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Investigation of Selenium Status in Hypo-, Hyper- and Euthyroid Children

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INVESTIGATION OF SELENIUM STATUS IN HYPO-, HYPER- AND EUTHYROID CHILDREN

by

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ABSTRACT

INVESTIGATION OF SELENIUM STATUS IN HYPOTHYROID, HYPERTHYROID AND EUTHYROID CHILDREN

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Old Dominion University, 1993
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Recently, it has been reported that human type I iodothyronine deiodinase, an enzyme important in the conversion of T_4 to T_3, is a selenoenzyme. Several studies have reported alterations in plasma selenium level in hypo- and hyperthyroid patients.

Using polarized Zeeman-effect atomic absorption spectroscopy, we measured selenium, zinc, copper and manganese concentrations in the plasma and/or red blood cells in children with or without thyroid disease being seen at the outpatient clinic at Children's Hospital of the King's Daughters. Children with thyroid disease were subdivided into untreated, treated and nonresponsive groups. Data were analyzed using Student's t-test and nonparametric correlation programs available in the SPSS program.

Significantly lower plasma selenium concentrations were found in untreated and treated hypothyroid patients when compared to control subjects. No other significant differences for plasma selenium and erythrocyte selenium concentrations were found among the other groups in both hypo- and hyperthyroid patients when compared to controls. No relationship was found between plasma selenium and

T_4 or TSH levels in hypothyroid patients. However, in hyperthyroid patients, plasma selenium levels correlated negatively to T_4 levels and positively with TSH levels.

Significantly lower plasma zinc levels were found in treated and nonresponsive hypothyroid patients. Significantly lower red blood cell zinc levels in untreated and nonresponsive hyperthyroid patients were also found. In addition, significant inverse correlations between red blood cell zinc and T_4 were observed in both hypothyroid and hyperthyroid patients.

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DEDICATIONS

To my husband, Yong Ming Tang.

To my father, Chang Zhai Sun.

To my mother, Jing Hua Liu.

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Chapter 1 Introduction

Background and History

Pathophysiology of Selenium

Selenium (Se) was first discovered to be an essential nutrient for animals by Schwarz and Foltz in 1957 (1). Since then, the role of Se in biological systems has been investigated repeatedly. Animals deficient in this trace nutrient exhibit poor growth, impaired neutrophil function and increased susceptibility to infection (2). In the human, studies have shown that selenium deficiency is a causal factor in a number of diseases. Keshan disease, for example, a cardiomyopathy frequently seen in children from Keshan county in China is a selenium-responsive condition. Lowered plasma selenium levels also are found in common diseases such as cirrhosis, cancers and coronary heart disease (3). Selenium deficiency can exacerbate goiter and hypothyroidism when concurrently associated with iodine (I) deficiency (4). Selenium deficiency has recently been common found to be in patients with AIDS and AIDS-related complex (5).

Early studies on the biochemical function of selenium focused on its relationship with vitamin E and its roles as an antioxidant. The discovery in 1973 that selenium was an essential constituent of glutathione peroxidase provided a potential mechanism through which the element could perform an antioxidant role

(6).

Since the late 1980s, it has been reported that a number of selenium functions are not associated with glutathione peroxidase activity. However, a number of biochemical and physiological effects of selenium could not be explained by the loss of selenium-dependent glutathione peroxidase activity alone. For example, in a 1980 study, selenium-deficient rats were shown to be extremely sensitive to diquat-induced liver injury (5). Doses of diquat which did not injure control rats caused massive liver necrosis and lipid peroxidation in selenium-deficient animals. Selenium injection increased glutathione peroxidase activity by only a trivial amount. More recently, studies in chickens have examined the effect of selenium intake on glutathione peroxidase activity and paraquat toxicity. Chickens fed a selenium-containing diet have reduced mortality due to paraquat, but selenium supplementation appeared to have no effect on plasma glutathione peroxidase activity. Recognition that selenium has biological activities which are not associated with glutathione has opened a new debate on other possible biochemical functions of selenium and has stimulated research in this area.

So far, thirteen types of selenium-containing proteins or protein subunits in the tissues of animals have been found (6). However, only three selenoproteins have been characterized in mammals: glutathione peroxidase, selenoprotain P, and type I iodothyronine deiodinase. It is generally believed that glutathione peroxidase is involved in protecting the cell membrane from peroxidative damage

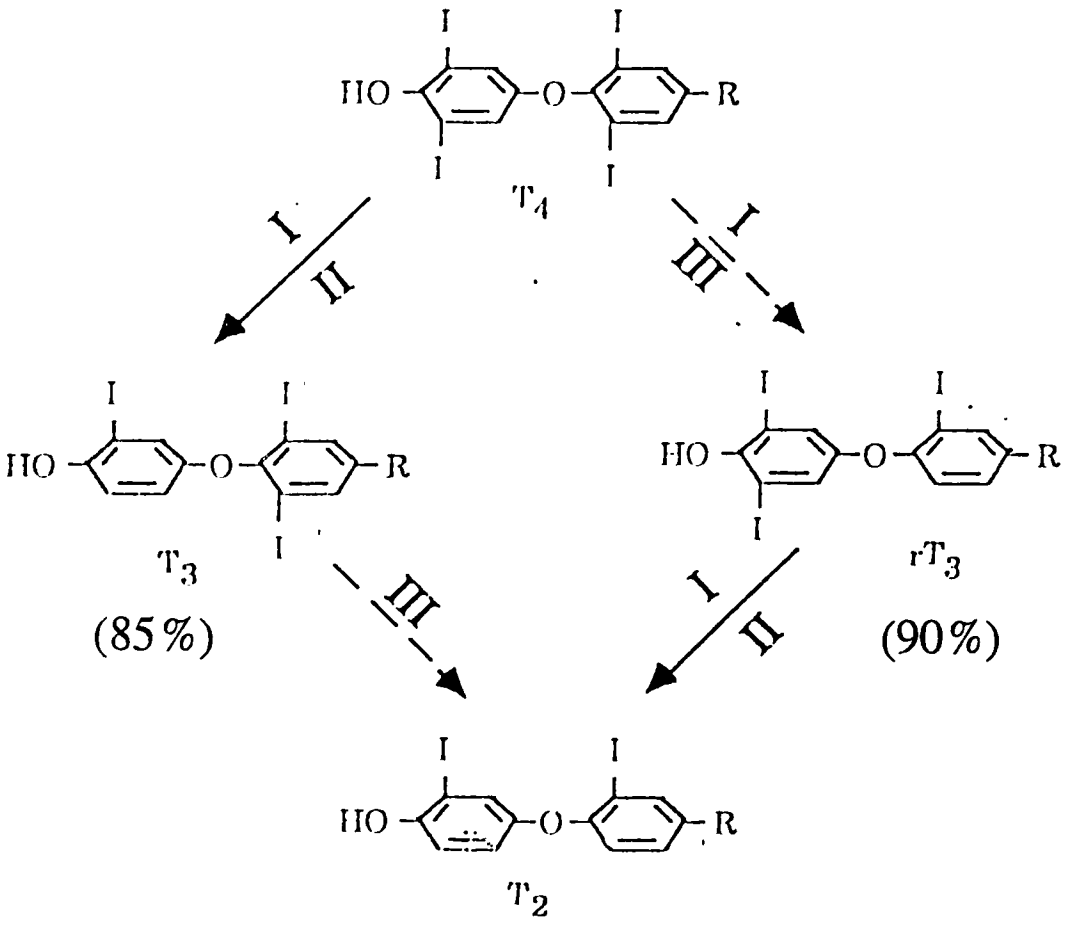
caused by lipid metabolism (6). Selenoprotein P, a newly discovered selenoprotein in rat plasma, may play a role in the transport of selenium, but further work is required to clarify its function (3).

Type I iodothyronine deiodinase has been characterized in human (7,8). Because type I iodothyronine deiodinase is one of the major enzymes involved in thyroid hormone metabolism, selenium has an important role in controlling thyroid hormone homeostasis. Adequate selenium nutrition is important for the normal function of type I iodothyronine deiodinase and maintenance of normal thyroid physiology (9).

Iodothyronine Deiodinases and Thyroid Hormones

Based on their location, function and substrate specificity (10), iodothyronine deiodinases can be divided into three types -- type I, type II and type III. Partial iodothyronine deiodinations catalyzed by iodothyronine deiodinases are shown in Figure 1.

Type I iodothyronine deiodinase is the major enzyme in liver, kidney, and also exists in skeletal muscle and thyroid gland. It can carry out both 5-and 5'-monodeiodination of 3,5,3',5'-tetraiodothyronine (T_4) to produce 3,5,3'-triiodothyronine (T_3) or 3,3',5'-triiodothyronine (reverse T_3 or rT_3). It can also carry out 5'-monodeiodination of rT_3 to produce 3,3'-diiodothyronine (T_2). Type I iodothyronine deiodinase relative substrate "preferences" are $rT_3 >> T_4 > T_3$



* $R = CH_2CHNH_2COOH$

(10). Type I iodothyronine deiodinase was identified as a selenium-containing enzyme in 1990 (7,8). It is responsible for more than 85% of the plasma T_3 produced in the peripheral tissues of mammals (11). Mutagenesis studies have shown that type I iodothyronine deiodinase is a member of the selenocysteine-containing oxidoreductase family (12). Because diiodothyronines (T_2s) apparently lack metabolic activity and the assays for them are less accurate than assays for T_3 and T_4, they have been studied less frequently. Thus, much less is known about the deiodination of T_2s than about the deiodination of T_4, T_3 and rT_3. Recent studies have only investigated the activating role of type I iodothyronine deiodinase in the production of T_3 (13).

The type II iodothyronine deiodinase is the major deiodinase found in brain, pituitary and brown adipose tissue (BAT). It appears to carry out only 5'-monodeiodination of T_4 or rT_3, and to produce T_3 or T_2 in these tissues (14). Type II iodothyronine deiodinase "prefers" T_4 over rT_3 (10). Type II iodothyronine deiodinase has different properties from type I iodothyronine deiodinase. It has been reported that type II iodothyronine deiodinase is not a selenoprotein and is only responsible for intracellular T_3 production in brain, pituitary and brown adipose tissue (15). The type II iodothyronine deiodinase is especially important in producing T_3 at the nucleus in the anterior pituitary cells to control thyroid stimulating hormone (TSH) synthesis and secretion (14).

Type III deiodinase enzyme is present in the sympathetic nervous system

and placenta, and catalyzes 5-monodeiodination of T_4 or T_3 to produce the inactive rT_3 or T_2. This enzyme "prefers" T_3 over T_4 (10). Because two inner-ring iodine atoms are required for activation of nuclear T_3 receptor, type III deiodinase causes the inactivation of either T_4 or T_3 (16). A summary of the features of the iodothyronine deiodinases are shown in Table 1.

T_4 is considered to be a prohormone, and is synthesized only in the thyroid gland. T_4 can be converted by type I and type II iodothyronine deiodinase 5'-monodeiodination to the only biologically active form of thyroid hormone, T_3. T_4 also undergoes 5-monodeiodination to produce the metabolically inactive rT_3. About 90% of circulating rT_3 is derived from type I ID 5-deiodination rather than from synthesis in the thyroid gland (9). T_3 and rT_3 also can be deiodinated by type III, I or II to produce T_2. Because T_2 is the inactive form of the thyroid hormone, and the method to assay it is less accurate, investigators have not studied it.

Thyroid hormone has multiple functions. It has a profound influence in stimulating normal growth and development. In humans, a deficiency of thyroid hormone in the newborn leads to severe intellectual and growth retardation as well as delayed maturation. Besides of its effect on growth and development, thyroid hormone stimulates cardiac contractility and heart rate. Thyroid hormone also stimulates the production of specific proteins, such as growth hormone in the pituitary and sex hormone-binding globulin by the liver (17). Abnormal thyroid

Table 1 Summary of the features of iodothyronine deiodinases

Type of ID	I	II	III
location	liver, kidney, skeletal muscle, thyroid gland	brain, pituitary, brown adipose tissue	central nervous system, placenta
function	5- and 5'-monodeiodination	5'-monodeiodination	5-mono deiodination
major substrates	rT_3 T_4	T_4 rT_3	T_3 T_4
products	T_3 rT_3 T_2	T_3 T_2	rT_3 T_2

status can lead to many biochemical and pathological changes in metabolism (11).

History of the Characterization of Type I Iodothyronine Deiodinase

Studies have shown that selenium deficiency impairs thyroid hormone metabolism by inhibiting the synthesis and activity of type I iodothyronine deiodinase (9). Because of the similarities in many of the biochemical changes found in hypothyroidism and in selenium deficiency, these findings led to the investigation of the effects of the selenium deficiency on thyroid hormone metabolism (11).

In 1987, Beckett et al. (2) first investigated the effect of selenium deficiency on plasma thyroid-hormone concentrations and on the hepatic production of T_3 from T_4 in vitro. They fed two groups of rats a selenium-deficient diet (containing < 0.005 mg of Se/kg diet) or a normal diet (containing 0.1 mg Se/kg diet) for 4-6 weeks. Then, they measured the plasma T_4, T_3, rT_3 concentrations, hepatic selenium-dependent glutathione peroxidase activity, and hepatic T_3 production in vitro. They found that selenium deficiency produced up to a 14-fold decrease in hepatic T_3 production from T_4 in vitro. The T_3 production rate couldn't be restored by the addition of a variety of cofactors such as selenite, selenate and selenomethionine. The impairment in hepatic T_3 production observed in selenium deficiency was reflected in the concentrations of thyroid hormones circulating in plasma, T_4 being increased by approximately 40% and T_3 being decreased by

30%. Selenium deficiency had no measurable effect on plasma rT_3 concentrations. They suggested that selenium deficiency produced an inhibition of both 5- and 5'-monodeiodination, and that selenium would need to be incorporated during enzyme synthesis in vivo for activity to be restored. Although at that time it was not understood that type I iodothyronine deiodinase was a selenoprotein, the work of Beckett et al. (2) served as the basis for later discoveries.

Two years later, Beckett et al. (14) performed a similar experiment with rats. They examined the inhibition of type I and type II iodothyronine deiodinases activities in rat liver, kidney and brain produced by selenium deficiency. They fed two groups of rats -- a selenium-deficient group and a control group -- with the same diet used in the earlier study (2) for 5 or 6 weeks. They then measured the T_3 levels in plasma, liver, kidney, brain, T_4 and TSH levels in plasma and glutathione peroxidase activities in plasma , whole blood, liver, kidney and brain. They found that the selenium deficiency in rats produced an inhibition of T_3 production when thyroxine (T_4) was added to homogenates of the brain, liver and kidney. Consumption of the selenium-deficient diet for 5 weeks produced a significant increase in plasma T_4 and a significant decrease in plasma T_3 in the rats compared to values from the control group. The mean plasma concentration of T_4 and T_3 in the selenium-deficient rats increased by 64% and decreased by 22%, respectively. These results confirmed the previous observations of Beckett et al. (2). The rate of T_3 production in incubations of liver homogenates was

approximately 3 times greater than the rate observed in the kidney homogenates. In both organs, selenium deficiency produced a significant decrease in T_3 production. In brain homogenates, consumption of the selenium-deficient diet for 6 weeks produced a significant decrease in T_3 production. Feeding of the selenium-deficient diet produced no significant effect on plasma thyroid stimulating hormone. The activities of glutathione peroxidase in all tissues decreased. Administration of 0.01 mg Se/kg body weight had no significant effect on the abnormal thyroid-hormone metabolism. But feeding of 0.2 mg Se/kg body weight restored T_3 production and produced significant increases in glutathione peroxidase activities in all tissues. They concluded that adequate dietary selenium concentrations were required for normal activity of the family of iodothyronine deiodinases, and suggested that deiodinase enzymes might be selenoenzymes or require a selenium-containing cofactor for their activity.

Further, Beckett et al. (18) examined the effects of concurrent iodine (I) deficiency on thyroidal iodine content in selenium-deficient rats. In this study, four group of rats were used with the following diets; (i) Se and I-supplemented (0.1 mg Se, 1 mg I/kg diet), (ii) Se-deficient (<0.005 mg Se/kg diet), (iii) I-deficient (<0.01 mg I/kg), (iv) Se and I-deficient (<0.005 mg Se/kg, <0.01 mg I/kg). After 7 weeks on the diets, the thyroid glands were removed under anesthesia, and the plasma was separated from the blood cells. They found selenium deficiency had no effect on the thyroid gland weight, but iodine

deficiency increased thyroid weight by 50%, and combined iodine and selenium deficiency increased thyroid weight by 45%. Significant increases in plasma thyroid stimulating hormone were produced by iodine deficiency, and concurrent selenium deficiency amplified this effect. Concurrent selenium deficiency produced a further significant decrease in thyroidal iodine when compared with animals deficient in selenium or iodine alone.

Additionally, Beckett et al. (4) conducted other studies both in rats and humans. They measured free T_4 and total T_3 concentrations in plasma, the concentration of selenium and the activity of glutathione peroxidase in whole blood from the rats. They found that the activity of type I iodothyronine deiodinase was markedly diminished in the kidneys of selenium-deficient rats due to the decreased synthesis. For the human studies, blood was taken from 106 consecutive patients presenting at a thyroid clinic. They have reported that whole blood selenium and glutathione peroxidase levels were altered with thyroid status. In the hyperthyroidism due to Grave's disease, the whole blood selenium concentration and the whole blood glutathione peroxidase activity were decreased when compared to euthyroid patients. In patients with Grave's disease who had been rendered euthyroid, blood selenium and blood glutathione peroxidase levels were not significantly different from the levels found in euthyroid patients. Beckett et al. suggested that hyperthyroidism resulted in a lowering of blood selenium and glutathione peroxidase levels.

Arthur (11) found that feeding a low iodine diet to rats resulted in a lower type I iodothyronine deiodinase activity in the liver. These result agree with those study (18) in which lower hepatic and renal type I iodothyronine deiodinase activities were found in iodine-deficient rats.

Behne and coworkers (19) in 1992 also studied the relationships between type I iodothyronine deiodinase and selenium metabolism in rats. They conducted experiments to determine the effect of low dietary iodine or selenium on the enzymatic activities of type I iodothyronine deiodinase and glutathione peroxidase, and to investigate the relationship between the tissue levels of selenium and these selenoenzymes at a high dietary intake of selenite or selenomethionine. They found that hepatic activities of type I iodothyronine deiodinase and glutathione peroxidase were considerably lower in selenium-deficient rats fed with 0.00 2 mg Se/kg diet as compared to the control animals fed a diet with 0.3 mg Se/kg diet. This is in accordance with the numerous studies on glutathione peroxidase and the recent results reported for type I iodothyronine deiodinase (2,8,20). Similar changes were observed in another study in which rats were fed a selenium-deficient diet for 5 weeks (21),

Behne and coworkers (20) also observed that an increase in dietary selenium above the normal amount (2 mg Se/kg diet) led to elevated tissue selenium concentrations in liver, thyroid, muscle and that retention was higher when the element was administered as selenomethionine than when it was given in an

inorganic form (selenite). Similar results were also produced by other investigations (22,23,24). Excessive selenium supply did not elevate the activities of hepatic glutathione peroxidase in the tissues, but caused a slight decrease. This result is not in agreement with previous results which reported that hepatic glutathione peroxidase activity of rats remained unchanged (24) or depressed (25) after excessive dietary selenium supply.

Finally, Behne et al. (20) also first studied the effect of high dietary selenium supply on the metabolism of type I iodothyronine deiodinase in rats. They found that the elevated selenium levels in the liver were not accompanied by an increase in the type I iodothyronine deiodinase activity, but actually resulted in a decrease. In the thyroid glands, the increase in the selenium supply had no significant effect on the concentrations of T_3 and T_4. They concluded that tissue levels of type I ID, similar to those of glutathione peroxidase, were kept within certain upper limits by homeostatic mechanisms. Excessive selenium supply did not further increase the activities of the selenoenzymes and might even have adverse, depressive effects. At high dietary intake, the tissue selenium content could not be considered an indication of selenoenzyme activities.

In 1992, Van Lente and Daher (26) evaluated plasma selenium concentrations in patients with sick euthyroid syndrome diagnosed by low serum T_3 concentrations. They measured concentrations of plasma T_4, T_3, TSH, selenium and albumin in their study. The selenium status in the patients with sick

euthyroid syndrome, untreated and treated hypothyroidism were compared with controls who were healthy. They found that selenium concentrations in plasma were significantly lower in hospitalized patients with either euthyroid sick syndrome or treated hypothyroidism than in patients with untreated hypothyroidism who were ambulatory. They concluded this decrease was due to nutritional effects. There was no significant, independent relationship between selenium and the thyroid function indices in their study. The strongest association was between serum albumin concentration and either selenium, T_3, or the molar ratio of T_3 to T_4.

Statement of the Problem

To summarize previous studies, two points can be made. First, type I iodothyronine deiodinase is selenium-containing enzyme. Selenium deficiency has been reported to cause abnormal thyroid hormone metabolism in laboratory animals. Second, most previous studies were performed using laboratory animals rather than humans. In the two published studies which investigated the relationship between plasma selenium concentrations and thyroid hormone status in humans (4, 26), altered selenium concentrations were found. One study (26) suggested that the lowered plasma selenium concentrations observed in their study subjects were most probably due to nutritional factors rather than hormonal effects. However, plasma selenium levels are not as suitable an indicator of nutritional

selenium status as is measurement of red blood cell (RBC) levels. RBC selenium levels are an integrated measure of selenium nutritional status over several months, while plasma levels reflect more immediate changes in selenium status. No study has been done investigating RBC selenium levels in hyper- or hypothyroid children or adults.

Is there a relationship between thyroid hormone status and selenium status (as assessed by plasma and/or RBC selenium levels) in humans? Furthermore, is there any relationship between selenium concentrations in these tissues and other trace elements reported to be altered by thyroid hormone status such as zinc, copper, and manganese? To examine the relationship between selenium and thyroid hormone status in hypothyroid, hyperthyroid and euthyroid children, selenium concentrations were measured by assaying selenium levels directly in plasma and red blood cells. Thyroid hormone status was determined by assaying plasma T_4 and TSH. Additionally, data on plasma zinc and copper, and red blood cell manganese and zinc for these subjects were available from unpublished studies, and we determined if concentrations of these trace metals were correlated with selenium levels in each of the diagnostic groups. These data were then examined statistically to determine significant correlations existed.

Chapter 2 Materials and Methods

Equipment

Selenium concentrations in plasma and red blood cells were determined using a polarized graphite furnace Zeeman-effect atomic absorption spectrophotometer (Model Z-8100, Hitachi Instrument Inc., Danbury, CT) equipped with tube-type pyrolytic graphite cuvettes, an optical pyrometer for furnace temperature control and an SS-200 autosampling system. The radiation source was an electrodeless discharge and additional direct current power supply (Hitachi Instruments Inc., Danbury, CT). Commercial grade argon gas was used as the carrier and shielding gas.

Hemoglobin concentrations were measured using a Spectronic 1001 spectrophotometer (Bausch & Lomb, Rochester, NY).

Eppendorf automatic pipettors (Brinkmann Instruments, Westbury, NY) and metal-free pipet tips (Bio Rad Scientific, Inc. Melville, NY) were used for all preparations of standards, controls and specimens.

Polystyrene 0.5 and 1.0 mL volume autosampler cups (Baxter Heath Çare Corporation, McGaw Park, IL) were used during the assays.

Blood samples were collected by the staff of the Children's Hospital of the King's Daughters (CHKD). EDTA-containing microtainers (Becton-Dickinson Co.

Inc., Cockeysville, MD) and stainless steel "butterfly" or vacutainer needles , or stainless steel lancets (Abbott Industries Inc., Chicago, IL) were used during blood collection.

Reagents

All reagents were prepared using deionized water obtained from a commercial 18 mega-ohm system (Millipore Systems, Commonwealth H_2O Inc.).

The selenium working standard was prepared from 10.00 g/L stock atomic absorption standard (National Institute of Standards and Technology, Gaithersburg, MD) as a 1:10 dilution followed by 1:1000 dilution. The final selenium working standard concentration was 1.000 g/L (1000 ppm).

The palladium (II) chloride (99% purity, Aldrich Chemical Co., Inc., Milwaukee, WI) solution was prepared by dissolving with stirring 0.1773 g palladium chloride in 540 μL concentrated hydrochloric acid (Analytical Reagent Grade, Baxter Health Care Corporation, McGaw Park, IL) and diluted with Milli-Q water to 100 mL. The final concentrations of palladium (II) chloride and hydrochloric acid solutions were 9.9 mmol/L and 65 mmol/L respectively. The palladium (II) chloride solution served as the matrix modifier. The function of palladium was to minimize volatilization losses of selenium at ash temperatures of up to 1400 ^{0}C, which are necessary for removal of most of the sample matrix.

Drabkin's solution was prepared by reconstituting Drabkin's Reagent (Sigma

Diagnostics, St. Louis, MO). A vial containing 100 parts sodium bicarbonate, 20 parts potassium ferricyanide, 5 parts potassium cynaide was diluted to 1.0 L using Milli-Q water. This reagent was stored in dark and used for the RBC hemoglobin determination. The cyanmethemoglobin standard (Sigma Diagnostics, St. Louis, MO) used for the hemoglobin assays had a concentration of 0.72 g cyanmethemoglobin/L.

Controls used in this study were:

1. Low, normal and high hematology controls (Fisher Scientific, Pittsburgh, PA)) which were used for hemoglobin assays.
2. Normal and high trace elements serum toxicology controls (UTAK Labs Inc., Canyon Country, CA) which were used for red blood cell and plasma selenium assays.
3. Control level I and level II sera (Centers for Disease Control, Atlanta, GA) were also used for RBC and plasma selenium assays.
4. Additionally, an EPA trace metals control and an in-house blood control were used for plasma and/or red blood cell selenium assays.

Subjects

Seventy-three children with thyroid disease and forty-eight euthyroid children served as the study subjects. According to their diagnoses, the thyroid patients were separated into two categories: hypothyroid (status 2) and

hyperthyroid (status 3). They were further subdivided into untreated (group 1), treated (group 2), or nonresponsive to treatment (group 3). Table 2 summarizes the classification of subjects in this study. Forty-eight patients who had normal thyroid function were used as controls.

This study was done retrospectively using blood collected during outpatient visits by patients to the CHKD Clinic. The selenium specimens were collected for other trace metal assays beginning 1984 through 1993.

Specimen Handling

Specimens for plasma and red blood cell assays were separated immediately after collection and stored frozen at -20 ^{0}C until assay.

Methods

Hemoglobin Determination

Red blood cell hemoglobin was assayed using the cyanmethemoglobin technique (28).

Selenium Determination Using Polarized Zeeman Effect Atomic Absorption Spectroscopy (PZAAS)

Plasma and red blood cell concentrations of selenium were measured using polarized graphite furnace Zeeman-effect atomic absorption spectroscopy and a

Table 2 Classification of subjects

status	thyroid status	number of patients
1 (control)	euthyroid	48
2	hypothroid	16[a] 21[b] 13[c]
3	hyperthroid	4 13 6

a untreated group
b treated group
c nonresponsive group

modified method as described by Pleban et al. (28) and Jacobson and Lockitch (29). Five aqueous selenium standard solutions were prepared to contain 0, 10, 25, 50, 75 μg Se/L by adding 0, 10, 25, 50, 75 μL of the selenium working standard into an appropriate amount of deionized water containing 400 μL palladium (II) chloride solution to give a final volume of one milliliter.

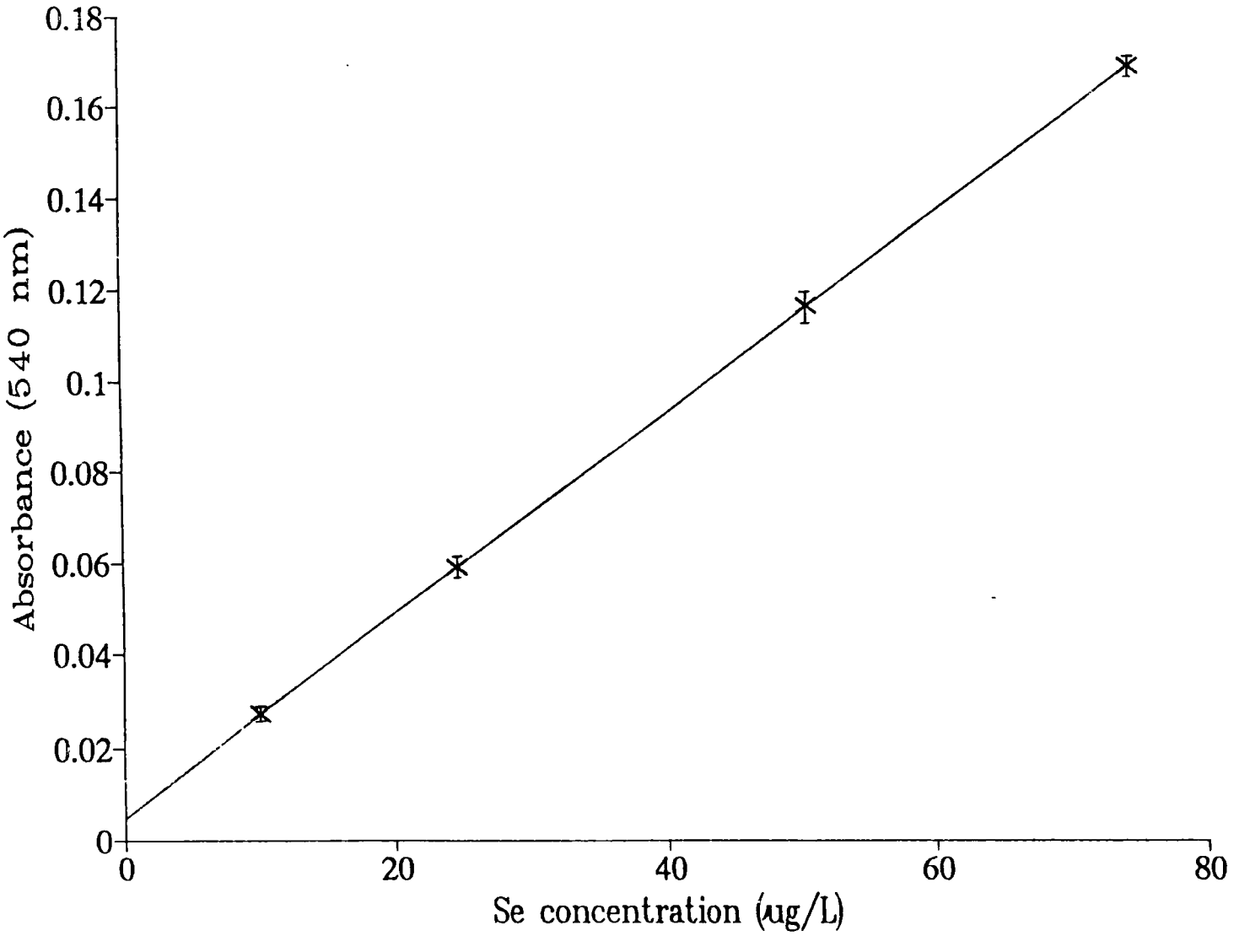
Controls and specimens solutions were prepared by diluting an aliquot of specimens with two aliquots of diluted palladium (II) chloride solution and two aliquots of deionized water. The dilution factor was five.

Standards, controls and specimens were placed in the autosampler wheel, a blank and the EPA controls were run after each 15 to 20 specimens. Blank and the EPA control were also assayed at the end of each run. Results of the analysis of blanks and controls were used to assess the precision and accuracy of the methodology.

To obtain the selenium concentration in the diluted specimen, the mean of the selenium absorbance from duplicate pipettings was compared to a matrix modifier based standard curve, and multiplied by the dilution factor. A representative curve is shown in Figure 2.

Complete methodology for selenium measurement is described in Appendix A.

Plasma zinc and copper, and RBC manganese and zinc concentrations (assayed using PZAAS) were provided by Drs. Pleban and Rohn. These data have


0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Absorbance (540 nm)
0
20
40
60
80
Se concentration (ug/L)

not been published.

Statistics

All data were analyzed using SPSS program to determine differences among the groups: untreated, treated, nonresponsive in each the diagnostic status patients and compared to the controls. The nonparametric correlation coefficients (Kendall Coefficients) were used to assess interrelations among variables --- plasma selenium, copper, zinc, T_4, TSH and red blood cell selenium, zinc, manganese. In addition, a two-tailed Student's t-test was used to compare means (when variances between groups were not significantly different).

Chapter 3 RESULTS

Accuracy and Precision

To insure good accuracy and precision of the results, the following controls were assayed in each run: normal and high trace elements serum toxicology controls, control level I and level II, EPA trace metals control and an in-house blood control. In addition, we checked the reproducibility of the results by assaying an EPA control between every 15-20 patient specimens and at the end of each assay. Additionally, we checked for contamination by running a blank at the beginning and end of each run. For the red blood cell assays, we prepared an in-house control which was collected and handled in the same manner as the specimen. No commercial control was available to check the accuracy of the red blood cell selenium assays. However, our methodology has performed well in a recent interlaboratory comparison survey for laboratories performing red blood cell selenium assays which has conducted by the Selenium Subcommittee of the International Union of Pure and Applied Chemistry (30). The statistical results for the control analysis of selenium assays are listed in Table 3 and compared to the reported values. Mean (SD) blank values were 0.226 (2) μg Se/L indicating minimal selenium contamination in the reagents. The mean (SD) selenium level of in-house blood control in this study was 46.6 (6) μg/g Hgb, while our results

Table 3 Statistical results for

selenium assays of controls in this study

controls	number	mean	SD	CV[c]	reported reference value mean (SD)
normal serum control[a]	14	106	11	10	96(10)
high serum control[a]	18	323	30	9	320(30)
level I[a]	9	114	10	9	116(14)
level II[a]	7	197	31	15	186(18)
EPA[a]	16	39	4	10	47(15)
in-house blood control[b]	15	46	6	13	45(3)

a: all units in μg Se/L
b: μg Se/g Hgb
b: coefficient of variation (defined as 100 time the standard deviation divided by the mean)

from previous study was 45.0 (3) μg Se/g Hgb (31). Selenium concentrations for all the controls were in the reported reference ranges. The coefficient of variation for the controls ranged from 9 to 15%. We obtained acceptable accuracy and precision.

Thyroid Hormones (T_4, TSH) Results

Because we did not have thyroid hormone data for the controls, we did not compare the concentrations of these hormones between controls and patient groups. Comparison of T_4 and TSH results to reported reference ranges were used to establish placement of each patient in a diagnostic group.

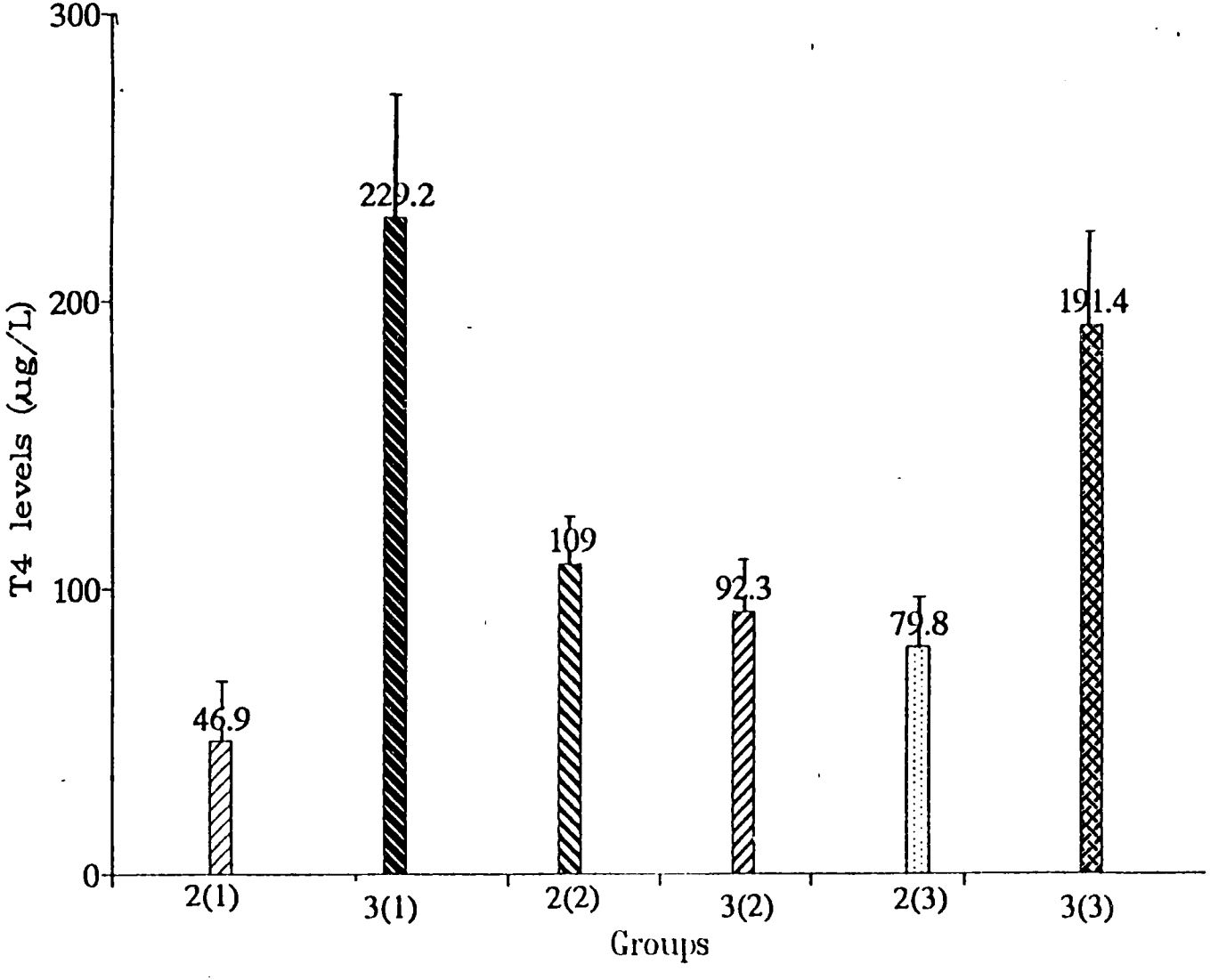
Hypothyroidism is a clinical state that develops whenever an insufficient amount of active thyroid hormone is available to tissues. Primary hypothyroid patients have a low plasma T_4 level combined with a high plasma TSH level. Hyperthyroidism refers to the clinical syndrome caused by an excess of circulating active thyroid hormone. Hyperthyroid patients have high plasma T_4 level and very low plasma TSH levels which are often is undetectable (32). T_4 and TSH reference ranges are 80.0-120.0 μg/L and 0.51-5.71 mU/L, respectively (33).

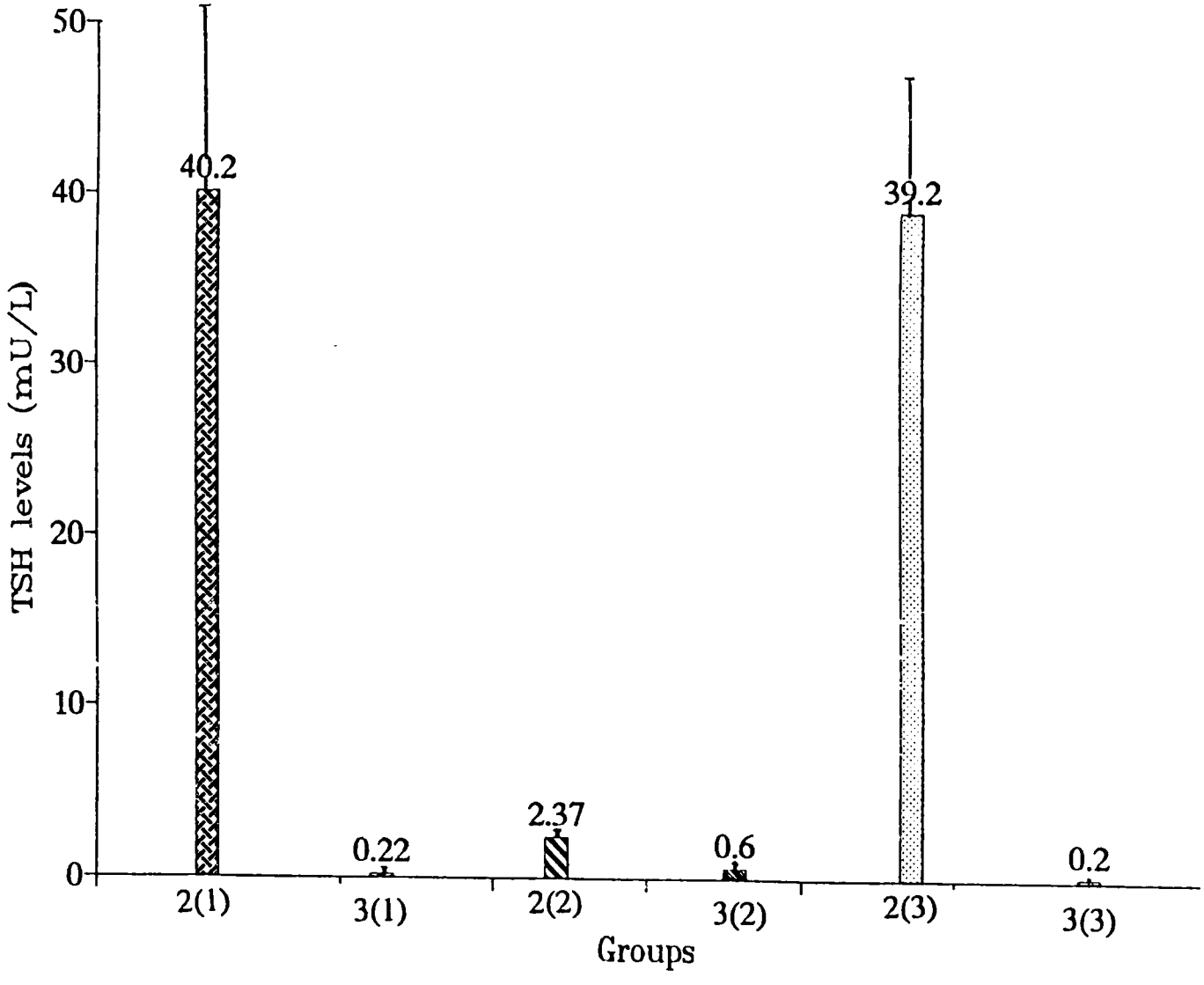
T_4 and TSH concentrations were measured in fifty and forty-seven specimens from hypothyroid patientsrespectively, thirty and sixteen specimens from hyperthyroid patients, respectively. Due to multicollections for some patients at different times, the patient number was not always the same as the number of

specimens. Each diagnostic status was classified into three groups called untreated (group 1), treated (group 2) and nonresponsive groups (group 3). Mean (SD) results of T_4 and TSH for hypothyroid and hyperthyroid patients are shown in Figures 3 and 4.

In hypothyroid patients, group 1 patients had lower mean (SD) T_4 levels [46.9 (28.9) μg/L] and higher mean (SD) TSH levels [40.2 (33.7) mU/L]. After treatment, mean (SD) T_4 levels went up [109.0 (36.4) μg/L] and mean (SD) TSH levels went down [2.37 (2.47) mU/L]. Both the levels were within the reference ranges. Group 3 patients still had lower mean (SD) normal T_4 level [79.8 (30.6) μg/L] and higher mean (SD) TSH levels [39.2 (30.6) mU/L].

In hyperthyroid patients, group 1 patients had elevated mean (SD) T_4 levels [229.2 (126.8) μg/L] and very low mean (SD) TSH levels [0.22 (0.14) mU/L]. After treatment, mean (SD) T_4 levels went down [92.3 (30.3) μg/L] and mean (SD) TSH levels went up [0.67 (0.63) mU/L], both mean levels were in the reference ranges. Group 3 still had high mean (SD) T_4 levels [191.4 (32.8) μg/L], and normal mean (SD) TSH levels [0.22 (0.14) mU/L]. The data for both T_4 and TSH in our groupings of hypothyroid and hyperthyroid patients were in agreement with the classification criteria for thyroid diseases.


300
200
100
0
T4 levels (µg/L)
46.9
229.2
109
92.3
79.8
191.4
2(1)
3(1)
2(2)
3(2)
2(3)
3(3)
Groups


50
40
30
20
10
0
TSH levels (mU/L)
40.2
0.22
2.37
0.6
39.2
0.2
2(1)
3(1)
2(2)
3(2)
2(3)
3(3)
Groups

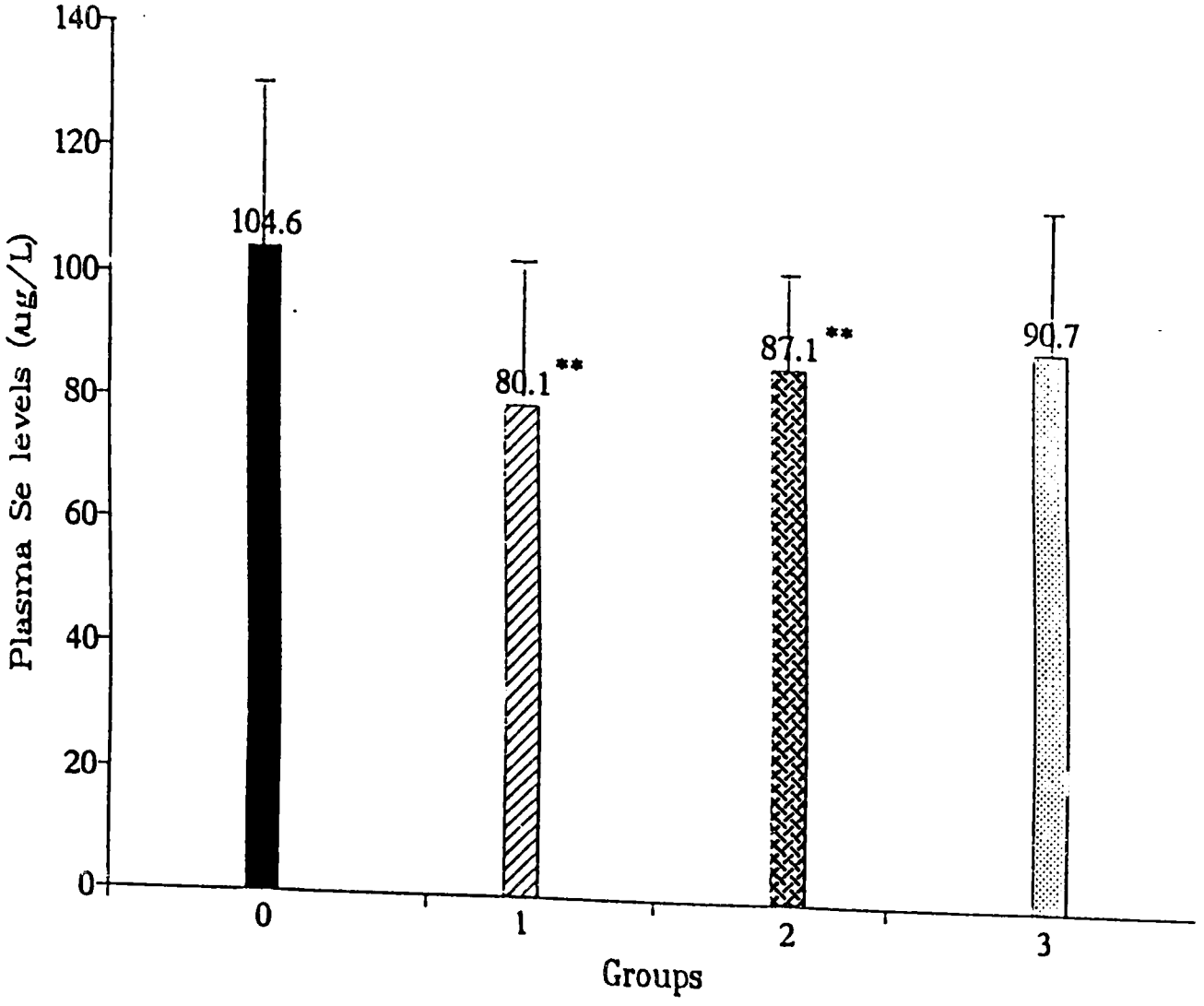
Selenium results

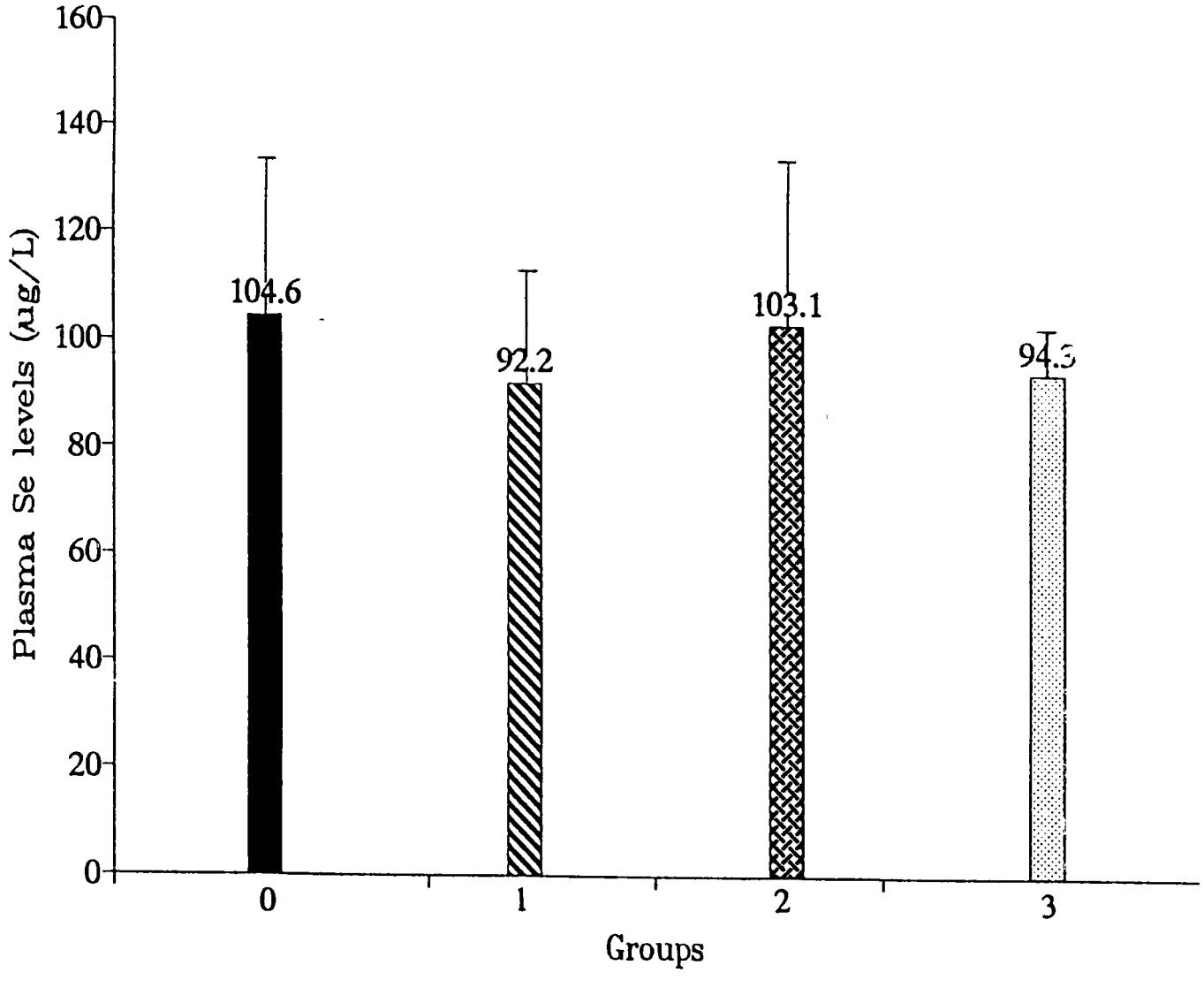
Plasma Selenium Levels

Forty-eight euthyroid patients served as controls (status 1) in this study. Mean (SD) plasma selenium level for the controls were 104.6 (30.6) μg/L.

We also assayed fifty hypothyroid patients (status 2) which were subclassified into untreated, treated and nonresponsive patients. Mean (SD) plasma selenium concentrations for these individuals are shown in Figure 5. When we compared mean plasma selenium concentrations for three groups of hypothyroid patients to the control selenium using the two-tailed Student's t-test, we found that the plasma selenium concentrations in untreated [80.1 (26.7) μg/L] and treated [87.1 (18.3) μg/L] groups were significantly lower than that in the control [104.6 (30.6) μg/L]. Response to treatment was assessed in hypothyroid patients by requiring TSH to be normal with the exception one of patient who was under the age of one year and had a diagnosis of congenital hypothyroidism. Nonresponsive patients had mean (SD) plasma selenium level of 90.7 (26.7) μg/L, which was not significantly different from the mean control level [104.6 (30.6) μg/L].

Plasma selenium concentrations were also measured in twenty-three hyperthyroid patients (status 3). For the hyperthyroid patients, subdivided into three groups, mean (SD) plasma selenium concentrations are shown in Figure 6. When we compared mean plasma selenium concentrations for these three groups


140
120
100
80
60
40
20
0
Plasma Se levels (ug/L)
104.6
80.1 **
87.1 **
90.7
0
1
2
3
Groups


160
140
120
100
80
60
40
20
0
Plasma Se levels (ug/L)
104.6
92.2
103.1
94.3
0
1
2
3
Groups

of hyperthyroid patients to the mean control selenium level using the two-tailed Student's t-test, we found that plasma selenium concentrations in untreated [92.2 (21.2) μg/L] and treated [103.1 (31.4) μg/L] hyperthyroid patients were not significantly different. The selenium levels in treated [103.1 (31.4) μ/L] group were very close to the control value [104.6, (30.6) μg/L]. Hyperthyroid patients which did not respond to treatment assessed by their T_4 levels (T_4 > 120.0 μg/L) had mean plasma selenium levels of 94.3 (8.8) μg/L.

Red Blood Cell Selenium Levels

We measured red blood cell selenium concentrations for total fifty hypothyroid patients (status 2). For three groups of hypothyroid patients, the mean (SD) red blood cell selenium concentrations for these children are shown in Table 4. When we compared mean (SD) red blood cell selenium levels of hypothyroid patients to the control levels using the two-tailed Student's t-test, we found that mean red blood cell selenium concentrations in group 1, 2 or 3 were not significantly different from the mean control levels.

Red blood cell selenium concentrations were measured in total a twenty-three hyperthyroid patients. For group 1, 2 and 3, means (SD) red blood cell selenium concentrations are shown in Table 5. Comparison of mean red blood cell selenium levels of all three groups of hyperthyroid patients to the mean control level using the two-tailed Student's t-test, indicated that red blood cell selenium

Table 4 Red blood cell selenium levels

in hypothyroid patients

groups	mean (SD) (μg Se/g Hgb)	specimen number	P[e] value
group 0[a]	0.570(0.261)	50	
group 1[b]	0.607(0.181)	15	0.532
group 2[c]	0.543(0.176)	30	0.477
group 3[d]	0.722(0.250)	13	0.066

a: control group
b: untreated group
c: treated group
d: nonresponsive group
e: probability (for t value calculated using two-tailed t-test)

Table 5 Red Blood Cell Selenium Levels

in Hyperthyroid Patients

groups	mean (SD) (μg/g Hgb)	specimen number	P[e] value
group 0[a]	0.570(0.261)	50	
group 1[b]	0.530(0.122)	4	0.599
group 2[c]	0.660(0.188)	20	0.099
group 3[d]	0.562(0.059)	5	0.942

a: control group
b: untreated group
c: treated group
d: nonresponsive group
e: same as Table 4

concentrations were not significantly different from the mean control level.

Other Trace Metal Concentrations

We assayed plasma zinc, copper and red blood cell zinc and manganese for fifty hypothyroid patients. Mean (SD) trace metals levels for hypothyroid patients are shown in Table 6. Comparison of the mean of trace metal levels for three groups hypothyroid patient groups to the control levels using the two-tailed Student's t-test, indicate that the mean plasma zinc concentrations in treated [77 (19) μg/L] and nonresponsive [78 (16) μg/L] groups were significantly lower than the mean control levels [93 (21) μg/L].

Trace metal concentrations were also assayed for twenty-three hyperthyroid patients. Mean trace metal levels for three groups of hyperthyroid patients are shown in Table 7. Comparison of the mean (SD) of trace metal levels for the three groups hyperthyroid patients to the mean control level [93 921) μg/L] using the two-tailed Student's t-test, shown treated hyperthyroid patients had significantly lower plasma zinc concentrations [73 (19) μg/L]. In addition, untreated [19 (3) μg/L] and nonresponsive [20 (6) μg/L] hyperthyroid patients had significantly lower red blood cell zinc concentrations than that oof the control [35 (7) μg/L].

Table 6 Mean (SD) Trace metal levels in hypothyroid patients

hypothyroid patients	plasma Zn	RBC Zn	plasma Cu	RBC Mn
group 0[a]	93(21)**	35(7)	107(23)	0.079 (0.024)
group 1[b]	81(21)	30(10)	93(36)	0.123 (0.092)
group 2[c]	76**(19)	35(14)	98(34)	0.087 (0.031)
group 3[d]	78**(16)	35(9)	103(27)	0.099 (0.063)

all units are μg/L for plasma assays and μg/g Hgb for RBC assays.
a: the control group
b: untreated group
c: treated group
d: nonresponsive group
**: significantly lower ($p < 0.017$)

Table 7 Mean (SD) Trace metal levels in hyperthyroid patients

hyperthyroid patients	plasma Zn	RBC Zn	plasma Cu	RBC Mn
group 0[a]	93[##](14)	35[**](7)	107(23)	0.079 (0.024)
group 1[b]	80(14)	19[**](3)	147(37)	0.110 (0.042)
group 2[c]	72[##](17)	29(11)	115(48)	0.097 (0.055)
group 3[d]	107(48)	20[**](6)	139(40)	0.085 (0.060)

** all units are μg/L for plasma assays and μg/g Hgb for RBC assays.
a: the control group
b: untreated group
c: treated group
d: nonresponsive group
##: significantly lower ($p < 0.001$)
**: significantly lower ($p < 0.03$)

Correlation Coefficients

Abnormalities in trace metal (Zn, Se, Mn, Cu) metabolism have been reported in patients with thyroid diseases, but data about interactions between thyroid hormones and trace metal levels in human are limited (34), we investigated the relationships for each diagnostic status between trace metal levels, T_4 and TSH. We grouped all diagnostic groups (1, 2, 3) for each thyroid disease state together, and calculated correlation coefficients to determine if significant relationships between the variables --- plasma Se, Zn, Cu, T_4, TSH and red blood cell Se, Zn, Mn existed. In the control group, the only significant correlation [correlation coefficient was 0.259 ($p=0.003$)] found was between plasma and red blood cell selenium.

Significant correlations found in hypothyroid patients are shown in Table 8. In hypothyroid patients, plasma and red blood cell selenium, plasma selenium and zinc, plasma selenium and red blood cell zinc concentrations were positively correlated. Red blood cell zinc and T_4, TSH and T_4, plasma copper and TSH, and red blood cell manganese and plasma selenium concentrations were negatively correlated.

Significant correlations found in hyperthyroid patients are shown in Table 9. In hyperthyroid patients, plasma selenium and TSH, red blood cell selenium and zinc, plasma zinc and T_4, plasma copper and T_4 concentrations were positively correlated. Red blood cell selenium and plasma zinc, red blood cell zinc and T_4,

Table 8 Significant correlations in hypothyroid patients

correlations	correlation coefficients
P Se and RBC Se	0.259, p= 0.003 (n=53)
P Se and P Zn	0.217, p=0.014 (n=50)
P Se and RBC Zn	0.177, p=0.034 (n=52)
RBC Zn and T_4	-0.205, p=0.021 (n=48)
TSH and T_4	-0.310, p=0.001 (n=47)
P Cu and TSH	-0.197, p=0.025 (n=48)
RBC Mn and P Se	-0.186, p=0.026 (n=53)

P=plasma

Table 9 Significant correlations in hyperthyroid patients

correlations	correlation coefficients
P Se and TSH	0.575, p=0.004 (n=13)
RBC Se and RBC Zn	0.232, p=0.042 (n=29)
P Zn and T_4	0.362, p=0.003 (n=30)
P Cu and T4	0.353, p=0.003 (n=30)
RBC Se and P Zn	-0.303, p=0.011 (n=29)
RBC Zn and T_4	-0.292, p=0.015 (n=29)
TSH and T_4	-0.316, p=0.050 (n=27)
P Se and T_4	-0.289, p=0.018 (n=27)

P=plasma

TSH and T_4, and plasma selenium and T_4 concentrations were negatively correlated.

To summarize the correlations in patients diagnosed with thyroid disease, we found that hypothyroid and hyperthyroid patients had consistent correlations between TSH and T_4, red blood cell zinc and T_4.

Chapter 4 DISCUSSION

We have found significantly lower plasma selenium concentrations in untreated and treated hypothyroid patients versus control. However, plasma and red blood cell selenium concentrations in other patients with thyroid disease were not significantly different from those found in the control children.

Our results for hypothyroid patients differed from those reported by Van Lente and Daher (27). In Van Lente and Daher's study, sick euthyroid and treated hypothyroid patients had significantly lower plasma selenium concentrations when compared to untreated hypothyroid patients and control subjects. In their study, untreated patients had lower plasma selenium levels when compared to those of the healthy control group, but the difference was not significant. However, the patients involved in the study of Van Lente and Daher were adult subjects with 65% of the untreated hypothyroid patients ambulatory, and 88% of the treated sick euthyroid patients hospitalized with other severe physical conditions (diabetic mellitus, surgery, etc.). In addition, Van Lente and Daher found that there was a significant positive correlation between serum albumin and plasma selenium. They suggested that the severe physical condition of treated hypothyroid and sick euthyroid patients caused a hypercatabolic state combined with poor nutritional

state and the loss of albumin in urine. Thus, the plasma selenium levels in these patients may have been decreased for these reasons. Also the blood volume in hospitalized patients is increased 10% due to immobilization. Therefore, plasma selenium levels which are based on plasma volume could have been lower due to hypervolemia. In our study, all patients were clinic outpatients, and their physical condition was probably not as severe as the subjects in the study of Van Lente and Daher. Furthermore, we studied children whose ages ranged from one to seventeen years old. The normal red blood cell selenium levels in our hypothyroid patients indicated that they were in good selenium nutrition. Red blood cell selenium levels in all groups of hypothyroid patients were not significantly different from controls suggesting that there was no difference in selenium nutrition between these groups. The major form of selenium in red blood cells is cellular glutathione peroxidase and is thought to represent a reserve of selenium that can be mobilized (35). These differences in the study subjects could explain why our results differed from those reported by Van Lente and Daher. The decreased plasma selenium levels found in our patients may actually be even lower since plasma volumes are reported to be decreased by 25% on the average in untreated hypothyroid subjects (36). Hypovolemia would cause the plasma selenium concentrations to be artifactually higher. The cause of the lowered plasma selenium concentrations is unclear, but mild albuminuria has been reported in untreated hypothyroid subjects (36). Perhaps lowered levels are due to urinary

losses, since glutathione peroxidase and selenoprotein P (the major selenium-containing proteins) have molecular weights similar to that of albumin. Alternatively, there may be a shift of selenium from the plasma to other tissue sites, or decreased synthesis of one or more of the selenium-containing proteins. Further work is necessary to determine the cause of the lowered plasma selenium levels in hypothyroid children.

Blood volume in untreated hyperthyroid patients is increased (36), which can cause plasma selenium levels to be artifactually lower in these patients. All groups of hyperthyroid patients did not have significantly different red blood cell selenium concentrations from controls. We can't say whether plasma selenium levels are affected by hyperthyroidism, because we were unable to adjust for the plasma volume increase (by ratioing selenium levels to albumin) expected in untreated hyperthyroid patients. Additionally, red blood cell selenium concentrations in hyperthyroid patients were not significantly different from the controls. These findings appear to be in opposition to those reported by Beckett et al.(4). They found lower whole blood levels which returned to control levels upon treatment. The patients in the study of Beckett et al. were diagnosed with Grave's disease, an autoimmune disorder. They found no differences in whole blood from hyperthyroid patients with other etiologies. We studied a small group of only four untreated patients and six nonresponsive patients, our small groups also did contain untreated hyperthyroid patients with Grave's disease. Moreover,

Beckett et al. used whole blood for their analyses. Anemia is reported to be common in untreated thyroid disease (36). Therefore, if whole blood selenium levels are not corrected for the hematocrit, they will appear lower since red blood cells contain a larger fraction of the selenium. The anemia would be resolved upon treatment and whole blood selenium would be expected to return to control levels.

Since 1988, many research articles (2,4,17,18,37) have been published and suggested that selenium deficiency affects thyroid hormone metabolism and activity of type I iodothyronine deiodinase. We did not measure the activity of type I iodothyronine deiodinase, and it is possible that alterations on the activity of this enzyme would be useful to interpret our results.

T_4 when converted to T_3 stimulates mRNA for type I iodothyronine deiodinase production, possibly to facilitate metabolism to the inactive T_2 forms. Therefore, severe selenium deficiency should affect the ability of hyperthyroid patients to metabolize T_4. But none of our study subjects appear to be severely selenium deficient as assessed by red blood cell levels.

In the investigation of correlations among variables, consistently negative correlation between TSH and T_4 has been seen in both hypothyroid and hyperthyroid patients. Negative correlation between TSH and T_4 is expected based on the negative feedback regulation of thyroid function (38). The strong positive correlation (0.575, $p=0.004$) between plasma selenium concentrations and TSH

levels in hyperthyroid patients is intriguing. However, since plasma selenium levels are positively correlated with plasma T_4, T_3 and TSH are positively correlated, this may explain our results. We found no relationship between plasma selenium level and TSH or T_4 in hypothyroid patients.

When compared to the control group, all hypothyroid patients had low plasma zinc concentrations, but only the treated and nonresponsive groups were significantly lower. Perhaps this reflects the shifting of zinc from plasma to tissues, which is frequently seen during growth spurts. Red blood cell zinc levels were not significantly different from controls, however, these levels were inversely related to T_4 levels in all hypothyroid patients.

All hyperthyroid patients had lower red blood cell zinc concentrations, but only untreated and nonresponsive patients had significantly lower levels compared to those seen in the controls. Theses results are in agreement with the study of Dolve et al. (34). In their study, they found that red blood cell zinc levels were significantly lower in hyperthyroid patients (not differentiated according to response to treatment).

We also found an inverse correlation between red blood cell zinc and T_4 concentrations in both hypothyroid and hyperthyroid patients. Most zinc in the red blood cell is present as the component of the isoenzyme carbonic anhydrase B (39) and thyroid hormone inhibits the synthesis of this enzyme (40). Hyperthyroid patients with high concentrations of T_4 will inhibit the synthesis of carbonic

anhydrase B, so the red blood cell zinc content is correspondingly decreased. Hypothyroid patients with low concentrations of T_4 will have higher synthesis of carbonic anhydrase B, and red blood cell zinc is correspondingly increased. Our results support this explanation. In all hypothyroid patients red blood cell zinc concentration was 33.6 μg/g Hgb. In all hyperthyroid patients, red blood cell zinc concentration was 23.2 μg/g Hgb. When compared to the hypothyroid patients, hyperthyroid patients had lower red blood cell zinc concentrations. But red blood cell zinc concentrations in hypothyroid patients were lower than the control (red blood cell zinc level for the control was 35.1 μg/g Hgb), suggesting that nutritional effects may have influenced our results.

Plasma copper is reported (36) to be increased in hyperthyroid patients and we found normal copper levels in all three groups of hyperthyroid subjects. Again a larger study group is needed to determine if thyroid patients have significantly increased plasma copper levels. We found a positive correlation between plasma copper and T_4 levels in hyperthyroid patients. Ninety-five percent of the plasma copper is present in ceruloplasmin, an antioxidant enzyme, and this may be a protective response to the hypercatabolic state induced by elevated T_4 levels.

In hypothyroid patients, we found a significant positive correlation between plasma concentration of selenium and zinc. Since both there metals may be loosely bound with albumin, urinary losses may be common explanation for this observation. Measurement of urinary levels of both these metals would answer

this question. The other relationships found between plasma selenium and RBC manganese and zinc in hypothyroid patients, RBC zinc and plasma and RBC zinc are less easy to explain.

Chapter 5 Conclusions

We found significantly lower plasma selenium concentrations in untreated and treated hypothyroid children when compared to euthyroid control children. Normal plasma and red blood cell selenium concentrations were observed in hyperthyroid patients regardless of treatment, when compared to control values. Plasma selenium was strongly correlated with TSH levels in hyperthyroid patients. No significant difference was found between RBC selenium concentrations in any of three hypothyroid and control groups, suggesting normal selenium nutritional status.

We also found that significantly lower plasma zinc in treated and untreated hypothyroid patients, and significantly lower red cell zinc in treated and nonresposive hyperthyroid patients, respectively. Additionally, red blood cell zinc and plasma T_4 concentrations were inversely correlated.

Because only few studies on the relationship between selenium and thyroid status have been done so far, further studies are needed in order to determine the effect of thyroid status on selenium metabolism.

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Appendix A

Selenium Assay in Serum/Plasma and Erythrocytes

Principle:

Serum/plasma or erythrocyte selenium concentration is determined directly in the specimen using a palladium matrix modifier. The metal is assayed using polarized Zeeman-effect flameless atomic absorption spectrophotometry.

Equipment:

Zeeman-effect atomic absorption spectrophotometer (Model Z8100) equipped with optical temperature monitor, and autosampling system (Model SS200) all from Hitachi, Ltd.

Pyrolytic graphite cuvets (180-7444)

Vortex mixer

Autosampler cups, 2.00 and 0.500 mL volume

Variable automatic pipettors (100-1000, 10-100 microliters)

Fixed volume pipettors (50, 100 microliters)

100 mL Nalge volumetric flask

Reagents, Controls:

Selenium Spectrometric Standard (10,000 mg/L, SRM 3149, NBS)

Palladium (II) Chloride (analytical reagent grade, Aldrich Chemical Co.). Prepare 9.4 mmol/L solution by dissolving 170 mg of the palladium chloride in about 75 mL of 18-meg-ohm deionized water and 540 microliters of concentrated HCl (analytical reagent grade, Fisher Scientific Co.) in a 100 mL volumetric, class A flask. Final concentration: 9.9 mmol/L $PdCl_2$ in 65 mmol/L HCl. Stir overnight, and when dissolved, dilute to 100 mL mark.

Seronorm Trace Element Lyophilized Reference Serum (normal control, Accurate Chemical Sciences Corp.). Reconstitute with 3 mL of deionized water.

Hemolysate Control (Erythrocyte Assays only)

Preparations prior to Analysis:

1. Insure that all reagents and controls are prepared and that expiration dates have not been exceeded.
2. Turn on power switch of atomic absorption spectrometer, and allow to initialize. From memory recall, enter program entitled "serum selenium" into memory. Call up "analytical conditions" screen and verify that correct program is in the memory.
3. Turn on Electrodeless Discharge Power Supply. Switch to HCA mode and

adjust wattage dial to read zero.

4. Press "condition set" button, adjust the wattage dial to 5 watt and allow the Se EDL to warm up for at least 45 minutes. Then follow wavelength calibrate procedure on "test modes" screen. Following wavelength calibration, proceed with the autosampler nozzle position check on "test modes" screen. Turn on cooling water, and argon gas. Follow optical pyrometer calibration procedure on "test modes" screen.

Specimen Collection:

Collect serum specimens in a 5 mL Sarstedt monovette syringe. Collect plasma/erythrocyte specimens in a "Trace Element Laboratory" microtainer containing 30 microliters of 30 mmol/L EDTA (purified). Specimens may also be collected in Becton-Dickinson Trace Element Vacutainers containing EDTA, heparin or with no additive. Specimens which are hemolyzed are unacceptable. Severely lipemic specimens must have chylomicrons removed by centrifugation at 13,000 g for 30 minutes. Inability to clear specimen in this manner makes it unacceptable for analysis. Optimal specimen volume: 0.500 mL serum/plasma of 0.5 mL packed erythrocytes. Minimum specimen volume: 25 microliters for single analysis.

Specimen Handling:

Separate serum/plasma from the erythrocytes within 4 hours of collection by centrifugation at 1000 g for 15 minutes. Transfer serum/plasma to a microcentrifuge tube and label with patient name, id, collection date. If erythrocyte selenium is to be assayed, add 750 microliters 18 megohm deionized water to packed erythrocytes remaining in microtainer and mix well (approximately fourfold dilution). For vacutainer collections, dilute 250 microliters of packed cells with 750 microliters of 18 megohm deionized water. Label appropriately. If specimens are to be stored longer than 24 hours before assay, freeze at -20 °C until assay.

Assay Procedure:

1. Prepare an intermediate working standard by first diluting 100 microliters of stock standard (NBS, 10,000 mg/L) with 900 microliters of 18 megohm deionized water to give 1.000 mL of solution. Vortex to mix. Transfer 100 microliters of this solution to a 100 mL Nalge volumetric flask containing 0.5 mL of 70% nitric acid and half-filled with deionized water. Mix contents and fill to mark with deionized water. Invert at least 13 times to mix. Label Selenium Standard, 1 ppm.
2. Prepare an aqueous standard curve by adding the following reagents to five labeled, acid-washed autosampler cups:

Cup Number	1	2	3	4	5
deionized water mL	0.600	0.590	0.575	0.550	0.525
Se Std, 1 ppm, mL	0.000	0.010	0.025	0.050	0.075
Pd Matrix Modifer	0.400	0.400	0.400	0.400	0.400
Concentration μg/L	0.0	10.0	25.0	50.0	75.0

3. Label an appropriate number of cups for specimens, blanks and controls. Fill out specimen manifest listing specimen id number. Pipet 50 microliters of blank, control or specimen into the appropriately labeled autosampler cup. Add 100 microliters each of Pd matrix modifer and deionized water. Place in autosampler wheel position indicated on manifest list. Place the standard curve cups followed by one blank and seronorm control in first seven positions and then specimens. Additional controls may be used (i.e. EPA 284; CDC sera, Levels I & II).
4. Depress "autosampler" button twice and insure that program includes 10 microliter sampling volume for both specimens and standards and default conditions for other parameters.
5. Depress "Analytical Conditions" key and insure that screen parameters are set as shown below:

lamp current	12.5 mA
wavelength	196.0 nm
slit width	1.3 nm
sample volume	10.0 uL
cuvette	pyrolytic tube
measurement	peak area

6. Depress "Graphite Furnace" key and insure that screen parameters are set as shown below:

Number	Stage	Temperature Start	Temperature End	Time (second)	Carrier Gas (ml/min)
1	dry	60	90	60	30
2	dry	90	120	40	30
3	dry	120	250	15	30
4	dry	50	50	4	30
5	ash	1200	1200	30	30
6	atom	2700	2700	5	0
7	clean	3000	3000	4	200

* Red blood cells were ashed at 1400°C for 40 seconds and cleaned for 7 seconds.

7. Depress the autozero button, and then the start button. If the cycle proceeds correctly, allow the sampler to continue beginning with the standard curve. At the completion of the standard curve analyses, check the correlation coefficient of the curve as printed out by the computer. The correlation coefficient must be greater than 0.999, before proceeding with the analysis. Remake the standards and repeat the analysis of the standard curve, if the correlation coefficient is less than 0.999.
8. If the standard curve is satisfactory, continue with the analyses, insuring that blanks and controls are within +/- 2 standard deviations of their respective means. Repeat the analyses if satisfactory values are not obtained before proceeding. Concentrations of all blanks, controls, or specimens may be read directly from the computer printout, in μg/L (ppb). Repeat analysis of specimens whose duplicate pipettings disagree by more that 15%.
9. For erythrocytes, divide the concentration obtained from the printout, by the respective hemoglobin concentration (see Hemolysate Hemoglobin Assay) in μg/L, to obtain the erythrocyte selenium concentration in μg/mg Hgb.

References:

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graphite-furnace atomic absorption spectroscopy with deuterium background correction and a reduced palladium modifier: age-specific reference ranges. Clin. Chem. 34, 709-714 (1988).