Iodine and selenium deficiency associated with cretinism in northern Zaire

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ABSTRACT  Selenium status was determined in an endemic-goiter area and in a control area of Zaire. Compared with the reference values of a noniodine-deficient area, serum selenium in subjects living in the core of the northern Zaire endemic-goiter belt (Karawa villages) was seven times lower in schoolchildren and still two times lower in cretins (P = 0.004). In a less severely iodine-deficient city of the same endemic (Businga), selenium status was moderately altered. RBC-GPX activity was linearly associated with serum selenium concentration up to a value of 1140 nmol/L and leveled off at ~15 U/g Hb at greater selenium concentration. At Karawa villages, selenium supplementation normalized both the serum selenium and the RBC-GPX. This combined iodine and selenium deficiency could be associated with the elevated frequency of endemic myxedematous cretinism in Central Africa.  Am J Clin Nutr 1990;52:1087-93.

KEY WORDS  Iodine deficiency, selenium deficiency, cretinism

Introduction

Endemic cretinism is associated with severe iodine deficiency. Since the description of McCarrison (1) in the Nepalese Himalayan valleys, two forms of this disease are classically distinguished. Endemic myxedematous cretinism is characterized by overt hypothyroidism, stunted growth, and variable intellectual development. Endemic neurological cretins show a severe neurological impairment (spastic diplegia and ataxic gait) and mental retardation frequently associated with deaf-mutism, normal growth, and normal thyroid function. More than 50% of cases of cretinism are neurological in Papua New Guinea (2), Latin America (3, 4), Vietnam (5), eastern China (6), Algeria (7), and Sicily (8), whereas neurological cretinism is uncommon in Central Africa.

Northern Zaire is covered by an endemic-goiter belt affecting 4 million people at a 65-85% goiter prevalence rate and at a 2-6% cretinism prevalence rate (9), mostly of the myxedematous form [78-85% of the cretins (10, 11)].

The reason for a variable distribution of both forms of cretinism remains unclear. It was recently proposed that a combined iodine and selenium deficiency could involve the thyroid invasion (12, 13) observed during the first years of life in myxedematous cretins of Zaire (14).

The aim of the present work was to determine the selenium status in a population living in the core of the endemic-goiter belt of northern Zaire (severe iodine deficiency in Karawa and moderate iodine deficiency in Businga) and in a Zairian area without endemic goiter (Kikwit). The selenium status in normal subjects and in cretins of the same villages was also compared.

Subjects and methods

The study was conducted in two rural villages close to Karawa [villages 1 (Boyalulia) and 2 (Botolo)] in the core of the endemic-goiter area of northern Zaire and in a small town located at 90 km from Karawa along the Ubangi River (Businga) at the border of the same endemic area. In the first two villages, 52 schoolchildren (aged 9-18 y) and 28 cretins (aged 3-25 y) were examined. Informed verbal consent was obtained according to local custom. (The village chief, the teachers, both parents, and the children were informed in local language by the local community health leader of the nature of the study.) Height was recorded and expressed as the percentage of the median height of normal age- and sex-paired subjects of the same area. At Businga biological samples were obtained from 30 hospitalized adults (21-42 years) during blood collections for medical checkups after informed verbal consent. In these three places iodized oil has been available

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to pregnant women attending the prenatal clinics since 1984. Nine medical and paramedical workers of a hospital in a non-iodine-deficient area (Kikwit, Bandundu region) volunteered as control subjects. The protocol was approved by the medical staff of the hospitals of Karawa and Kikwit, by the medical authorities of the Ecole de Santé Publique of Kinshasa, and by the ethical committee of the Erasme University Hospital, Brussels.

A trial of selenium supplementation was organized in the two villages close to Karawa (Fig. 1). Before supplementation, serum selenium and RBC-GPX were measured in all the subjects (group A). The schoolchildren and the cretins were supplemented for 2 mo with selenium [50 μg Se/d per os (po) as selenomethionine (Wassen International, Leatherhead, Surrey, UK)] in village 1 (group B) or with placebo in village 2 (group B'). The group of schoolchildren and cretins of each village was subdivided for the next 4 mo into a supplemented subgroup (100 μg Se po/d as selenomethionine) and a placebo subgroup [village 1, 6 mo selenium (group D) and 2 mo selenium plus 4 mo placebo (group E); village 2, 6 mo placebo (group F) and 2 mo placebo plus 4 mo selenium (group C)].

Each child received 15 tablets every 2 wk. The compliance was verified by counting the remaining tablets every 2 wk and it was considered sufficient for all subjects (≥ 12 tablets consumed/2 wk). Selenium supplementation was interrupted 17 d before the end of the 6-mo study because of logistical difficulties that impeded the supply of selenium tablets. According to the initial results of thyroid function, iodized oil [0.5 mL Lipiodol per os one time (Guerbet, Aulnay-sous-Bois, France)] was administered at the end of the 2-mo study period to all schoolchildren and to the cretins.

Blood samples were collected in tubes with adenine citrate dextrose as anticoagulant for erythrocyte enzyme activity measurement, in tubes treated with ethylenediamine tetraacetic acid-K3 for determination of hematological indices and for measurement of erythrocyte selenium concentration, and in plain tubes for measurement of serum thyroid indices and serum selenium concentration. All samples were immediately stored in a refrigerated box and kept at −4 °C until their arrival at Brussels the same day. Erythrocyte lysate, plasma, and sera were kept within 48 h. Erythrocyte enzyme activity was determined on selenium concentration. All samples were immediately stored in tubes for measurement of serum thyroid indices and in plain tubes treated with ethylenediamine tetraacetic acid-K3 for determination of hematological indices and for measurement of serum total and free thyroxine (T₄, FT₄), total and reverse triiodothyronine (T₃, rT₃), thyroxine-binding globulin (TBG), and thyrotropin (TSH) concentrations by radioimmunoassay in duplicate with commercial kits (for T₄, FT₄, T₃, and TSH, Diagnostic Product Corp, Los Angeles; TBG, Behring, Marburg, FRG; rT₃, Serono Diagnostic, Chavannes-de-Bogis, Switzerland). Urinary iodide concentration was measured as previously reported (14). Hemoglobin A, A₂, and S were determined by electrophoresis on agarose, and the various fractions were calculated by densitometry (Beckman, Fullerton, CA). Selenium was measured by spectrophotofluorimetry in London (15).

Erythrocyte glutathione peroxidase (RBC-GPX) and glutathione reductase were measured according to Beutler after elimination of leukocytes and platelets (16). Glucose-6-phosphate dehydrogenase and pyruvate kinase were measured with commercial kits (Boehringer, Mannheim, FRG) in Leuven, Belgium.

Values are expressed as means ± 1 SD in the text and in the tables and as x ± 1 SEM in the figures. Serum TSH and urinary iodide concentrations are expressed as geometric mean (geometric x ± 1 SD) in text and tables. The comparison of means was performed by analysis of variance (ANOVA) and by one-way ANOVA when repeated measures were obtained in the same group. The comparison of frequencies was performed by the chi-square test. The regression was calculated by a model of linear regression (y = ax + b). Multifactorial analysis was performed by ANOVA after subdividing the independent variables into tertiles (ie, same number of subjects in the three groups) and by multiple-regression analysis. The multifactorial analysis was limited to the subjects of the endemic area who were relatively homogeneous concerning the variance of the biological variables (homoscedasticity). All statistical procedures were conducted with the Statistical Package for Social Sciences (SPSSPC+, SPSS, Inc, Chicago).

**Results**

Table 1 shows the baseline epidemiological and biological values in the schoolchildren and cretins of Karawa and in the adult subjects of Businga and Kikwit. Mean urinary iodide was high at Kikwit (4.53 μmol/L) and moderately low at Businga (0.38 μmol/L; P < 0.001); it was extremely low at Karawa and similar in schoolchildren (0.20 μmol/L) and in cretins (0.16 μmol/L). The indices of thyroid function followed the same geographical trend. Compared with the normal values observed in Kikwit, the adult patients of Businga were euthyroid but an
In Karawa the normal schoolchildren presented a clear pattern of juvenile hypothyroidism and the cretins showed biological signs of extremely severe hypothyroidism. On the other hand, the mean erythrocyte hemoglobin (hemoglobin S and increased concentrations of hexose-6-phosphate dehydrogenase deficiency and of abnormal hemoglobin A2 consistent with a β-thalassemia trait) were similar in the four groups.

Serum selenium concentration (Fig 2) was similar in schoolchildren and in cretins of Karawa [343 ± 176 nmol/L (± 1 SD = 52) vs 443 ± 188 nmol/L (n = 23), P > 0.1] and was markedly lower in both groups than in adult patients of Businga [753 ± 355 nmol/L (n = 29), P < 0.001] or than in volunteers of Kikwit [2555 ± 347 nmol/L (n = 9), P < 0.001]. In Karawa the RBC-GPX concentration was almost twice as low in cretins as in schoolchildren [1.7 ± 1.8 U/g Hb (n = 27) vs 3.3 ± 2.4 U/g Hb (n = 46), P = 0.004] and was markedly lower than in Businga (8.7 ± 4.7 U/g Hb (n = 25), P < 0.001) or at Kikwit [15.0 ± 2.2 U/g Hb (n = 8), P < 0.001]. This last value was similar to that of 20 adult Belgian control subjects (15.0 ± 2.8 U/g Hb).

Erythrocyte [red blood cell, (RBC)] selenium concentration was also measured. It was similarly extremely low in schoolchildren [304 ± 133 nmol/L RBC (n = 27)] and in cretins [279 ± 156 nmol/mL RBC (n = 12)] of Karawa compared with non-deficient areas.

The relationship between the individual values of serum selenium and of RBC-GPX is given in Figure 3. In the low-selenium range (<1140 nmol/L), the relationship between both variables fit a linear-regression curve well. At greater selenium concentrations, the values of RBC-GPX were leveling off at a normal value.

Multifactorial analysis (Table 2) of the effect of iodine and selenium on thyroid hormones (before selenium supplementation) was limited to the subjects of the endemic area to ensure a relative homogeneity of variance. As expected, the main effect of iodine was observed in ANOVA and multiple regression. A supplementary effect of RBC-GPX determined by ANOVA was statistically significant only for serum TSH (P < 0.05); in a multiple-regression analysis a supplementary effect of RBC-GPX was observed for serum T3, FT4, and TSH. The effect of serum selenium was not significant in the ANOVA or in the multiple regression. The addition of age in the multifactorial analysis, which is known to affect the thyroid indices (17), did not disclose a significant effect and did not modify the effect of iodine or RBC-GPX (data not shown).

Table 3 shows the evolution of the selenium status in the subjects of Karawa. After 2 mo of selenium supplementation (group B), serum selenium was normal whereas RBC-GPX reached about half the normal value. As a result of the accidental interruption of selenium supplementation 17 d before the end of the study (see Methods), the mean serum selenium concentrations at 4 (group C) and 6 mo (group D) of selenium supplementation were intermediate between those before and those after 2 mo of selenium supplementation. By contrast, RBC-GPX continued to increase and reached a normal mean concentration after 6 mo. The subjects supplemented for 2 mo with selenium and thereafter for 4 mo with placebo (group E) presented RBC-GPX concentrations similar to those observed at 2 mo, whereas

![Image](https://www.ajcn.org/doi/abs/10.3942/ajcn.2009.01089)
Selenium deficiency has been associated in China with two endemic diseases, a cardiomyopathy called Keshan disease and an osteoarthropathy called Kashin-Beck disease (19). Serum selenium in these affected populations was still lower than the

![FIG 3. Relationship of RBC-GPX and serum selenium in normal schoolchildren and cretins of Karawa, northern Zaire (crosses and open circles), in adult hospitalized patients of Businga, northern Zaire (closed triangles), and in adult volunteers of Kikwit, central Zaire (open triangles). The analyses were performed before selenium supplementation. The hatched area represents the range of values in European countries. The equation of the linear regression for the selenium values <1000 nmol/L is $y = 0.021x - 4.8$ ($r = 0.63; P < 0.001$); for the selenium values >1000 nmol/L, the coefficient of the slope was not statistically different from zero and it was considered that the linear regression could fit the equation $y = \text{constant}$ ($r = 0.05; P > 0.5$).

![FIG 2. Comparison of individual values and mean concentrations (±1 SEM) of erythrocyte glutathione peroxidase (RBC-GPX) and of serum selenium in normal schoolchildren and in cretins of Karawa, northern Zaire (severe iodine deficiency), in adult hospitalized patients of Businga, northern Zaire (moderate iodine deficiency), and in adult normal volunteers of a noniodine-deficient area (Kikwit, Central Zaire).]

serum selenium had decreased markedly to values barely greater than before supplementation.

After 2 mo of selenium supplementation, the serum selenium increase in cretins ($1724 ± 547 \text{nmol/L, } n = 9$) was more pronounced than in schoolchildren [944 ± 285 nmol/L ($n = 23$), $P = 0.002$]; the same was true for RBC-GPX [10.33 ± 5.31 U/g Hb ($n = 9$) vs 5.76 ± 2.19 U/g Hb ($n = 24$), $P < 0.05$]. After 4- and 6-mo supplementation, the evolution of serum selenium and RBC-GPX was similar in cretins and in schoolchildren.

Serum selenium and RBC-GPX were not modified in the placebo group [serum selenium at 2 mo, 479 ± 233 nmol/L ($n = 24$), and at 6 mo, 301 ± 111 nmol/L ($n = 6$); RBC-GPX at 2 mo, 3.0 ± 2.7 U/g Hb ($n = 27$), and at 6 mo, 3.5 ± 3.3 U/g Hb ($n = 8$)].

**Discussion**

These data clearly document a very severe selenium deficiency in the core of the northern Zaire goiter belt. At Karawa the mean concentrations of serum selenium were three to seven times lower than in nondeficient areas (18). They were definitely lower than in New Zealand (mean serum selenium ~ 600 nmol/L), considered to be marginally selenium deficient, and they were barely greater than the values observed in some areas of China considered to be severely selenium deficient (18).

**TABLE 2**

Multifactorial analysis of the effect of iodine (urinary iodide) and of selenium status [serum selenium and red blood cell glutathione peroxidase (RBC-GPX)] on thyroid indices by ANOVA and multiple-regression analysis*

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Serum T4</th>
<th>Serum FT4</th>
<th>Serum T3</th>
<th>Serum TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis of variance: main effects (F values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary iodide</td>
<td>13.5‡</td>
<td>12.4‡</td>
<td>1.4</td>
<td>8.1†</td>
</tr>
<tr>
<td>RBC-GPX</td>
<td>1.6</td>
<td>2.7</td>
<td>1.2</td>
<td>4.6‡</td>
</tr>
<tr>
<td>Serum selenium</td>
<td>1.3</td>
<td>1.9</td>
<td>0.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Multiple-regression analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$ of iodine</td>
<td>0.42‡</td>
<td>0.51‡</td>
<td>0.07</td>
<td>-0.58‡</td>
</tr>
<tr>
<td>Multiple $r$ of iodine + RBC-GPX</td>
<td>0.48‡</td>
<td>0.59‡</td>
<td>0.09</td>
<td>0.67‡</td>
</tr>
<tr>
<td>F value (multiple $r$ vs $r$)</td>
<td>14‡</td>
<td>24‡</td>
<td>1.5</td>
<td>29‡</td>
</tr>
</tbody>
</table>

* The effect of age and serum selenium was not significant ($P > 0.3$) in the ANOVA or in the multiple-regression analysis.
‡ $P < 0.001$.
§ $P < 0.05$.
value of the subjects of Karawa (152 nmol/L) (18). All children studied, even those with undetectable concentrations of RBC-GPX, were in good health; nevertheless, in the absence of specific clinical criteria, it is not possible to rule out the possibility that some cases of cardiomyopathy in northern Zaire, a disease frequently observed in tropical areas, were actually misdiagnosed cases of Keshan disease.

The public health benefit of selenium supplementation is recognized in the extreme environmental conditions of China (19). By contrast, in New Zealand, where the selenium deficiency is moderate, the benefit of supplementation in humans remains uncertain and controversial (20).

At Karawa the severity of the deficiency is intermediate between these two situations. As expected, selenium supplementation corrects the altered selenium status. The discrepancy between the evolution of serum selenium and that of RBC-GPX activity after 2 mo of supplementation is best explained by the different metabolic half-lives of the two pools of selenium (1 d for serum selenium, 30 d for RBC-GPX) (18). After 6 mo of supplementation, the RBC-GPX was entirely normal. Metabolic studies have shown that some tissues release their selenium content very slowly; this can also explain the stability of RBC-GPX during 4 mo after stopping selenium supplementation (18).

In the present study, as one progressed from a noniodine-deficient area (Kikwit) to an intermediate-severity goiter area (Businga) and to a severe iodine-deficient area (Karawa), there was a progressive severity of selenium deficiency, assessed either by serum selenium or RBC-GPX. The subjects of these three places were heterogeneous concerning the range of age and the life conditions (schoolchildren, cretins living in rural villages, hospitalized patients, and hospital volunteers). Despite this evident bias, we believe that the variables measured accurately reflect the selenium status of the general population. The effect of age on the selenium status in the range studied (3–42 y) is negligible, at least in nonselenium-deficient populations (21). These populations live almost exclusively on locally produced foods, whatever the socioeconomic level. The staple food is based on cassava and maize in the three places studied. The selenium content of these products is directly dependent on the geographical nature of the soil (22). Great variations in selenium status were observed in places that are close together (eg, Karawa and Businga are 90 km apart).

The geographical association of two trace element deficiencies raises two issues: 1) Is it specific to Central Africa? 2) Does it imply an interaction between the two trace elements acting on thyroid function?

Concerning the first question, the data on selenium status in endemic-goiter areas are scarce. Besides the previously described deficiency in eastern Zaire (13), New Zealand and Finland are moderately or marginally selenium deficient (19), and the iodine supply in these countries is rather high (23, 24). The iodine-deficient areas of China overlap only for a small part with selenium-deficient areas (Qinghai province) (25). Interestingly, myxedematous cretinism is more frequent in this province than in eastern China (Guizou, Heilongiang, and Shanxi provinces), where neurological cretinism is predominant. Low selenium status has also been reported in proteoenergetic malnutrition [Guatemala (26), Eastern Zaire (27), Jamaica (28), and Sudan (29)] without evidence of associated iodine deficiency. Clinical examination of the children in the present study and previous investigations demonstrated their relatively good nutritional protocaloric status (30). Selenium concentration in another iodine-deficient population was found to be normal (Velingara, Senegal, 1116 ± 156 nmol/L whole blood (n = 20); AT Diplock and JN Lazarus, unpublished observations, 1988). In summary, a geographical association of selenium and iodine deficiency is certainly not the rule.

Concerning the second issue, it is known that hydrogen peroxide generated at the apical membrane of thyroid cells is necessary to oxidize tyrosyl residues of thyroglobulin in the formation of thyroid hormones (31). The synthesis of an excess of hydrogen peroxide in a stimulated gland and a lack of hydrogen peroxide detoxifying enzyme would lead to a progressive deleterious effect on the gland (12). The geographical association described here is one more argument in favor of this hypothesis (ie, that combined iodine and selenium deficiency causes an irreversible loss of thyroid function); it does not constitute a proof. Animal experiments have clearly established interactions of selenium with thyroid hormones. The effect of selenium on the thyroid function could be mediated by a modulation of the deiodinases converting T4 into T3 and into rT3 in extrathyroidal tissues (32). The precise mechanism by which selenium deficiency could interact with thyroid function may be more complex than initially postulated.

Multifactorial analysis (ANOVA, multiple regression) of the respective effects of iodine and selenium on thyroid function showed the main effect of iodine on T4, FT4, and TSH. A supplementary effect of RBC-GPX was seen by multiple regression
for the same thyroid indices and by ANOVA for TSH. The multiple-regression procedure treats all the individual data whereas the ANOVA treats the data grouped in classes (tertiles in the present analysis). This difference in statistical procedure could explain that the multiple-regression analysis was a more powerful test to detect an effect of RBC-GPX on thyroid function. Nevertheless, these data should be considered cautiously and further investigation is required to define a possible effect of selenium status on thyroid function in humans. The geographical association of cretinism and selenium deficiency in this study, in eastern Zaire (Idjwi Island, Kivu Lake) (13), and in the province of Qinghai, China (25), may represent the first clues that combined iodine and selenium deficiency exert an irreversible deleterious effect on the thyroid function in at least some subjects.

It was also speculated that genetic diseases known to be associated with an increased sensitivity to oxidant stress and frequent in black populations could be a predisposing factor for cretinism. This seems not to be the case because glucose-6-phosphate dehydrogenase deficiency, β-thalassemia trait, and sickle cell anemia trait were not more frequent in cretins. The RBC-GPX concentration in cretins was twice as low as in schoolchildren despite similar serum selenium concentrations. RBC-GPX activity is determined genetically (33) and a lower enzymatic activity in cretins could be a marker of this disease; however, after selenium supplementation, enzymatic activity was restored to normal in cretins, rendering this hypothesis unlikely. It is more likely that the lower RBC-GPX reflected a lower selenium supply in cretins; RBC-GPX is a better indicator of long-term selenium supply than is serum selenium. From the present data it is not possible to determine why the selenium supply in cretins should be lower; small differences in accessibility to some foods of handicapped children could be an explanation. Severe hypothyroidism in six subjects of a nonselenium-deficient country (Belgium) did not modify the RBC-GPX activity (\(\bar{x} = 17.8 \pm 2.3\) U/g Hb).

In conclusion, severe selenium deficiency was documented in the core of the endemic-goiter belt of northern Zaire, which reinforces the hypothesis of an association with endemic myxedematous cretinism. The clinical and public health benefit of selenium supplementation in this area remains to be determined.

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