentrations of thyrotropin and free thyroxine were within the normal range, whereas serum thyroglobulin concentration, urinary iodine concentration and thyroid volume were indicative of moderate to severe iodine deficiency. At the end of the study all indicators had improved significantly, except thyroid volume which had decreased in the supplemented group only. The supplemented group also still had significantly lower serum thyroglobulin and higher urinary iodine concentrations than the non-supplemented group.
Conclusion: Serum thyroglobulin and urinary iodine concentrations are the indicators most influenced by a changing iodine supply. Current normal reference ranges of serum concentrations of thyrotropin and free thyroxine are too wide for detecting iodine deficiency in this age group.

INTRODUCTION

In the context of initiatives to achieve universal salt iodization, a number of population-based studies have addressed the question of which indicators best reflect improvement in iodine status and thyroid function (1-4). While thyrotropin (TSH) is widely used to screen neonates for congenital hypothyroidism, there have been doubts about its specificity in older children and adults when assessing hypothyroidism induced by iodine deficiency (5). Several studies have shown that while urinary iodine concentrations and thyroid volumes were indicative of iodine deficiency in the populations studied, both serum TSH and free thyroxine (FT4) concentrations were within normal ranges (2,3). Thyroid volume, serum thyroglobulin (Tg) concentration, and urinary iodine concentration have all been suggested as useful indicators for measuring improvement in iodine status following iodine prophylaxis. However, all three have their own characteristics and limitations. Normal ranges for thyroid volume have been established (6,7), but this indicator reflects long-standing hypothyroidism and does not respond rapidly to changes in iodine status. Serum Tg concentration is thought to respond quickly to stimulation of the thyroid, increasing when iodine supply to the thyroid is depleted, and returning to normal levels when the supply is sufficient. However, Tg immunoassays show large inter-laboratory and inter-assay variability, which makes it difficult to establish a universal normal range and cut-off points for distinguishing between different degrees of iodine deficiency (8,9). Urinary iodine concentration is not a direct measure of thyroid function, but reflects recent iodine intake and thyroid hormone catabolism. Thus, population groups, even if currently found to be in the mildly deficient to normal range of urinary iodine concentration, may still experience serious functional consequences of iodine deficiency in the preceding period.

As part of a study on iodine status and mental performance in schoolchildren aged 7-10 y in an iodine deficient area of Benin, West Africa, the five indicators mentioned above were used to measure the effects of changing iodine supply on iodine status and thyroid function , both at the beginning of the study in 1995 and one year later, when iodized salt had become available to the population. The aim of this study was to evaluate the suitability of these indicators under conditions of increasing iodine supply by comparing their responses and examining their interrelationships.

MATERIALS AND METHODS

Study area and subjects

The study was carried out in four villages in the district of Basila, province of Atacora, in northern Benin, where prevalence rates of goiter among schoolchildren aged 6-12 yr varied from 20 to 60 % (Doh, A. and Ategbo, EA. Prévalence de la carence en iode dans l'Atakora; unpublished report, 1994). The villages had neither electricity nor clean drinking water. The population was mainly engaged in subsistence farming. Polygamy was common and levels of parental education were low (Table 1).

Children from standards 2 and 3 in the four primary schools in the study area were considered for enrolment. In two of the four schools, girls were not selected as they already had received an oral dose of iodized oil in the previous year. A total of 211 children were selected. As 13 children had left school or moved out of the area by the end of the intervention period, data from 198 children are presented.

The study was approved by the health and education authorities of the province of Atacora, Benin and by the Medical Ethics Committee of the Division of Human Nutrition and Epidemiology of Wageningen University. The aim of the study was explained to local administrative and traditional authorities, parents and teachers. Having obtained verbal approval from local authorities, the parents and the parents-teachers association, all children selected were examined by a physician. Several children with skin or respiratory infections, and malaria were treated. No children were excluded on health grounds.

Table 1. General characteristics of the study population [1)]

Characteristics of subjects	
N (males/females)	198 (168/30)
Age (y)	8.9 ± 1.2
Height-for-age (Z-score)	-1.64 ± 0.96
Weight-for-age (Z-score)	-1.34 ± 0.72
Education (y)	2.8 ± 0.8
Characteristics of family	
Family size (n)	14.4 ± 7.6
Education of parents (y)	
- father	1.4 ± 0.8
- mother	1.2 ± 0.6
Size of landholding (ha)	3.1 ± 2.5

[1)] Results are expressed as mean ± SD, except for the number of subjects

Study design

The study was set up as a randomized double-blind placebo-controlled intervention. Following baseline measurements children were stratified by school, school class, and sex and subsequently matched on the basis of similar age and height-for-age. From each pair of children, one child was randomly allocated to one of two groups. The groups were then randomly allocated to receive either a dose of iodized oil (Lipiodol UF 7; 540 mg I/mL) or a placebo (poppyseed oil), both provided by Guerbet Laboratories (Aulnay-sous-Bois, France) and administered as a single oral dose (1.0 mL) with a Swift 7 dispenser (English Glass Company, Leicester, UK) in January 1996. The codes were broken after the completion of the final test.

In this paper we describe the performance of different indicators in a group which had received an iodized oil supplement ("supplemented group") and in a group which had received a placebo ("non-supplemented group"). In addition both groups had access to iodized salt for the last 6 mo of the 10 mo observation period, as iodized salt (containing approximately 50 ppm KIO_3) began to appear in the markets in the study area alongside non-iodized salt about 3-4 mo after supplementation.

Somatic and biochemical indicators

Blood, urine and anthropometric variables as well as thyroid volume were measured at baseline in October-November 1995. All measurements were repeated in October-November 1996. Additional urine samples were collected one week and 5 mo after supplementation, i.e. in January and May 1996.

Venous blood was drawn from the antecubital vein, immediately followed by the application of one drop of whole blood onto a filter paper card (Schleicher & Schuell, grade 903; Keene, NH., USA). These cards were air-dried for 1 to 2 h and packed in polyethylene bags before being frozen. Hemoglobin was assessed using a portable hemoglobinometer (Hemocue®, Helsingborg, Sweden). Serum samples were prepared and frozen before being transported. Casual samples of urine (ca. 25 mL) were collected early in the morning to which some crystals of thymol were added. Blood spot cards, and frozen samples of urine and serum were transported to the Micronutrient Research Laboratory, University of Ghana at Legon, Accra, Ghana for analysis of urinary iodine (chloric acid digestion followed by Sandell-Kolthoff reaction (10); TSH in blood spots (Spectra Screen™ Dried Blood TSH EIA Kit; IEM diagnostika, Reflex Industries, Santee, CA, USA) and serum ferritin (ELISA), within the next 6 to 9 mo. Frozen serum samples were also transported to the Laboratory for Endocrinology and Radiochemistry, Academic Medical Center, Amsterdam, the Netherlands, for assessment of Tg (RIA), FT4 (time-resolved fluoroimmunoassay; Delfia™, Wallac Oy, Turku, Finland) and TSH (immunoluminometric assay; Brahms Diagnostica Gmbh, Berlin, Germany). The coefficients of intra-assay and inter-assay variation were: 3.6 % and 6.4 % for FT4, 2.4 % and 4.5 % for TSH and, depending on the concentration, 6-7 % and 7-10 % respectively for Tg.

A subsample of the sera (n=23) was analysed for selenium concentration at Rowett Research Institute, Aberdeen, U.K. using a fluorimetric assay method with diaminonapthaline complexing and an International Atomic Energy Agency blood standard (IAEA, Vienna).

Anthropometric measurements were made in duplicate. Height was measured to the nearest mm, using a microtoise (Stanley Tools ®, Besançon, France). Weight was measured to the nearest 0.25 kg using a spring scale. Thyroid volume was measured by one investigator only (TvdB) with a portable ultrasound scanner (Aloka SSD 500; Aloka Co.Ltd, Japan) with a 5 MHz transducer.

Data analysis

Prints of the ultrasound images of the thyroid glands were examined by a paediatric thyroidologist (T.V.) and either accepted or rejected for inclusion in the data set on the basis of criteria pertaining to clarity of the surface outlines and proper positioning of the gland. Thyroid volumes were calculated using the formula: Volume of one lobe (mL) = 0.479 x max. thickness x max. width x max. length (cm)(6). Body surface area was calculated using the following formula: Body surface area (m^2) = $W^{0.425}$ x $H^{0.725}$ x 71.84 x 10^{-4}, where W is the weight in kg and H the height in cm (11).

Anthropometric indices were calculated using Epi-Info (version 6.02; CDC, Atlanta, GA, USA).

The Kolmogorov-Smirnov test was used to determine whether variables were normally distributed. Variables were log-transformed if not normally distributed. Student's t-test was used to assess differences between groups. The paired samples t-test was used to assess changes in the variables over time. Correlation and multiple regression analyses were carried out to determine the interdependence of the indicators used. Factor analysis (12) was applied to examine underlying structures in the data set and determine whether the variables used could be combined into a single or a reduced number of composite measures representing "iodine status", as has also been done for iron status (13).

All data were processed and analysed using SPSS/PC software (SPSS-Windows 8.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Anthropometric characteristics

Of the children, 33 % had z-scores for height-for-age < -2 SD of NCHS reference (14), indicating stunted growth in this population, while 17 % had low weight-for-age (z-score <-2 SD of NCHS reference). One year later these proportions were 33 % and 20 % respectively. Older children were more malnourished than younger children (data not shown).

Biochemical and ultrasound characteristics at baseline

Based on criteria established by WHO/Unicef/ICCIDD (14), the baseline urinary iodine concentration of 0.16 µmol/L (20.3 µg/L) indicated that the study population as a whole could be considered moderately to severely iodine deficient (Table 2). Mean serum TSH and FT4 concentrations were within the normal reference range (15-17), but 4.5 % of the children had both a low serum concentration of FT4 (< 10 pmol/L) and a high serum concentration of TSH (> 4.4 mU/L). Approximately 15 % of the children had a serum FT4 concentration < 10 pmol/L, while 11 % of the children had a serum TSH concentration > 4.4 mU/L. Of the different indicators measured, serum Tg concentration was significantly correlated with all other indicators, except TSH in blood spots (Table 3). Serum TSH concentration was significantly correlated with all other indicators except thyroid volume. When related to body surface, thyroid volume measurements showed that 51.5 % of the children had goiter, i.e. a thyroid volume above the upper limit of normal. Thyroid volume was significantly correlated with body surface (r =0.271; *P* =0.002), but not with age. The various indicators measured showed a somewhat poorer iodine status and thyroid function in older children than in younger children, but the differences were significant (*P* < 0.05) only for urinary iodine concentration (data not shown).
The mean serum selenium concentration in a subsample of 23 children was 28 ± 12 µg/L..

Regression analysis with thyroid volume as the dependent variable and the other iodine indicators as the independent variables did not result in a meaningful regression variate. When Tg was taken as the dependent variable, all other indicators, except thyroid volume were significant predictor variables (*P*< 0.05). This regression variate may be presented as: $Y= 2.825 +0.237\ X_1 - 0.025\ X_2 - 0.210\ X_3$; where Y = log (Tg), X_1 = log (TSH); X_2 = FT4; X_3 = log (urinary iodine concentration). Model R^2 = 0.323; adjusted R^2 = 0.308. $F_{[5, 143]} = 22.24$, $P < 0.0001$ (for the units used, see Table 2).

Table 2. Biochemical characteristics of study population (n= 198) [1)]

	Baseline	*End of study*
Serum thyrotropin concentration (mU/L) [2) 4)]	2.1 (1.5; 3.4)	1.4 (1.1; 1.9)
Serum thyroglobulin concentration (pmol/L) [4)]	215 (135; 362)	95 (66; 140)
Urinary iodine concentration (μmol/L) [4)]	0.16 (0.06; 0.42)	0.67 (0.30;1.25)
Serum free T4 concentration (pmol/L) [4)]	12.0 ± 2.4	14.2 ± 2.3
Thyroid volume (mL) [3) 4)]	5.5 (4.5; 6.9)	5.0 (4.0; 6.0)
Blood hemoglobin concentration (g/L) [4)]	114.5 ± 12.0	117.9 ± 10.3
Serum ferritin concentration (μg/L)	46.9 (28.4; 71.8)	49.2 (25.4; 99.5)

1) Results are expressed as median (25th, 75th percentiles) or as mean ± SD.
2) The baseline serum concentration of thyrotropin was measured in only 154 subjects
3) Measurements of thyroid volume met the criteria as described under *Data analysis* in 99 subjects
4) Baseline/final values are significantly different ($P<0.05$); paired samples t-test (non-normally distributed variables were log transformed)

Biochemical characteristics at end of the study

By the end of the study period all indicators showed significant improvement in the study population as a whole (Table 2). The proportions of children with a low serum concentration of FT4 or a high serum concentration of TSH had decreased to 2 % and 1 % respectively. The proportion of children with goiter had decreased to approximately 27 %. Correlations between the different variables were no longer significant, except for the negative correlations between serum Tg and urinary iodine concentration and between thyroid volume and serum TSH concentration (Table 3).

Factor analysis, followed by oblique rotation produced two components with Tg and urinary iodine concentration loading on the first component and FT4 loading on the second component. TSH loaded initially on both components, but at the end of the study it loaded primarily on the second component (Table 4). Very similar results were found when younger and older children (above and below the median age) were compared.

Table 3. Correlations between indicators of iodine status [1] before and after the study

	Pearson correlation coefficients [2]											
	1 [3]		2		3		4		5		6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1. Tg (serum)	1.00	1.00										
2. TSH (serum)	0.43***	0.12	1.00	1.00								
3. TSH (whole blood spots)[4]	0.07	n.a.	0.33***	n.a.	1.00	n.a.						
4. Free T4 (serum)	-0.34***	-0.02	-0.34***	-0.02	-0.20 **	n.a.	1.00	1.00				
5. Thyroid volume	0.27 **	0.07	0.16	-0.17*	0.07	n.a.	-0.06	-0.03	1.00	1.00		
6. Urinary iodine concentration	-0.50***	-0.26***	-0.37***	-0.08	-0.09	n.a.	0.21	0.03	-0.14	-0.06	1.00	1.00

[1] Non-normally distributed variables were log-transformed
[2] Significance of correlation coefficients: * $P<0.05$; ** $P<0.01$; *** $P<0.001$
[3] Horizontal numbers 1-6 refer to the numbered indicators in the left column
[4] TSH in whole blood spots was assessed only at baseline; n.a. = not available

Table 4. Oblique-rotated factor matrix of indicators of iodine status [1] before/after the study

	Oblique-rotated Loadings [2]					
	Factor 1		*Factor 2*		Communality [3]	
	Before	After	Before	After	Before	After
Serum thyroglobulin concentration	0.82	0.71			0.68	0.53
Serum thyrotropin concentration	0.66		-0.55	-0.61	0.56	0.46
Serum free thyroxine concentration			0.95	0.83	0.90	0.73
Urinary iodine concentration	-0.86	-0.78			0.78	0.61
					Total	Total
Sum of squares (Eigenvalue)	2.11	1.28	0.82	1.06	2.92	2.34
Percentage of trace [4]	52.8	32.0	20.5	26.5	73.3	58.5

[1] Non-normally distributed variables were log-transformed

[2] The loading refers to the correlation of the variable and the factor; only loadings ≥ 0.50 are included

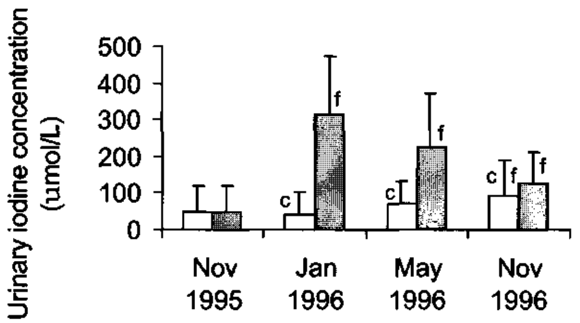
[3] Communality refers to the amount of variance in a variable that is accounted for by the two factors together

[4] Proportion (%) of the total variance to be explained

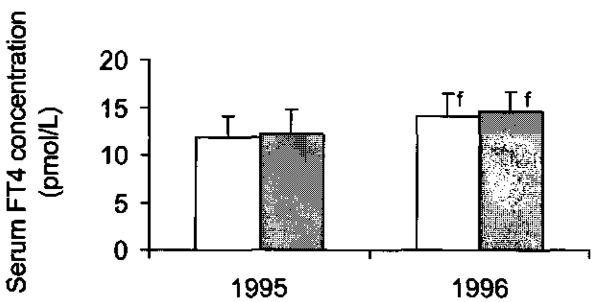
Supplemented vs. non-supplemented groups

Initially the supplemented and non-supplemented groups were fully comparable. Following the oral administration of the iodized oil, the mean urinary iodine concentration in the supplemented group improved (Fig. 1a). The difference between the two groups became smaller when 3-4 mo later the whole population began to have access to iodized salt and the mean urinary iodine concentration improved in the non-supplemented group. At the end of the study (12 mo after the baseline survey; 10 mo after supplementation) the supplemented and the non-supplemented groups did not differ in serum TSH and FT4 concentrations or thyroid volume, but differed in serum Tg concentration and urinary iodine concentration ($P<0.0001$; Fig 1 A-E). Both in the supplemented and in the non-supplemented groups all indicators except thyroid volume improved significantly during the study period. Thyroid volume improved significantly only in the supplemented group. At the end of the study supplemented and non-supplemented groups also differed with respect to the correlations between serum concentrations of TSH and Tg: 0.03 (NS) vs. 0.23 ($P<0.05$) respectively, and between urinary iodine concentration and serum Tg concentration: -0.06 (NS) vs. -0.30 ($P<0.01$) respectively (data not shown).

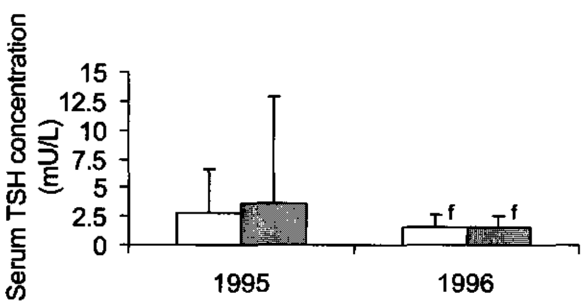
A


Urinary iodine concentration (umol/L)
500
400
300
200
100
0
c
f
Nov 1995
Jan 1996
May 1996
Nov 1996

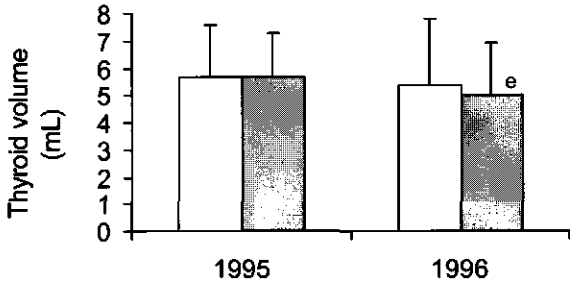
B


Serum FT4 concentration (pmol/L)
20
15
10
5
0
f
1995
1996

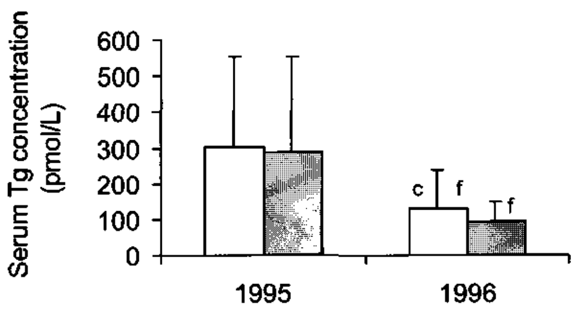
C


Serum TSH concentration (mU/L)
15
12.5
10
7.5
5
2.5
0
f
1995
1996

D



E



Legend Figure 1

Indicators of iodine status and thyroid function in children who received a placebo (open bars) or a single oral dose of iodized oil (closed bars) in January 1996. Values are means + SD. Both groups had access to iodized salt during the last 6 mo of the follow-up period of 10 mo. Panel A: *Urinary iodine concentration* (n = 198 in 1995 and in 1996). Panel B: *Serum FT4 concentration* (1995: n = 193; 1996: n = 198). Panel C: *Serum TSH concentration* (1995: n = 154; 1996: n = 198). Panel D: *Thyroid volume* (1995: n = 131; 1996: n = 146). Panel E: *Serum Tg concentration* (1995: n = 194; 1996: n = 198). Statistical significance of difference with placebo group at same time point using Student's t-test is indicated with: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$. Statistical significance of difference with values in 1995 of same group using paired t-test is indicated with: d, $P < 0.05$; e, $P < 0.01$; f, $P < 0.001$.

DISCUSSION

Serum Tg and urinary iodine concentration were the most appropriate indicators for measuring change in iodine status in this population. The change in iodine supply to this population through iodized oil supplementation and consumption of iodized salt was reflected in considerable changes in the concentration of iodine in urine and of Tg in serum (300 % and 56 % respectively) and to a lesser extent in the serum concentrations of TSH and FT4 (33 % and 18 % respectively). Serum Tg concentration and urinary iodine concentration were closely associated not only when iodine deficiency was moderate to severe, but also when it was mild. Moreover, under conditions of moderate to severe iodine deficiency, serum Tg concentration was correlated with all other indicators of thyroid function except TSH in blood spots. As shown by others (18) serum Tg concentrations are raised as a consequence of elevated serum TSH concentrations, but, unlike TSH and FT4, Tg is not involved in any feedback regulatory mechanisms. Thus both urinary iodine concentration and Tg are independent measures of iodine supply to the body.

Serum concentrations of TSH, the recommended indicator for use in screening newborn infants, and of FT4 cannot be regarded as appropriate indicators for detecting moderate to severe iodine deficiency in children of this age. Based on these concentrations, the iodine status of the study population would have been classified as within the normal reference range (14-17), both at the beginning and at the end of the study period. Under the conditions of low iodine intake as found in our study area, it is plausible to assume that thyroid hormone production has been affected negatively, causing a temporary increase in serum TSH concentration. This could induce initially increased bloodflow through the thyroid and subsequently hypertrophy and hyperplasia. These mechanisms would result in increased production of thyroxine (T4). Once T4 production has increased to satisfactory levels, serum TSH concentrations would fall. This may explain why mean serum concentrations of FT4 and TSH were found to be within the normal range. However, changes in serum FT4 and TSH concentrations following increased iodine intake were still significant. Moreover, the proportions of children whose serum concentrations of FT4 were too low or whose serum concentrations of TSH were too high were significantly diminished. This suggests that the normal reference range of these indicators may be too wide to detect mild to moderate degrees of iodine deficiency, or alternatively, that conclusions with respect to thyroid function under such conditions may not be drawn on the basis of results of just these two indicators.

As suggested by several authors (19-22), the "normal" concentration of FT4 may also have been the result of a lower deiodination of thyroxine because of a concurrent severe selenium deficiency. In a subsample (n=23) of our study population the mean serum selenium concentration was only 28 ± 12 μg/L, while in studies in healthy child populations in Europe values were found to range from 60 to over 100 μg/L (22). A normal serum concentration of FT4 in its turn could be accountable for the normal serum concentration of TSH.

While mean serum TSH concentration at baseline was within the normal range, TSH measurements in whole blood spots showed that 42 % of the children had concentrations > 5mU/L whole blood. Although there are doubts about the applicability of the latter cut-off point in age groups other than neonates (14), this rate may still be considered high and concurs with our data on goiter rates and urinary iodine concentration which indicated a moderate to severe public health problem. However, the correlation between the concentrations of TSH in serum and whole blood, although significant, was not very high (r = 0.33) . Furthermore, while initial serum TSH concentration was correlated with all other indicators except thyroid volume, the TSH concentration in blood spots was only correlated with FT4. Therefore the validity of the filter paper method for whole blood TSH may be questioned.

Comparison of the performance of the various indicators in the supplemented and non-supplemented groups, 10 mo after supplementation, shows that thyroid volume and serum concentrations of TSH and FT4 were not significantly different between these two groups. As the whole population began to have access to iodized salt about 3-4 mo after supplementation, children in the supplemented group did not maintain their better iodine status, except with respect to the concentration of Tg in serum and iodine in urine. It would only be a matter of time for the non-supplemented children to fully catch-up with the supplemented children. It is noteworthy however, that even after both groups have had access to iodized salt for about 6 mo, serum Tg concentration and urinary iodine concentration were still significantly better in the supplemented group. This further supports our proposition that Tg and urinary iodine concentration are more sensitive indicators than TSH or FT4.

Correlations between all indicators of iodine status are shown to decrease considerably when iodine status improves. At the end of the follow-up period they were still stronger in the non-supplemented group than in the supplemented group. When serum indicators of iodine status reach normal values and show less variation, correlations between indicators are likely to disappear.

Over the study period all indicators except thyroid volume, changed significantly in the study population as a whole. Thyroid volumes were significantly reduced in the supplemented group only. Earlier studies have shown different rates of reduction of thyroid volume following iodized oil administration, some showing a rapid response (23,24), others a more modest or gradual response (2,25,26). Reduction in goiter sizes and goiter rates following the introduction of iodized salt have generally been modest. In a study in Italy (27), iodized salt prophylaxis prevented development of goiter in children born after the introduction of the salt, but was less effective in reducing goiter size in children born earlier. In a recent study carried out in South Africa (28), the prevalence of goiter was not reduced one year after the introduction of mandatory salt iodization, while urinary iodine concentration was indicative of an improved iodine supply. Similar results were also reported from Indonesia (29). Therefore thyroid volume is not appropriate as an indicator for measuring change in iodine status in the short term.

Current recommendations suggest that thyroid volumes be related to body surface rather than to age in populations with a high prevalence of malnutrition, because of its effect on growth and development of the thyroid gland (7). This was confirmed in our study, in which the older tertile was significantly more undernourished (lower weight-for-age) and stunted (lower height-for-age) than was the younger tertile, thus providing an explanation why thyroid volume was not related to age but was related to body surface. The age differences in degree of malnutrition probably may be ascribed to a period of severe drought and hunger in the study area in 1987.

The results of this study show that " iodine status", just like status of iron or vitamin A, is a concept not easily captured in one indicator. It may well be that a concomitant selenium deficiency would explain the relatively high serum concentration of FT4 and the relatively low serum concentration of TSH in this iodine deficient population.

In population-based studies, depending on the age of the target group, different combinations of indicators should be used. Under the conditions as found in Benin, serum Tg concentration together with urinary iodine concentration are in our opinion the best combination of indicators for schoolchildren aged 7-10 y.

Acknowledgements

We are grateful to D. Alnwick (then at Unicef, New York) for providing us with the portable ultrasound scanner used in this study. We also thank Dr. J.R. Arthur, Rowett Research Institute, Aberdeen, U.K. for carrying out selenium analyses in a subsample of sera from our study population.

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Iodine deficiency and elevated total serum homocysteine concentration: a double burden for children in Benin?

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Submitted

ABSTRACT

Elevated serum homocysteine concentration is considered a risk factor for cardiovascular disease and colon cancer with genetic, dietary and pathological conditions, including hypothyroidism, playing a role. Serum homocysteine concentration was previously shown to be increased in a goitrous population in West Africa. Our study assessed serum total homocysteine (tHcy) concentration in 188 schoolchildren, aged 7-11 y, in an iodine deficient area and examined whether there is an association with iodine status. Children (n=101) received a single oral dose of iodized oil, containing 540 mg I, while 97 children received a placebo. Three to four months after supplementation, the whole population began to have access to iodized salt. Biochemical indicators (urinary iodine concentration and serum concentrations of thyrotropin, free thyroxine and thyroglobulin) were measured at the beginning and end of the study, while serum tHcy concentration was measured at the end only. The geometric mean serum tHcy concentration was 8.2 μmol/L (range 2.1- 14.9 μmol/L). Children with higher serum thyrotropin concentrations or with goiter tended to have higher serum tHcy concentrations (NS). The findings suggest that populations suffering from consequences of malnutrition in terms of quantity and quality may also be more prone to risks associated with elevated concentrations of tHcy.

INTRODUCTION

Hyperhomocysteinemia is seen as an independent risk factor for cardiovascular disease in adults (1,2). An elevated serum or plasma concentration of total homocysteine (tHcy) is associated with genetic, pathological and dietary factors, including low intake of the B-vitamins: folic acid, vitamin B_6 and vitamin B_{12}. As homocysteine is metabolized either by the remethylation or the transsulfuration pathways, enzyme deficiencies in these pathways may obstruct these metabolic processes.

Primary hypothyroidism has been associated with an increased risk for cardiovascular disease through different mechanisms, including hyperhomocysteinemia and the occurrence of atherogenic lipid abnormalities, notably the elevation of total cholesterol and a change in proportions of high-density and low-density lipoproteins (3). Hypothyroidism may lead to a decrease in hepatic levels of enzymes involved in the remethylation pathway, methylene tetrahydrofolate reductase in particular (4), which could result in hyperhomocysteinemia. Thyroxine was shown to be effective in the treatment of hyperhomocysteinemia in one study among hypothyroid patients (5), while in another study thyroxine administration only decreased fasting but not postload plasma tHcy concentration (6).

Ingenbleek *et al* were the first to describe elevated serum concentrations of homocysteine in a population with endemic goiter in West-Africa (7). These authors noted that in their study population, which consisted of a control group and 3 goitrous groups, based on a classification into 3 increasingly severe stages of goiter, homocysteine concentration was significantly higher when goiter stage was higher. Higher goiter stages were also characterised by higher serum concentrations of thyrotropin (TSH) and lower serum concentrations of free thyroxine (FT_4). Whether this phenomenon is generally present in iodine deficient hypothyroid populations to our knowledge has not been studied. Moreover the questions whether, at which age, and under which conditions children become predisposed to elevated serum tHcy concentration has also not yet been answered. At the same time, few studies have addressed the establishment of a normal reference range for tHcy concentrations in children (8,9).

Therefore, as part of a study on iodine status and mental performance of school children aged 7-11 y in an iodine deficient area in northern Benin, serum tHcy concentration was measured with the aim to assess its distribution chracteristics and to examine its relationship with indicators of thyroid function and iodine status.

SUBJECTS AND METHODS

Study area and subjects

The study was carried out in 198 children from 4 villages in the province of Atacora, northern Benin, where iodine deficiency was a problem of public health importance. The socio-economic conditions were poor. There was neither electricity nor clean drinking water. The population was mainly engaged in subsistence farming. The main staples eaten were maize, millet, sorghum, yam and cassava. These formed part of almost every meal and were prepared in various ways. The main meal was generally accompanied by a relish, made of red peppers, onions, garlic, mustard from the locust bean seed, condiment cubes, oil and salt. If available, tomatoes or okra might be added and, if the family could afford it, a small quantity of dried fish or meat. Apart from onions, tomatoes and okra, other vegetables were hardly eaten.

Children from grades 2 and 3 in the 4 primary schools in the study area were considered for enrollment. The study was approved by the health and education authorities of the province of Atacora, Benin and by the Medical Ethics Committee of the Division of Human Nutrition and Epidemiology of Wageningen University. Having obtained verbal approval from local authorities, the parents and the parents-teachers association, all children selected were examined by a physician. Children with skin or respiratory infections, and malaria were treated, but no children were excluded on health grounds.

Study design

A baseline survey was carried out in October-November 1995, just before supplementation, and included anthropometric measurements (weight and height) and collection of urine and blood samples. These measurements were repeated in the same period in 1996. Approximately 3-4 mo after the start of the observation period of 10 mo, the population began to have access to iodized salt. Thus, at the end of the observation period the study population consisted of one group of children who had received iodine supplementation 10 mo earlier and one group who had received a placebo, while both groups had had access to iodized salt for a period of approximately 6-7 mo.

Somatic and biochemical indicators

Anthropometric measurements were made in duplicate. Height was measured to the nearest mm, using a microtoise (Stanley ®, Besançon, France). Weight was measured to the nearest 0.25 kg using a spring scale. Casual urine samples were collected early in the morning for determination of iodine concentrations. Venous blood was drawn from the antecubital vein for assessment of thyroglobulin (Tg), FT_4 , TSH, ferritin and tHcy concentrations in serum and hemoglobin in whole blood. Whole blood was collected in vacutainer tubes and allowed to clot for 30-40 min at room temperature before being centifuged, after which the samples were put on cold packs. Procedures followed and assay methods used for assessment of the variables related to iodine status and thyroid function have been described elsewhere (10). Serum tHcy concentrations were assessed in the laboratory of the Division of Human Nutrition and Epidemiology, Wageningen University, using an HPLC-FD assay in combination with SBD-F derivation (11). The inter- and intra-assay coefficients of variation of this assessment were 6 % and 3 % respectively. Because of a shortage of serum, tHcy concentration could only be measured in 188 serum samples obtained at the end of the observation period.

Data analysis

To check the normality of data, the Kolmogorov-Smirnov test was used. Pearson correlation coefficients were calculated for the relationship between normally distributed variables. Variables were logtransformed if not normally distributed. All data were processed and analysed using SPSS-pc software (SPSS-Windows 8.0; SPSS Inc., Chicago, IL, USA). Anthropometric indices were calculated using Epi-Info (version 6.02; CDC, Atlanta, GA, USA).

RESULTS

Somatic and biochemical characteristics

Compared to a reference population (12), the mean Z-scores for height-for-age (HFA) and weight-for-age (WFA) were -1.60 ± 0.93 SD and -1.36 ± 0.71 SD respectively. The proportion of children who were stunted (Z-score for HFA <-2 SD) at the beginning of the study was one-third, while 17 % were underweight (Z-score for WFA <-2 SD) and 2 % were wasted (Z-score for weight-for-height <-2 SD). These proportions did not change significantly during the study.

At the start of this study the study population was moderately to severely iodine deficient (median urinary iodine concentration, 0.16 μmol/L). As the whole population began to have access to iodized salt, the iodine status of the placebo group also improved. The whole study population was mildly iodine deficient by the end of the observation period, 10 mo after supplementation (median urinary iodine concentration, 0.67 μmol/L; Table 1). Initially one-third of the children were anemic (hemoglobin <110 g/L), while at the end of the study 19 % of the children were anemic.

With respect to serum tHcy concentration, three observations fell outside the range of mean + 4 SD and were excluded. Serum concentrations of tHcy ranged from 2.1 to 14.9 μmol/L. The mean concentration was 8.5 ± 2.3 μmol/L (Table 2). The geometric mean concentration was 8.2 μmol/L. There was a tendency towards increased serum tHcy concentration in tertile groups with higher serum TSH concentration Similarly, children who were classified as having goiter on the basis of WHO normative values for thyroid volumes in relation to body surface (13), tended to have higher serum tHcy concentrations. These differences were however not significant (data not shown). Concentrations of serum tHcy were not correlated with age, but were weakly, but significantly correlated (Pearson) with WFA (r = 0.163; P = 0.026), weight (r = 0.176; P = 0.016) and with hemoglobin concentration (r = 0.210; P = 0.004).

Table 1. Biochemical characteristics of study population (n = 198) [1)]

	Baseline	*End of study*
Serum total homocysteine concentration (μmol/L) [2)]	n.a.	8.5 ± 2.3
Serum thyrotropin concentration (mU/L) [3)]	2.1 (1.5; 3.4)	1.4 (1.1; 1.9)
Serum thyroglobulin concentration (pmol/L) [4)]	215 (135; 362)	95 (66; 140)
Urinary iodine concentration (μmol/L) [4)]	0.16 (0.06; 0.42)	0.67 (0.30;1.25)
Serum free T4 concentration (pmol/L) [4)]	12.0 ± 2.4	14.2 ± 2.3
Thyroid volume (ml) [4) 5)]	5.5 (4.5; 6.9)	5.0 (4.0; 6.0)
Blood hemoglobin concentration (g/L) [4)]	114.5 ± 12.0	117.9 ± 10.3
Serum ferritin concentration (μg/L)	46.9 (28.4; 71.8)	49.2 (25.4; 99.5)

1) Results are expressed as median (25th, 75th percentiles) or as mean ± SD.
2) Measured in 188 subjects.
3) At baseline measured in only 154 subjects.
4) Baseline/final values are significantly different (P<0.05); paired samples t-test (non-normally distributed variables were log transformed).
5) Results of measurements in 99 subjects.

Table 2. Comparison of published data on plasma/serum concentration of tHcy in children with data from Benin[1)]

Country	Population	Age (y)	Plasma/ serum	tHcy (µmol/L)
Belgium (8)[2)]	White	5-9	Plasma	6.2
	"	10-14	"	7.1
Spain (14)	White	0-10	Plasma	5.8
Norway (15)	White	8-12	Plasma	5.3
South Africa (16)	White	7-15	Plasma	5.1
	Black	"	"	5.8
USA (9)	White	13-14	Serum	5.0
	Black	"	"	5.5
Benin	Black	7-11	Serum	8.2

[1)] All measurements made with HPLC-FD
[2)] Reference in parentheses.

DISCUSSION

This study demonstrates that serum tHcy concentrations in this child population are higher than those reported from Belgium, the USA, Spain, Norway and South Africa (8,9,14-16). The children in our study, even though still mildly iodine deficient based on their mean urinary iodine concentration, may be considered euthyroid on the basis of median serum concentrations of TSH and FT_4. This may explain why we found no significant correlations between serum tHcy concentration and indicators of thyroid function.

However, the Beninese children included in our study may be considered poorly nourished. This condition may not only interfere with amino acid metabolism and thus influence serum tHcy concentration directly, but also with various vitamin- or mineral-dependent biochemical pathways, which may affect thyroid homeostasis (17,18). In their goitrous patients Ingenbleek *et al* (7) noted that methionine concentrations remained normal but that serum tHcy concentration was higher when goiter stage was higher. These authors ascribed this phenomenon to functional impairment of hepatic enzymes in the transsulfuration pathway, such as cystathionine β-synthase, as a result of food deprivation. Whereas Ingenbleek *et al* considered the poor nutritional status of their study population as the key factor responsible for aggravating goitrogenic processes as well as the downregulation of protein metabolism, more recently other authors (5,6) have emphasized the role of hypothyroidism per se in affecting folate metabolism and thus the downregulation of the remethylation pathway of homocysteine metabolism. While the relatively high serum concentrations of tHcy in our study population probably may not be ascribed to hypothyroidism, the very low intake of animal products as well as vegetables and thus of sources of folic acid and vitamins B_6 and B_{12}, may very well be implicated.

In view of the importance attached to high serum concentrations of tHcy, the existing lack of standardization of assessment methods and normal reference data for children is remarkable (19,20). The agreement among assessment methods is insufficient to allow these to be used interchangeably (20). Among-laboratory variations within one method can exceed among-method variations (21). Moreover, although serum tHcy concentrations are slightly higher than those in plasma (22), they are sometimes used interchangeably. Leaving blood samples to clot at room temperature for up to 40 min as we did, may have increased serum tHcy concentrations in our samples, because of an ongoing production of homocysteine in the erythrocyte and increased export to the extracellular compartment (23). When blood was kept at room temperature, Osganian *et al* (9) reported a 5 % increase in serum tHcy concentration during a period of 20 min, while Fiskerstrand *et al* (22) reported a 20-60 % increase during a period of 4 to 24 hr. On the basis of these findings we estimate that hemolysis may have contributed to an increase in serum tHcy concentration of 5-10 % in our samples.

A few studies have shown that blacks have higher mean tHcy concentrations than whites (Table 2). Whether genetic differences or whether differences in diet and lifestyles are accountable for this difference is not known. Poor nutritional status, including protein-energy malnutrition and micronutrient deficiencies, may affect homocysteine metabolism either directly or indirectly through interference with thyroid homeostasis. The schoolchildren which we studied in rural West Africa suffer from multiple deficiencies, which not only may affect their present functional performance but may also predispose them to chronic diseases, such as coronary heart disease, later in life. Further studies are warranted to elucidate the interactions between hypothyroidism, deficiencies of various nutrients and the incidence and prevalence of elevated serum concentrations of tHcy.

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Iodine deficiency and school behavior of children in Benin

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ABSTRACT

Iodine deficiency may have adverse effects on mental performance, learning and behavior. To examine the question whether improvement of iodine status reverses such negative effects, a study was carried out among iodine deficient schoolchildren aged 7-11 years, in grades 2 and 3 in northern Benin. The study was set up as a double-blind placebo-controlled intervention, involving the administration of a single oral dose of iodized oil or a placebo. However, during the intervention period the iodine status of the control group was also improved as the population began to have access to iodized salt. In this paper we describe the use of a School Behavior Checklist in assessing consequences of improvement of iodine status for four dimensions of the Big Five personality model, i.e. extraversion, conscientiousness, agreeableness and emotional stability. The fifth dimension, a description of intellectual behavior, is not included in the checklist. The teachers of the children included in the study completed the checklist at the beginning and at the end of the intervention period. The responses of the teachers at baseline demonstrated a good similarity with the four-factor model on which the checklist is based. Reliability coefficients were good for the scales for conscientiousness and extraversion, but less satisfactory for agreeableness and emotional stability. During the intervention period the marks for school achievement as well as the mean scores for emotional stability, agreeableness and extraversion decreased significantly in the study group as a whole. However, only in the younger children was the change in emotional stability and agreeableness associated with change in iodine status. No correlations were observed between the children's school marks in 1995 and those in 1996. This study provides a basis for further research that should be aimed at refinement and adaptation of the tool for use in the educational context as found in West Africa as well as at the further elucidation of the relation between iodine status, thyroid function and personality traits.

INTRODUCTION

The relationship between protein-energy malnutrition, often occurring in conjunction with other specific nutrient deficiencies, ill-health and poor socio-economic conditions on the one hand and cognitive development on the other hand has been studied extensively (1). The effects of malnutrition on children's social and emotional functioning have received less attention, although there is evidence from animal and human intervention studies that malnutrition is associated with poor attention, lack of social responsiveness, low activity level, and changes in characteristics related to affection and emotional control (2). These " non-cognitive" aspects of behavior may well have an important role to play in cognitive development as mediators of learning processes.

Deficiencies of some micronutrients, such as iron, iodine and possibly zinc, are also associated with altered behavioral and cognitive development, the severity of which depends on time of onset and duration of the deficiency. Iron deficiency anemia has been shown to pose a high risk of impaired development and behavioral differences in children (3). The reasons for these phenomena are thought to include permanent changes to neuromaturation and altered neurotransmitter function, but also poor environments, and "functional" isolation (4). Anemic children show increased wariness, hesitance and fearfulness and interact less with their environment. Moreover they are less successful in eliciting caretaker responses.

Severe iodine deficiency during intrauterine and early postnatal life is known to impair permanently a child's potential for cognitive development. Correction of iodine deficiency later in life may however still have positive effects on cognitive performance in schoolchildren (5,6). In some ecological studies aspects of behavior such as motivation were also shown to differ between iodine replete and iodine deficient children. According to Tiwari and colleagues (7) iodine deficient children are slow learners with a concurrent low "motivation to achieve". These authors ascribed poor performance to both neurologic impairment and paucity of psychologic stimulation. However, iodine deficiency is generally found in backward areas, which in itself may account for differences in the learning environment and learning attitude. In a study by Huda et al. (8), which controlled for such environmental differences, biochemical hypothyroidism as a result of iodine deficiency was shown to be significantly associated with school performance and cognition.

In studies in children with sporadic congenital hypothyroidism, differences in timing and duration of thyroid hormone deficiencies were shown to be associated with different cognitive and behavioral impairments. These ranged from neuromotor, visuomotor and visuospatial skills, to speech and language, attention and memory skills (9-11). Even though the hypothyroidism had been diagnosed and treated soon after birth, a group of Canadian children with congenital hypothyroidism still scored significantly lower at 13 years of age on general IQ tests than controls (11). Similarly, both overt and subclinical hypothyroidism in adults appear to be associated with neuropsychological deficits, especially with respect to attention, verbal and visual recall and reaction time (12) as well as mood disturbances (13).

The aims of our study in an iodine deficient area of Benin were to examine whether mental abilities of iodine deficient schoolchildren may be improved by supplementation with iodine as well as to examine whether changes in their iodine status are associated with changes in school achievement and behavior. To this end, a series of mostly non-verbal mental tests, including a concentration test was administered.

School behavior of individual children was assessed by their teachers, at the beginning and end of the study period, using a checklist developed by Resing et al.(14). This checklist helps to describe school behavior in terms of four main factors or scales: agreeableness, extraversion, emotional stability and conscientiousness.

SUBJECTS AND METHODS

Study area and subjects

The study was carried out in the 4 primary schools in 4 villages in the province of Atacora, northern Benin, among children of grades 2 and 3, aged 7-11 y, with a mean of 2.8 yr of education. The study area was underdeveloped. There was neither clean drinking water nor electricity. The schools were poorly equipped and had very few written or visual teaching materials. The mean level of education of the parents of the children included in the study was very low (1.3 y; Table 1). Most parents were engaged in subsistence farming.

Study design

The study was set up as a randomized double-blind placebo-controlled intervention. In January 1996 children were given a single oral dose of either iodized oil (Lipiodol UF 7; 540 mg I/mL) or a placebo (poppyseed oil), both provided by Guerbet Laboratories (Aulnay-sous-Bois, France). A baseline survey was carried out just before supplementation, in October-November 1995, including the administration of mental development tests, anthropometric measurements and collection of urine and blood samples as well as completion of school behavior checklists by the teachers of the children. All these measurements were repeated in the same period in 1996. Additional urine samples were collected one week and 5 months after supplementation which took place in January 1996.

Approximately 3-4 months after the start of the observation period of 10 months the population began to have access to iodized salt. Thus, by the end of the observation period the study population consisted of one group of children who had received iodine supplementation 10 months earlier and one group who had received a placebo, while both groups had had access to iodized salt for a period of approximately 6-7 months.

Somatic and biochemical indicators

Anthropometric measurements were made in duplicate. Height was measured to the nearest mm, using a microtoise (Stanley ®). Weight was measured to the nearest 0.25 kg using a spring scale. Venous blood was drawn from the antecubital vein for assessment of concentrations of free thyroxine (FT4), thyroid stimulating hormone (TSH), thyroglobulin (Tg) and ferritin in serum and hemoglobin in whole blood. Casual urine samples were collected for determination of iodine concentrations. Procedures followed and assay methods used for assessment of these variables have been described elsewhere (6).

School behavior assessment and school achievement

The school behavior checklist (Schobl-R) has been developed as a tool to assist teachers in assessing the social and emotional functioning of their pupils and relating the outcome to their learning performance (14,15). The checklist is based on the "Big Five" dimensions of personality (16-18), but excludes one of the five dimensions, i.e. a description of intellectual behavior. Factor analysis following the pilot-testing in large primary school populations including groups of children with learning problems and groups with a different ethnic background, resulted in 4 factor scales. These scales comprise: extraversion, agreeableness, conscientiousness, and emotional stability.

The behavior checklist consists of a questionnaire comprising a series of 42 questions pertaining to different behavioral characteristics of the child. On a 6-point scale teachers are asked to indicate the degree to which a given statement was characteristic of the child. This questionnaire was completed for each child both at the beginning and at the end of the observation period. Examples are the following statements:

Is a noisy child	*3 2 1 - 1 2 3*	*Hardly says a word*
Is easily upset	*3 2 1 - 1 2 3*	*Is inperturbable*

In addition to the behavior checklist yearly report marks of the children were collected. As part of a battery of mental tests which has been described elsewhere (6), a concentration test was administered.

Data analysis

The Kolmogorov-Smirnov test was used to check whether the distributions of the scores on the variables were normal. Nonnormally distributed parameters were log-transformed. Data are reported as mean and SD for normally distributed parameters and as median and 25 th - 75 th percentile for nonnormally distributed parameters. A factor analysis was carried out to verify whether the responses by the school teachers show an underlying structure corresponding with the 4 dimensions of personality on which the checklist is based. Cronbach's alpha was computed to asses the consistency of the 4 scales, at the level of the individual teachers as well as for the entire data set. Correlation coefficients (Pearson, Spearman) were calculated between schoolbehavior scales and indicators of iodine status at the beginning and at the end of the observation period. Multivariate regression analysis was used to identify significant predictors of changes in school behavior. Anthropometric indices were calculated using Epi-Info (version 6.02; CDC, Atlanta, GA, USA). All data were processed and analysed using SPSS-pc software (SPSS-Windows 8.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Initially 211 children were enrolled in the study. Thirteen children had left school or had moved out of the area by the end of the observation period. For 1 child the questionnaire was not completed. This child was excluded from the study. Therefore the study population described here comprises 197 children aged 7-11 yr at the beginning of the study.

Socio-economic characteristics and nutritional status

Socio-economic data of the children and their families are presented in Table 1. The proportion of girls in the study population was only one-sixth. In 2 out of the 4 schools, all girls were excluded from the study as they had been given an iodine supplement the previous year. Moreover, girls were underrepresented in the schools. Polygamy was common and people lived in extended families (defined here as the number of people eating from the same meal). The study population could initially be classified as chronically malnourished with a high degree of stunting: one third of the children had Z-scores for height-for-age below -2 SD of the reference population (19). The study population was also moderately to severely iodine deficient (Table 2). While anthropometric indices did not change significantly, most children showed clearly improved iodine status by the end of the study period.

Table 1. General characteristics of the study population at baseline[1)]

Characteristics of subjects	
n (males/females)	197 (167/30)
Age (yr)	8.9 ± 1.2
Height-for-age (z-score)	-1.64 ± 0.96
Weight-for-age (z-score)	-1.34 ± 0.72
Education (yr)	2.8 ± 0.8
Characteristics of families	
Family size (n)	14.5 ± 7.6
Education of parents (yr)	
- father	1.4 ± 0.8
- mother	1.2 ± 0.6

[1)]Results are presented as mean ± SD, except for the number of subjects

Table 2. Biochemical characteristics of the study population (n = 197) [1)]

	Baseline	*End of study*
Serum thyrotropin concentration (mU/L) [2) 4)]	2.1 (1.5; 3.4)	1.4 (1.1; 1.9)
Serum thyroglobulin concentration (pmol/L) [4)]	215 (135; 362)	95 (66; 140)
Urinary iodine concentration (μmol/L) [4)]	0.16 (0.06; 0.42)	0.67 (0.30;1.25)
Serum free T4 concentration (pmol/L) [4)]	12.0 ± 2.4	14.2 ± 2.3
Thyroid volume (mL) [3) 4)]	5.5 (4.5; 6.9)	5.0 (4.0; 6.0)
Blood hemoglobin concentration (g/L) [4)]	114.5 ± 12.0	117.9 ± 10.3
Serum ferritin concentration (μg/L)	46.9 (28.4; 71.8)	49.2 (25.4; 99.5)

[1)] Results are expressed as median (25th, 75th percentiles) or as mean ± SD.
[2)] The baseline serum concentration of thyrotropin was measured in only 154 subjects
[3)] Measurements of thyroid volume met the criteria as described under *Data analysis* in 99 subjects
[4)] Baseline/final values are significantly different ($P<0.05$); paired samples t-test (non-normally distributed variables were log transformed)

School achievement

As part of the normal procedures for assessing school achievement, children were given marks by their teachers for 5 subjects: arithmetic, reading, history, geography and French. No correlations were observed between marks for the different subjects in 1995 and those in 1996. There were also no correlations between the sums of the marks in 1995 and those in 1996. These sums, which are used to decide whether a child proceeds to the next grade or not, were significantly lower in 1996 than in 1995 ($P < 0.0001$). It was only amongst the group of children who failed at least once in these two years ($n = 30$) that the sum of the marks obtained in 1995 and 1996, awarded by the same teacher, correlated well ($r = 0.691$; $P = 0.002$). Moreover, no significant correlations were observed between the sums of the marks in either 1995 or 1996 with performance on the mental tests. The change in sums was not associated with the change in any of the parameters of iodine status or thyroid function. The change in the sums was positively correlated with the change in scores on the concentration test ($r = 0.221$; $P = 0.002$).

School behavior

A factor analysis of the set of responses of the teachers on the Schobl-R checklist given at baseline showed a reasonable agreement with the 4-factor structure on which the checklist was based (Table 3). This pattern was less clear when the second series of responses was examined. At that point in time conscientiousness, agreeableness and extraversion emerged again as separate factors, but more emotional stability items were loading on the factors for agreeableness and extraversion.

Individual scores on the 4 behavior scales were computed by adding up the scores on the different questionnaire items belonging to that scale. The higher the score, the more positive was the appreciation of that aspect of behavior of the child. Mean scores on all four scales went down over the intervention period. The changes were significant ($P < 0.05$) in the total group for extraversion, agreeableness and emotional stability, but the degrees of change differed by age group (Table 4).

The intercorrelations between the four scales, agreeableness, extraversion, conscientiousness and emotional stability both at the beginning and at the end of the observation period are given in Table 5. The comparative fit index and Bentler-Bonett normed fit index were 0.914 and 0.897 respectively, indicating a high degree of agreement between the correlation matrices at baseline and at the end of the study period. The major differences are presented by the intercorrelation between conscientiousness on the one hand and agreeableness and emotional stability on the other hand.

At the beginning of the study the internal consistencies of the 4 scales as measured by Cronbach's alpha are high for conscientiousness and extraversion and somewhat lower for emotional stability and agreeableness (Table 6). These reliability coefficients remained almost the same during the intervention period, except for the reliability coefficient for emotional stability which had decreased.

Correlations between results for the 4 behavior scales in 1995 and 1996 were significant and ranged from $r = 0.173$ ($P = 0.015$) for emotional stability to $r = 0.317$ ($P < 0.0001$) for conscientiousness.

Table 3. Varimax rotated 4-factor[1)] matrix for behavior checklist[2)] (n = 197)

Items Schobl-R[3)]	I	II	III	IV
Always shows immediately whether he/she likes a classmate	0.54			
Acts excessively cheerful	0.68			
Tries to get as much attention from teacher as possible	0.60			
Always greets the teacher enthusiastically	0.56			
Talks loudly	0.69			
Blurts out	0.40			
Is confidential with all children	0.62			
Always shows that he/she is there	0.44			
Acts disorderly in order to make other children laugh	0.33		0.63	
Is a noisy child			0.70	
His/her thoughts are easily read	0.76			
Is always cock of the walk			0.76	
Only needs one warning		0.60		0.33
Can work a long time while sitting in the same position		0.60		
Is so focused on work that he/she does not notice the activities around him/her		0.76		
The results of his/her work are always predictable	0.37	0.30		
Tackles problems all by him/herself		0.53		
Can work alone easily	0.43	0.62		
Is completely absorbed by the work		0.65		
Works thoroughly	0.35	0.74		
Does exactly what is being asked		0.67		
Imposes too high demands or his/her own work		0.57		
Always wants to finish or change his/her work if it's time	0.38	0.62		
Keeps working even if problems occur				
Thinks he/she knows better than others			0.65	
Reacts strongly to, even harmless, teasing	0.34		0.36	
Easily takes something away from another child				
Always asks for more	0.62			
Doesn't let others use something that is his/hers			0.66	
Always looks for largest piece in case of treats	0.69			
Seldom meets wishes of other children			0.56	
Easily criticizes proposals of classmates			0.60	
Gets angry when classmate tries to correct him/her				
Wants to know what he/she will get in return when somebody is asking for something			0.42	
Is easily upset				0.60
If something unexpected happens, he/she is usually more confused than others				0.39
Always directly approaches teacher if teased	0.59		0.31	
Takes corrections from teacher too personally	0.38			0.41
Gets really upset if teased				0.55
In case of small injury, he/she directly approaches teacher	0.58			
Gets teary eyes in case a story is told in which something bad happens to somebody				0.55
Never takes something lightly				0.42

[1)] I = Extraversion; II = Conscientiousness; III = Agreeableness; IV = Emotional stability

[2)] Only loadings ≥ 0.30 are shown.

[3)] Only one, shortened description of each item.

Table 4. Significance of change in scores on schoolbehavior scales by age over the study period (P-values; Paired-samples t-test; 2-tailed)

	Age (mo)		Total
	< 105 (n =95)	≥ 105 (n =102)	(n =197)
Extraversion	0.922	0.008	0.036
Agreeableness	0.002	0.329	0.006
Conscientiousness	0.829	0.019	0.063
Emotional stability	0.008	0.018	< 0.000

Table 5. Correlation coefficients (Spearman)[1] between schoolbehavior scales in total group (n = 197), before and after the intervention period

	1		2		3		4	
	Before	After	Before	After	Before	After	Before	After
1.Extraversion	1.00	1.00						
2.Agreeableness	-0.47***	-0.40***	1.00	1.00				
3.Conscientiousness	0.11	0.06	0.20**	-0.08	1.00	1.00		
4.Emotional stability	-0.19**	-0.28***	0.28***	0.32***	0.08	-0.23***	1.00	1.00

[1] *P < 0.05; ** P < 0.01; ***P < 0.001

Table 6. Reliability coefficients of schoolbehavior scales (Cronbach's alpha), before and after the intervention period (n = 197)

	Before	After
Extraversion	0.81	0.83
Agreeableness	0.63	0.64
Conscientiousness	0.85	0.84
Emotional stability	0.66	0.47

School behavior and iodine status

With respect to initial iodine status, weak but significant correlations were found between:

- extraversion and thyroglobulin (r = -0.17 ; $P = 0.018$)
- emotional stability and FT4 (r = -0.20 ; $P = 0.006$)

At the end of the observation period the only significant correlation between iodine variables in blood or urine and behavior was the one between FT4 and emotional stability (r = -0.16; $P = 0.027$). No association was observed between behavior factors and anthropometric variables or family characteristics such as educational levels of father and mother and family size.

Multiple regression analysis was used to identify the significant predictors of the changes in these three scales. Predictors applied were: age, height-for-age, weight-for-age, school, educational level of father, blood hemoglobin concentration, change in serum concentrations of FT4, Tg and TSH and change in urinary iodine concentration. With respect to the change in agreeableness in the younger group, the log-transformed changes in serum concentrations of Tg and TSH were the only significant predictors ($P < 0.0001$ and $P = 0.002$ respectively), accounting for approximately 17 % of the variance ($R^2 = 0.191$; adjusted $R^2 = 0.168$; $F_{[2, 69]} = 8.161$, $P = 0.001$). The regression coefficients (β) were 0.514 and -0.427 respectively. For the change in emotional stability in the younger age group, the log-transformed changes in serum concentrations of Tg and TSH were the only significant predictors ($P < 0.030$ and 0.023 respectively), accounting for 6 % of the variance (Model $R^2 = 0.086$; adjusted $R^2 = 0.060$; $F_{[2, 69]} = 3.259$, $P = 0.044$). The correlation coefficients were 0.313 and -0.329 respectively. Thus a decrease in serum TSH concentration was associated with an increase in agreeableness and emotional stability, while a decrease in serum Tg-concentration was associated with a decrease in agreeableness and emotional stability.

In the older age group no meaningful regression variates could be formed with these predictors for the changes in emotional stability, extraversion or conscientiousness. The scale for conscientiousness was significantly correlated with results on the concentration test for both the younger and the older group (total group: $r = 0.296$; $P < 0.0001$).

DISCUSSION

The results from this study suggest that several aspects of school behavior in our study population were affected upon improvement of iodine status. Although a causal relationship could not be established, as iodine status of the control group had also improved, in younger children (< 105 mo) an improvement of serum TSH concentration contributed to improvement in agreeableness and emotional stability, whereas an improvement of serum Tg concentration contributed to a decline in agreeableness and emotional stability, as assessed by their teachers. The influence of change in indicators of iodine status on behavioral change in the older children was less pronounced.

In studies concerning the relationship between behavioral characteristics and thyroid function, serum concentration of thyroxine (FT4 or total T4) or TSH are often the indicators of choice (7,11,20,21). Although serum FT4 concentration was found to be negatively correlated with emotional stability, both initially and at the end of the observation period, the change in its concentration during the observation period was not associated with the changes seen in school behavior in the same period. Changes in serum concentrations of Tg and TSH were more closely associated with changes in behavior, possibly because changes in these indicators during the observation period were proportionally much larger than in FT4. Moreover, although the serum FT4 concentration in the study population increased significantly during the observation period, it was within the normal range, both at the beginning and at the end of the observation period. It is remarkable that the association between FT4 and emotional stability was found to be negative. In a study amongst children with congenital hypothyroidism, it was noted that serum T4 concentration was negatively associated with attention (11). It is conceivable that children with higher FT4 levels are less apathetic, show greater excitability and are more easily distracted. Such a phenomenon would also explain why an improvement, i.e. a decrease, in serum Tg concentration contributed to a decrease in agreeableness and emotional stability in the younger children. However, as the reliability coefficients for the scales for agreeableness and emotional stability were not satisfactory, the association between changes in scores on these scales and change in iodine status must be interpreted with caution.

Although the scale for conscientiousness comprises some items on concentration, the scores on these items did not change significantly during the observation period. The separate concentration test however did show a positive influence of an improvement in iodine status (6). This concentration test was positively and significantly associated with conscientiousness.

It is remarkable that at the end of the intervention period the teachers found the younger children on average less agreeable and less emotionally stable, while the older children were found to be not only less emotionally stable but also less extravert and less conscientious. The test-retest correlations were weak, but significant. The reliability coefficients for the different scales were good for extraversion and conscientiousness, but less satisfactory for agreeableness and emotional stability. Factor analysis of the responses of the teachers at the end of the study, as well as the change in the intercorrelations between the behavior scales at the beginning and end of the study indicated that the mutual relations between the scales had changed. The scale for emotional stability in particular changed during the intervention period. These findings are difficult to interpret. Several explanations may be possible: 1) The children in general may have become less apathetic and more lively or restless, because of the change in iodine status. In a crowded classroom with a traditional style of teaching this may be less appreciated. 2) The size of grade 2 varied from 40 to 60 children in the 4 schools. The large size of the the groups being taught by one teacher also makes an assessment of the child's behavior rather difficult. In determining the child's rating on the items in the questionnaire, the teacher has to have a "reference child" in mind, with whom he compares the child being rated. In a situation where almost everybody has undergone some change, as was the case in the study population, this reference child may have changed as well, causing all ratings to shift in the same direction. 3). The fact that marks for school achievement did not show any correlation between one year and the next, suggests that the teachers had difficulty in assessing children's overall performance. Again, the large classes, the virtual absence of study materials and the fact that teachers themselves often have had relatively little professional training may have contributed to this situation. In addition, the absence of a correlation may be due to the fact that these marks were given by different teachers. For the children who repeated a class and who were given marks by the same teacher, correlations were high ($r = 0.69$). In this context the fact that school marks were significantly lower in 1996 than in 1995 is also difficult to interpret. There were no significant differences in marks between standards 2 and 3 in 1995, or between standards 3 and 4 in 1996. There were also no significant differences in marks between teachers either in 1995 or in 1996.

Although the Big Five personality structure is considered to provide a model that is universally applicable, it still remains the question whether teachers across different cultures interpret or appreciate aspects of school behavior in a similar way. As a tool to assess different personality traits in a different culture, the checklist would need further fine tuning, especially with regard to the scales for agreeableness and emotional stability, as these 2 factors emerged less clearly as independent factors from the factor analysis and had lower reliability coefficients.

The findings imply that the results of the behavior assessment questionnaire must be interpreted with caution. However, in view of the considerable changes in biochemical indicators that took place during the observation period, it is likely that behavioral changes in the children did occur. The behavior of younger children in particular appears to be susceptible to changes in iodine status. In nutrient supplementation studies such "side-effects" generally receive little attention. They may however affect learning processes, whether temporary or permanently, and thus merit further investigation. To our knowledge this is the first study that has attempted to measure changes in personality traits in relation to change in iodine status, on the basis of 4 of the 5 dimensions of the "Big Five". Thus it provides a starting point for further research that should be aimed at refinement and adaptation of the tool for use in the educational context as found in West Africa as well as at the further elucidation of the relation between iodine status, thyroid function and personality traits.

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The role of iodine in mental and psychomotor development of children: an overview[1]

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Submitted

[1] An earlier version of this paper appeared in: Shresta, R.M. 1994. Effect of iodine and iron supplementation on physical, psychomotor and mental development in primary school children in Malawi. PHd thesis Wageningen University. It has now been revised in collaboration with the previous authors (Shrestha, West)

INTRODUCTION

A wealth of studies has addressed the relationships between nutritional status, both pre- and post-natally, and growth and development of infants and children. The notion that deficiencies of nutrients may also affect mental development got a firm foothold in the 1960's, following the studies by Cravioto and de Licardie (1,2) and others (3,4) on the relationship between PEM (protein-energy malnutrition) and mental performance. Studies into the effects of micronutrient deficiencies soon followed suit. Even though the relationship between iodine deficiency and cretinism was known for a much longer time, the first studies into iodine deficiency and mental performance of non-cretinous populations also date from this period. Research on the association between cognition and iron deficiency anemia only started in the 1970's.

The concept that various degrees of iodine deficiency may lead to a wide range of aberrations in physical and mental development was clearly presented by Hetzel (5). His paper helped to bring about an interest in the consequences of iodine deficiency in terms of a country's development potential, beyond the research community. Even though goiter and cretinism had disappeared in several countries in Europe and Latin America with the introduction of iodized salt, several scientific research questions remained. For instance, the worldwide magnitude of iodine deficiency disorders (IDD) and the specific role of iodine deficiency in mental and psychomotor development were not fully known. Moreover, given that nutrient deficiencies have a negative effect on mental and psychomotor development, the question arises to what extent such effects are reversible. Deficiency of a specific nutrient at a critical time may produce specific lesions in the brain and perhaps other regions of the central nervous system resulting in permanent mental retardation. Whether these lesions affect specific mental abilities, the efficiency of information processing, or a combination of these two options is not clear (6). In addition, stimulation from other environmental factors and adaptive mechanisms, referred to as plasticity of mental development, including individual differences in response to external factors, also play an important role in determining the eventual impact of a nutrient deficiency. In this context the concepts of *critical point* and *catch-up behavior* have been introduced. As far as critical point is concerned, both the magnitude of the insult in relation to the individual's ability to withstand the insult and its timing with respect to the individual's developmental phase are involved.

Apart from these conceptual issues, there are also methodological difficulties in assessing the effect of nutrient deficiencies in general and iodine deficiency in particular on mental development. On the one hand, there are problems related to the tests of mental function with respect to sensitivity, specificity and validity. Similarly, the appropriateness of using test materials and procedures, designed for use in developed societies, in the typically non-Western context of most of the iodine deficiency studies may be questioned. On the other hand, criteria for assessment of iodine status and thyroid function both at the population and individual level are not unequivocal.

Conceptual framework

Malnutrition with respect to macronutrients and micronutrients impinges on the individual's growth and development and its effects are observed throughout the life cycle, possibly starting as early as conception. Deficiencies of protein and energy and of iron and iodine during the prenatal period have been linked with a high incidence of spontaneous abortions and low-birth-weight children, and compromised immune competence and mental performance (7). The effects of such deficiencies during the postnatal period have been linked with stunting, wasting, inappropriate immune response resulting in increased incidence and severity of infections, and impaired physical and mental development (8).

During the prenatal period, the fetus is totally dependent upon the mother for the supply of nutrients. After birth, nutrients continue to be supplied by the mother through breastfeeding but this dependence decreases with time. As the child grows, various environmental and social factors, such as access to food, exposure to infection, parental income and education, social stimulation and interaction, and services including education and health, contribute to shaping a child's development. It is within this context that mental and psychomotor development are influenced by iodine nutriture. Based upon empirical data, the relationship between various environmental factors, including iodine supply, and mental development can be conceptualized as outlined in Figure 1.

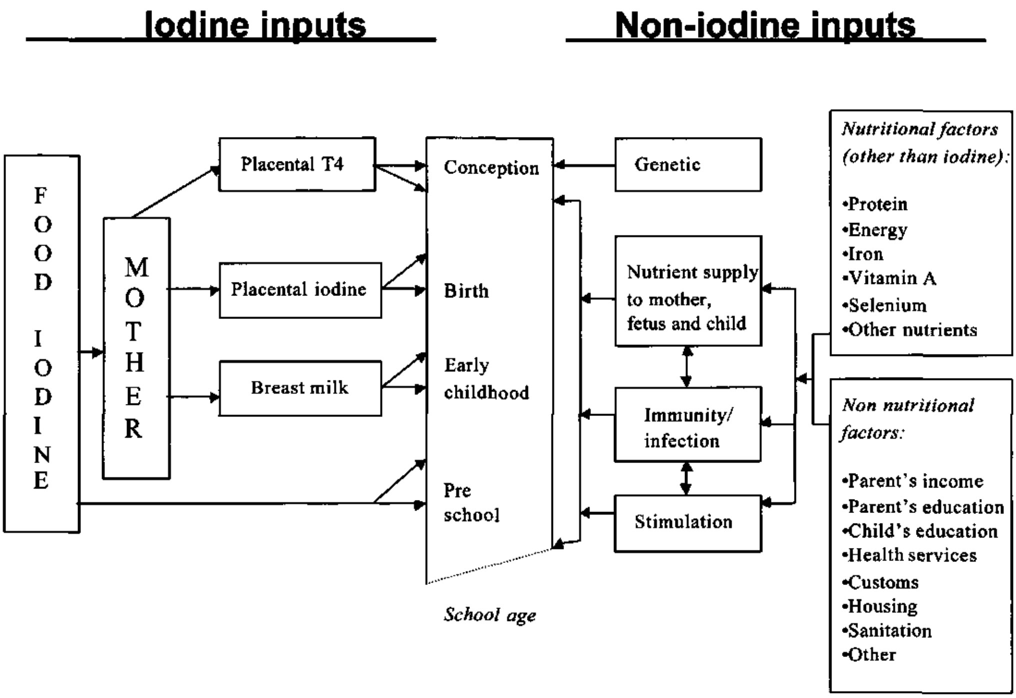

Figure 1. Pathways of iodine and non-iodine inputs during fetal and child development.

Iodine supply, thyroid function and mental development

Iodine exerts its function in the body only when it is incorporated into the thyroid hormones thyroxine (T4) and triiodothyronine (T3). The pathways and control mechanisms involved in iodine metabolism are reasonably well understood (9) as is the physiological role of the thyroid gland in relation to various body functions. Initially the fetus is dependent on the mother for preformed T4 but after about 18-22 weeks, the fetus gradually becomes more independent of the mother for T4 as it starts to synthesize its own from iodine supplied via the placenta (10). However, newborns with a total organification defect, who cannot synthesize their own thyroid hormones, had cord serum T4 concentrations of about 20-50 % of those of normal newborns, an indication that transplacental transfer of maternal T4 continues until birth (11).

There is adequate evidence to link iodine deficiency with impairment of the maternal and fetal endocrine system (12-14) and consequent mental deficiency in the new-born, which in its most severe form leads to cretinism. Iodine deficiency can also cause less obvious intellectual deficits in non-cretins (5,15-19). Whether such individuals are called sub-cretins (18) or mentally sub-normal is a matter of definition. Differences in severity and timing of fetal exposure to iodine deficiency may lead to different developmental outcomes (10,20).

Apart from the question raised in the introduction with respect to reversibility a number of other fundamental, interrelated questions still remain to be answered: 1) is there an age-limit beyond which mental deficits are irreversible? 2) is there a threshold in duration and extent of exposure beyond which mental deficits are irreversible? 3) which mechanisms are involved in reversing mental deficits? 4) what role does post-natal iodine deficiency play in mental development?

Iodine deficiency and cretinism

Goiter and cretinism have been the subject of speculation and study for a few thousand years. Research into the origin of these phenomena was accelerated in the wake of a vivid scientific interest in human anatomy in the 17th century. Observations on cretinism and goiter were collected in Diderot's Encyclopedia in 1754. In that same year, the word *cretinism* appeared in print, in an article by one of the co-editors of the encyclopedia, describing the condition of an "imbecile who was deaf and dumb and had a large goiter hanging down the waist" (21).

Cretinism as described by McCarrison in 1908 (22) includes mental retardation, deaf-mutism, and characteristic spastic or rigid neuro-motor disorders, abnormal somatic development with disproportionate body and facial characteristics, growth retardation and hypothyroidism. Using the broad terminology of McCarrison, initially two different types of cretinism were defined: *neurological* and *myxedematous cretinism* (23,24). A third type that has been introduced since then is the *mixed type,* presenting combinations of symptoms of neurological and myxedematous cretinism (25,26). Neurological and myxedematous cretinism differ in terms of timing of the insult of iodine deficiency to the developing brain. Low serum concentrations of maternal T4 early in pregnancy, i.e. before the onset of fetal thyroid function, may result in the neurological type, a condition which is irreversible. Neurological cretins may be born euthyroid. Myxedematous cretinism may originate later in pregnancy or post-natally, when maternal and/or fetal hypothyroxinemia persist (10,27). This form of cretinism which is often characterized by congenital hypothyroidism, may be reversed to some degree by T4 or by iodine provided that it is sufficient to produce normal levels of T4.

As patterns and intensity of neurological, intellectual and audiometric deficits often seem to be common to all types of endemic cretins, the usefulness of categorizing cretins into the conventional types has been questioned (25,28). Moreover, in the past decade, several studies have drawn attention to the combined roles of iodine and selenium deficiency, together with dietary thiocyanate overload in the etiology of the different manifestations of endemic cretinism (29-34).

With the spontaneous disappearance of endemic cretinism and decline in deaf mutism in Italy and Switzerland following the introduction of iodized salt, controlled trials were commenced in 1966 in Papua New Guinea (Studies 1-5 in Table 1) and in Ecuador (Studies 6-9) to see whether endemic cretinism could be prevented by supplementation with iodine. The studies carried out by Pharoah and colleagues (Studies 1-5) were based upon a single dose of 4 ml of Neohydriol ® (containing approximately 400 mg I /mL) or placebo given intramuscularly to persons > 12 y or half of this dose to persons < 12 yr. Iodized oil or saline was given to alternate families in the study area, thus covering more than 16,000 people living in 27 villages. Longitudinal observation of children born to mothers in these villages has provided extensive data on various aspects of iodine deficiency disorders and the effects of iodine supplementation (35). A total of 946 children were born, 412 to treated mothers and 534 to the saline group mothers. Seven cretins were born to the iodine-treated mothers of whom 6 were conceived prior to treatment (the time of treatment in relation to conception was not known in the seventh) while 26 cretins were born to the untreated mothers of whom 5 were conceived prior to injection. This work clearly demonstrated that iodine deficiency is the prime cause of endemic cretinism and that endemic cretinism may be prevented by iodine supplementation prior to or early in pregnancy.

Iodine status and mental performance in non-cretinous populations

The above-mentioned studies not only looked into occurrence and prevention of endemic cretinism, but also addressed the question whether mental and psychomotor performance of non-cretinous populations are affected by iodine deficiency and to what degree. They were the first of a variety of studies on this topic, which can be classified into two main categories: observational and intervention studies. The observational studies may be subdivided into ecological and case-control studies. The studies were carried out in Papua New Guinea, Ecuador, Chile, Indonesia, Spain, China, Zaire, Bolivia, Malawi, India, Italy, Bangladesh and Benin using a variety of tests to measure mental and psychomotor development. The study sites, target groups, design, tests used and results are presented in Table 1.

Ecological studies

Ecological studies compare population groups who live in different "ecologies" or different physical environments, but who are not expected to differ with respect to other variables, such as health, education and socio-economic status. The ecological studies discussed in this paper have compared populations in iodine deficient and iodine sufficient areas (Studies 11, 13, 16, 18 & 19). In 1976, Bleichrodt and colleagues (Study 11) compared children aged 6 to 20 yr in an iodine deficient and an iodine-sufficient village in

Indonesia with similar social and economic backgrounds. They also compared children under the age of 12 yr in iodine deficient and iodine-sufficient areas in Spain. From 18 different tests for general intelligence and motor skills, appropriate tests were chosen for different age categories. In Indonesia, the authors found no difference in general intelligence in the youngest age group while a significant difference was noted in non-intelligence tests such as concentration, perception and motor skills which are all independent of educational background. In Spain, in the iodine deficient children <2.5 yr, mental development was significantly lower but there was no difference in psychomotor development while for the 2.5-5 yr (Spain) and 6-12 yr groups (Spain and Indonesia), both mental and several aspects of psychomotor development (reaction time and eye-hand coordination) were lower.

In an ecological study in China, Boyages et al (Study 13) compared the IQ scores of several groups (7-14 yr & 28-35 yr) from iodine deficient and iodine-sufficient rural areas with urban controls. The lowest IQ scores were found in the population from the iodine deficient rural area with the iodine-sufficient rural areas and urban controls being successively higher. In the iodine deficient villages, the authors were able to link the low IQ scores with low audiometric ability and presence of abnormal neurological signs.

Ma and colleagues summarized 14 studies conducted in 13 different localities in China involving iodine deficient and comparable iodine-sufficient areas (presented here as Study 16). These studies, which were based upon the Stanford-Binet test and Wechsler Intelligence Scale for Children (WISC), showed a considerably lower IQ among iodine deficient populations. Mild psychomotor defects and hearing impairment were also recorded in iodine deficient populations.

Tiwari et al (Study 18) made a comparison between children (aged 9-12 yr and 12-15 yr) from villages with mild iodine deficiency and severe iodine deficiency in terms of performance on several learning tests as well as motivation to achieve. Children from the mildly iodine deficient villages learned faster and were more motivated to achieve than their peers from the severely iodine deficient villages.

Similarly, children from mildly iodine deficient and iodine-sufficient villages in Italy were compared by Aghini Lombardi and colleagues with respect to performance on subtests of the WISC-Revised (block design and coding test) and simple reaction time (Study 19). No difference was observed on the WISC-R tests, but children from iodine deficient villages showed a significantly delayed reaction time.

Case-control studies

Case-control studies compare individuals (cases) with matched peers from the same population (controls), who differ from each other with respect to only one condition or variable. The case-control studies discussed in this context, compared goitrous and non-goitrous children (Study 10), children born before or after an iodine control programme was introduced (Study 19) or children with high vs. low serum thyroxine concentration (Study 20). In a study in Chile (Study 10) in which schoolchildren (age not reported) with and without goiter were matched for age and sex, those children without goiter scored higher on various tests used. In addition, there was a positive correlation between iodine status, as measured by goiter size and serum concentrations of T3 and T4, and IQ score using the WISC and the Koppitz test. The children were similar in nutritional status as measured by height-for-age, weight-for-height and history of pre- and post-natal illness. The authors concluded that a moderate prevalence of goiter alters IQ, suggesting that the deficit in IQ increases with increased severity of iodine deficiency. This conclusion was substantiated by the fact that the IQ of this group of children was not as low as that of children in more severe iodine deficient areas. The study however, did not show any difference in visio-motor coordination between the goitrous and non-goitrous children. The authors suggested that visio-motor coordination would only be affected in areas of severe iodine deficiency.

In the study in Italy mentioned above (Study 19), a comparison was also made between children born before or after iodine prophylaxis, matched with children from an iodine-sufficient area. A delayed reaction time was observed in children born before iodine prophylaxis.

In Bangladesh, children with low serum T4 concentrations were matched with children with high serum T4 concentrations and compared on a wide range of mental and psychomotor tests (Study 20). The euthyroid group had higher scores on the Wide Range Achievement Test (measuring schooling-related performance). For all but one of the cognitive function tests, differences were not significant. However, after combining the tests into 2 principal components (cognitive and motor performance) through factor analysis, a significant effect of the T4 status on cognitive performance was shown.

Intervention studies

The intervention studies discussed here comprise studies in which specific target groups were supplemented with iodized oil. All intervention studies were carried out in iodine deficient areas. Some of the intervention studies were implemented using a control group from within the same area (Studies 4, 5, 14, 15, 17, 21 & 22) while some were implemented using a control group from an adjacent or more distant area (Studies 6-9 & 12). In some of the intervention studies, mothers were dosed with iodized oil (Studies 4, 5, 7-9, & 14) while in other studies the children were given iodized oil (Studies 6-8,12,15,17, 21 & 22).

The results of the research work carried out by Pharoah, Connolly and colleagues in a population in Papua New Guinea were analyzed both cross-sectionally (Studies 1 -3) and as intervention studies (Studies 4 & 5). In the cross-sectional analysis, psychomotor performance of children, measured using a selection of tests, was correlated with maternal T4 during pregnancy. The authors concluded that maternal T4 and not T3 may be essential for normal neurological maturation of the fetus before the thyroid of the fetus becomes functional. In Study 4, psychomotor performance was measured in children aged 11 yr in 1978 and 15 yr in 1982, but no differences were found between children of mothers who received iodine during pregnancy and those of mothers who did not. Of a group of 208 children aged 6-12 yr, 115 were born to iodine-treated mothers and 79 to the saline group, 14 of whom were cretins (36). The smaller number of children in the saline group was attributed to the high infant and child mortality rate in this group. The authors reported (Study 5) no difference between the two groups in grip strength or movement speed as measured by tapping and dotting and by nutting bolts. Although no differences were noted in gross motor skills, the treated group was better on the speed of movement tests of pegboard and bead threading, indicating that finer motor tasks, demanding speed as well as accuracy, were improved in children born to iodine-sufficient mothers.

Another series of intervention studies (Studies 6-9) was also started in Ecuador in 1966 when the entire population of one village (Tokachi) was injected with iodized oil while a second village (La Esperanza) was kept as a control. Women of child-bearing age and children born since 1966 in the test village were re-injected in 1968. In 1968, a group of 6-10 yr old children, injected with iodized oil in 1966 were compared with children from the control village using the Goodenough Draw-A-Man test (Study 6). The treated children had higher scores than did children from the control village but with a 2-tailed Student's t-test, the difference was only significant for girls. Using a one-tailed t-test, which could perhaps be justified if an improvement in performance was to be expected, the difference was significant for the boys as well. The authors conclude that the lack of significance could be due to the small sample size and limited number of tests. However, the results are of considerable clinical significance as they point to the possibility of improving mental function in children living in endemic iodine deficient areas as late as the age of 8 yr.

In another study in the same villages (Study 7), the authors compared the mental development test scores between children who received iodine through the mother during the last phase of pregnancy, during lactation and also directly by intramuscular injection and children who received iodine through the mothers throughout the intra-uterine period, during lactation and directly by intra-muscular injection. These children were also compared with children of similar age and sex in the control village. The authors concluded that iodine prophylaxis early in fetal life prevented mental retardation while supplementation late in fetal life or after birth did not prevent mental retardation.

Trowbridge (Study 8) compared three groups of children in Tokachi (test village) according to the time of their conception with children in La Esperanza (control village). In the test village, Group 1 received iodine post-natally; Group 2 were born within 9 months of the start of iodine supplementation; while Group 3 were born 9 to 18 months after the iodized-oil campaign commenced. The scores of the three groups of children using a modified version of the Stanford-Binet Intelligence test were compared among themselves and with scores of children from the control village in each group. There was no significant difference between the test and control villages on mean IQ score. However, in the test village, the IQs of children in Group 1 were significantly lower than those in Group 2 and 3.

In 1981 Fierro-Benitez et al (Study 9) compared school performance of 128 children born to mothers injected with iodized oil with 293 children born to control mothers. The authors found no difference in the age of admission to school. However, a higher proportion of children born to treated mothers were found to be in higher grades than children from the control village: 64 % of children of treated mothers repeated a class compared with 80 % of children of untreated mothers. The authors also administered various tests, which measure verbal, numerical and spatial ability, immediate memory, perception, reasoning, visual memory, speed and accuracy. Scores obtained were not significantly different between the two groups for the Terman-Merrill, WISC and Goodenough tests. While the treated children scored higher on the Goddard and Bender-Gestalt tests, on the Raven Colored Progressive Matrices test they had lower scores than untreated children. The authors concluded that the lower scores of untreated children in the Goddard and Bender-Gestalt tests are an indication of the effect of delayed maturation of regions of the nervous system dealing with psychomotor development. In a paper discussing the population referred to in Study 9, Fierro-Benitez and colleagues (37) reported that, 21 years after the initial intervention commenced, children born to mothers injected with iodized oil were doing better generally in life, having good jobs, good school records, and were able to migrate to urban areas while the less-gifted children, born to iodine deficient mothers not treated with iodine remained in the countryside. These conclusions are somewhat tenuous because getting a job, education performance, ability to migrate and social class involve complex social dynamics, but do give an indication of the improvement in life which can come through supplementation of mothers with iodine.

In an intervention study in Spain started in 1982 (Study 12), children who received iodized oil in 3 iodine deficient villages were compared with untreated children with similar educational and nutritional status as measured by weight-for-height in 2 villages about 40 kms away. No differences were observed in mental and psychomotor performance between the two groups after a follow-up period of 32 months.

In a double-blind intervention study (Study 14) in Ubangi, Zaire, Thilly and colleagues injected mothers with iodized oil and placebo between 1973 and 1977 and followed thyroid function, and psychomotor and somatic development of their offspring. They reported lower birth weight and later psychomotor development in children under two years born to untreated mothers (38) as evaluated by the Brunet-Lézine scale. Thirty-nine children born to treated mothers and 36 children born to untreated mothers were tested at age 4-9 months, 10-15 months and 16-23 months using the Brunet-Lézine scale (not all children were tested at all ages). The authors reported that Development Quotients of children from untreated mothers were consistently inferior to those from treated mothers (statistically significant for age 4-9 mo and for the group as a whole).

Thilly and colleagues (39) also examined whether hypothyroidism in children can be prevented by correcting iodine deficiency during pregnancy in a study of 671 children aged 0 to 7 years, whose mothers were given either iodized oil or placebo during the fifth month of pregnancy. The concentrations of iodine in urine and of T4 in serum of children born to the mothers who received the placebo were low throughout with about 10 % showing clinical signs of hypothyroidism. Among the treated group of children up to the age of 2 yr, only one case of clinical hypothyroidism was noted. Between the age of 2 and 4 yr, there was a gradual decrease in T4 levels, comparable to levels in children born to untreated mothers. Thilly et al (7) also reported that the magnitude of the thyroid anomalies observed in newborns was directly related to those of the mothers living in iodine deficient areas. Thus they concluded that thyroid insufficiency in pregnancy in regions with severe endemic goiter can increase risk of hypothyroidism and associated psychomotor defects in new-borns. These findings are in contrast to those reported by Pharoah et al (40) and Fierro-Benitez et al (19) who showed that correction of iodine deficiency after the fifth month of pregnancy did not have any beneficial effects on newborns in terms of psychomotor development. Thilly and colleagues (41) attributed this inconsistency to other factors such as goitrogens in the diet.

Bautista et al carried out the first double-blind, placebo controlled intervention study in which children and not their mothers were dosed with iodine (Study 15). In this study in Tiquipaya, Bolivia, they administered 1 mL of iodized oil orally (Ethiodol ®, containing 460 mg I/mL) or its placebo to 200 school children aged 66-144 mo with goiter but no signs of protein-energy malnutrition. Urinary iodine concentration, thyroid size and height were measured and physical examinations made every 6 months. A full-scale assessment including intelligence tests (Stanford-Binet and Bender-Gestalt) was repeated after 22 months. During the study urinary iodine concentrations increased and goiter sizes decreased in both treated and control groups. There were no significant differences in mental development between the groups. Although the authors reported a correlation between goiter reduction and improvement in IQ scores, this study did not answer the question, whether correction of iodine deficiency could improve intelligence.

In a study carried out in Malawi (Study 17), iodine supplementation to primary school children was found to have a marked significant impact on mental development as measured by verbal fluency, exclusion, visual memory, verbal meaning, quantity and closure, one year later. The effects were quite dramatic especially with respect to verbal fluency. If the effects observed (0.9 SD) were applied across the full range of mental development tests used in measuring IQ, the improvement observed would correspond to an increase in 10-15 points. The performance on physical and psychomotor tests were less dramatic with significant improvement being seen only for eye-hand coordination (as measured by a ball throwing exercise), hand grip and sitting/standing. No effects were observed in the tapping, balancing, peg-board and reaction time tests.

On the other hand, Huda and colleagues (Study 21) found no effect of treatment on either mental or psychomotor performance over a period of 4 months. They carried out a double blind, placebo-controlled study in Bangladesh among schoolchildren in grades 1 and 2, in which children were dosed orally with 400 mg iodine (lipiodol) and were subjected to a wide range of mental and psychomotor tests.

The results of a study carried out by the authors in Benin (Study 22) lend support to the findings of Study 17. In a double blind placebo-controlled intervention study, children (aged 7-11 yr) were dosed orally with 1 mL iodized oil, (Lipiodol, 540 mg I/mL) or placebo and were retested 10 months after supple-mentation. Even though 3-4 months after supplementation the whole study population began to have access to iodized salt, there remained considerable differences in iodine status within the total group. Children whose urinary iodine concentration improved, showed significantly greater improvement on the combination of mental tests used than children whose urinary iodine concentration did not change.

The iodine intervention studies referred to above point to an effect of iodine deficiency on mental performance, either through the interference of maternal hypothyroxinemia with the development of the fetal nervous system or through inadequate production of thyroid hormones by the child itself. However, there may be additional mechanisms for the impairment of mental development, for instance through inadequate hearing which may limit social interaction and may lead to late learning. Several studies have reported higher mean hearing thresholds in apparently normal individuals living in iodine deficient areas as compared to hearing thresholds of people living in iodine-sufficient areas (25,42-44). In Benin (45), hearing thresholds were shown to be associated with iodine status as measured by serum Tg concentration, while hearing thresholds were negatively associated with performance on mental tests. People with hearing impairment generally are not less intelligent than people with normal hearing. However, if schoolchildren are subjected to tests which measure schooling-related learning, differences in performance might be due to hearing deficits. On the other hand, if tests are used which have been developed to measure "fluid" intelligence[1)], which is independent from education, differences in hearing capacity would not explain any differences in test performance. In that case, hearing defects may have originated concurrently with other injuries to the development of the nervous system, but are not the cause of poor performance.

[1)] "Fluid" intelligence stands for the adaptive process of apprehending an unfamiliar configuration and rearranging it to satisfy some requirement, as opposed to "crystallized" intelligence which refers to an almost automatic response to a task, as it closely corresponds to past practices and experiences.

CONCLUSION

The fact that iodine deficiency affects mental and psychomotor development of children is no longer an issue. The data from Papua New Guinea, Zaire and Ecuador have demonstrated that correction of iodine deficiency before or early in pregnancy improves mental and psychomotor performance of offspring. The evidence is clear that frank cretinism cannot be fully reversed, although there are reports, mainly from China, that treatment of cretins can result in some recovery in functions such as hearing and reproductive performance. Almost all the ecological studies demonstrate significant differences between children from iodine deficient areas and those from iodine-sufficient areas in terms of IQ, aspects of psychomotor development and learning abilities as demonstrated by school achievement. The methodological limitations of these studies however, and in particular the question of comparability of study areas, preclude the drawing of conclusions with respect to cause and effect relationships. Similarly, the case-control studies indicate differences in mental performance, some aspects of psychomotor performance and school achievement between goitrous and non-goitrous children or children with low versus high serum T4 concentrations.

The relatively large number of intervention studies differ in terms of target group (mothers or children; different age groups), severity of iodine deficiency, study design (use of control groups from within or outside the same community; some studies used a placebo, others did not; some designs were double-blind, others were not), types of tests used and duration of follow-up period. In a meta-analysis Bleichrodt and Born (46) combined 18 studies, including 10 studies described in this paper (intervention studies 1, 6-9, 12, 14,15), an ecological study (Study 11) and a case control study (Study 10). The results demonstrated that the iodine deficient and the iodine-sufficient groups were 0.9 SD or 13.5 IQ points apart. However, this meta-analysis which combined various study designs, degrees of iodine deficiency and mothers and children of various age groups, did not address the issue of reversibility of the negative effects of iodine deficiency.

Only 4 studies have applied a double blind placebo-controlled design to examine whether negative effects of iodine deficiency in children can be reversed. Two of these, the studies in Bolivia and Benin, encountered an improvement in iodine status of the control group during the follow-up period. Still, the study in Bolivia did show that there was a significant association between decrease in goiter size and increase in IQ, 22 months after supplementation (47). In Benin, children whose urinary iodine concentration increased, showed greater improvement in mental performance than children whose urinary iodine concentration did not improve, 10 months after supplementation (48). The study in Malawi clearly demonstrated an improvement in mental performance and several aspects of psychomotor performance as a result of iodine supplementation 1 yr earlier. A similar study in Bangladesh however did not show any difference in performance on cognitive and psychomotor tests between supplemented and non-supplemented children 4 months after supplementation (49). It is conceivable that the follow-up period in the Bangladesh study was too short to detect differences in performance on the types of tests used.

Thus the evidence that supplementation of iodine deficient schoolchildren with iodine results in improvement in mental and psychomotor development is not yet conclusive. The questions raised in the introduction, which are highly relevant from a public health point of view, remain: is subtle damage produced by iodine deficiency, less than that required to produce cretinism, reversible and if so, under which conditions and up to what age. The question with respect to the specific role of postnatal iodine deficiency in mental development has not been addressed. In addition, the studies done so far do not unequivocally answer the question whether some specific mental abilities are more readily affected by supplementation than others or whether factors such as restoration of alertness, mental speed and concentration following supplementation play a role in improving mental and psychomotor performance.

There is therefore a clear need for further study in this area. Replications of the double blind placebo-controlled studies should take place in several settings with different levels of iodine deficiency, in different age groups and with different follow-up periods, as well as with a comprehensive set of tests. Although ethical objections to such studies are likely to be raised, the authors feel it is ethical to carry them out in primary school children as long as there is no conclusive evidence that negative effects of iodine deficiency in this age group may be reversed. Until now the prime target for iodine supplementation programs are girls and women of reproductive age. Obviously, if women are not reached prior to conception, their iodine deficiency should be treated as a matter of urgency. It is however not yet clear which policy should be followed for children. Confirmation that the mental and psychomotor performance of iodine deficient children may be influenced positively by improving their iodine status would provide a much stronger justification to extending iodine supplementation programmes also to children in areas where iodised salt is not readily accessible. Up until now, there is no concrete scientific evidence for the need to reach children in these conditions, except to reduce their goiter rates.

The progress made with salt iodisation initiatives makes further trials in children increasingly difficult. However, there are still many communities and people within communities which have insufficient access to the amounts of iodine required for adequate thyroid function and thus for living up to their full physical and mental potential.

Study No.	Reference; Study site	Age yr, (n)	Study design
1	Pharoah et al (50) Papua New Guinea	10-12 (20)	Mothers injected in 1966 with iodized oil (4mL) or placebo and children tested in 1980-81; analysed as cross-sectional study
2	Connolly & Pharoah (51) Papua New Guinea	14-16 (22)	Children of treated mothers referred to above, tested in 1985; analysed as cross-sectional study
3	Pharoah et al (40) Papua New Guinea	6-12 (208)	Population as above, children tested in 1978; analysed as cross-sectional study
4	Pharoah et al (52); Papua New Guinea	11 & 15 (28-30)	Population as above, children tested in 1978 and 1982; analysed as intervention study
5	Connolly et al (36); Papua New Guinea	Children, < 12 (194)	Population as above, children tested in 1978; analysed as intervention study (treated, n = 115; control, n = 79)
6	Dodge et al (53) Ecuador	6-10 (96)	Intervention study: children in one village (n = 51) injected with iodized oil (2mL) and compared after 2 yr with those in control village (n = 45)
7	Fierro-Benítez et al (19) Ecuador	Children age > 40 mo (150)	Intervention study: mothers injected with iodized oil (4mL) during early (n = 41) or late pregnancy (n = 26); matched children in control village (n = 83) injected with iodized oil (0.5mL)

Tests used	Results
Pegboard, bead threading, peg frame, Pacific design construction test	Psychomotor performance correlated with maternal total T4 during pregnancy
Pegboard, bead threading	Psychomotor performance correlated with maternal total T4 during pregnancy
Hand grip, dotting, bolt nutting, pegboard	Psychomotor performance correlated with maternal total T4 but not total T3 during pregnancy
Hand grip strength, tapping, dotting, bead threading, pegboard, Pacific design construction test	No difference between children of treated (n = 11-13) and untreated mothers (n = 15-18)
Hand grip strength, tapping, dotting, bolt nutting, bead threading, pegboard	No difference in hand grip strength , tapping, dotting, and bolt nutting between two groups; children born to treated mothers performed better in bead threading and pegboard (speed/accuracy tests)
Goodenough Draw-A-Man	Mental development of treated children tended to be better than controls; statistically significant only for girls using two-tailed test and for boys only when a one-tailed test was used
Stanford-Binet	Children of mothers treated early in pregnancy had signifantly better mental performance than children of mothers injected late in pregnancy and children from control village

Study No.	Reference; Study site	Age yr, (n)	Study design
8	Trowbridge (54); Ecuador	2-8 (125)	Intervention study: mothers injected with iodized oil (4mL): children born 0-9 mo before (n = 35); 0-9 mo afterwards (n = 44); 9-18 mo afterwards (n = 46). Children compared with controls from non-suppl. Villages
9	Fierro-Benítez et al (55); Ecuador	8-15 (421)	Intervention study: mothers in one village injected with iodized oil (4mL); children (n = 128) compared with matched children (n = 293) in control village
10	Muzzo et al (56); Chile	School-aged children (90)	Case control study: comparison between goitrous (n = 42) and non-goitrous (n = 48) children matched for nutritional status
11	Bleichrodt et al (57); Indonesia and Spain	Indonesia 6-20 (245); Spain 0-12 (355)	Ecological studies: comparison between children in iodine deficient and non-deficient areas
12	Bleichrodt et al (58); Spain	6-12 (205)	Intervention study: children given one dose (2mL) iodized oil in one village (n = 103) and compared with untreated children (n = 102) in matched village, 32 mo after supplementation
13	Boyages et al (59); China	7-35 (369) 270 children 99 adults	Ecological study: comparing individuals in 3 areas differing in access to iodized salt: rural areas with and without iodized salt, urban areas with iodized salt

Tests used	Results
Stanford-Binet	Treatment of mothers prior to conception or during gestation signifantly improved sub-sequent mental performance of children compared to children who were not treated until after birth. No significant dif-ferences in IQ between children from test and control villages
School performance, Terman-Merrill, Goddard, Goodenough Draw-a-Man, Wechsler, Bender-Gestalt, Raven (not all subjects undertook all tests)	Children from treated mothers had better school performance, psychomotor development indices (Goddard and Bender-Gestalt) and Goodenough Draw-a-Man test; no significant difference in Terman-Merrill, Wechsler and Raven tests
Wechsler, Bender-Gestalt, Koppitz, pegboard	Performance of non-goitrous children higher than goitrous children, no difference in visio-motor co-ordination and hand skills
Maze, fluency, visual memory, figural unit, hand grip, figure comparison, exclusion, pegboard, balancing, tapping, Bender-Gestalt	Subjects from iodine deficient areas: < 2.5 yr (Spain), difference in mental, no difference in Raven and psychomotor scores; 2.5-5 yr and 6-12 yr (Spain and Indonesia), lower mental and psychomotor scores; 13-20 yr (Indonesia), lower mental scores; no difference in eye-hand coordination
As above	No effect of treatment
Hiskey-Nebraska, Griffiths mental development scales	Mental performance in urban children > rural children with iodized salt > rural children without iodized salt

Study No.	Reference; Study site	Age yr (n)	Study design
14	Thilly et al (7); Zaire	4-23 mo (75)	Double blind intervention study: mothers injected (4mL) with iodized oil (n = 36) or placebo (n = 39) at 28 w gestation
15	Bautista et al (47); Bolivia	5-12 (200)	Double blind, placebo-controlled intervention study; children with thyroid enlargement dosed orally (1mL) with iodized oil (n = 100), placebo (n = 100); tested after 22 mo
16	Ma (18); China	Primary school children (4392)	Ecological studies: comparison between children in 13 iodine deficient areas with those in control areas
17	Shrestha et al (60); Malawi	6-8 (241-321) (to be modi-fied)	Double-blind placebo-controlled study: children dosed orally with iodized oil (1mL) or placebo
18	Tiwari et al (61); India	9-12; 12-15 (200)	Ecological study: comparison between children in villages with mild iodine deficiency (MID) and severe iodine deficiency (SID)
19	Aghini Lombardi et at (62); Italy	7-12 (270)	Ecological study: comparison between children from villages with mild iodine deficiency and iodinesufficient villages Case-control study: comparison between children born before or after iodine prophylaxis matched for age and sex with children from iodine-sufficient area

Tests used	Results
Brunet-Lezine	Mental performance higher in children aged 4-8 mo but no difference in children aged 10-15 mo and 16-23 mo.
Stanford-Binet, Bender-Gestalt	Goiter rate lower in treated group, no difference in physical and mental per-formance and school per-formance Significant asso-ciation be-tween decrease in goiter size and increase in IQ
Stanford-Binet, Wechsler	Children from iodine deficient areas had lower IQ's
Fluency, exclusion, verbal meaning, visual memory, closure, hand grip, sitting-standing, pegboard, balancing, ball throwing, tapping, reaction time	Mental development, hand grip, sitting-standing, and eye-hand co-ordination improved in treated children, no difference in other psycho-motor tests
Maze learning test, verbal and pictorial learning test and achievement motivation scale	MID children performed better on maze learning and pictorial learning tests than SID children and learned faster on all tests; MID children were more motivated to achieve
Block design (WISC-R), coding test (WISC-R), simple reaction time	No difference between children from iodine deficient and iodine-sufficient villages on block design and coding tests; reaction time sig-nificantly delayed in children from iodine deficient villages Delayed reaction time in children born before iodine prophylaxis

Study No.	Reference; Study site	Age yr (n)	Study design
20	Huda et al (63); Bangladesh	School Children; Grades 1 and 2 (340)	Case-control study: comparison of children with low T4 with children with high T4, matched for school and grade
21	Huda et al (49); Bangladesh	School Children; Grades 1 and 2 (305)	Double blind, placebo-controlled intervention study; children dosed orally with 400 mg lipiodol
22	Van den Briel et al (48); Benin	7-11 (196)	Double blind, placebo-controlled intervention study; children dosed orally with iodized oil (1mL; 540 mg) or placebo; 3-4 mo after supplementation, iodized salt was introduced in the study area

Test used	Results
Wide Range Achievement Test (WRAT), (reading, spelling, arithmetic), verbal fluency, digit span, visual search, French learning test, Corsi blocks, Raven Coloured Progressive Matrices, symbol modalities test, modified Stroop test, upper limb speed and dexterity, pegboard	The euthyroid group had better scores on the WRAT test than the hypo-thyroid group. For the cognitive function tests differences were not sig-nificant, except for the French learning test. Factor analysis revealed 2 principal com-ponents (general cognitive and motor). A significant effect of thyroid group on the general cognitive factor was shown.
Same as in study no. 20, except WRAT test	No difference between treated and untreated children on any of the cognitive and motor tests.
Block design (WPPSI), closure, concentration, ex-clusion, fluency, mazes, hand movements Raven, coloured progressive matrices	Children whose urinary iodine concentration improved, showed significantly greater improvement on the com-bination of tests used than children whose urinary iodine concentration did not change

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8

General Discussion

In this thesis a study has been described which was set up to examine whether iodine supplementation improves mental performance of iodine deficient schoolchildren in Benin. This chapter evaluates the contribution this work has made to the present body of knowledge on the relationship between iodine deficiency and mental performance. It also identifies gaps in knowledge and areas for future research and discusses policy issues and recommendations.

Does iodine supplementation of iodine deficient children improve their mental performance and, if so, how?

In Chapter 1 various factors were identified which may have a bearing on growth and physical and mental development of children. In order to establish the influence of iodine supplementation on mental performance, a double-blind placebo-controlled study would be the most powerful method. Thus, in our study children were stratified by school, school class, and sex and subsequently matched on the basis of similar age and height-for-age. From each pair of children, one child was randomly allocated to one of two groups, which were then randomly allocated to receive iodine supplementation or a placebo. However, as explained earlier, iodized salt was introduced in the study area three to four months after supplementation, necessitating a reformulation of the study hypotheses. As the whole study population had begun to have access to iodized salt, the original study design with a control group consisting of children, who had not received any iodine, was no longer valid. It was therefore decided to take children whose iodine status had not improved, as measured by urinary iodine concentration, as the control group. This condition applied to about one third of the total group. This "unchanged" group had higher initial mean scores on variables reflecting iodine status and thyroid function than the "improved" group (Chapter 2). Based on an initial median urinary iodine concentration of 77.9 μg/L, the "unchanged" group could be considered mildly iodine deficient. At the end of the study period this same group was still mildly iodine deficient (median urinary concentration 85.4 μg/L). Initially, the "improved" group was severely iodine deficient (median urinary iodine concentration 12.0 μg/L), while at the end of the study period it was mildly iodine deficient (median urinary iodine concentration 85.5 μg/L). As the change (delta) in performance on a series of test items was the outcome variable of choice rather than an absolute score, this approach is justified in our opinion (Chapter 2).

Our results show that over an observation period of 10 months, children whose iodine status improved as measured by urinary iodine concentration also showed a greater improvement in scores on all mental performance tests than children whose iodine status showed little or no change (Chapter 2). Similarly, when compared to the "unchanged" group, the "improved" group performed significantly better on the tapping test, a test measuring manual speed and accuracy (P=0.007; data not presented). Improved children were also faster on the simple reaction time and choice reaction time tasks than the unchanged children, but the differences were not significant (P=0.060 and P=0.054 respectively; data not presented).

As discussed in Chapter 7, only very few double-blind placebo-controlled studies have been carried out in children and these have produced different results. Two studies, including ours, encountered an unexpected influx of iodine into the area. A major research question, i.e. whether a mental deficit, resulting from iodine deficiency may be reversed, is therefore not yet answered unequivocally.

Potential mechanisms

With respect to the mechanisms that may be involved in a reversal of mental deficits, there is also still a clear need to explore different avenues further. This applies both to the domain of psychology and to that of neurology. Different theories of intelligence have led to different types of studies into the effects of nutrient deficiencies on mental performance. Basically two main classes may be distinguished: psychometric studies and those based on information processing theories (1-3). Psychometric studies deal with differences in task performance, which abilities are measured by a test or which combination of abilities is measured (1). Factor analysis is a key instrument in organizing abilities at different levels and in different structures. Most studies into the consequences of iodine deficiency on mental and psychomotor performance, including ours, may be classified as psychometric studies. In our study we administered a series of tests, covering as much as possible the primary mental abilities as identified by Thurstone (4) and Ekstrom et al (5) (Chapter 2). Thurstone's verbal and number abilities were not included. The test battery thus mostly tapped abilities in the domain of "fluid" intelligence, as opposed to "crystallized" intelligence [1].

Studies based on information processing theories, in analogy with computer operations, attempt to understand the mental processes that underlie task performance. Such research thus deals with the processes at work between perception and action. In this context six "performance components" have been identified: encoding, inference, mapping, application, justification and response (3). Individual variation in the efficiency of the various steps in information processing is thought to be affected by various nutrient deficiencies and this in turn may be reflected to a certain degree in task performance as measured by various mental tests (2). Besides "lower-level" processes, reflecting the influence of biological or physiological factors, "higher level" processes, reflecting socio-cultural variables, experience and learning play a key role in determining mental performance. Both types of processes are involved in mental performance and interact in a number of ways (2). Examples of such interaction in mental development of children under conditions of iron deficiency anemia have been described by several authors (6-11). In the children studied, not only the child's physiological status, but also its interaction with environmental variables, with caretakers in particular, were shown to be determinants of the final outcome. Anaemic children for instance show increased wariness, hesitance and fearfulness. They interact less with their environment and are less successful in eliciting caretaker responses.

With respect to consequences of iodine deficiency, the efficiency of information processing has not been studied extensively except for reaction-time tests. Similarly, the effects of for instance behavioral changes such as apathy, on interaction with the environment and thus on learning and psycho-social development have not been given much attention.

Furthermore, our knowledge of what happens to brain development and function in humans under conditions of iodine deficiency is incomplete. It is based to a large extent on animal studies. The knowledge acquired from animal models cannot always be extrapolated readily to human beings because of the differences between man and animals

[11] Definitions of fluid and crystallized intelligence have been given in Chapter 1.

in phylogenetic characteristics as well as in psycho-sensory and social responses to external stimuli. Experiments with sheep, marmosets and rats have shown that iodine deficiency is related to fetal development as indicated by reduced brain weight with reduced numbers of cells, reduced birth weight and delayed fetal maturation (12-14). Both in humans and animals transplacental transfer of maternal thyroxine (T4) to the fetus is crucial for the normal development of its central nervous system. In rats, even mild iodine deficiency in the mothers, leads to reduced availability of T4 for the fetus (15,16). T4 is needed for conversion, through de-iodination, into triiodothyronine (T3), which regulates transcription through thyroid hormone receptors in the developing brain and other tissues following a precise development schedule (17). Alterations in availability of T3 may lead to alterations in expression and/or stability of specific mRNA's. This again may lead to alterations in specific protein synthesis and thus to specific neuro-anatomical abnormalities (18).

It does not seem likely that the neuro-anatomical lesions in the central nervous system, resulting from pre-natal iodine deficiency is completely reversible. In that respect, lessons may be learned from experiences with sporadic congenital hypothyroidism and timing of treatment. While early diagnosis and adequate treatment with thyroxine (T4) may normalize most functions of the central nervous system, the neuropathological effects of severe fetal hypothyroidism do not seem to be reversed completely by postnatal T4 treatment (19-21). Similarly, studies into maternal thyroid dysfunction during pregnancy have shown negative effects on the neuropsychological development of the child (22-26). As noted in some of the retrospective surveys however, not all women had been treated timely or adequately, while some were not treated at all.

Assuming that most children with subclinical signs of iodine deficiency do not have any specific gross pathology of the central nervous system, the effects of iodine deficiency on mental performance may be general rather than focal. If this is so, measuring specific abilities, as used in IQ batteries, according to some authors may be less useful than measuring variables that reflect general brain damage, such as mental speed, attention and working memory (2).

In our study, concentration was improved in the children whose iodine status, as measured by urinary iodine concentration, was improved. This may have contributed to a better performance on the other mental tests. However, the change was small and by itself not significant. Moreover, one of the children's personality traits, conscientiousness, which also comprises concentration was not changed in the perception of their teachers (Chapter 6). In a study by Tiwari et al. (27), children from iodine deficient areas were less motivated to achieve and were also described as apathetic. It may well be that an increase in iodine supply to iodine deficient children makes them more alert to their environment and to tasks assigned to them. A much more positive attitude towards life and disappearance of lethargy following introduction of iodized salt, has been described for a whole village in China (28). As a clinician might say: a hypothyroid child is ill, if it is given medication, it will show an immediate change in its behaviour. At the beginning of our study most children were not biochemically hypothyroid as mean serum concentrations of free thyroxine (FT4) and thyroid stimulating hormone (TSH) were within the normal range. Only a small proportion (4.5 %) had both a low FT4 and high TSH concentration, while approximately 15 % had a low FT4 concentration and 11 % a high TSH concentration.

Finally, higher hearing thresholds have been associated with iodine deficiency (29-33). In our study in Benin children with poorer iodine status, as measured by serum concentrations of thyroglobulin (Tg), could hear less well, especially in the higher frequency range. Moreover, hearing thresholds were negatively associated with performance on mental tests (Chapter 3). Thus the question may be raised whether a deficit in hearing capacity plays a mediating role in affecting mental performance. It is conceivable that children with defective hearing lag behind in mental development through slow learning and interaction with the environment. For that reason such children might differ from children with normal hearing in terms of school achievement or performance on schooling-related tests. However, the tests we administered were not measuring this type of "crystallized" intelligence, but rather the "fluid" type of intelligence. Moreover, the hearing deficits in our study group were small and did not impede normal interaction in any noticeable manner. Thus we feel that it is unlikely that defective hearing would provide an explanation for poorer performance on mental tests. Consequently, the negative association found between hearing thresholds and test performance therefore most probably does not reflect a cause-effect relationship but rather points to a common origin.

Assessing iodine status

A recurrent problem in the range of studies carried out on iodine deficiency and mental performance, including our study, is the choice of age-specific indicators and cut-off points for the assessment of iodine status, and thus of comparablity (Chapter 4). A number of studies have used T4, while in other studies urinary iodine concentration and Tg were used. In the age-group we studied, there is as yet no universally accepted single indicator for " iodine status", while cut-off points enabling different degrees of iodine deficiency to be distinguished are based on populations rather than on individuals. In a number of the ecological studies discussed in Chapter 7, iodine deficiency is treated as a dichotomous variable rather than a continuous variable: groups compared are considered to be either iodine deficient or iodine sufficient. In both groups the iodine status of individuals may vary considerably. This may provide an explanation why in a number of studies differences between groups in terms of mental or psychomotor performance were not significant.

On the basis of our work, we suggest that more than one indicator is needed to be able to assess iodine deficiency, particularly in conditions where the deficiency is moderate to mild, and have suggested that serum thyroglobulin and urinary iodine concentration be taken as the indicators of choice (Chapter 4). Classification into different degrees of iodine deficiency still remains problematic however, as urinary iodine concentration may vary on a daily basis, depending on the intake and the time of day, while thyroglobulin assay methods and reference materials are not yet universally standardized.

Ensuring a better future for children in rural northern Benin

Iodine deficiency is often found in areas where most of the food consumed is produced locally and the market infrastructure is very limited. The site that was selected for this study fell into this category. The population did not have access to sufficient food in terms of quantity and quality throughout the year. Moreover, educational, health and sanitary facilities were poor.

The nutritional status of the child population studied was characterized by a relatively high degree of stunting, iron deficiency anemia, iodine deficiency and possibly selenium deficiency (Chapters 2-4). Moreover, these children also appeared to have elevated serum concentrations of total homocysteine (Chapter 5), although in the absence of a normal reference range for children, this could not be proven. These nutritional problems - iodine deficiency, iron deficiency and stunting in particular- further compounded by the effects of socio-economic deprivation, all may affect mental development of children negatively.

Another clearcut obstacle to the development of children is the poor quality of education (Chapter 6). The observation that there is no significant correlation between children's marks in one year with those of the next year is cause for concern. Similarly, no significant correlation between performance on the mental tests and school achievements was observed (Chapter 6). The intra-observer (teacher) agreement as shown for children who repeated a class, was good, while the inter-observer agreement between teachers was very low. This implies that teachers applied their own standards in assessing school achievement of children, but that these standards were not "standardized".

The relative weight of these two major obstacles to socio-economic progress, iodine deficiency and poor education, is hard to assess. Moreover, these two factors may not act independently. As shown in studies on iron deficiency anemia and on protein-energy malnutrition, the effects of nutritional deficiencies on learning and mental development may be moderated by psycho-social stimuli and interaction with family, teachers, etc. (11,34-36). Thus, solving the problem of iodine deficiency is essential, but if the quality of education is not improved, children may still not live up to their full potential.

Policy implications

To address the above mentioned issues, a two-pronged approach would be required. The first and cheapest approach comprises measures aimed at ensuring access to and adequate intake of iodine by the population of Benin, mothers and children in particular. Secondly, it is essential that attention be given to upgrading educational services.

Iodization of salt has received world-wide recognition as the most cost-effective means of controlling IDD. A unique form of collaboration has emerged in the past decade: partnerships between the private sector (salt producers), international non-governmental agencies (ICCIDD, Kiwani's International), UN Agencies (UNICEF, WHO, FAO) and governments with the aim to achieve universal salt iodization. Almost 80 % of governments in African countries with an IDD problem now have adopted legislation to facilitate the process of universal salt iodization while about 70 % of households in Africa now have access to iodized salt (37).

In Benin a resolution was signed by 5 ministers (Health, Commerce and Tourism, Industry, Finance, Rural Development) in November 1994, banning the import of non-iodized salt and prescribing the levels of iodine in salt at all stages in the production and marketing chain. Iodized salt was not yet available in the north at the end of 1995. However, in the course of 1996, iodized salt began to be distributed in the study area by women traders who obtained their supplies from an extension worker. In a small market survey carried out at the end of 1996, two types of salt were found on the market: iodized salt coming through the extension worker with an iodine concentration of approximately 50 ppm, and non-iodized salt coming from across the border from Togo.

To determine the potential contribution of iodized table salt to the iodine requirements of adults and children in northern Benin, discretionary salt intake of women and their male children aged 9-12 years was measured in one of the study villages in 1995 (38); Appendix 1). The mothers were shown to have a total salt intake of 9.0 ± 2.9 g/d and their sons had an intake of 5.7 ± 2.8 g/d. Discretionary salt contributed about 50 % of total salt consumed in diets of both sons and their mothers. Based on the median discretionary salt intake of the women in this study, the iodine content of the table salt used should be 38 ppm to meet their daily iodine requirements. For their sons it should be 52 ppm (Table 1;App.1). The stipulation, as laid down in the above-mentioned Beninese resolution, that at retail level salt should contain 30-50 mg of iodine per kg, thus in practice might not be adequate, especially not for children. The lower cut-off point probably is too low in view of the fact that further losses of iodine are likely to occur at household level, during storage and cooking. Therefore either the requirement for iodine content of the salt at retail level should be set at a higher level, or other potential vehicles for the supply of additional iodine should be identified. Condiment cubes (Maggi cubes ®, Nestlé S.A., Vevey, Switzerland and similar products from other producers) are an important source of salt in the diet of the majority of the rural people in Benin. These cubes are added habitually to the sauces that are eaten with the main meals (and thus might be regarded as discretionary salt as well). They would therefore provide an excellent vehicle for iodine fortification.

The same study also points to the great variability in total and discretionary salt intake between different cultures, necessitating a differentiated approach. Our study was based on a small sample, thus the findings must be interpreted with caution. However, in the framework of universal salt iodization initiatives, discretionary salt intake as well as salt intake from non-discretionary or semi-discretionary sources, such as condiment cubes, should be examined regularly to ensure and maintain adequate intake.

Recent surveys in Benin (37) suggest that about 90 % of households now have access to iodized salt. As the surveys were done in areas that could be reached relatively easily with the "Thyromobil" [2] it is likely that in the more disadvantaged areas in the North, this percentage is still considerably lower. Moreover, salt samples examined were shown to have variable contents of iodine. New developments in salt iodization processes, e.g. the binding of iodine to salt through a chelation-process, are expected to prevent loss of iodine in the marketing chain as well as during cooking. Such developments will help to reduce the need for monitoring losses in specific brands of salt. However, as long as such processes are not or cannot be widely adopted by the numerous small salt producers in West Africa, monitoring of iodine concentrations at various points in the chain from production to consumer remains crucial (39).

As many food products, including salt, are traded across borders, there clearly is also a need for neighbouring countries to collaborate on harmonizing their legislation concerning iodized salt, including regulations with respect to the level of iodine fortification, as well as on enforcement. Similarly, inter-country collaboration is needed on monitoring procedures and on the development of a network of regional laboratories and the establishment of a reference laboratory. Furthermore, on a country basis, taxes and

[2] The ThyroMobil is a van equipped with a sonographic device and facilities for assessment of urinary iodine concentration and iodine content of salt. It is made available by ICCIDD and the Merck company to assist countries in assessing progress towards universal salt iodization.

duties on import of iodate and equipment should be minimized. Appropriate labelling and social mobilization strategies need to be developed (40) to ensure that universal salt iodization initiatives are sustained and that consumers actively seek to buy iodized salt.

Now that salt iodization as a means of controlling IDD appears to be well on its way in Benin, ways should be found to invest more in another determinant of progress: schooling. This applies not only to the physical infrastructure, buildings and teaching aids, but also to the quality of teacher training and supervision. Efforts must be strengthened to keep children in school for a longer period and attract those who do not enroll in school at all. Just now a lot of human potential is still left undiscovered.

Conclusions and recommendations

As discussed in this chapter as well as in Chapter 7, there is still a considerable gap in our knowledge with respect to the question whether deficits in mental and psychomotor performance of schoolchildren as a result of iodine deficiency, may be reversed. At the same time, the conditions under which mental deficits may be reversed (age of child, timing, duration and severity of iodine deficiency) as well as mechanisms by which reversibility may be realized, all need further study as well. Such studies are increasingly difficult to implement, because of the progress made with universal salt iodization initiatives and because of ethical objections that are likely to be raised. There is so far no "hard" evidence to justify iodine supplementation to primary schoolchildren in situations where access to iodized salt is inadequate. This is particularly true in situations of moderate to mild iodine deficiency. Any motivation to supplement in these situations may be based on principles of equity or entitlement, but is not based on scientific grounds.

Apart from priorities for research, there are priorities for programmes and policies. Full access to iodized salt is a matter of priority, even in the most backward areas, but at the same time monitoring of iodine content at various points in the production-consumption chain remains essential. Similarly, food consumption patterns, including salt intake from various sources need to be (re-) assessed on a regular basis.

In order to be able to better utilize the human resource potential for further development of the country, the next step would be improving the quality of education.

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Appendix 1

Determination of discretionary salt in rural Guatemala and Benin in order to determine the level of iodine fortification of salt required to control iodine deficiency disorders: Studies using lithium-labeled salt.

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American Journal of Clinical Nutrition 1998; 68:636-641

Abstract

The use of discretionary salt, which is salt added during cooking and at the table, as a suitable vehicle for iodine has been assessed by measuring salt consumption using the lithium-marker technique in rural areas of Guatemala and Benin. In both countries, we studied boys, aged 6 to 12 y, and their mothers. Subjects used lithium-labeled salt after all normal salt was removed from the households. In Guatemala, 24-h urine samples for 9 mother-son pairs were collected at baseline and on Days 7, 8 and 9 during the use of lithium-labeled salt. Total maternal salt intake averaged 5.2 ± 1.7 g/d (mean ± SD) of which 77 ± 24% came from discretionary sources whereas Guatemalan boys consumed a total amount of 1.8 ± 0.6 g salt/d of which 72 ± 12% came from discretionary sources. In Benin, urine collection from 13 mother-son pairs took place at baseline and on Days 5 and 7. Beninese mothers had a total salt intake of 9.0 ± 2.9 g/d and their sons, 5.7 ± 2.8 g/d; discretionary salt contributed 52 ± 14% and 50 ± 13% respectively of total salt consumed. Therefore, fortification of household salt appears to be an appropriate method of controlling iodine deficiency in both countries, although for Benin fortification of other salt sources could be considered.

INTRODUCTION

Iodine deficiency disorders (IDD) constitute one of the major public health problems in the developing world, with more than one billion people at risk. Retarded physical and mental development are important health consequences of lack of iodine in the diet (1). For adult women, the recommended daily iodine intake is 150 μg (200 μg during pregnancy) and it is 120 μg for children (7-12 y) (2). The natural iodine content of food depends on the iodine content of the soil in which it is grown. For people living in an iodine-deficient area, it is impossible to improve their iodine status by selecting foods grown locally.

In the countries involved in this study, Guatemala and Benin, iodine deficiency is still a problem of concern. While Guatemala has had a history of salt fortification for decades, a goiter rate of 20% was found in school children in 1988 (3). Benin has no program for fortifying salt until lately and in recent studies goiter rates of 45-60% have been found, which indicates that Benin is a country with a severe public health problem with respect to IDD (4).

Fortification of salt has proven to be an effective measure to combat iodine deficiency. Salt is a basic ingredient of almost every meal worldwide, so a very large target group can be reached. There are various technologies for fortifying salt with iodine; all are quite simple and inexpensive (5). In order to determine the level of iodine supplementation required for cooking and table salt, we need to know how much salt from these discretionary sources is consumed.

Several attempts have been made to assess the dietary intake of salt in individuals in different countries. These estimates are usually based on individual responses to questionnaires, national food consumption statistics, or figures for household salt purchase (5). In some developing countries, calculations of potential consumption of iodized salt are based on food balance sheet data, that is: total consumption of salt divided by the population. All these methods are suspected of inaccuracy because no allowances are made for salt losses during cooking or on the table or for the use of salt for other purposes, such as for animal feed and de-icing (6).

A relatively new method for measuring the actual consumption of salt for the individual is the lithium-marker technique (6-12). The principle of this method, first introduced by James and colleagues, is the labeling of household salt with a known amount of lithium. A metabolic study showed that 93% of the lithium in labeled salt is excreted in urine, 1.7% in feces and 1.7% in sweat (7). Because of the high proportion of lithium excreted in urine, the level of which stabilizes after six days, it is possible to calculate the consumption of labeled household salt. Leclerq et al (13) simplified the original design by showing that, at a constant intake, the excretion of lithium measured over 3 days after stabilization provides a good estimation of intake.

The recently released Guatemala Micronutrient Survey (14) estimated that the average daily household salt intake per capita is 11 g/d in rural areas. In this study, salt consumption was estimated by using information on the amount of salt purchased for consumption by the whole family, the number of family members and the time in which the amount purchased was consumed. For Benin, no research on salt consumption has been done as far as we are aware. Mandated by their respective governments, the iodine content of salt is 30 to 100 μg/g in Guatemala and 30 to 50 μg/g in Benin. The present studies were designed to determine the average amount of salt consumed by individuals in these two different developing countries using the lithium-marker technique. Labeling with lithium instead of iodine has the advantage that lithium is not taken up by the body and that there is no or relatively little interference from other sources. There is some consumption of iodized salt already by the populations in both Guatemala and Benin. In this way, information was obtained about the potential contribution of discretionary salt, which could be fortified, to the iodine supply in Guatemala and Benin. The usefulness of the lithium-marker technique in these countries will also be discussed.

SUBJECTS AND METHODS

Subjects

In Guatemala, nine children aged 6 to 9 y and their mothers participated in the study, carried out in January and February 1996. All lived in Buena Vista, a hamlet of San Pedro Sacatepéquez, where the ambient temperature is 12 - 25 °C during the first two months of the year. Although it is only about 20 km from the capital, the area is still very rural. The subjects were selected non-randomly by seeking families with a son in the target age-range and, asking them to participate after an explanation of the study. In Benin, the selection of subjects was based on the population of an ongoing study with school children in the rural village of Pénessoulou, Sous-Préfecture of Bassila in the province of Atacora. After an information meeting for parents, thirteen children (aged 8 to 12 y) and their mothers were selected. The fieldwork here also took place in January and February 1996, and the ambient temperature during these months was 10 – 32 °C. The parents' consent was obtained after they had been given a full explanation of the study in their own language, which is Spanish or Cakchiquel in Guatemala and Anii in Benin. The Guatemalan study was approved by the CeSSIAM Ethical Committee on Studies with Human Subjects and the Beninese study by the Medical Ethics Committee of the University of Benin in Cotonou.

Preparation of the labeled salt

The lithium-labeled salt given to the subjects was prepared by AKZO-Nobel (Hengelo, The Netherlands), according to the method described by Sanchez-Castillo et al (8). Sodium chloride (NaCl) and lithium carbonate (Li_2CO_3) was mixed in the proportion of 92 g Li_2CO_3 per kg salt. Portions of this mixture were placed in alumina crucibles (Alsint Haldenwanger, Berlin, Germany) and heated in a furnace at 900 °C. After cooling, the labeled salt was milled into fractions of different size. From this premix, 2 kg were mixed with 18 kg of normal, unlabelled household salt. Of this 20 kg, one half was used in Guatemala, and the rest in Benin. The grain size of the salt in Guatemala was set at 300-800 μm, as the general salt in Guatemala is quite coarse, while for Benin the grain size was 150-630 μm.

The limits of the lithium content were checked, after packing the salt in plastic bags of 250 g each, by taking samples of 10 g from every 4th bag and sending these to Wageningen Agricultural University for analysis. The Guatemalan samples showed a mean content of 1,575 mg Li per kg of salt (CV: 3.7%) and the Beninese samples 1,579 mg Li per kg (CV: 19.6%). Unfortunately the CV of the lithium content of the salt in Benin was somewhat large, probably due to settling out of the lithium in the stored salt.

Experimental design

The experimental period lasted ten days for each family in Guatemala and seven days in Benin. On Day 0, one day before the start of the intervention, the subjects collected urine for 24 h. As water and some vegetables may be a source of lithium (7) this was used to measure the basal excretion of lithium as well as sodium and chloride. During the experimental days, the families used the lithium-labeled salt instead of their usual household salt. In both countries, all pre-existing salt in the households was purchased so that no unlabeled salt was available in the households during the study. In Guatemala, 24-h urine samples were collected on Days 7, 8 and 9 for measurement of urinary lithium, sodium and chloride excretion while using the labelled salt. In Benin, this was done on Days 5 and 7.

Urine collections

Labeled plastic bottles were distributed to the mothers and children for collection and storage. Smaller bottles were also used to collect urine at school (with the help of the teachers), the field or the market. Thymol was used as a preservative (ca. 1 g per bottle) in Benin. Urine collections were planned so that they did not take place during the menstrual period of the women. On the day the collection began, the first urine voided on rising was not collected, but all subsequent urine voided over the next 24-h period, ending with the first urine voided on the following day, was collected. Total volume of the urine was calculated by weighing it on a digital balance, assuming that the density of urine is 1.000 kg m^{3}. Then, the urine was transferred in duplicate into plastic, screw top test tubes. The aliquots were placed in a freezer within 24 h of collection. All urine samples were sent to the laboratory of the Division of Human Nutrition and Epidemiology of Wageningen Agricultural University and stored at −18 °C until further analysis.

Analysis of urine samples

Urinary sodium and lithium were measured on an atomic absorption spectrophotometer (Perkin-Elmer 2380, Norwalk, Connecticut, USA). Chloride was measured using a coulometric method with a Chloor-o-counter (type 77, Marius, Utrecht, The Netherlands). All measurements were carried out in duplicate.

Calculations

Because of possible intake of sodium as the sodium monoglutamate or other non-chloride sodium sources in foods, total salt intake was calculated from the urinary output of chloride, assuming that non-sodium forms of dietary chloride were negligible. The lithium output derived from the labeled salt was calculated from the mean on Days 7 to 9 (Guatemala) or Days 5 and 7 (Benin), corrected for the background lithium excretion on Day 0. Leclercq et al (13) reported that 95 ± 6% (mean ± SD) of lithium consumed is excreted in urine. Discretionary salt consumption was calculated by dividing the excretion of lithium (g/d) for each day urine was collected from each subject by the proportion of lithium in the salt. Individuals' means were calculated for all days of complete urine collection. Finally, the means of groups of mothers and of children were calculated. Discretionary salt consumption was expressed as a percentage of total salt intake.

Statistics

All statistics were carried out using SAS, version 6.09 (15). All variables were checked for normality using tests for skewness, kurtosis, Shapiro/Wilk, Stem Leaf and Normal probability plots. Because all variables were found to be reasonably normal distributed, the differences between groups and days were compared using Student's t-test.

RESULTS

The mean ages of the Guatemalan mothers and children were 37.4 and 8.1 y, respectively; for the Beninese, these were 38.0 and 9.7 y, respectively. Beninese mothers had a mean height of 1.58 ± 0.03 m (mean ± SD) and their weight was 58.5 ± 10.9 kg. Their sons had a height of 1.26 ± 0.06 m and a weight of 24.1 ± 3.0 kg. Anthropometric data for the Guatemalan subjects are not available.

In Guatemala, 56 of the 72 urine collections (78%) were complete. The 16 collections reported as incomplete were distributed over 11 subjects. In Benin, all 78 collections (100%) were complete. The collections were classified as complete based on self-report criteria. The urinary volume, ion excretion and data on salt intake are shown in Table 1. Mothers had a total salt intake of 5.2 ± 1.7 g/d (mean ± SD) in Guatemala and of 9.0 ± 2.9 g/d in Benin based on chloride excretion. Discretionary salt contributed 77 ± 24% of the total salt ingestion of Guatemalan mothers and 52 ± 14% of that of Beninese mothers. Guatemalan children consumed a total amount of 1.8 ± 0.6 g salt/d of which 72 ± 12% came from a discretionary source. For Beninese children, the mean total salt intake was 5.7 ± 2.8 g/d. Discretionary salt contributed 50 ± 13% to their total intake. In both countries, total salt intake and discretionary salt intake were lower in the juvenile group than in the maternal group ($P < 0.01$). No significant differences were found for mean sodium and chloride excretion during the experimental days compared to the baseline values, except for Guatemalan boys, where absolute excretion of both sodium and chloride was 40% lower ($P<0.05$) during the experimental days (Table 1). Also, urine volume was significantly ($P<0.01$) lower in Guatemalan boys during days 7 – 9 compared to the baseline collection. The molar concentration of sodium and chloride was not different between days.

No significant differences in salt intake between Beninese mothers and their sons were found when their body weights were taken into account. Total salt intake on a body weight basis for Beninese women was 0.16 ± 0.05 g/kg bodyweight and for their sons 0.23 ± 0.10 g/kg bodyweight. In both countries, no significant differences between mothers and children were found in the proportion of discretionary salt. In Guatemala, the difference in mean urine volume between mothers and children was significant ($P<0.001$). Because salt intake is not always normally distributed, it may be better expressed by median values, which is also included in Table 1. As the data are normally distributed, mean and median are very similar.

Table 1. Daily ion excretion and total and discretionary salt intake in mothers and their sons in Guatemala and Benin (mean ± SD)

	Guatemala				Benin			
	Day 0		Mean Days 7-9[1]		Day 0		Mean Days 5 and 7[2]	
	Mothers (n=8)	*Sons (n=7)*	*Mothers (n=9)*	*Sons (n=9)*	*Mothers (n=13)*	*Sons (n=13)*	*Mothers (n=13)*	*Sons (n=13)*
Mean urine excretion (L/d)	1.18±0.37	0.71±0.09	1.10 ± 0.24	0.56 ±0.24[b,*]	1.13 ± 0.40	0.82 ± 0.28	1.20 ± 0.36	0.74 ± 0.21
Mean ion excretion (mmol/d)								
Sodium	89 ± 28	53 ± 20	93 ± 31	33 ± 12[a]	146 ± 46	97 ± 40	143 ± 48	118 ± 43
Chloride	89 ± 36	51 ± 16	88 ± 29	31 ± 11[a]	148 ± 51	102 ± 38	140 ± 51	124 ± 38
Lithium	0.00±0.01	0.00±0.01	0.89 ± 0.43	0.28 ± 0.12	0.00 ± 0.02	0.00 ± 0.02	1.01 ± 0.49	0.86 ± 0.40
Mean estimated salt intake[3] Total (g/d)	-	-	5.2 ± 1.7 (5.3)	1.8 ± 0.6[+] (1.8)	-	-	9.0 ± 2.9 (8.0)	5.7 ± 2.8[+] (5.1)
Discretionary (g/d)	-	-	3.9 ± 2.0 (3.4)	1.3 ± 0.6[+] (1.3)	-	-	4.7 ± 1.9 (3.9)	2.9 ± 1.9[+] (2.3)
Discretionary (%)	-	-	77 ± 24 64)	72 ± 12 (72)	-	-	52 ± 14 48)	50 ± 13 (45)

1) Calculated over 3 days.
2) Calculated over 2 days.
3) Medians in parentheses.
a and b Significant differences between experimental days and baseline for a $P<0.05$ and b $P<0.01$.
* and + Significant differences between mothers and children for * $P<0.001$ and + $P<0.01$.

Table 2. Comparison of published estimates of salt intakes (mean ± SD) assessed by the lithium-labeling method as reported in the literature

Site	Subjects	N	Total salt	Discretionary salt		Reference
			g	g	%	
U.K.	Adult men	33	10.6 ± 0.55	1.22	11	Sanchez-Castillo et al, 1987 (9)
U.K.	Adult women	50	7.4 ± 0.29	0.91	12	Sanchez-Castillo et al, 1987 (9)
Italy	Adult men	7	9.35 ± 1.75	2.63 ± 1.17	32 ± 12	Leclerq et al, 1990 (13)
Italy	Adult men	71	11.2 ± 3.4	4.0 ± 2.3	36 ± 17	Leclerq et al, 1991 (16)
Italy	Adult women	7	7.66 ± 2.81	2.16 ± 0.82	32 ± 15	Leclerq et al, 1990 (13)
Italy	Adult women	78	9.4 ± 3.3	3.8 ± 2.4	39 ± 18	Leclerq et al, 1991 (16)
Italy	Children 8-12 y	5	6.14 ± 2.10	1.64 ± 0.82	24 ± 11	Leclerq et al, 1990 (13)
Italy	Children 8-12 y	121	7.7 ± 2.5	2.5 ± 1.3	34 ± 15	Leclerq et al, 1991 (16)
Benin	Adult women	13	9.0 ± 2.9	4.7 ± 1.9	52 ± 14	Present study
Guatemala	Adult women	9	5.2 ± 1.7	3.9 ± 2.0	77 ± 24	Present study
Benin	Boys 8-12 y	13	5.7 ± 2.8	2.9 ± 1.9	50 ± 13	Present study
Guatemala	Boys 6-9 y	9	1.8 ± 0.6	1.3 ± 0.6	72 ± 12	Present study

Table 3. Comparison of the iodine content of salt mandated by the government, recommended by WHO (5) and the desirable content based on salt consumption in Guatemala and Benin

	Guatemala	Benin
Mandatory iodine content of salt (mg/kg)	30 – 100	30 - 50
WHO recommendation (mg/kg)	40 – 60[1]	20 – 30[2]
Desirable iodine content of salt, based on median salt intake of women (mg/kg)	44	38
Desirable iodine content of salt, based on median salt intake of children (mg/kg)	92	52

[1)] Assuming a daily salt consumption of 5 g/person the recommendation for retail sacks varies from 40 mg/kg for the cool dry climate and 60 mg/kg for the warm moist climate. In Guatemala both climates can be found.
[2)] Assuming a daily salt consumption of 10 g/person the recommendation varies from 20 mg/kg for the cool dry climate and 30 mg/kg for the warm moist climate. Both climates can be found in Benin.

DISCUSSION

In the work of James and colleagues (12), the application of the lithium-marker technique was aimed at providing advice to individuals at risk of hypertension. Such people need to know how to reduce salt intake, especially which sources of salt in food should be reduced. In the present studies, we have extended the application of this technique to the estimation of adequate fortification levels for iodine in commercial salt supplies in order to control IDD in a developing country setting.

Moreover, in contrast to the situation in the United Kingdom (10), where only 15% of the sodium excreted came from salt used for cooking and for adding at the table (discretionary salt), the corresponding figures for the specific populations involved in Guatemala and Benin were 75% and 50% respectively. Although the precision of our studies was low because of the small number of subjects studied, our estimates are 3 to 5 times higher than the proportion of discretionary salt in the United Kingdom (10). They almost certainly reflect real differences from the United Kingdom studies and a difference between the subjects in Guatemala and Benin. We speculate that the practices contributing to the relatively lower contribution of discretionary salt per se in Benin is the use of condiment cubes (Maggi cubes ®, Nestlé S.A. cubes, Vervey Switzerland, and similar products from other manufacturers). These are used in sauces for eating with staple foods and are rich sources of salt.

With the present study, there are now four reports of data on salt intake of populations of four discrete societies studied using a lithium-labeled salt technique (Table 2). The first two studies were from European countries in temperate climates, and involved adults in both, as well as children in Italy. With Guatemala and Benin, we add data from tropical latitudes and involving two groups of mothers and their male offspring. As shown in Table 2, the Beninese women in our study consumed more salt than European women, whereas Guatemalan mothers consumed less. Salt intakes from all sources for Beninese children are the equivalent to that of adult Guatemalan women. The lowest average daily salt intakes yet documented by this method are those of the children from Guatemalan households, at about 2 g per day.

Physiological adaptation, expressed through cultural factors, however, may explain more of the two-fold difference in total salt intake across these two nations. Indeed, both are in the tropical latitudes, but the indigenous population of Buena Vista live in the highlands, where the ambient temperature is 12-25 °C in the first two months of the year, and humidity is low. The Beninese community of Pénessoulou is close to sea-level in a climate that is both humid and hot, with ambient temperatures in January and February of 10-32 °C. It may be that higher salt intakes are physiologically appropriate to compensate for higher evaporation losses in the hotter African setting.

Another notable finding is that the discretionary salt consumption estimates are much lower by this urinary excretion method than those based on other methods for assessing salt intake (14,17). The question arises as to how much of this difference can be explained by incompleteness of urine collections. Without the benefit of external markers, such as para-amino benzoic acid (PABA) (18) or intrinsic markers, such as creatinine from endogenous muscle turnover, it is difficult to verify the completeness of urine collections. We opted to address the issue of collection losses by relying on self-reported criteria. As all urine collections that were reported as incomplete were left out of the calculations and the measured 24-h volumes of urine between the women (Table 1) are not different, we feel that the differences between the lithium-marker technique and other methods of estimating discretionary salt intake are not based on a major error in the lithium-marker technique. Of all population groups only the Guatemalan boys showed a decrease in absolute excretion of sodium and chloride during the experimental days, coupled with a decreased urine volume and steady sodium and chloride concentrations. This may have been caused by incomplete collections of urine during the experimental days. This would lead to an underestimation of the salt consumption by this group of about 40%, but even if corrected by this proportion, salt consumption would still be quite low. In addition, the estimate of the proportion of discretionary salt consumed is not affected by incomplete collection of urine, as shown by Leclercq et al (13). Overall, subjects did not change their salt intake habits, although the lithium-labeled salt was less coarse than the salt they were used to. Though the authors are aware of the limits of this study, the results are nevertheless striking. For Guatemala, the national discretionary salt intake per capita estimated by data on household salt purchases is 11 g (14) whereas we found it to be 4 g for adult women, based on the urinary lithium-marker technique. Even if we underestimated the salt consumption by 50% due to incomplete collections, salt consumption would still be much lower than expected from estimates using other methods.

In Table 3, we illustrate a comparison of the mandatory iodine content of salt in Guatemala and Benin with the recommendations of WHO. We have undertaken an exercise of assigning the levels of iodine that would be needed in the salt of the respective areas to meet the daily recommended intakes of 150 μg for adult women and 120 μg for children in the present age-range. A number of assumptions and caveats are worth examining. The first is that there are no systematic errors and that the estimates of total salt intake based on chloride excretion and of discretionary salt intake are accurate. Although in both countries the same original mix was used, the lithium in the salt in Benin was variable (CV: 19.6%). This might have been a cause of error in the outcome of the calculations for Benin. Losses of lithium in sweat in England during wintertime were small as determined by Sanchez-Castillo et al (7), but it is likely that Guatemalan and Beninese people will sweat more due to the climate and exertion. However, analysis of sweat of physically active people, after ingestion of lithium, showed that sweat is not an important route of lithium excretion (19).

Beyond the specifics of the accuracy of methods would be the issue of how prudent it is to generalize our particular values beyond the two isolated rural populations in their respective nations. In this regard, there is a premise that the rural populations will be more vulnerable to IDD than urban ones. It would then be important that the two areas chosen -- Buena Vista in Guatemala and Pénessoulou in Benin -- be typical, rather than atypical, of the dietary total and discretionary salt use patterns of the rural societies of the respective nations. Finally, beyond the representative nature of the populations are issues based on the sample-sizes that our limited supply of lithium-labelled salt permitted us to study. It can be asked whether the total of 26 person-days of excretion covered by the Beninese design for each age-group and the 27 person-days of excretion per age-group in Guatemala were sufficiently representative to reflect the usual, year-round pattern for the respective localities. On a person-to-person basis, the same concern can be voiced for the true stability of two days of observations in Benin and the three days in Guatemala, in order to define "usual" intakes for individuals reflecting the intake of individuals throughout the year.

Assuming the aforementioned assumptions and premises are valid, the recommendable fortification levels of iodine for the 3.4 and 3.9 g/day median discretionary salt intakes for the women of Guatemala and Benin in order to achieve their 150 μg/d would be 44 and 38 μg/g respectively (Table 3). Based on this common experience of the central tendency hypothesis, the iodization levels to protect children would be 92 and 52 μg/g in the respective nations. The currently mandated levels for both nations would embrace these estimates of iodine fortification, but the WHO range would not be protective.

In conclusion, fortification of household salt is a promising approach to overcoming IDD in the rural areas of both countries. Nothing in the present study challenges this tenet. However, we can conclude that the real salt consumption varies considerably between countries and can be much lower than expected from existing research. It is obvious that this has a direct effect on iodine intake of a population. Therefore, we think it is necessary to examine salt intake patterns carefully within the framework of iodine fortification programs.

Acknowledgments

We want to thank Dr. Ivania Mena and the mothers' committee of Buena Vista (Guatemala) for all the help they gave during the fieldwork. Thanks to Dr. Eric Ategbo for making the study in Benin possible. For making available lithium-labeled salt we want to thank Ir. Monique Veenendaal from AKZO-Nobel, Hengelo, The Netherlands. Also a word of thanks to Dr. Paul Hulshof and Frans Schouten for carrying out the chemical analyses.

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SUMMARY

The relationship between iodine deficiency, goiter and cretinism has long been known. However, the concept that iodine deficiency may lead to a broad spectrum of problems, ranging from abortion and stillbirth to goiter, deafness and cretinism as well as less obvious mental deficits in non-cretinous populations, evolved only relatively recently. Since the early 1980's these are collectively called iodine deficiency disorders (IDD).

A wide range of studies has been carried out to examine the relationship between iodine deficiency and physical and mental development in different population groups. An overview of these studies is given in **Chapter 7**. The first controlled trials were set up by the end of the 1960's to see whether endemic cretinism could be prevented by the administration of iodized oil to women before or during pregnancy. Results were unequivocal: endemic cretinism is prevented by supplementation with iodine prior to or early in pregnancy. Subsequently the question was addressed to what extent different degrees and duration of maternal iodine deficiency were reflected in the mental and psychomotor performance of non-cretinous children at various stages in their lives. Several observational and intervention studies also focused on the association between iodine status of children later in life or changes therein and their mental and motor performance. Although most of the observational studies demonstrated significant differences in mental and psychomotor performance between iodine deficient and iodine replete children, intervention studies in children produced mixed results. By 1994, the question whether deficits in mental performance as a result of iodine deficiency may be reversed had been dealt with in only 2 double blind placebo-controlled studies. One of these studies demonstrated a clearcut improvement, the other study encountered an improvement of iodine status of the control group, but still demonstrated an association between decrease in goiter size and increase in IQ.

Therefore, our study was designed to address the question whether mental performance of iodine deficient schoolchildren is improved by iodine supplementation. In addition other aspects associated with iodine deficiency were studied, including behavioral change and hearing thresholds and their relation with mental performance. Different indicators were used to describe iodine status and thyroid function, both at the beginning and at the end of the study: serum concentrations of free thyroxine (FT4), thyroid stimulating hormone (TSH) and thyroglobulin (Tg), urinary iodine concentration and thyroid volume. The study was carried out in an iodine deficient area of northern Benin between 1995 and 1996. The study was set up as a double blind, placebo-controlled study, involving supplementation of children with a single oral dose of iodized oil (540 mg iodine) or a placebo (poppyseed oil) in January 1996. Approximately 4 months later iodized salt was introduced into the area. This resulted in an improvement of the iodine status of the control group as measured by serum concentrations of FT4, Tg and TSH as well as urinary iodine concentration. Thus, at the end of the study, children were divided into two groups: one group of children whose iodine status, as measured by urinary iodine concentration, had improved and one group of children whose urinary iodine concentration had remained unchanged.

For assessing mental performance of the children a series of tests was chosen from the African Child Intelligence test, developed for use in East Africa, as well as some other "culture-fair" tests of general intelligence, such as the block design test from the Wechsler Intelligence Scale for Children and the Raven test. These tests do not measure any schooling-related skills or "crystallized intelligence", but rather "fluid intelligence", i.e.

the ability to reason by analogy, to apprehend an unfamiliar configuration and to construct a solution. Results showed that an improvement in iodine status as measured by urinary iodine concentration, was reflected in a significantly improved performance on the combination of tests, 10 months after supplementation (**Chapter 2**). Children whose urinary iodine concentration remained basically unchanged showed less progress in performance, even though their iodine status as measured by several variables was on average better than that of their improved counterparts, both initially and at the end of the study. This finding suggests "catch-up" of the iodine-deficient children.

Hearing thresholds were shown to be associated with iodine status as measured by serum Tg concentration (**Chapter 3**). Children with higher, i.e. less favorable, serum concentrations of Tg had higher hearing thresholds than children with lower serum concentrations of Tg. In other words: they could hear less well, especially at higher frequencies. Moreover significant negative correlations were found between hearing thresholds and performance on all mental tests except the Raven test. Thus, children who performed better on the tests could also hear better. As the hearing impairment was small and the mental tests did not address schooling-related learning, it is unlikely that children performed less well because of hearing deficits. Moreover, children with lower hearing thresholds did not differ from children with higher hearing thresholds in terms of their initial serum concentration of Tg or urinary iodine concentration. At the end of the study however children with lower hearing thresholds had a lower, i.e. better, serum concentration of Tg and higher urinary iodine concentration than the children with higher hearing thresholds. This suggests that the differences in hearing thresholds result from a recent differentiation in iodine status.

Several studies have shown that, while median urinary iodine concentration and thyroid volume were indicative of iodine deficiency in the populations studied, mean serum concentrations of TSH and FT4 were found to be within the normal range. In our study we evaluated the suitability of serum concentrations of Tg, TSH and FT4, thyroid volume and urinary iodine concentration as indicators of change in iodine status and thyroid function under conditions of an increase in iodine supply (**Chapter 4**). Initial mean serum concentrations of TSH and FT4 were within the normal range, whereas serum Tg concentration, urinary iodine concentration and thyroid volume were indicative of moderate to severe iodine deficiency. As iodized salt had become available in the study area a few months after supplementation with iodized oil, both the supplemented group and the non-supplemented group showed significant improvement on all variables studied with the exception of thyroid volume, which decreased in the supplemented group only. Thyroid volume and serum concentrations of TSH and FT4 were not significantly different between the supplemented and non-supplemented groups 10 months after supplementation. The supplemented group still had significantly lower serum Tg and higher urinary iodine concentrations than the non-supplemented group. The latter two indicators would be the most suitable for measuring change in iodine status, but methods for estimating serum Tg concentration need to be standardized before this indicator can be used widely in iodine deficiency control programmes.

Hypothyroidism has been associated with an increased risk for cardiovascular disease through different mechanisms, including hyper-homocysteinemia. We assessed the serum concentration of total homocysteine (tHcy) at the end of the intervention period and examined whether there is an association with iodine status (**Chapter 5**). The geometric mean serum tHcy concentration of 8.2 μmol/L (range 2.1- 14.9 μmol/L) was high as compared to findings in other countries. However, as there is not yet a normal reference range for tHcy in children, it is not possible to judge whether or not tHcy was elevated in this group. Children with higher serum TSH concentrations or with goiter tended to have higher serum tHcy concentrations than children with lower serum concentration of TSH or without goiter, but the differences were not significant. At the time of tHcy measurement, the children were still mildly iodine deficient, based on their median urinary iodine concentration, but might be considered euthyroid, based on mean serum concentrations of TSH and FT4. This may explain why we found no significant association between tHcy and indicators of thyroid function.

To examine the question whether an improvement of iodine status is associated with behavioral change, a School Behavior Checklist was used. This checklist has been developed for assessing four of the "Big Five" dimensions of personality, i.e. extraversion, conscientiousness, agreeableness and emotional stability (**Chapter 6**). The list was completed by the teachers of the children included in the study, both at the beginning and at the end of the study. Factor analysis of the responses of the teachers at baseline indicated a good agreement with the four-factor model on which the checklist is based. The reliability coefficients for the scales for agreeableness and emotional stability proved to be less satisfactory than those for conscientiousness and extraversion. No correlation was found between the children's marks in 1995 and those in 1996. At the end of the study, the sums of the marks for school achievement as well as the mean scores on the behavior scales for extraversion, agreeableness and emotional stability had decreased significantly in the group as a whole. No association was found between the decline in school achievement and change in iodine status. The change in iodine status was found to contribute to change in emotional stability and agreeableness in the younger children only.

In conclusion it may be said that a major research question, i.e. whether a mental deficit resulting from iodine deficiency may be reversed and if so, through which mechanisms, has not yet been answered unequivocally. Such studies are increasingly difficult to carry out in view of salt iodization programmes being implemented all over the world, but remain pertinent. Iodine status was improved considerably in our study population, not only because of the supplementation with iodized oil, but also because of an increase in access to iodized salt. Data collected in 2000 show that about 90 % of households in Benin have access to iodized salt. However, close monitoring of iodine intake and supply, especially in more backward areas, remains crucial.

Apart from iodine deficiency, another obstacle to socio-economic progress in the underprivileged parts of the country is the poor quality of education. In order to ensure that children live up to their full potential, access to iodized salt is a must, but so is the provision of good education.

SAMENVATTING (Dutch Summary)

Het verband tussen jodiumgebrek, struma (krop) en cretinisme is al lange tijd bekend. De opvatting dat een tekort aan jodium kan leiden tot een breed scala aan problemen, van abortus en doodgeboorte tot krop, doofheid en cretinisme alsook tot minder in het oog vallende beperkingen in mentaal functioneren bij niet-cretins, werd echter pas vrij recent gemeengoed. Sinds het begin van de tachtiger jaren worden deze afwijkingen als gevolg van een jodiumtekort samengevat met de Engelse term "Iodine Deficiency Disorders" (IDD).

Een hele reeks studies is uitgevoerd om het verband te onderzoeken tussen jodiumtekort en lichamelijke en geestelijke ontwikkeling in verschillende bevolkingsgroepen. Een overzicht van deze onderzoeken wordt gegeven in **hoofdstuk 7**. De eerste gecontroleerde studies werden tegen het eind van de zestiger jaren uitgevoerd om te onderzoeken of endemisch cretinisme voorkomen kon worden door het toedienen van gejodeerde olie aan vrouwen voor of tijdens de zwangerschap. De resultaten waren eenduidig: cretinisme wordt voorkomen door jodiumsuppletie vóór of in een vroeg stadium van de zwangerschap. Vervolgens onderzocht men in welke mate verschillende gradaties en duur van jodiumtekort bij de moeder van invloed waren op het mentaal en psychomotorisch functioneren van hun ogenschijnlijk normale kinderen in verschillende levensfases. Diverse observatie- en interventiestudies richtten zich toen ook op het verband tussen (veranderingen in) jodiumstatus en mentaal en psychomotorisch functioneren van kinderen op oudere leeftijd. Hoewel de meeste observatiestudies significante verschillen aantonen tussen kinderen die jodiumdeficiënt zijn en kinderen die dat niet zijn, waren de resultaten van de interventiestudies niet eenduidig. In 1994 waren er nog maar 2 dubbelblinde placebo-gecontroleerde onderzoeken uitgevoerd naar de vraag of een achterstand in mentaal functioneren als gevolg van een jodiumtekort reversibel is. Een van deze onderzoeken toonde een duidelijke verbetering aan als gevolg van jodiumsuppletie van schoolkinderen, het andere onderzoek kreeg te maken met een onverwachte verbetering van de jodiumstatus van de controle-groep, maar toonde wel een verband aan tussen een afname van de omvang van de schildklier en een stijging in IQ.

Onze studie was derhalve opgezet om te onderzoeken of het mentaal functioneren van jodiumdeficiënte schoolkinderen verbeterd wordt door jodiumsuppletie. Bovendien werden andere aspecten van het functioneren van kinderen die mogelijkerwijs beïnvloed worden door jodiumtekort en die eventueel ook een interactie vertonen met mentaal functioneren, zoals gehoor en gedrag, meegenomen in het onderzoek. Verschillende indicatoren werden gebruikt om de jodiumstatus en schildklierfunctie te beschrijven, zowel aan het begin als aan het eind van de studie: concentraties in serum van vrij thyroxine (FT4), thyrotropine of schildklier stimulerend hormoon (TSH) en thyroglobuline (Tg), de concentratie van jodium in urine en het schildkliervolume. Het onderzoek werd uitgevoerd in een jodiumdeficiënt gebied in noord-Benin tussen 1995 en 1996. Het onderzoek was opgezet als een dubbelblind, placebo-gecontroleerd onderzoek, waarbij kinderen eenmalig een orale dosis gejodeerde olie (540 mg jodium) danwel een placebo (papaverzaadolie) kregen in januari 1996. Ongeveer 3-4 maanden later werd gejodeerd zout geïntroduceerd in het onderzoeksgebied. Dit leidde tot een verbetering van de jodiumstatus van de controle (placebo) groep, zoals bleek uit de serum concentraties van FT4, Tg en TSH alsook uit de concentratie van jodium in de urine. De kinderen werden aan het eind van het onderzoek dientengevolge in 2 groepen ingedeeld: een groep kinderen van wie de jodiumstatus, bepaald aan de hand van de jodiumconcentratie in urine, verbeterd was en een groep kinderen bij wie de jodiumconcentratie in urine niet veranderd was.

Voor het vaststellen van het mentaal functioneren van de kinderen werd een aantal testen genomen uit de Afrikaanse Kinder Intelligentie Test, ontwikkeld voor gebruik in Oost Afrika, alsook enkele algemene, zogenoemde "culture-fair" intelligentie-tests, zoals de blokken-test uit de Wechsler Kinder Intelligentie Test en de Raven test . Deze testen meten geen onderwijs-gebonden vaardigheden ("crystallized intelligence"), maar veeleer "fluid intelligence", ofwel de mate waarin een kind redeneert op basis van analogieën en daarmee een onbekend vraagstuk kan doorgronden en met een passend antwoord kan komen. De resultaten laten zien dat, 10 maanden na de suppletie, kinderen met een verbeterde jodiumstatus, gemeten aan de hand van jodiumconcentratie in urine, een significant grotere vooruitgang op de mentale testen boekten dan de kinderen bij wie de jodiumstatus niet veranderd was **(hoofdstuk 2)**. Deze laatste groep kinderen toonde een veel kleinere vooruitgang, ofschoon hun jodiumstatus gemiddeld genomen beter was dan die van hun "verbeterde" maatjes, zowel aan het begin als aan het eind van het onderzoek. Deze bevinding suggereert een inhaalslag ("catch-up") van de jodiumdeficiënte kinderen.

De gehoordrempels bleken geassocieerd te zijn met jodiumstatus gemeten aan de hand van de concentratie van Tg in serum **(hoofdstuk 3)**. Kinderen met hogere, dwz. minder gunstige, concentraties van Tg in serum hadden hogere gehoordrempels dan kinderen met lagere Tg-waarden, oftewel: zij konden minder goed horen, vooral bij hogere frequenties. Bovendien werden er significante negatieve correlaties gevonden tussen gehoordrempels en scores op alle mentale testen, behalve de Raven test. De kinderen die het beter deden op de testen, hadden lagere gehoordrempels. Aangezien de mate waarin het gehoor was aangetast, beperkt was en de mentale testen niet onderwijs-gebonden waren, is het niet waarschijnlijk dat de kinderen het op de testen minder goed deden vanwege eventuele hoorproblemen. Bovendien verschilden de kinderen in de tertielen met de hoogste danwel laagste gehoordrempels niet van elkaar voor wat betreft de concentraties van Tg in serum of van jodium in urine aan het begin van de studie. Aan het eind van het onderzoek echter hadden de kinderen met lagere gehoordrempels een lagere, ofwel betere, concentratie van Tg in serum en een hogere jodiumconcentratie in de urine dan kinderen met hogere gehoordrempels. Dit suggereert dat de verschillen in gehoordrempels voortkomen uit een recente differentiatie in jodiumstatus.

Verschillende studies hebben aangetoond dat terwijl de mediaan-waarden voor jodiumconcentratie in urine en schildkliervolume indicatief waren voor een jodiumtekort in de onderzochte populaties, de gemiddelde concentraties van zowel TSH als van FT4 in serum normaal waren. In onze studie onderzochten we de geschiktheid van de concentraties van Tg, TSH en FT4 in serum, de concentratie van jodium in urine alsook het schildkliervolume als indicatoren van veranderingen in jodiumstatus en schildklierfunctie in een situatie van toename in jodiumvoorziening **(hoofdstuk 4)**. De concentraties van TSH en FT4 in serum vielen binnen de normale range, terwijl de concentraties van Tg in serum en die van jodium in urine, alsmede het schildkliervolume wezen op een matig tot ernstig jodiumgebrek. Nadat er enkele maanden na aanvang van de studie gejodeerd zout in het onderzoeksgebied werd geïntroduceerd, toonden zowel de gesuppleerde als de placebo-groep een significante verbetering van alle indicatoren, met uitzondering van het schildkliervolume, dat alleen significant afnam in de gesuppleerde groep. Tussen de gesuppleerde en de niet-gesuppleerde groep bestonden 10 maanden na de suppletie geen significante verschillen voor wat betreft het schildkliervolume en de serumconcentraties van TSH en FT4. De gesuppleerde groep had echter nog wel een significant lagere concentratie van Tg in serum en een significant hogere concentratie van jodium in urine dan de niet-gesuppleerde groep. De twee laatstgenoemde indicatoren zijn naar onze mening dan ook het meest geschikt voor het meten van veranderingen in jodiumstatus. De methoden voor de bepaling van de serum Tg concentratie moeten echter gestandaardiseerd worden voordat toepassing in bevolkingsonderzoek zinvol is.

Hypothyreoïdie wordt geassocieerd met een verhoogde kans op hart- en vaatziekten, waarbij verschillende mechanismen een rol kunnen spelen, waaronder hyperhomocysteinemie. In ons onderzoek is aan het eind van de interventieperiode de serumconcentratie van totaal homocysteine (tHcy) bepaald en onderzocht of er een associatie is met jodiumstatus (**hoofdstuk 5**). Het gevonden geometrisch gemiddelde voor de serum tHcy concentratie van 8.2 µmol/L (spreiding 2.1-14.9 µmol/L) was hoog in vergelijking met bevindingen in andere landen. Daar er echter nog geen normaal-waarden bestaan voor tHcy in kinderen, is het niet mogelijk om aan de gevonden waarde een oordeel te verbinden. Kinderen met hogere TSH-waarden of met een groter schildkliervolume hadden hogere serumconcentraties van tHcy, maar de verschillen waren niet significant.

Om te onderzoeken of een verbetering in jodiumstatus ook geassocieerd is met een verandering in gedrag, werd een Schoolgedrag Beoordelingslijst gebruikt. Deze lijst is ontwikkeld voor het beschrijven van 4 van de "Grote Vijf" persoonlijkheidsdimensies, te weten: extraversie, werkhouding, aangenaam gedrag en emotionele stabiliteit (**hoofdstuk 6**). De vijfde dimensie, intelligent gedrag, is hierin niet opgenomen. Tevens werden de schoolcijfers van de kinderen verzameld. De gedrag beoordelingslijst werd ingevuld door de onderwijzers van de kinderen, zowel aan het begin als aan het eind van de studie. Een factoranalyse van de antwoorden van de leerkrachten aan het begin van de studie gaf een goede overeenkomst met het 4-factoren model waarop de lijst is gebaseerd. Aan het eind van het onderzoek, bleken de geaggregeerde schoolcijfers alsook de gemiddelde scores op de gedragsschalen voor extraversie, aangenaam gedrag en emotionele stabiliteit significant gedaald te zijn. Er werd geen verband gevonden tussen de cijfers van de kinderen in 1995 en die in 1996. Er werd evenmin een verband gevonden tussen de afname in schoolresultaten en de verandering in jodiumstatus. De verandering in jodiumstatus droeg voor een klein, doch significant deel bij aan de verandering in emotionele stabiliteit en aangenaam gedrag bij alleen de jongere kinderen.

Concluderend kan gesteld worden dat een belangrijke onderzoeksvraag, namelijk of een achterstand in mentaal functioneren als gevolg van jodiumdeficientie reversibel is, en zo ja, via welke mechanismen, nog niet eenduidig beantwoord kan worden. Ofschoon steeds moeilijker vanwege de zoutjoderings-programma's die overal ter wereld worden opgezet en uitgevoerd, blijft het doen van dergelijk onderzoek relevant. De jodiumstatus van onze onderzoekspopulatie verbeterde aanzienlijk, niet alleen vanwege de suppletie met gejodeerde olie, maar ook vanwege de toegenomen toegang tot gejodeerd zout. Recente gegevens uit Benin (2000), tonen aan dat ongeveer 90% van de huishoudens toegang zou hebben tot gejodeerd zout. Met name in slecht bereikbare gebieden zijn er echter nog steeds mensen die verstoken zijn van dit "kostbare" element. Een nauwlettend toezien op een adequate jodiumvoorziening en opname blijft derhalve cruciaal.

Afgezien van jodiumdeficientie lijkt een ander obstakel voor sociaal-economische vooruitgang in de minder bevoorrechte gebieden van Benin de slechte kwaliteit van het onderwijs te zijn. Voor een optimale ontwikkeling van kinderen is niet alleen toegang tot gejodeerd zout een "must", maar ook tot goed onderwijs.

Acknowledgements

Over the past 7½ years, as this thesis came to life, I have faced the challenge of combining diverse roles and responsibilities as a manager, an "older" AIO, a wife, a mother, a daughter, sister, friend, colleague. Occasionally I have had to neglect some of these roles. I am indebted to the patience and understanding of those who bore this occasional neglect and hope that they will judge this ultimate result as sufficient compensation.

I could not have accomplished this work if it were not for the support of my husband Bart, son Vincent, daughter Suzanne, parents, close relatives and friends. Throughout the years, you all demonstrated an unlimited confidence that this undertaking would turn out well and was worth pursuing. This left me no choice but to continue.

Trust and inspiration characterized the collaboration with my promotors, Professor Jo Hautvast, Professor Clive West and Professor Nico Bleichrodt.

Jo, after having been one of the first nutritionists to graduate from your department, I am now one of the last to obtain a PhD degree under your supervision. In the 28 years between these two events, you even were my boss for some years. When at some point in time, I suggested that I was still interested in doing a PhD study, there was not a shred of doubt in your response: the appointment to discuss the details of getting started was made immediately. I have learned a lot from you in your various roles as professor, boss, supervisor and coach and always valued your capacity for being unconventional, thinking strategically and seizing opportunities when they arise.

Clive, your enormous drive and dedication to education and research is exemplary for all your PhD candidates. I could not have wished for a more competent superviser. You demand a lot from your students, but even more from yourself, and so nobody has any problems with the occasionally delayed appointment or the disappearnce of a manuscript in the enormous piles on your desk. Supervising an older person like me, who may think she knows better, is not always easy. Yet I learned from you a tremendous amount about scientific research as well as about personal dedication. I would eagerly ask ask you to be my supervisor again if the opportunity were there.

Nico, after your earlier adventures with resarchers from Wageningen in Malawi, it did not take long to get you interested and involved in our study in Benin. Your optimism, your perpetually good mood, the ample time that you made available for me to discuss methods and papers, your presence in Benin to train the testers, all these inputs have helped me greatly. I hope that you look back on this period with satisfaction and that we have an opportunity to work together again.

In Benin I was able to build on the excellent cooperation that already existed between the Division of Human Nutrition and Epidemiology, Wageningen University and the Department of Nutrition and Food Science of the Faculty of Agriculture, National University of Benin. I fully relied on the support of Dr. Eric Ategbo. Eric, the preparations in the field, the contacts, the logistical support, the recruitment of staff, I left it all to you and your staff in Cotonou -Waliou, Romain and others- and you never once failed. Merci pour tout. Thank you also for being my paranimf.

Another crucial contact in Cotonou, a person who was always supportive and hospitable when we fled the hardship of the countryside was Erik Frederiks, liaison for Wageningen University with the University of Benin. Erik, thanks for sharing the luxury

of a house with a shower and a well-filled refrigerator. Thank you also for the much more important fact that you took me to the hospital just in time.

Quite a number of people were actively involved in the actual field work. In 1995: the Dutch MSc students Marja Rozendaal and Monique Gerichhausen and the Beninese colleagues Basile, Damien, Pierre and Charlemagne. In 1996 the MSc students Marja Klijn and Almi Hanselman and the Beninese colleagues Damien and Pierre again as well as Alex and Jean. They all worked hard on administrating the mental and psychomotor tests, collecting data on socio-economic characteristics, food habits, children's age, hearing thresholds, etc. Apart from doing a lot of work, the "girls", the two Marja's, Monique and Almi were sociable and indispensable companions. Pierre Koukoui deserves an extra word of thanks, because he was an accurate administrator and stayed on after everybody left to finalize the work in the field all by himself.

Joke Barendse from the division of human Nutrition and Epidemiology came over to help with collecting blood samples and proved to be an expert with children. Moreover, she gave moral support when the going was tough and was a wonderful companion in our women's house in Penessoulou.

The mayor of Penessoulou, the "chef traditionel", the teachers, the parents of the children and finally the children themselves were always helpful. At times, it was not easy to fully explain the objectives of the study or to allay the high expectations for what a new project was expected to bring. Our worlds were totally different, our collaboration was nevertheless excellent. Affsa and Alassane played a fabulous role in taking good care of us and in sharing with us the "in's and out's" of village life.

The blood samples of "our" children were spread all over the world so to speak: I would like to thank dr. Asibey-Berko and mr. Edmund Danso of the Micronutrient Research Laboratory of the University of Ghana at Legon, Accra; Dr. Erik Endert of the Laboratory for Endocrinology and Radiochemistry of the Academic Medical Center of the University of Amsterdam; Dr. John Arthur of Rowett Research Institute, Aberdeen, UK and staff of the laboratory of the Division of Human Nutrition and Epidemiology of Wageningen University: Peter van de Bovenkamp, Paul Hulshoff, Pieter Versloot and Truus Kosmeijer for doing all the analyses and Jan Harryvan and Joke again for giving me advice on the equipment needed for collecting blood under difficult tropical conditions.

Unicef New York through its country office in Benin made available the portable ultrasound scanner. Dr. Tom Vulsma from the Academic Medical Center was so kind to spend time during weekends to analyse the printed images of the children's thyroid glands and advise me on their interpretation. The discussions Clive and I had with him and Dr. Jan de Vijlder from the same center on the interpretation of our data and the paper that was to based on these were challenging and most useful.

Similarly the discussions, especially in the early stages of this project, with Fons van de Vijver were stimulating and helpful with respect to the study design and analysis of the results.

For statistical analyses I had to rely on other people as well: dr. Peter Dekker from the Department of Work and Organisational Psychology, Free University, Amsterdam, spent quite some time in trying to unravel some of the results. Other colleagues from the Division of Human Nutrition and Epidemiology, Jan Burema, Ben Scholte en Dirk Joghems helped me with the statistics, hardware and software needed for data analysis. Grietje van der Zee helped ordering and shipping all the goods needed in the field.

Other people in the department: prof. Frans Kok, Lidwien van der Heijden, Lous Duym, Marie Jansen, Fré Pepping and many others made me feel at home on the 3rd floor of the Biotechnion, by treating me as a colleague within the division. (Fré, thanks for always telling me the latest news). Adel den Hartog and Jupie Zwartz, both retired from the division, have always been stimulating by showing a sincere interest in my work.

This research would not have been possible if it were not for the financial support of the Nestlé Foundation and for Wageningen University, which some years ago started a special programme entitled "Onbezoldigd promoveren", specifically aimed at financing PhD research for female graduates of the university so as to have a better gender balance amongst PhD-holders of this university. Marlou Hautvast was the one to point out this opportunity to me. Marlou, thank you also for the moral support you have given over many years, both when we were colleagues at IAC and afterwards.

At IAC, many colleagues have often inquired about the progress that I was making and have shown true concern for the workload that was on my shoulders. Some people have done more than that: Annemarie Mijers, Wim Holleman and Jos Mooij have actually helped to get tables typed and manuscripts mailed in time or to get references complete.

I myself was a paranimf to my paranimf, Marianne Stasse. She has been and is an example to me both in her professional and personal life. She has had to endure more set-backs in life than most other people, but she has always remained cheerful and truly interested in what people around her are doing.

My son Vincent has been a great help with corrections and the final lay-out of this document. Neither of us will forget the time we spent during the weekends in the fall of this year at the Biotechnion. My nephiew Hugo Grondel helped out when my RSI threatened to become serious. Meanwhile Suzanne has kept our house clean for the past few years. Thus I am back where I started: with the family. My parents have instilled the feeling in me that women are not in any way inferior to men and have always encouraged me in my ambitions and Bart has kept me going. Without his continued support I would not be writing these final words of my thesis.

About the author

Tina van Ingen was born on August 7th, 1948, in Doorwerth, The Netherlands. In 1966 she obtained her gymnasium-ß diploma from the "Christelijk Lyceum" in Arnhem. The following year she spent in Wyoming, Michigan, USA as an exchange student. In 1967 she started studying at the (then Agricultural) University of Wageningen, initially in the Home Economics programme. One year later, when the new MSc programme on Human Nutrition was established, she switched to this programme, specialising in Biochemistry, Human Physiology and Nutrition. During her studies she spent six months at Columbia University, New York, N.Y. working on a study on enzymatic markers of malnutrition in rats. In January 1974 she graduated with the first batch of four nutritionists from Wageningen University and subsequently started to work at Nijmegen University, Department of Preventive Dentistry, where she was involved in the Nijmegen GVO-project (development of a health education programme for primary schools) and teaching nutrition to dentistry students.

From 1976 to 1983 she lived with her husband in Malaysia, Tanzania and Germany, where she took up various free lance, local assignments and consultancies. In 1982 she started teaching the topic "nutrition in the tropics" in the T.K.O.- courses (tropencursus voor kader in ontwikkelingswerk). In 1985 she started working for the International Course on Food Science and Nutrition (ICFSN), a project financed by Nuffic, through Wageningen University. She joined the International Agricultural Center (IAC) in 1988, when the ICFSN project was integrated into IAC's regular training programme. She was responsible for various international nutrition courses and was engaged in the development of nutrition training capacity in a number of countries. In addition she carried out missions to evaluate projects, including training activities, on behalf of different donors. Her work took her to: Benin, Botswana, Burkina Faso, China, Egypt, Ethiopia, Indonesia, Kenya, Lesotho, Malawi, Malaysia, Niger, Pakistan, the Philippines, Tanzania, Thailand, Zambia and Zimbabwe.

Since 1997 she is head of the department of Agricultural Production and Nutrition and member of the Management Team of IAC. She is also responsible for a programme of collaboration with the Institute of Food, Nutrition and Family Science, University of Zimbabwe and is a member of the Academic Advisory Committee of the Regional Training Programme on Food and Nutrition Planning, based at the University of the Philippines at Los Baños.

She has worked on the research described in this thesis since 1994 on a part-time basis.

She is married to Bart van den Briel and has two children, Vincent (1977) and Suzanne (1979).

The work described in this thesis was a collaborative effort of the Division of Human Nutrition and Epidemiology and the Département de Nutrition et des Sciences Agro-Alimentaires, Faculté de l' Agriculture, Université Nationale du Benin.
Financial support for this research from the Nestlé Foundation- for the study of problems of nutrition in the world- and Wageningen University is gratefully acknowledged.

Cover design: T. van den Briel
Printing: Grafisch bedrijf Ponsen & Looijen BV, Wageningen, the Netherlands