Low selenium status in the elderly influences thyroid hormones

Oliviero OLIVIERI, Domenico GIRELLI, Margherita AZZINI, Anna Maria STANZIAL, Carla RUSSO, Massimiliano FERRONI* and Roberto CORROCHER

Institute of Medical Pathology, Chair of Internal Medicine, University of Verona, Italy, and *Public Health Service, Verona, Verona, Italy

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1. Iodothyronine 5'-deiodinase, which is mainly responsible for peripheral triiodothyronine (T₃) production, has recently been demonstrated to be a selenium-containing enzyme. In the elderly, reduced peripheral conversion of thyroxine (T₄) to T₃ and overt hypothyroidism are frequently observed.

2. We measured serum selenium and erythrocyte glutathione peroxidase (as indices of selenium status), thyroid hormones and thyroid-stimulating hormone in 109 healthy euthyroid subjects (52 women, 57 men), carefully selected to exclude abnormally low thyroid hormone levels induced by acute or chronic diseases or caloric restriction. The subjects were subdivided into three age groups. To avoid conditions of undernutrition or malnutrition, dietary records were obtained for a sample of 24 subjects, randomly selected and representative of the whole population for age and sex.

3. In order to properly assess the influence of selenium status on iodothyronine 5'-deiodinase type I activity, a double-blind placebo-controlled trial was also carried out on 36 elderly subjects, resident at a privately owned nursing home.

4. In the free-living population, a progressive reduction of the T₃/T₄ ratio (due to increased T₄ levels) and of selenium and erythrocyte glutathione peroxidase activity was observed with advancing age. A highly significant linear correlation between T₄, T₃/T₄ and selenium was observed in the population as a whole (for T₄, R = -0.312, P < 0.002; for T₃/T₄ ratio, R = 0.32, P < 0.01) and in older subjects (for T₄, R = -0.40, P < 0.05; for T₃/T₄ ratio, R = 0.54, P < 0.002).

5. The main result of the double-blind placebo-controlled trial was a significant improvement of selenium indices and a decrease in the T₄ level in selenium-treated subjects; serum selenium, erythrocyte glutathione peroxidase activity and thyroid hormones did not change in placebo-treated subjects.

6. We concluded that selenium status influences thyroid hormones in the elderly, mainly modulating T₄ levels.

INTRODUCTION

Full activity of the thyroid hormones requires the deiodination of thyroxine (T₄) to triiodothyronine (T₃). T₃ is produced by the thyroid gland but results mainly from the peripheral deiodination of T₄ catalysed by iodothyronine 5'-deiodinase (ID) [1]. Three isoenzymes of iodothyronine 5'-deiodinase have been identified [1]: type I (ID-I) is found in the liver, kidney and thyroid; ID-II is abundant in the brain, brown adipose tissue and pituitary; and ID-III is present in the brain and placenta. ID-I, which is mainly responsible for peripheral T₃ production, has recently been demonstrated to be a selenium (Se)-containing enzyme [2, 3]; this observation agrees with previous reports that a Se-deficient diet inhibits ID-I in rats [4, 5].

Advanced age is a well-recognized condition characterized by important changes in thyroid function [6]. In addition to a high frequency of overt hypothyroidism [6], reduced iodine uptake, weight, colloid content, follicular volume of the gland and reduced peripheral conversion of T₄ to T₃ with a lower T₃/T₄ ratio are usually observed in elderly euthyroid people [7]. Numerous features of the aging process associated with impaired or slowed cellular functions to some extent mimic those of hypothyroidism, so it is sometimes very difficult to clinically distinguish the manifestations of the illness from those related to aging per se. As a result of these similarities, the concept of aging as a sort of 'tissue hypothyroidism' has also been proposed [7].

No studies relating Se status, thyroid function and aging in humans have been published to date. However, since Se status tends to decline in people over 60-65 years of age [8, 9], reduced ID-I activity due to impaired Se availability may explain the
reduction in the serum $T_3/T_4$ ratio observed in elderly people. To test this hypothesis, we investigated the relationships between age, Se status and thyroid hormones in three groups of free-living healthy subjects of different age, matched for sex distribution. In addition, in a smaller group of institutionalized elderly subjects, we investigated the effects of Se supplementation on thyroid hormones.

**MATERIAL AND METHODS**

**Subjects**

An initial age-based, sex-balanced selection of 500 subjects was obtained by applying tables of random numbers to the population of Nove (a village near Vicenza, northern Italy) appearing on the electoral register.

A further selection was performed in order to study the consequences of aging without the confounding effects of chronic or acute diseases that might affect levels of both Se and circulating thyroid hormones. Thus, very strict criteria were adopted to define the 'healthy' population. We therefore excluded all subjects known to be suffering from hypertension, diabetes, hyperlipidaemia, liver disease, neoplastic disease, renal disease, endocrinological or immunological diseases, coagulative disorders or acute intercurrent illness. Three seemingly euthyroid subjects with thyroid-stimulating hormone (TSH) values higher than 5 m-units/l were also excluded. One hundred and nine subjects (52 women, 57 men) were finally admitted to the study. None was institutionalized or on a special diet, and none was taking preparations containing multivitamins and/or trace elements. Four subjects were excluded from the study early on because of intercurrent acute illness interfering with oral absorption of the tablets. All the other subjects consumed their habitual diet, prepared in the kitchen of the nursing home, and had no known nutritional problems during the study.

The subjects were randomly allocated to the Se (Se+) or placebo (Se-) group, according to the number of their bedroom. Every day for 3 months, one tablet of sodium selenite (100 pg) or placebo (starch) was taken during breakfast, under the supervision of a nurse. Neither the patient nor the investigator knew which treatment was allocated and only at the end of the study was the randomization code broken. Thirty six subjects (Se - group, 14 women and 3 men; Se+ group, 14 women and 5 men) concluded the study and their results were suitable for statistical evaluation. No side effects were recorded during the study. Blood samples for measuring Se parameters and thyroid hormones were collected before and after the supplementation period (3 months). Informed consent was obtained from all subjects and the study was approved by the local ethics committee.

**Biochemical analysis**

Blood samples were collected after overnight fasting and processed within 1 h. Haemogram was automatically measured using the Technicon H-2 System (Technicon Instruments, Tarrytown, NY, U.S.A.). Plasma cholesterol and serum albumin were determined using a Technicon DAX 96 automated analyser (Technicon Instruments). Serum $T_3$, $T_4$, free thyroxine ($FT_4$) and TSH were assayed by a chemiluminescence immunoassay system (LIA-mat, Byk-Sangtec Diagnostica, Germany) [10, 11]. For the quantitative determination of thyroxine-binding globulin (TBG), a radioimmunoassay kit (Gamma Dab125 TBG Radioimmunoassay, Sorin Biomedica, Vercelli, Italy) was employed. To prepare haemolysates for enzyme assays, the erythrocytes were washed and then filtered in cellulose. For serum Se assay, blood was collected in trace-element-free vacutainer tubes, containing no additives, for metal and metalloid determinations (Becton Dickinson, Rutherford, NJ, U.S.A.).

**Erythrocyte glutathione peroxidase assay.** Activity of erythrocyte glutathione peroxidase (GSH-Px, EC 1.11.1.9), which accurately reflects the Se intake over 2–3 months [8], was measured in 50 µl of cell lysate, according to the Günzler method, using tert-butylhydroperoxide as acceptor substrate, as previously described [12]. GSH-Px activity was expressed as IU, defined as µmol of NADPH oxidized per min per g of Hb (IU/g Hb).

**Se assay.** Serum Se was determined as previously
Table 1. Main features of free-living subjects, subdivided according to age groups. Statistical significance: *p < 0.05 for group I compared with group II; **p < 0.05 for group I compared with group III. Comparisons between age groups were performed using analysis of variance and differences at the 5% significance level were assessed by Tukey's test. Means not sharing a common superscript symbol are not significantly different.

<table>
<thead>
<tr>
<th>Age (years)</th>
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<th>Group III</th>
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<tr>
<td>20-44</td>
<td>23.1 ± 3.2</td>
<td>25.6 ± 2.5</td>
<td>&gt; 65</td>
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<td>25-39</td>
<td>25.8 ± 2.0</td>
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<td>24.0 ± 2.8</td>
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<td>60-79</td>
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<td>80+</td>
<td>21.5 ± 2.3</td>
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Table 2. Thyroid parameters and indices of Se in free-living subjects. Statistical significance: *p < 0.05 for group I compared with group III; **p < 0.05 for group I compared with group II. Comparisons between age groups were performed using analysis of variance and differences at the 5% significance level were assessed by Tukey's test. Means not sharing a common superscript symbol are not significantly different.

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Fig. 1. Correlation between serum Se and T₄ in a free-living population as a whole.

**RESULTS**

**Population study**

Table 1 gives the main features of the subjects, subdivided according to the various age groups. All our subjects had normal nutritional indices such as body mass index, Hb, packed cell volume and serum albumin; dietary records showed no significant differences between the groups.

Levels of thyroid hormones, TSH, T₃/T₄, and serum Se and erythrocyte GSH-Px activity of the subjects, subdivided according to age groups, are listed in Table 2. Erythrocyte GSH-Px and the T₃/T₄ ratio were lower in older (group III) than in younger subjects (group I). Serum Se was lower and T₄ was higher in group III compared with the other two groups. T₃, FT₄ and TSH levels were similar in all groups (Table 2).

In the population as a whole, serum Se was positively correlated with the T₃/T₄ ratio (R = 0.32, P < 0.01) and negatively correlated with T₄ (R = −0.31, P < 0.01; Fig. 1). When the analysis was performed separately for the different age groups, a significant correlation was found only for subjects in group III (for T₃/T₄; R = 0.54, P < 0.002; for T₄, R = −0.40, P < 0.05; Fig. 2), who also had lower Se values. The T₃/T₄ ratio and erythrocyte GSH-Px activity were also correlated (R = 0.50, P < 0.002) in the group III subjects.

described [12], using hydride generation atomic absorption spectrometry on serum stored at −20°C until analysed. Briefly, 1 ml of serum was treated by wet digestion in nitric acid/hydrochloric acid (1:5:5, v/v). Se concentrations were determined using a G.B.C. 902 atomic absorption spectrophotometer equipped with a hydride generation system and a selenium hollow cathode lamp. Sodium borohydride was used as reductant. A batch of Seronorm trace element serum (Nycomed Pharma As, Oslo, Norway) with a defined Se concentration (mean recommended value 1.19 μmol/l, range 1.17–1.22; mean value obtained in our laboratory 1.20 μmol/l) was used as a control reference. The within- and between-run coefficients of variation were 1.7% and 4%, respectively.

**Statistical methods**

Statistical analysis was carried out with an Apple Macintosh SE/30 computer using the Systat 5.0 statistical package. The data are given as means ± SD. Comparison of the various parameters among age groups was performed using one-way analysis of variance; differences at the 5% significance level (P < 0.05) were assessed by Tukey’s post hoc probability test for multiple comparisons. Paired Student’s t-test was used to compare the same parameters before and after supplementation in Se- or placebo-treated subjects. Simple correlations were determined using Pearson’s correlation coefficient.

**RESULTS**

**Population study**

Table 1 gives the main features of the subjects, subdivided according to the various age groups. All our subjects had normal nutritional indices such as body mass index, Hb, packed cell volume and serum albumin; dietary records showed no significant differences between the groups.
Supplementation trial

Table 3 gives the main features of institutionalized subjects who underwent the trial. Serum Se, erythrocyte GSH-Px, T₃, T₄, FT₄ and T₃/T₄ were generally very low in these institutionalized subjects, while TSH levels were still normal.

After supplementation, parameters of Se status strongly improved in Se-treated (Se = +61%, GSH-PX = +80.9%) patients, but did not change in the placebo-treated subgroup (Table 3). The only noteworthy modification in thyroid hormones induced by Se supplementation was a significant decrease in T₄ levels (−7.5%); this change was not due to a parallel decrease in TBG, the major T₄-binding protein in serum (Table 3). No changes in hormonal parameters were observed in the placebo-treated subgroup (Table 3).

DISCUSSION

After the recent demonstrations that ID-1 is a selenocysteine-containing enzyme [2, 3], increasing attention has been focused on the relationships between Se status and thyroid hormones. Pathological conditions affecting thyroid function such as Graves' disease [13] and hypothyroidism [14–16] have been studied, but there has been little investigation of the topic in healthy humans, particularly in relation to aging. Our study aimed to investigate the topic by two different approaches: firstly, to evaluate age-related changes in Se status and thyroid hormones and their possible correlation in a free-living healthy population; secondly, to verify the consequences on thyroid hormones of a Se supplementation trial in a smaller sample of institutionalized elderly people with a poor Se status.

Since serum levels of thyroid hormones could be lowered by a great variety of acute or chronic diseases or calorie restriction in seemingly euthyroid individuals [7], we devoted particular care to the selection of free-living subjects. With the strict criteria adopted, our aim was to avoid possible changes in thyroid hormone concentrations which might reflect diseases associated with age, rather than being an effect of aging per se. Nutritional state was also checked: all our subjects had normal nutritional indices such as body mass index, Hb, packed cell volume and serum albumin; moreover, a 7-day food record randomly obtained for a representative sample of subjects showed similar calorie intake without any significant differences between the age groups (Table 1).

We found a low T₃/T₄ ratio, high T₄ and reduced Se and erythrocyte GSH-Px activity in the older subjects; moreover, T₄ and T₃/T₄ were significantly correlated with Se. Although these results supported the hypothesis that an impaired Se status may explain the hormonal changes observed in elderly people, conceivably via impaired activity of the Se-containing enzyme ID-1, direct evidence of a causative link was still lacking.

For this purpose, a double-blind placebo-controlled supplementation trial was carried out, whose main result was a significant decrease in T₄ levels in Se-treated subjects (Table 3).

In this case, we adopted three criteria for the selection of subjects: no evidence for gastrointestinal or other diseases interfering with absorption of tablets by the oral route, a poor Se status and a constant diet during the trial (for the latter reasons we chose very old and institutionalized people). All subjects included in the study met all these requirements. As reported in Table 3, the subjects presented a very poor Se status. Compliance of the subjects to the Se treatment was proved by the remarkable improvement of Se indices in Se-treated subjects, whereas in placebo-treated subjects the same parameters remained unchanged (Table 3). Moreover, the diet provided by the nursing home was constant over a period of 3 months.

However, in these very old subjects (85 ± 7 years; twenty eight subjects were more than 80 years of age) a generalized reduction of thyroid hormone synthesis and an impaired nutritional state was evident (Table 3). In these respects, the institutionalized subjects were very different from the free-living older subjects of group III (mean age 76 ± 6 years; only five were more than 80 years of age). In particular, Se deficiency was more severe and T₄ levels were lower than in the subjects of group III.

Clearly, these two groups of elderly people were representative of two different steps in the aging process in that a progressive impairment of several functions, i.e. thyroid function, occurs. In institutionalized subjects of a more advanced age and poorer nutritional status, the functional decline of thyroid gland (the only source of T₄) determined a significant reduction of circulating T₄ levels, whereas in free-living elderly people thyroid hormone synthesis was still effective and T₄ levels were high.
In our institutionalized elderly people, the recovery of an adequate Se status produced a 7.5% decrease in T₄ levels in spite of impaired thyroid hormone synthesis. This percentage could probably be higher in subjects (as for example in our free-living elderly people) presenting normal or increased availability of T₄, the substrate for ID-I. Supposing that the increase in T₄ levels observed in the free-living subjects of group III (mean T₄ = 104 nmol/l), compared with that of groups I (mean T₄ = 85.1 nmol/l) or II (mean T₄ = 92.9 nmol/l), was only due to an impaired ID-I activity, the percentage increase in T₄ values dependent on impaired ID-I function could be estimated to be between 10.6 and 18%. Therefore, a 7.5% reduction in T₄ levels obtained after Se supplementation is a noteworthy result, provided that institutionalized elderly people had a T₄ reduction of 19.6–26.3% in comparison with free-living subjects of group I or II, respectively.

Results of the Se supplementation trial suggest that T₄ is the most sensitive parameter to Se repletion and in conditions where thyroid T₄ synthesis is blunted, such as in very advanced age. To the best of our knowledge, this is the first study demonstrating that Se supplementation modifies the parameters of thyroid function in euthyroid elderly people. Previous observations by other authors are in agreement with the present results. In animals [17–19] and in children [20], low Se status was demonstrated to lower the T₃/T₄ ratio, mainly through an increase in T₃ rather than by affecting T₄ levels. In Se-deficient children, affected by phenylketonuria, after 46 weeks of Se supplementation the only modification was a significant decrease in T₄, which increased back to the initial values within 3 weeks after stopping supplementation [21]. The only apparent discrepancy between our results and previous findings concerns the results of the fraction free of T₄. FT₄ levels were unchanged after 3 months of Se supplementation in our elderly subjects; conversely, in rats [22] and in children [20], changes in Se status give rise to changes in both total T₄ and FT₄. We do not have any logical explanation for this discrepancy, except the obvious difference of species and age of our subjects.

In this and in other studies, TSH levels were not affected by impaired Se status [4, 15, 20, 22], suggesting normal T₃ and deiodinase activity within the pituitary, since inadequate T₃ concentrations act as a signal for TSH secretion. In rats, aging was reported to be associated with reduced ID-I activity in liver and thyroid, but with normal circulating TSH levels, normal T₃ and increased deiodinase activity within adenohypophysis [23].

In conclusion, a definite percentage of circulating T₄ is sensitive to the variations of Se, the trace element cofactor of ID-I. Due to the high frequency of Se deficiency in elderly subjects, abnormalities in T₄ levels as a result of impaired peripheral deiodinase activity are probably frequent in older people, but are clinically underscored because T₃ and TSH values are normal.

The long-term consequences of Se depletion on thyroid metabolism during the aging process remain to be established and to be evaluated in terms of preventive action and health advice. In fact, a Se deficiency-induced increase in T₄ may have inhibitory effects on ID-II [24], whose activity is the major determinant of T₃ levels in the neuronal and pituitary cells. Further investigations, specifically addressed to the effects of long-term Se supplementation, will be necessary to clarify this potentially very important point.

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