EFFECTS OF DIETARY SELENIUM DEPLETION AND OF DIETARY SELENIUM AND IODINE INTERACTIONS ON THYROID HORMONE METABOLISM OF RAT

by Christine Hotz

A thesis submitted to the Deparment of Foods and Nutrition
in partial fulfillment of the requirements
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A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

EFFECTS OF DIETARY SELENIUM DEPLETION AND OF DIETARY SELENIUM AND IODINE INTERACTIONS ON THYROID HORMONE METABOLISM OF RAT

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Iodine is a structural component of thyroid hormones and its availability through the diet can alter thyroid hormone production. Selenium has a secondary role in regulating thyroid hormone metabolism via the activity of type I deiodinase and possibly through the activity of thyroidal selenium dependent glutathione peroxidase. Two experiments were designed to, first, study the effects of selenium depletion alone and, second, to study the interactions of dietary iodine and selenium on thyroid hormone metabolism. In addition, one method development study was carried out to improve the analysis of the type I deiodinase selenoenzyme activity, which is involved in regulating the balance of active and inactive thyroid hormone forms in circulation.

In the first experiment, selenium depletion had no significant effect on circulating thyroxine levels. Both sex- and tissue-specific responses to selenium depletion were observed in the parameters measured. The results demonstrated the ability of rats, with previous selenium stores, to maintain type I deiodinase activity and thyroxine levels when fed a moderately low dietary selenium level for a prolonged period.

In the second experiment, iodine and selenium demonstrated interactive effects on both plasma thyroxine and thyroidal glutathione peroxidase activity. When both iodine and selenium were deficient, circulating concentrations of thyroid hormones were maintained at control levels. The possibility that a dual deficiency may be dangerous in stressed conditions, such as fetal development and growth, was not dismissed, as the protective activity of glutathione peroxidase in the thyroid during iodine deficiency appeared to be limited by the availability of selenium. A high amount of iodine in the thyroid may also have toxic effects, and if GSH-Px is compromised during severe selenium deficiency, thyroid tissue damage may ensue.

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I. LITERATURE REVIEW

INTRODUCTION

Iodine deficiency and its associated disorders are one of the most important, global nutritional problems of our time. Although knowledge of the disorders and ways to eradicate them are quite extensive, millions of people continue to be at risk of iodine deficiency. Due to the structural need for iodine in thyroid hormones (TH), iodine deficiency disorders result from alterations in TH metabolism and balance. The general roles of TH are in physical and neurological growth and development. An estimated 4-5% of the world's population are affected by iodine deficiency disorders (Lamberg 1993).

Several regions of iodine deficiency are also deficient in selenium. Selenium has a secondary role in the regulation of TH metabolism; it is required for the activity of 3'5' Type I deiodinase, whose main function is to convert the inactive TH form to the active form. A dietary deficiency of this element thus can alter the balance of active and inactive TH forms. The selenium dependent glutathione peroxidase enzyme also has a special protective role in the thyroid gland that is related to TH synthesis and in this respect its activity is also involved in TH metabolism. It has been hypothesized that in geographical regions that are deficient in iodine, a concurrent selenium deficiency can increase or modify the symptoms associated with iodine deficiency (Dumont et al 1994).

IODINE AND IODINE DEFICIENCY DISORDERS

Iodide ions occur in variable amounts in soil. Within areas of similar geological

iodine status, fish, dairy products, and to a lesser degree, meat, contain more iodine relative to plants; the actual concentrations will vary according to the amount of available iodine in the area of production. Iodine content of freshwater typically reflects that of the soils it resides in, and can serve as a minor source of dietary iodine. The main cause of inadequate iodine consumption and thus iodine deficiency disorders (IDD) is the geological absence of iodine in the soil. Such soils exist in specific regions of all continents, typically in inland or mountainous regions (Merke 1984). Though the problem has been corrected in many developed nations, an estimated 1 billion people are still at risk of IDD (Maberly 1994), and most are concentrated in Africa, Asia, the Andean regions of South America, and still in some parts of Europe.

The clinical manifestations of IDD are varied and are related to the stage of development at which they occur. Goitre, the compensatory enlargement of the thyroid, can appear from the neonatal period to adulthood while cretinism, a condition of compromised physical and mental development, results from deficient supply of maternal TH to the fetus. Due to the requirement for adequate TH for proper fetal and infant neurological development, and the different severities at which iodine deficiency can occur, varying degrees of intelligence, neuronal, and audiometric disorders are likely produced more frequently than the state of cretinism itself. Abortions, stillbirth, and infant mortality are also increased by iodine deficiency. The social and economic implications of iodine deficiency may thus be underestimated as these states are more difficult to define and may be less easily attributable to low iodine status (Pfannenstiel 1984). Iodine deficiency and the associated disorders have been previously reviewed by

Lamberg (1993), and Hetzel (1990).

Boussingault, a French chemist studying in South America, was the first to make the connection between lack of iodine and the prevalence of goitre (Merke 1984). This discovery, made through his comparative observations of goitrous and non-goitrous populations, led him to strongly suggest to authorities the use of iodine prophylaxis for treatment of goitre. As early as 1835, natural, iodine-containing salt was sent to goitrous regions and was reported to successfully reduce goitre.

Within the last century, developed nations have been mostly successful in eliminating the iodine deficient state. Since the first large scale human studies conducted by Marine and Kimball in Akron, Ohio in 1917 most iodine supplementation trials have reduced the incidence of goitre and prevented cretinism (Filetti et al 1984). In many countries this success has led to the widespread practice of iodine fortification of table salt. The Swiss Ministry of Health was the first to approve iodine fortification of table salt in 1922 (Matovinovic 1984). Currently in industrialized nations, additional sources of iodine resulting from modern processing procedures include bread, where potassium iodate is used as a dough improver, dairy products, where iodophores are used as disinfectants for milk production and storage equipment, and various other foods which contain erythrosine, an iodine containing food colorant (Filetti et al 1984).

Although policies regarding supplementation may exist in developing countries, adverse political and economic conditions have acted as constraints to their implementation or enforcement. Maberly (1994) commented on the many logistical problems that have been encountered in the use of iodized salt. These include poor

product quality, storage, packaging, and methods of transportation which can lead to reduced retention of iodine.

Other methods of iodine prophylaxis have been used successfully to reach endemic areas where salt fortification is not feasible. Iodized oil has been administered via intramuscular injection or as an oral dose in gel capsule form. These single large doses are advantageous as they remain effective from one to several years, depending on the dose size. The slow release of iodine from the stored fatty acids makes thyrotoxicosis a rare side effect (Filetti et al 1984).

THYROID HORMONES

Details of thyroid hormones and their synthesis have been reviewed in a text by McNabb (1992). The thyroid gland actively accumulates and oxidizes iodide ions. Thyroid cells synthesize thyroglobulin, a glycoprotein which is secreted into thyroid follicles and is stored as a mass termed "colloid". Tyrosine residues contained in the thyroglobulin protein structure are the target of iodination, and form the basic structure of the thyroid hormones. Tyrosine iodination is catalyzed by thyroperoxidase, which oxidizes the iodide ions using locally generated H_2O_2 as an electron acceptor. The tyrosine residue ring structure is either mono- or di- iodinated and two of these iodinated residues are coupled to form a thyroid hormone molecule; this coupling is also catalyzed by thyroperoxidase using H_2O_2 as cofactor. The coupling of two di-iodinated tyrosine residues produces tetraiodothyronine (thyroxine, T4) while the coupling of one di-iodinated and one mono-iodinated tyrosine residue produces triiodothyronine (T3). The

direct production of T4 is thought to be 10x greater than direct T3 production, at least when iodine is sufficient.

In the thyroid, thyroglobulin and attached thyroid hormones enter the follicle cells by pinocytosis, these vesicles fuse with lysosomes, the hormones are cleaved, and are then released into circulation. The majority of the circulating thyroid hormones are transported bound to plasma proteins, mainly thyroxine-binding globulin. Prior to being taken up by target tissue cells, TH are released from the transport protein as free T4 and T3. After entering target cells they may again be bound to proteins for storage before being released in the cell for use. Although T3 is bound with less affinity to the transport and storage proteins than T4, T3 has a much higher affinity to thyroid hormone receptors, resulting in a higher turnover rate of T3. TH activate the transcription process and ultimately upregulate carbohydrate, protein and lipid metabolism, therefore controlling basal metabolic rate. TH also regulate thermogenesis and are required for growth and development of central nervous system tissues.

Although T4 is the most abundant TH form released by the thyroid into circulation, its function is considered to be that of a prohormone. To become biologically active, T4 must first be converted to T3 by deiodination. T3 has specific cell receptor sites and is found at a much lower concentration in the plasma. Reverse T3 (rT3) is an inactive TH form that is also produced by the thyroid.

DEIODINASE ENZYMES

Specific enzymes exist in many tissues which catalyze deiodination of TH. The

activities of these deiodinase enzymes function to maintain a balance of active and inactive TH in circulation and within tissues. Type I and Type II deiodinases (DI-I and DI-II, respectively) catalyze the cleavage of an iodide ion from the 5', outer ring positions, as well as from the 3' position. **Figure 1** shows the deiodination reaction of T4 to T3 where one iodine atom is removed from the 5' position of the tyrosine residue. DI-I is present in many tissues but its activity is highest is in liver, kidney, and thyroid. DI-I activity in tissues peripheral to the thyroid is believed to provide up to 80% of the T3 in circulation for use by other tissues. Its activity can also function to inactivate T3. The order of preference of substrates for DI-I is:

$$rT3 >> T4 >> T3$$

DI-II is most active in the cerebral cortex, pituitary, and brown adipose tissue and also functions in these tissues to deiodinate T4 to T3 at the 5′ or 3′ position. The T3 resulting from DI-II activity is believed to remain within the tissue of origin for local use, not contributing to the circulating pool of T3. Its order of preference for substrates is:

$$T4 > rT3 >> T3$$

Additional deiodinase activity also exists which catalyzes the release of I^- from the 5 or 3, inner ring positions of TH. Although often referred to as Type III Deiodinase, Köhrle (1994) cautions that this enzyme should not be identified as a separate enzyme as the 5 deiodination reaction has yet to be associated with an enzyme distinctly different from DI-I. The 5 deiodination reaction occurs in most tissues but has greatest activity in the central nervous system, placenta, and skin. Both T4 and T3 are substrates for this activity and are deiodinated to produce the inactive TH forms rT3 and

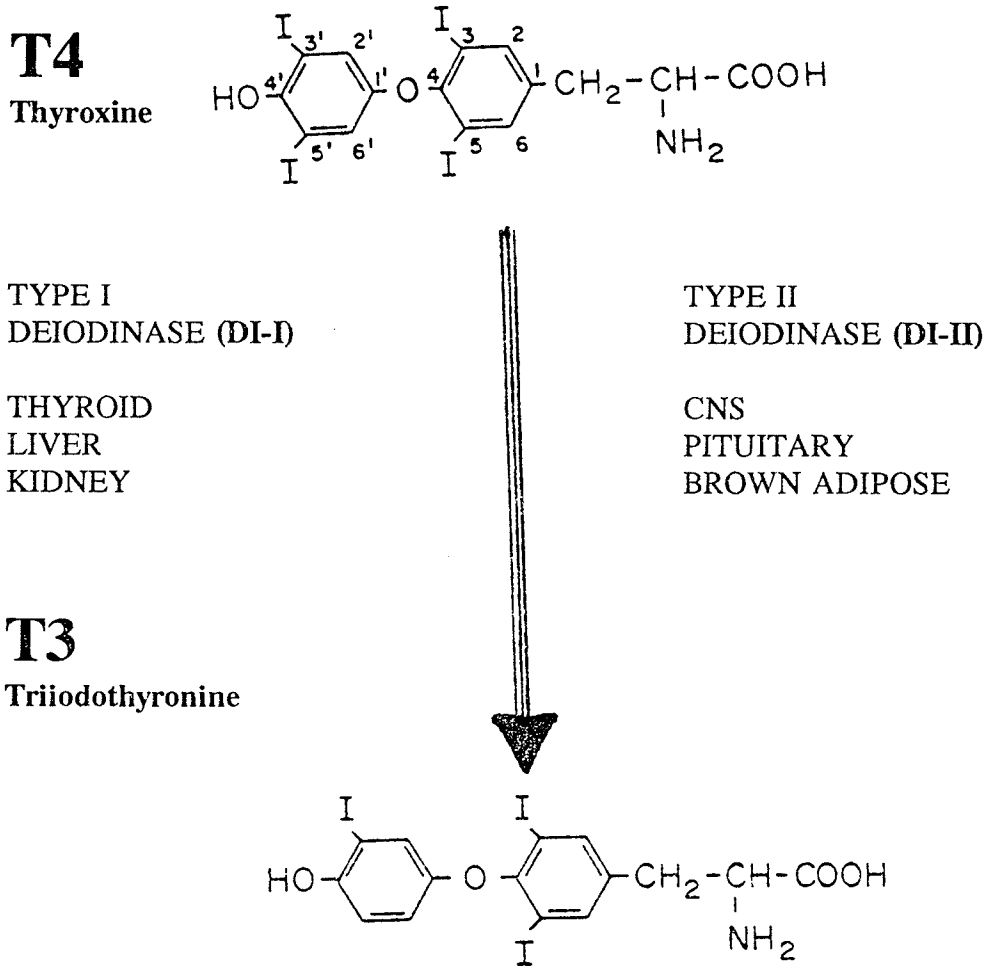

Figure 1. The deiodination of T4, the inactive thyroid thyroid hormone form, to T3, the active form, is catalyzed by either the Type I or Type II deiodinase enzymes. In both cases, the iodine ion is removed from the 5' position of the outer tyrosyl residue ring structure.

T2 respectively. The 5'-, 3'- deiodinating activity of DI-I and DI-II, and the 5-, and 3-deiodinating activity of the 'Type III' enzyme probably function to regulate local T3 levels by preventing its inappropriate accumulation. The deiodinase enzymes and their activities have previously been reviewed in detail (Köhrle 1994).

Although DI-I activity in peripheral tissues is believed to account for 80% of the circulating T3 in euthyroid conditions, this widely accepted value has been questioned. Chanoine et al (1993) have provided evidence that the rat thyroid gland is responsible for at least 55% of the circulating T3 through its own internal DI-I activity. Thyroidectomized rats, after being supplemented with thyroxine to maintain normal plasma T4 levels, showed a 55% decrease in plasma T3, suggesting the peripheral organs have a lesser role in maintaining circulating T3 than previously believed. Thus, the relative importance of peripheral DI-I activity has not been firmly established.

REGULATION OF THYROID FUNCTION

Thyroid hormone production is regulated by thyroid-stimulating hormone (TSH), also known as thyrotropin. TSH is secreted by the pituitary gland in response to low circulating T4 concentration and its general effect is to stimulate TH production and secretion. The thyroidal response includes increased rate of iodide uptake, iodination and coupling of the tyrosine residues, proteolysis of the stored thyroglobulin, and secretion of the cleaved TH. The rapid effects result from cAMP mediated reactions. The secretion of TSH from the pituitary may also be regulated by thyrotropin-releasing

hormone, produced by the hypothalamus in response to circulating TH concentration. The thyroid gland can also regulate its own activity in response to iodine concentration and this process is referred to as autoregulation. Both high and low iodine intake affect thyroid functioning and, as in response to TSH, autoregulatory responses affect almost every aspect of TH functioning (Ingbar 1984). Thyroidal regulation has been outlined in Guyton (1992).

CLINICAL ASPECTS OF IODINE DEFICIENCY

Iodine deficiency perpetuates the hypothyroid condition. Hypothyroidism in humans and rats is characterized by decreased plasma T4 concentration and elevated circulating TSH. Prolonged hypothyroidism results in thyroid hypertrophy. In experimental animals, this is identified by increased thyroid size and weight and, in humans, is clinically detected by thyroid palpation and classified as goitre.

In rats, plasma T3 concentration is often found to remain stable during iodine deficiency (Abrams and Larsen 1973, Beckett et al 1993, Meinhold et al 1993), and has also been shown to slightly increase (Meinhold et al 1992). Plasma T3 concentrations in humans have also been found to remain normal or increase during iodine deficiency. This probably reflects the human ability to compensate for lower T4 levels over years of low iodine intake via thyroid autoregulation. Evidence for this comes from the development of hyperthyroidism in long time residents of iodine deficient areas that were given iodine supplementation (Clugston and Hetzel 1994). The possible danger of a high iodine dose in newborns has also been raised (Lamberg 1993).

OTHER FACTORS RELATED TO IODINE DEFICIENCY DISORDERS

Clearly there are factors other than iodine intake which can affect TH metabolism. Genetic variation appears to have caused a differential response of individuals to iodine supplementation (Cowan et al 1965, Filteau et al 1994, Cooper et al 1993). Diet restriction has been shown to decrease circulating TH concentration in rats (Pascual-Leone 1994). Iron-deficiency anemia has been shown to impair TH status (Beard et al 1989). The consumption of goitrogens, a variety of substances that either block the uptake of iodine by the thyroid or the synthesis of TH, can also add to the problem of IDD and increase the requirement for iodine. Cassava is an example of a goitrogenic food and is of special concern because it is a staple food in some regions of Africa and throughout the tropics where iodine deficiency occurs. Another important dietary factor in the stasis of TH metabolism is selenium status. This factor will be considered in detail and forms a focus of research in this thesis.

SELENIUM AND SELENIUM DEFICIENCY

Dietary sources of selenium in decreasing order of concentration are meat, cereals, dairy products, and fruits and vegetables. As with iodine, selenium content of foods will vary with the amount of selenium available in the soil. Both selenium deficient and selenium toxic soils exist in different parts of the world. Selenium deficient soils can be found in areas of China, Africa, New Zealand, Europe, and North America. In China, two diseases have been associated with selenium deficient regions. Keshan disease is characterized by cardiomyopathy, predominantly affecting children and women

of child bearing age. Kashin-Beck disease is characterized by osteoarthritis that afflicts adolescents and pre-adolescents. Although selenium may not be the only factor involved in the development of these diseases, selenium deficiency at least predisposes an individual to them. Changes in TH metabolism in these severely deficient areas has not been an area of focus, but such changes may be secondary to the effects caused by these other diseases.

Selenium is physiological active in the form of selenoenzymes where selenium exists as a selenocysteine residue. Selenium may also be present in proteins in an inactive form as selenomethionine, where it simply takes the place of methionine and may become available for active use through protein turnover. Several tissue selenocysteine containing proteins have been identified but only a few have had their specific biochemical function described in detail (Behne et al 1988). What is known about these selenoproteins is that selenocysteine is incorporated by specific mechanisms, and their distribution within various tissues is homeostatically controlled. The retention factor for one selenoprotein can vary between tissues and the retention factor of different selenoproteins can vary within the same tissue (Behne et al 1988).

The enzymatic function of one of these selenoproteins has already been discussed; the selenoprotein DI-I has a pivotal role in regulating the circulating TH concentration. The selenoprotein with the most well documented activity is selenium dependent glutathione peroxidase (GSH-Px). This enzyme has an antioxidant function; it catalyzes the conversion of reduced glutathione to the oxidized form, concomitantly neutralizing H_2O_2 and other hydroperoxides, and preventing the generation of other oxygen-derived

free radicals and lipoperoxides. This enzyme is present in cells and plasma, and the largest pool of activity occurs in liver and erythrocytes (Behne and Wolters 1983). Due to the large amount of H_2O_2 produced in the thyroid for TH synthesis, it is evident that there is a special need for GSH-Px activity within the thyroid gland. As a result, selenium studies that focus on the effects of dietary selenium intake on TH metabolism by measuring the response of DI-I should also consider GSH-Px activity response, not only as a marker of selenium status, but as a possible modulator of TH production (Golstein 1988). Selenium metabolism and the effects of selenium deficiency have been previously reviewed (Levander and Burk 1990).

IODINE AND SELENIUM: INTERACTIONS WITH THYROID HORMONE METABOLISM

Two distinct forms of cretinism have been defined. One is the neurological type cretinism, characterized by mental retardation, neurological defects, deaf-mutism, and goitre. The other is the myxedematous type cretinism characterized by impaired growth, mental retardation and hypothyroidism, with less goitre and often, in fact, degenerated thyroid tissue. The main difference between these two types of cretinism is that in neurological cretinism impaired neurological development is due to inadequate maternal T4 supply to the fetus, whereas, in myxedematous cretinism mental retardation and impaired growth may result from thyroid absence or failure in the fetus or infant itself (Corvilain 1993). In some populations, an overlap of the two types cretinism may occur.

Although in both cases, iodine deficiency is the primary cause of the disorders, the hypothesis is that concurrent selenium deficiency induces the thyroid failure in myxedematous cretins. The geological absence of selenium from areas with a high incidence of myxedematous type cretinism has been established in Idjwi Island and the Ubangi, Zaire. It is also known that the thyroidal production of H_2O_2 for TH synthesis is increased during iodine deficiency (Corvilain et al 1991). However, selenium deficiency would decrease activity of thyroidal GSH-Px, thus allowing cytotoxicity and tissue damage to occur, ultimately resulting in thyroid failure. Thyroid degeneration was found to occur prenatally or in early infancy, during times of thyroid stress. This theory has been reviewed by Dumont et al (1994).

Another consequence of selenium deficiency is the decreased activity of DI-I, which can result in increased circulating T4 concentration and decreased T3 in rats (Arthur et al 1992). Selenium deficiency may in fact be a protective feature in iodine deficient, pregnant women and reduce the incidence of fetal neurological damage. As the main TH substrate in the central nervous system is T4, increasing maternal circulating T4 concentration with selenium deficiency when it is already low due to iodine deficiency, could bring it above the critical level needed for proper neurological development. This has brought about a caution against supplementing with selenium, when iodine is also deficient, especially in pregnant women (Contempré et al 1991).

STUDY RATIONALE

In order to understand the pathogenesis of the different types of cretinism, it is

necessary to increase our knowledge of the way selenium status affects parameters related to TH metabolism and to determine the way selenium interacts with iodine in altering these parameters. Two experimental studies were designed to increase our understanding of these issues. Additionally, a reproducible assay for the measurement of DI-I activity was developed to aid in the achievement of these objectives.

One of the critical roles of selenium in TH metabolism is through the activity of the DI-I enzyme. Various methods have been used to determine its activity in tissues, however, methodological variations, which include assay substrate type, use of an enzyme activator, and method of product separation, have made it difficult to compare results between studies. Due to the interest in DI-I in TH regulation, it was not only necessary to adopt a good, working assay for use in this study but it was timely to put forth an efficient and reliable method in detail that could be repeated by other researchers to produce more comparable results. The result of this work is presented in part II of this thesis.

Prior to looking at the interactions of iodine and selenium, it seemed important to develop a better understanding of how selenium deficiency affects the clinical aspects of TH metabolism on its own and how these effects relate to other more familiar biochemical indices of selenium status. The second study of this thesis looked at the effects of selenium depletion on rats over time, and considered many biochemical factors and how they differed between males and females.

Finally, more information is needed to better understand the complex interrelationships between iodine, selenium, and TH metabolism. In the third study of

this thesis, the clinical outcomes of concurrent selenium and iodine deficiencies and the parameters which respond interactively were studied. An additional objective was to determine the consequences of imbalanced intakes of the two elements were determined, such as when one or the other is provided as a supplement when both were previously deficient. This study used low, normal, and high intakes of dietary iodine and selenium by rats in a 3 x 3 factorial design to characterize each of these situations biochemically and to elucidate their possible roles in pathologies related to TH metabolism. This study would also serve to provide information on the consequences of imbalanced dietary intake of iodine and selenium, such as when one element is provided as a supplement when both were previously deficient.

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II. METHOD DEVELOPMENT

A METHOD FOR THE DETERMINATION OF TYPE I IODOTHYRONINE DEIODINASE ACTIVITY IN LIVER AND KIDNEY USING ^{125}I LABELLED REVERSE TRIIODOTHYRONINE AS A SUBSTRATE

INTRODUCTION

The deiodinase enzymes catalyze the deiodination of thyroid hormone molecules. Physiologically, they function to regulate the balance of the various thyroid hormone species which have different biological activities. Type I iodothyronine deiodinase (DI-I, E.C. 1.11.1.8, iodide peroxidase) catalyzes the release of an iodide ion from the 5′ or 3′ outer ring positions of reverse T3 (rT3), tetraiodothyronine (thyroxine, T4), or triiodothyronine (T3), in the following order of preference :

rT3 > > T4 > T3 (Körhle 1994).

DI-I activity is found in many tissues including liver, kidney, and thyroid. Type II deiodinase is located mostly in the central nervous system, pituitary, and brown adipose tissue. It prefers T4 as substrate and is believed to function in producing T3 for local use only (Körhle 1994). DI-I in liver, kidney, and thyroid provides the majority of circulating T3, the most biologically active thyroid hormone form, for use by other tissues. DI-I is a seleno-enzyme containing one seleno-cysteine residue at its catalytic

site (Berry et al 1991). Dietary Se deficiency has been shown to decrease DI-I activity in various tissues (Beckett et al 1987, 1989, DePalo et al 1994, Vadhanavikit and Ganther 1993). The Se requirement for normal DI-I activity is tissue specific. Liver has a higher Se requirement to maintain activity than does the thyroid (Vadhanavikit and Ganther 1993). The relative activities of DI-I in liver and kidney are unclear; one study reports activities approximately 3 times greater in liver than in kidney (Beckett et al 1989) while another reports greater activity in kidney (DePalo 1994). The need for further study in this area exists.

The method reported in this paper measures DI-I activity directly using 5′[^{125}I]rT3 as the substrate. The reaction mixture containing the $^{125}I^-$, released by DI-I activity, is separated on an ion-exchange column. Following elution, the non-reacted rT3 substrate and other deiodination products remain bound to the resin while the released $^{125}I^-$ passes through, is collected in the filtrate, and counted. Activity is expressed as pmol I^- released per mg tissue protein per minute of reaction.

Variations in the techniques of previous methods include substrate type and concentration, concentration of enzyme activator, incubation time, reaction stop solution composition, the reaction product measured, and the method for separating and quantifying reaction products. A method for detecting DI-I activity which standardizes the assay conditions that effect response has yet to be established and described in detail. This method is a modification of that developed by Leonard and Rosenberg (1980) and that used by Olin, Walter, and Keen (1994). This method consolidates the efficiencies of previous methods, choosing a moderate incubation time, a safe and consistent stop

solution, and, additionally, reduces handling by performing the ion-exchange chromatography within a centrifuge filter unit. Reproducibility of such a widely used assay should also be documented.

METHODS

Apparatus: Tissues were homogenized using a Kinematica Polytron (#CH-6010, Brinkman Instruments, Rexdale, ON). Non-sterile, nylon, 0.45 μm pore size Lida centrifuge filter units fitted with 3 mL receiver tubes were obtained from Chromatography Sciences Company (#7011-06, St. Laurent, PQ, Canada). Dowex-50W strongly acidic cation exchanger was obtained from Sigma Chemical Co. (#50X2-200, St. Louis, MO). Radioactivity was measured on an Auto-Gamma 5000 series gamma counter system (model #5530, Packard Instrument Company, Downers Grove, IL). An Abbott VP Supersystem autoanalyzer equipped with a 550/650 nm filter (#8088-26, Abbott Laboratories Ltd., Mississauga, ON) was used in total protein determination.

Reagents:

All chemicals were obtained from ICN Biochemicals, Inc., Aurora, OH, unless otherwise indicated.

Tissue Homogenization solution: HEPES buffer solution, 100 mM, was prepared by dissolving 5.958 g HEPES acid and diluting to 250 mL with demineralized water (DMW)(A) and dissolving 2.603 g HEPES, sodium salt and diluting to 100 mL with DMW (B). Solution B (approximately 70.5 mL) was added to 250 mL solution A

to obtain pH 7.0 at 20°C. A 1,4-dithiothreitol (DTT, #100597) 100 mM solution was made by dissolving 151.24 mg DTT and making to 10 mL with DMW. The final tissue homogenization solution (1 mM DTT, 10 mM HEPES buffer, 320 mM sucrose) was prepared by mixing 54.77 g sucrose, 50.0 mL 100 mM HEPES buffer (pH 7.0), and 5.0 mL 100 mM DTT solution and diluting to 500 mL with DMW. The homogenization solution was made fresh weekly and stored at 4°C.

^{125}I-rT3 incubation solution: The incubation solution (0.005 μM ^{125}I-rT3, 0.495 μM 5′-L-rT3, 2mM 1,4 DTT, 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA) was prepared as follows. 10 mg L-3, 3′, 5′-, reverse T3 (rT3) (#642515, Calbiochem, LaJolla, CA) was dissolved in 100 mL 50% (v/v) n-propanol/water to produce a 153.6 μM rT3 stock solution. Dissolution was aided by use of an ultrasonic bath. A 100 mM EDTA solution was prepared by dissolving 0.372 g EDTA, disodium salt and making to 10 mL with DMW. The potassium phosphate buffer was prepared by dissolving 2.722 g KH_2PO_4 and making to 100 mL with DMW (A) and dissolving 9.128 g K_2HPO_4 and making to 200 mL with DMW (B). Solution B (approximately 142 mL) was mixed with solution A to obtain a potassium phosphate buffer (200 mM) of pH 7.0 at 20°C. Phosphate buffer (5.0 mL), 200 μL DTT solution, 100 μL EDTA solution, 32.2 μL rT3 solution, and 100 μL ^{125}I-rT3 (specific activity >750 μCi/μg, radiochemical purity >95%, #NEX109-0142, DuPont NEN Research Products, Mississauga, ON, Canada) were mixed and diluted to 10 mL. Incubation solution was made fresh weekly and stored at 4°C.

T4/PTU stop solution: A 100 μM stock solution of L-thyroxine, sodium salt, pentahydrate (T4, #103073), was prepared by dissolving 8.89 mg in 100 mL 50% (v/v) n-propanol/water. A 6-n-propyl-2-thiouracil (PTU, #102748) stock solution, 100 μM, was prepared by dissolving 3.40 mg PTU in 200 mL demineralized water. The final stop solution (10 μM T4, 10 μM PTU) was prepared fresh weekly by mixing 2.5 mL of each stock solution and diluting to 25 mL with 25% (v/v) n-propanol/water, and was stored at 4°C.

Ion Exchange Column Preparation: Dowex-50W resin was equilibrated by filter-washing with 10% (1.74 M) acetic acid (500 mL acetic acid per 100 g resin). A resin slurry was prepared fresh weekly (stored at 4°C) by weighing 65.04 g washed resin into 124.96 g of 10% acetic acid and mixing thoroughly. The slurry was mixed intermittently while adding 2 mL to each filter unit column, fitted with a 2 mL collection vial. Units were centrifuged (1,500 X g, 5 minutes) and the eluant discarded. The resulting column bed had an approximate volume of 1 mL.

Tissue Preparation: Tissues were homogenized in cold DI-I homogenization solution at a 1:39 and 1:24 dilution (w/v) for livers and kidneys respectively. Homogenates were centrifuged (1500 X g) for 10 minutes at 4°C. Floating debris was removed by aspiration and the supernatant aliquotted to 2 mL screw-capped tubes. Homogenates were recentrifuged (20,000 X g) for 5 minutes at 4°C, floating debris removed, supernatant decanted into 2 mL plastic tubes and used for the DI-I assay.

Thyroids were homogenized in glass-tube homogenizers at a 1:19 (w/v) dilution. The whole homogenate was used for analysis.

Type I Deiodinase Assay: Aliquots (100 μL) of ^{125}I-rT3 incubation solution were heated in 2 mL screw-capped tubes at 37°C for 20 minutes. The pre-incubated tubes were removed from the oven, 20 μL vortexed tissue homogenate was added, the mixture was vortexed, and the tubes returned to 37°C. Total reaction time was 11 minutes. The reaction was stopped by flooding the reaction mixture with 200 μL of cold T4/PTU stop solution and vortexing. For each run, reactions for 8 samples and 1 blank, all in duplicate, were begun and stopped at 20 second intervals. Centrifuge filter units, fitted with 3.0 mL receiver tubes, were loaded with 80% (256 μL) of the stopped reaction mixture and centrifuged (1500 X g) for 5 minutes at 4°C. Columns were eluted twice with 1.0 mL 10% acetic acid and recentrifuged as above after each addition. The receiver tubes containing the total filtrate were removed, capped, and the released $^{125}I^-$ counted in a gamma counter. Blanks were produced using the same assay procedure but substituting 20 μL homogenization solution for sample. Empty receiver tubes were used to measure background radiation and 100 μL ^{125}I-rT3 was used to calculate total substrate activity. Samples were corrected by subtracting the amount of $^{125}I^-$ released from homogenization solution blanks. Counts per minute were automatically corrected for ^{125}I isotope decay.

Protein Determination: Total protein content of tissues was determined using

a copper-based biuret reagent (Abbott QuickStart® Total Protein, #LN 5A13-22, Abbott Laboratories, Mississauga, ON, Canada), and read on an Abbott VP Supersystem autoanalyzer. Low and high total protein standards of 2 and 8 mg · mL^{-1} were used (Agent®, Abbott Laboratories, Mississauga, ON, Canada). Tissue protein concentrations were analyzed using the same homogenates as used in the DI-I assay and results were expressed as mg protein · mL^{-1}. The dilutions used produced homogenates with protein concentrations of approximately 3.5 - 4.5 mg protein · mL^{-1}, unless otherwise stated.

Calculations: The method assumes homogenous distribution of ^{125}I-labelled and non-labelled rT3 in the incubation solution. Calculations were performed as follows, where:

TC = total counts, obtained from 100 μL ^{125}I-rT3 incubation solution

BC = blank counts, obtained from 20 μL reacted homogenization solution

SC = sample counts, obtained from 20 μL reacted sample homogenate

SC_c = sample counts corrected for 80% of total filtrate volume used in gamma counting

= (SC x 100/80) - (BC x 100/80)

SA = specific activity

$$= \frac{\text{total moles rT3 in 100 } \mu\text{L incubation solution}}{\text{TC}}$$

= 50 pmol/TC

$$\text{DI-I activity} = \frac{\text{SCc(cpm)}}{\text{sample volume } (\mu\text{L})} \times \text{SA(pmol/cpm)} \times \frac{1}{\text{protein (mg/mL)}} \times \frac{1000}{\text{time (min)}}$$

$$= \text{pmol } I^- \text{ released} \cdot \text{mg protein}^{-1} \cdot \text{minute}^{-1}$$

RESULTS

Reproducibility: Day-to-day reproducibility data were determined by the analysis of liver homogenates and are summarized in Table 1. A 1:39 (w/v) liver homogenate was prepared and frozen in aliquots at -80°C. Aliquots were analyzed on 8 different days over a one month period and produced results with a coefficient of variation of 13%. Reproducibility was also checked using homogenates prepared from different sections of the same liver (Table 1). Analysis of 7 such homogenates over a period of 4 days produced results with a coefficient of variation of 22%. Within-run reproducibility was checked by pooling duplicate values for 15 samples. Coefficients of variation for duplicability averaged 10%, ranging from 0.30-25%. Coefficient of variation for protein determination from the homogenates used in day-to-day analysis was found to be 3%.

Linearity: The assay following the described protocol was found to be linear between 7 and 15 minute incubation times ($r^2=0.99$, $p<0.0005$). An 11 minute incubation time was chosen as it was in the centre of the linear portion of the curve.

Linearity was also determined using 3 different dilutions of liver and kidney homogenates. Livers were analyzed for DI-I activity and protein concentration as 100%,

70% and 40% dilutions of a 1:19 (w/v) homogenate. Recoveries were calculated as the percent of DI-I activity for each dilution over the mean DI-I activity value for all dilutions and are shown in Table 2. Results for 5 liver samples prepared as such, produced a range of 80-132% recovery, and indicated acceptable linearity within this dilution range. Kidneys were analyzed as 70%, 40%, and 10% dilutions of a 1:9 (w/v) homogenate. Results for 3 dilutions of 2 samples produced a range of 80-118% recovery from dilutions (Table 2). Typical plots of the linearity of DI-I activity over a range of protein concentrations are shown in Figure 1 for liver (A) and kidney (B).

Typical values: Samples were collected from rats that were fed for 6 weeks, from weanling, diets containing the recommended amounts of vitamins and minerals. Livers and kidneys from male and female rats, and thyroids from male rats were analyzed by this method and results are shown in Table 3. No significant differences were found between male and female livers. In kidneys, however, females had approximately 50% less mean DI-I activity than males. The coefficients of variation, ranged from 28-43%, and suggested substantial individual variation in activity, over and above that expected from day-to-day variation.

Table 1

Day-to-Day Reproducibility of DI-I Activity Assay

	n	Mean±SD (pmol $I^- \cdot min^{-1} \cdot mg\ pro^{-1}$)	Range	CV%
Liver homogenate[1]	8	5.1±0.7	4.2-6.3	13
Liver sections[2]	7	2.0±0.5	1.5-2.8	22

[1] A 1:39 (w/v) liver homogenate was prepared and aliquots were stored frozen at -80°C. Individual aliquots were thawed and analyzed on 8 different days over a one month period.

[2] A 1:29 (w/v) liver homogenate was prepared from each of 7 different sections from one liver and homogenates were analyzed individually over a period of 4 days. Liver sections were stored at -80°C.

Table 2

Linearity of DI-I assay over varying protein concentrations[1]

Tissue	*n*	Protein[2] ($mg \cdot mL^{-1}$)	% Recovery Mean±SD	% Recovery Range
Liver	15	2.8-7.6	100±14	80-132
Kidney	6	1.0-8.0	100±14	80-118

[1] Samples of liver or kidney homogenate were diluted and samples containing different concentrations of protein were analyzed for DI-I activity.

[2] Protein concentration was determined on each dilution level of the tissue homogenate used in the DI-I assay.

Table 3

DI-I Activity[1] in Various Tissues of Rat[2]

Tissue	Sex	*n*	Mean±SE	Range
Liver	Male	10	5.6±0.8	2.9-10.8
Liver	Female	10	5.5±0.5	3.3- 7.4
Kidney	Male	10	6.3±0.8	2.3- 9.9
Kidney	Female	10	3.1±0.3	1.3- 4.6
Thyroid	Male	3	0.9±0.2	0.6- 1.2

[1] DI-I activity expressed as pmol $I^- \cdot min^{-1} \cdot mg\ protein^{-1}$.

[2] Tissue samples were obtained from Sprague-Dawley rats that were fed for 6 weeks, from weanling, a semi-purified diet containing the recommended amounts of vitamins and minerals.

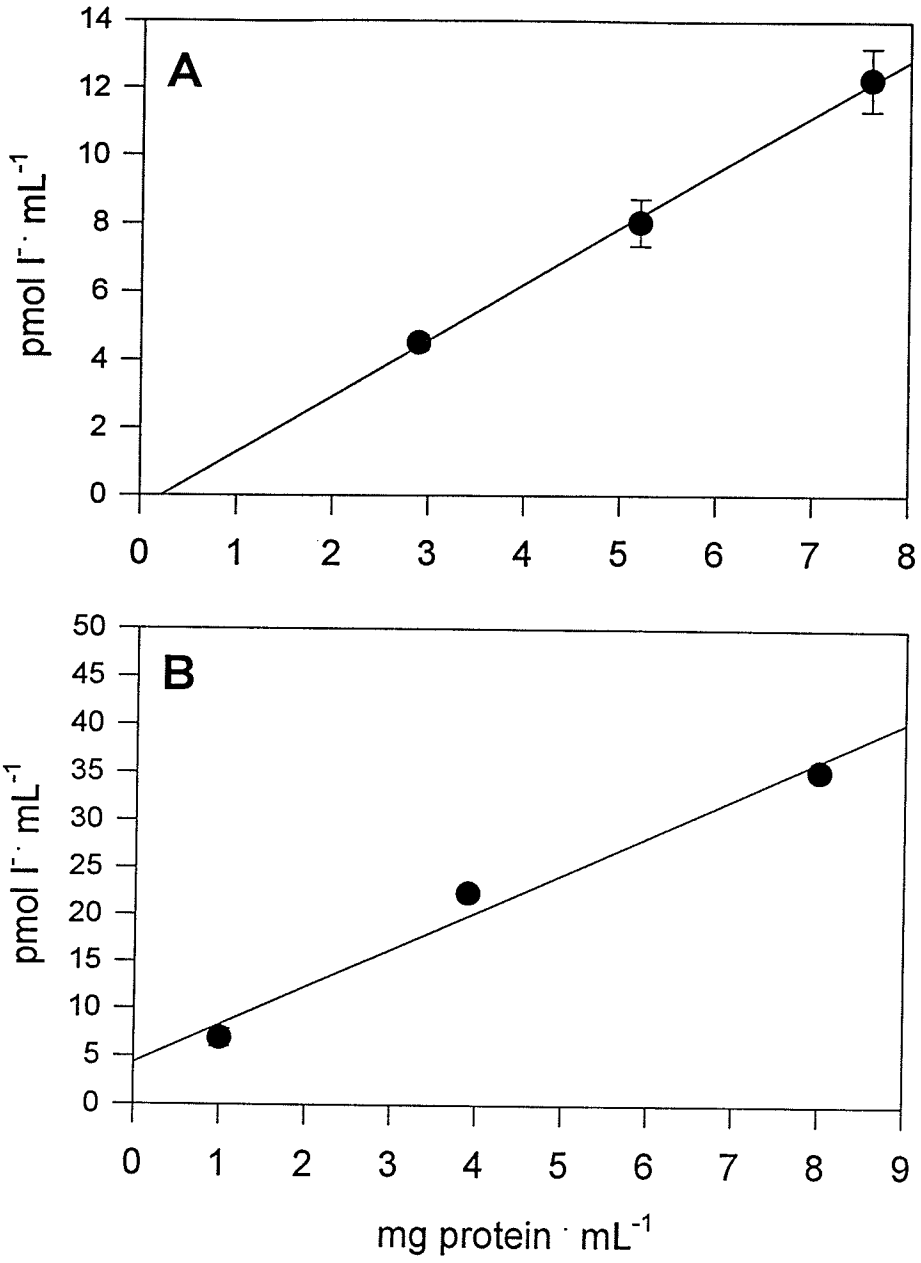

Figure 1. Typical plots showing the DI-I activity of liver (A) and kidney (B) homogenates at three different protein levels. Samples were prepared and analyzed for DI-I as described in the text.

DISCUSSION

Although methods for analysis of DI-I activity have previously been reported, a detailed, standardized description for the method is lacking. In recent years, several methods, with numerous modifications, have been used to detect tissue DI-I activity. This large variety has prevented the comparison of results amongst studies with similar objectives, notably selenium depletion studies. The intention of the present method development was to consolidate the effective procedures of previous methods to produce an efficient assay that can be easily reproduced.

Several studies have used the production of T3 from T4 substrate as an indicator of DI-I activity (Beckett et al 1993, Behne et al 1992, Meinhold et al 1993). Use of rT3 as a substrate is more efficient as it is found nearer the end of the deiodination reaction cascade, is the preferred substrate for DI-I activity (Köhrle 1994), and produces equivalent amounts of 3,3'T2 and I^- in these assays (Leonard and Rosenberg 1980, McCann et al 1984, St. Germain 1988). In methods that use T4 as substrate, residual rT3 may act as contaminant, competing with added T4 (Leonard and Rosenberg 1980). The quantification of T3 as reaction product may therefore underestimate DI-I activity.

DI-I activity may be more efficiently determined by quantifying the release of I^- from the substrate. When the production of iodothyronines is used to determine activity, the reaction products must be subjected to separation and subsequent quantification. Radioimmunoassay (Beckett et al 1987, 1989, Behne et al 1992, Meinhold et al 1993), and thin layer chromatography followed by autoradiography (Beckett et al 1992, 1993, Leonard and Rosenberg 1978) have been used, but these require substantial handling and

consequently introduce greater potential for experimental error. Ion-exchange chromatography has been used successfully to separate released $^{125}I^-$ from iodothyronine substrate (Leonard and Rosenberg 1980, Leonard et al 1981, Olin et al 1994,Sawada et al 1986, Visser et al 1982). This study used centrifuge filter units containing the resin bed ion-exchange medium. This method proved to be convenient, rapid, and accurate as centrifugation allowed elution to occur rapidly, and the collection vial containing the total filtrate was directly loaded into the gamma counter. Two elutions were sufficient to extract the released $^{125}I^-$; the second elution extracted only a further 1-5% activity (data not shown).

5'DI-I is located in the cytosolic side of the endoplasmic reticulum membrane in liver and in the cytosolic side of the plasma membrane in kidney (Köhrle 1994). Many methods have used the microsomal fraction of homogenates to measure DI-I activity in liver (Vadhanavikit and Ganther 1993) kidney (Leonard and Rosenberg 1978, 1980), and cerebral cortex (Visser et al 1982) while others, including the present method, have used whole homogenates of liver (Becket et al 1989, McCann et al 1984, Meinhold et al 1993, Olin et al 1994, St. Germain 1988), kidney (Beckett et al 1989, St. Germain 1988) and cerebral cortex (Beckett et al 1989, Leonard et al 1981, McCann et al 1984, St. Germain 1988). Microsomal fractions of liver contain greater DI-I activity than does the cytosolic fraction on a per protein basis (DePalo et al 1994, Sawada et al 1986), however, activity in whole homogenates produces consistent results.

DTT is a thiol activator of DI-I activity and is included in the homogenization solution. DI-I activity rate was previously shown to be constant over 2-30 minutes

incubation time for 0.5 μM ^{125}I-rT3 and 0.5 mM DTT at 37°C (Leonard and Rosenberg 1980). The rate of release of $^{125}I^-$ from rT3 increases with increasing concentration of DTT, as well as with increasing concentration of rT3 substrate (Leonard and Rosenberg 1980, Visser et al 1982). Release of I^- over time was linear for each of the DTT and rT3 concentrations tested. Incubation with 0.5 μM rT3 (0.495 μM rT3 + 0.005 μM ^{125}I-rT3) substrate and 2 mM DTT over 5 minutes has been used successfully (Olin et al 1994). In the present study, the latter concentrations of rT3 and DTT were used, but the total incubation time was extended to 11 minutes.

Addition of 1 mM EDTA to the incubation solution was found to decrease the non-specific release of I^- into the reaction mixture (McCann et al 1984) and was incorporated in the incubation solution of this method. DI-I activity was stopped using a 1:1 solution of 10 μM PTU and 10 μM T4. PTU is a persistent inhibitor of DI-I activity and inhibition increases in the presence of T4 (Leonard and Rosenberg 1980). T4/PTU solution is safer, and of more consistent composition than a human serum stop solution which has been used by others (Olin et al 1994, Visser et al 1982).

DI-I activity, as determined by this method, increases linearly with increasing protein concentration of liver and kidney homogenates (Figures 1A and B). Typical values were determined in this study at a protein concentration of 3-4 mg $\cdot$ mL^{-1}, producing results in the approximate range of 1-10 pmol I^- released $\cdot$ mg $protein^{-1} \cdot min^{-1}$. When rT3 was used as a substrate in other studies, values obtained have ranged from 0.1-100 pmol $I^- \cdot$ mg $pro^{-1} \cdot min^{-1}$.

Day-to-day variation from analysis of frozen (-80°C) aliquots of the same

homogenate produced results with a 13% coefficient of variation. Variation in DI-I activity was increased to 22% when different homogenates from the same liver were used. The increase in the coefficient of variation over that which was found to be produced by day-to-day variation may be attributed either to inconsistency of homogenates or a heterogenous distribution of activity in the liver. Preliminary results indicated that homogenization time (20 - 60 sec) was not a source of this variation.

The present method is simple and very sensitive, where 14-16 samples and 1 blank, in duplicate, can be analyzed per run. Sample sizes as small as 10 μL have been analyzed for the DI-I activity portion of the assay, and 25 μL for total protein analysis, making the procedure useful for the analysis of small tissues such as thyroid. The method presented in this paper has several other advantages. The use of highly purified, 5'-labelled ^{125}I-rT3 substrate yields reproducible results and the reaction is conveniently monitored, particularly when the ion exchange separation is performed using centrifuge filter units. The incubation solution includes 2 mM DTT as enzyme stabilizer, and 1 mM EDTA to decrease non-specific release of I^-. Finally, the modified stop solution containing PTU and T4 is safer and of more consistent composition than those containing human sera. Results from the analyses by this method yielded coefficients of variation for day-to-day reproducibility of 10-13%. Results derived from the various methods previously used are uncomparable and heighten the demand for a standard method to be adopted for DI-I activity determination, particularly given its importance in selenium and iodine metabolism and in the control of thyroid hormone levels.

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III. EXPERIMENT 1

TYPE I DEIODINASE AND GLUTATHIONE PEROXIDASE DEMONSTRATE TISSUE- AND SEX-SPECIFIC RESPONSES TO MODERATE SELENIUM DEPLETION IN MALE AND FEMALE RATS

INTRODUCTION

The trace element selenium (Se) is present in tissues in various forms of selenoproteins. Two of these whose enzymatic functions are well-established are Se-dependent glutathione peroxidase (GSH-Px, EC 1.11.1.9), involved in the cell antioxidant system, and Type I, 5'-deiodinase (DI-I, EC 1.11.1.8), which catalyzes the conversion of thyroxine (T4), the pro-hormone form of thyroid hormone, to the biologically active form triiodothyronine (T3, Köhrle 1994). Several other selenoproteins have been identified, although few have been characterized with specific biochemical functions. The tissue concentration of Se in the rat varies with tissue type (Behne and Höfer-Bosse 1984). The responses of both GSH-Px and DI-I enzymes to Se depletion are also tissue specific phenomena. Their responses have been studied in liver (Beckett et al 1987, Arthur et al 1990), between liver and kidney (Beckett et al 1989), and between liver and thyroid (Vadhanavikit and Ganther 1983, Beech et al 1995, Behne and Kyriakopoulos 1993). From these studies it can be summarized that DI-I is more resistant to Se depletion than GSH-Px, that the activities of these enzymes are more resistant to depletion in kidney than in liver, and that activities in thyroid are much more resistant to depletion than in liver.

Few studies have compared Se distribution between males and females. Relatively high Se content has been found in testes and ovaries suggesting distribution may be under hormonal control (Behne and Wolters 1983). Plasma GSH-Px activities were found to be reduced by Se depletion more quickly in males than females (Hill et al 1987). Thompson et al (1995) compared the responses of GSH-Px to Se depletion in males and females. However, comparisons of the relative susceptibilities of GSH-Px to Se depletion in different tissues could not be made between sexes as the animals were severely Se deficient and almost all tissues examined had extremely low Se content and GSH-Px activity.

Due to the role of DI-I in thyroid hormone metabolism and the biological importance of maintaining stable circulating concentrations of thyroid hormones, Se depletion studies have also focused on the responses of T4 and T3. Most often, plasma T4 increased when Se was depleted and this has been attributed to decreased DI-I activity (Arthur et al 1990, Beckett et al 1987, 1989, 1992, Thompson et al 1995, Vadhanavikit and Ganther 1993). Although elevated T4 was most often accompanied by decreased plasma T3 (Arthur et al 1990, Beckett et al 1987,1989, 1992, Thompson et al 1995), circulating T3 has also been found unchanged (Beckett et al 1989, Meinhold et al 1992, 1993, Vadhanavikit and Ganther 1993). Vadhanavikit and Ganther (1993) attributed these conflicting results to differences in the form and amount of dietary Se used. Arthur et al (1990) have suggested that changes in thyroid hormone concentrations are early consequences of Se depletion. This statement may apply only to a rapid or severe Se depletion treatment. The increased retention and redistribution of Se with low Se intake

(Behne and Höfer-Bosse 1984) and conflicting results in T3 response, suggest the possibility that changes observed in thyroid hormone concentration are an acute response, and that these changes may stabilize in the longer term as homeostatic functions are established.

The present study was conducted to establish whether or not the patterns of Se distribution and the response of selenoenzymes to Se depletion in female rats follows those patterns already established in males and to clarify the early responses of GSH-Px and DI-I to Se depletion, including the effect on the amount of circulating T4. The study design used rats that were initially provided with a Se sufficient diet, and then subsequently fed a Se deficient diet so that the responses to Se depletion could be measured over time.

MATERIALS AND METHODS

Animals and Diets: The following study protocol was approved by the Health Protection Branch Animal Care Committee, Ottawa, ON, Canada. Male and female weanling Sprague-Dawley rats (Charles River Canada, St. Constant, PQ, Canada) were raised for 6 weeks in individual stainless steel cages with free access to a nutritionally complete diet (AIN-76A, American Institute of Nutrition 1977) and demineralized water. After 6 weeks, 10 male and 10 female rats were necropsied to determine baseline parameters of Se metabolism. The remaining rats were randomly divided into 2 groups for each sex: one group was continued on the control diet (basal diet plus 0.1 mg Se/kg diet as selenite) and the other was provided the same basal diet without added Se (0.035

mg Se/kg diet, by analysis). Body weight and food consumption were determined weekly. Male (5) and female (5) rats from each diet group were necropsied at 8, 10, 12, 14, and 16 weeks into the study. During each study week, food source was removed the afternoon prior to necropsy. Rats were anesthetized with 5% isofluorane (500 mL/minute oxygen) and necropsied by exsanguination via the abdominal aorta. Whole blood was collected and liver, kidney, and thyroid were removed and stored at -80°C until the time of analysis. Thyroids were weighed immediately after removal.

Materials: Concentrated acids and solvents were of the highest purity available and were purchased from BDH Incorporated, Toronto, Ontario or J.T. Baker Inc, Phillipsburg, NJ. Chemicals used in the analysis of GSH-Px activity and tissue Se were purchased from Sigma Chemical Co., St. Louis, MO, unless otherwise stated. Chemicals used in the DI-I assay were purchased from ICN Biochemicals except for ^{125}I-reverse T3 (specific activity >750 μCi/μg, radiochemical purity $>95\%$, #NEX109-0142, DuPont NEN Research Products, Mississauga, ON, Canada) and L-3, 3′, 5′-, reverse T3 (#642515, Calbiochem, LaJolla, CA). GSH-Px activity, hemoglobin, and total protein analyses were automated on an Abbott VP Supersystem autoanalyzer (#8088-26, Abbott Laboratories Ltd., Mississauga, ON, Canada).

GSH-Px Activity Analysis: GSH-Px activity was determined in erythrocytes, liver, kidney and thyroid. Erythrocytes (approximately 25-30 μL) were separated from whole blood, washed with 1 mL cold saline solution (0.15 mol/L NaCl) and centrifuged for 3 minutes (20 000 x g). The pellet was resuspended and lysed in 1.0 mL glutathione reagent (2 mmol/L reduced glutathione, free acid, crystalline, in 10 mmol/L potassium

phosphate buffer, pH 7.0). The lysates were diluted 1:7 with glutathione reagent and stored at -80°C until analyzed. Tissues were homogenized 1:10 (wet weight:volume) in cold 0.2% Triton X-100 and homogenates diluted in 150 mmol/L phosphate buffer (pH 7.0) in the following proportions: liver and kidney 1:10, thyroid 1:6. Se-dependent GSH-Px activity was analyzed using an automated modification of the method of Paglia and Valentine (1967) at 37°C using a 340/380 nm filter (Friel et al 1993). The reagent consisted of 150 mmol/L potassium phosphate buffer (pH 7.0), 5 mmol/L EDTA, 0.5 mmol/L sodium azide, 2 mmol/L reduced glutathione, 0.24 mmol/L NADPH (Sigma #N-6505), and 1 U/mL glutathione reductase (Sigma #G-4751). The substrate used was 0.3 mmol/L tert-butyl hydroperoxide. Reagent and substrate were prepared fresh daily. Hemoglobin was analyzed using Drabkins reagent (Sigma Diagnostics, #525-2) in an automated assay at 30°C with a 415/450 nm filter. The assay was standardized with lyophilized human hemoglobin, with 0.36 and 1.09 μg/L as low and high standards. Total protein content of tissues was determined using an Abbott QuickStart® Total Protein kit (#LN 5A13-22, Abbott Laboratories, Mississauga, ON, Canada) following manufacturer's instructions. Low and high total protein standards of 2 and 8 mg/mL were used (Agent®, Abbott Laboratories, Mississauga, ON, Canada). Homogenates from the GSH-Px analysis, after further dilution with glutathione reagent (liver, 1:4; kidney, 1:5; thyroid, 1:6), were used for protein analysis. Results were expressed as mU GSH-Px/mg hemoglobin or /mg protein, where 1 Unit of activity catalyzed the oxidation of 1.0 mmol of reduced NADPH/minute.

Tissue Se analysis: Tissue Se was determined using wet ash digestion and

fluorometric analysis following the method of L'Abbé et al (in press 1996). Tissue samples (0.5-1.0 g) were digested in 6.0 mL nitric, 5.0 mL sulphuric, and 2.0 mL perchloric concentrated acids by leaving overnight at room temperature, heating at low-medium temperature (to avoid charring) and gradually increasing to high heat to distill off nitric and perchloric acids. Digests were cooled and boiled for an additional 5 minutes after addition of 250 μL 30% hydrogen peroxide. The latter step was repeated 4 times in total and cooled digests were then made up to 10.0 g with distilled, demineralized water. Sample tubes containing 1.0 g digest, 1.0 mL H_20, 2.5 mL 40% ammonium hydroxide, 1.0 mL EDTA (20 mM), and 0.5 mL diaminonaphthalene reagent (6.32 mM, fresh washed with cyclohexane) were incubated at 60°C for 1 hour. After cooling, 5.0 mL cyclohexane was added and tubes were rotated for 15 minutes. Se concentration was determined from the organic layer on a spectrofluorophotometer (Shimadzu, #RF-5000, Mandel Scientific Company Ltd., Guelph, ON) at 365 nm excitation and emission at 520 nm. Selenium standards (200-1250 ng/L) were prepared from a SPEX plasma standard (SPEX Industries Inc, Edison, NJ) and a standard reference material (bovine liver, #1577b, U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was used for quality control.

DI-I Activity Analysis: DI-I activity was determined in liver and kidney using a modified method of Olin et al (1994). Full details of the procedure have been described elsewhere (Hotz et al 1996). Tissues were homogenized in a cold solution containing 1 mmol/L dithiothreitol, 10 mmol/L HEPES buffer (pH 7.0), and 320 mmol/L sucrose (liver 1:39, kidney 1:24 wet weight:volume). Homogenates were centrifuged

(1500 x g) for 10 minutes at 4°C and the supernatant recentrifuged (20 000 x g) for 5 minutes at 4°C. A supernatant aliquot (20 μL) was added to 100 μL ^{125}I-reverse T3 solution (0.005 μmol/L ^{125}I-reverse T3, 0.495 μmol/L 5'-L-reverse T3, 2 mmol/L 1,4 dithiothreitol, 100 mmol/L potassium phosphate buffer, pH 7.0, 1 mmol/L EDTA) and incubated at 37°C for 11 minutes. The reaction was stopped using 200 μL stop solution containing 10 μmol/L T4 and 10 μmol/L propyl-thiouracil. The stopped reaction mixture was added to a centrifuge filter unit containing 1 g Dowex-50W resin and centrifuged 5 minutes at 4°C. Columns were eluted with 1.0 mL 10% acetic acid and recentrifuged (2x). Counts in total filtrate were measured on an Auto-Gamma 5000 series gamma counter system (#5530, Packard Instrument Company, Downers Grove, IL). Results were expressed as pmol I^- released $\cdot$ minute^{-1} $\cdot$ mg protein^{-1}. Total protein was determined using the same homogenates used for the DI-I assay, by the procedure described above.

Statistical Analysis: In Table 1, data are reported as mean $\pm$ SD (n=10) and significant differences between males and females were determined using a paired *t*-test. In Table 2, data are expressed as mean $\pm$ SD, and as the percent change from controls. Results were considered to be non-significant (NS) if the difference between Se depleted and control groups during the entire depletion phase (weeks 8-16) was determined to be non-significant ($p > 0.05$) by repeated measures ANOVA. For all groups, n=5. In the figures, data points represent group means with SEM error bars. Significant differences between means for control rats and Se depleted rats at each study week were determined using a paired *t*-test. Statistical analyses were performed using the statistical software package CSS: Statistica (StatSoft, Inc., Tulsa, OK).

RESULTS

The Se status of males and females fed the control diet for 6 weeks was compared and results are shown in **Table 1.** Females had 1.7x greater liver Se concentration than males, while the Se content of kidney was not significantly different between sexes. GSH-Px activity in liver and kidney followed a similar pattern to tissue Se; females had 1.7x greater activity in liver than males, while activity in kidney was comparable between sexes. GSH-Px activity of erythrocytes and thyroid were not significantly different between males and females. Males had 2x greater DI-I activity than females in kidney while activities in liver did not differ. Serum T4 tended to be higher in males than females, although this difference was not statistically significant ($p > 0.05$).

The effects of Se depletion on rats at week 16 (**Table 2**) are presented as the mean $\pm$ SD, and as the percent change from controls. Percentages are shown only for those parameters which were found to be significantly different ($p \leq 0.05$) between Se depleted and control rats over the entire depletion phase (weeks 8-16), as determined by repeated measures ANOVA. Se depletion did not produce any differences in body weight, food consumption, or thyroid weight (results not shown). Liver Se concentration decreased to a greater degree in males (17%) than females (9%) while kidney Se decreased to the same degree in both sexes (10%) for Se depleted rats, as compared to controls. Liver GSH-Px activity, similarly to tissue Se, decreased more in males (28%) than in females (10%). The reverse was true for kidney GSH-Px, as the decrease in activity of females (28%) was greater than the decrease seen in males (19%). Male GSH-Px activity in liver and kidney decreased by a greater percentage than did

tissue Se content. This pattern was true only in the kidney of females. A decrease in erythrocyte GSH-Px activity in males and females (12%) occurred in Se depleted rats, while activity in thyroid was unaffected by depletion.

The effects of Se depletion on body weight (**Figure 1**), tissue Se content (**Figure 2**), tissue GSH-Px activity (**Figure 3**), tissue DI-I activity (**Figure 4**), and serum T4 concentrations (**Figure 5**) were examined. Hepatic DI-I activity did not respond overall to Se depletion (Figure 4A). However, at week 8, the Se depleted group (male and female combined) had significantly higher activity than controls, followed by a stabilization in activity during the following weeks. Kidney DI-I activity in males showed no change while activity in females actually increased (48%) in the Se depleted group.

Although serum T4 did not change significantly in animals fed the Se deficient diet compared to controls, the trend in their response over the depletion period is noteworthy. After 2 weeks on the Se deficient diet (week 8 of the study), the serum T4 concentration of both males and females tended to be higher than that of the controls. By week 10 in males and by week 16 in females however, the response to the low Se diet seemed to change, and the T4 levels of the Se depleted groups tended to be lower than the control levels.

Table 1

Selenium status of male and female rats[1]

Parameter	Tissue	Male	Female
Body Weight, g		354±34	229±18*
Food Consumption, g		746±78	588±44*
Tissue Se, $\mu g \cdot mg\ tissue^{-1}$	Liver	546±61	904±96*
	Kidney	781±53	829±100
GSH-Px, $mU \cdot mg\ protein^{-1}$	Liver	376±92	643±67*
	Kidney	210±37	212±41
	Thyroid	19±5	17±3
GSH-Px, $mU \cdot mg\ hemoglobin^{-1}$	Erythrocyte	224±32	248±19
DI-I, $pmol\ I^- \cdot min^{-1} \cdot mg\ protein^{-1}$	Liver	5.6±2.4	5.5±1.5
	Kidney	6.3±2.7	3.1±1.1*
T4, $nmol \cdot L^{-1}$	Serum	65±19	40±9

[1]Comparison of Se status of male and female rats fed a Se supplemented diet (0.1 mg Se/kg diet) for 6 weeks. Results are expressed as mean ± SD and significance (*, $p \leq 0.05$) was determined by paired t-test. For all groups n=5, except T4 where n=4.

Table 2

Se status of Se depleted rats and percent change from control[1]

Parameter	Tissue	Male		Female	
		Mean ±SD	% change	Mean±SD	% change
Tissue Se, μg	Liver	517 ± 99	17%↓	929 ± 95	9%↓
· mg tissue^{-1}	Kidney	1000 ± 98	11%↓	938 ± 99	9%↓
GSH-Px, mU	Liver	338 ±103	28%↓	781 ±108	10%↓
· mg protein^{-1}	Kidney	226 ± 67	19%↓	172 ± 52	28%↓
	Thyroid	17 ± 3	NS	16 ± 7	NS
GSH-Px, mU					
· mg hemoglobin^{-1}	Erythrocyte	302 ± 50	8%↓	288 ± 17	16%↓
DI-I, pmol I^-					
· min^{-1} · mg protein^{-1}	Liver	4.6± 2.2	NS	5.9± 1.7	NS
	Kidney	4.5± 1.2	7%↑	2.0± 0.9	48%↑

[1]Results are from rats fed a control diet (basal plus 0.1 mg Se/kg diet) for 6 weeks then switched to a low Se diet (0.035 mg/kg diet) for 10 weeks (week 16). Data are expressed as mean ±SD, and as the percent change from controls. Results were considered to be non-significant (NS) if the difference between Se depleted and control groups during the entire depletion phase (weeks 8-16) was determined to be non-significant ($p > 0.05$) by repeated measures ANOVA. For all groups, n=5.

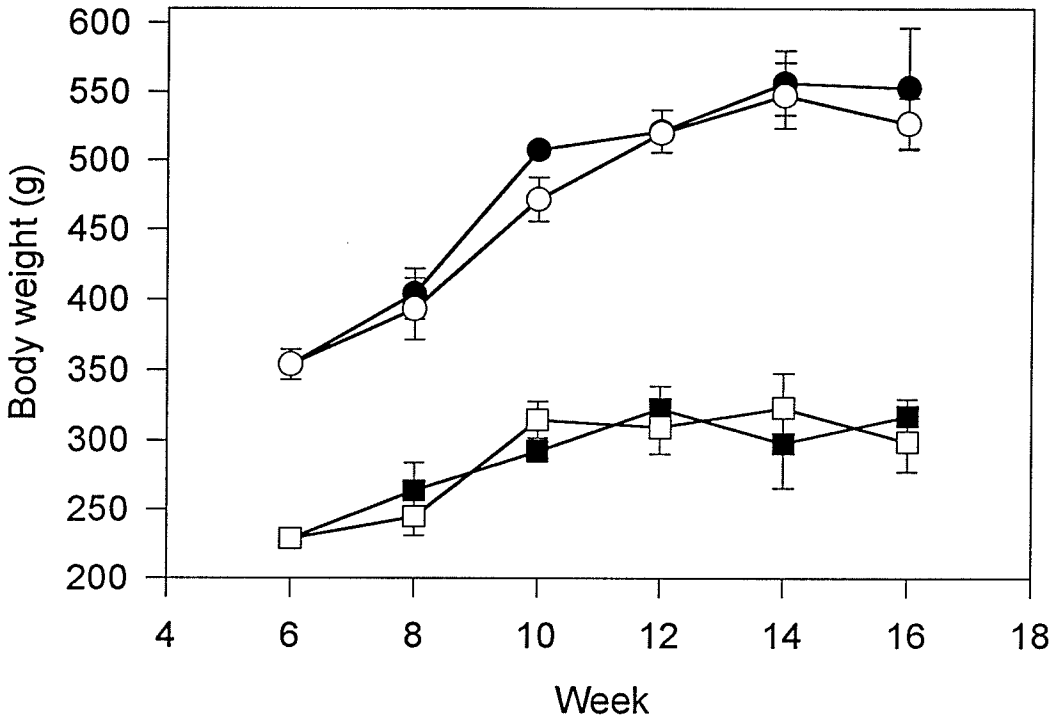

Figure 1. Body weights of rats fed either a control diet or a Se deficient diet. Data points represent mean±SEM, where n=10 at week 6 and n=5 at weeks 8-16. Means for male controls are shown as ●, male Se depleted rats as ○, female controls as ■, and female Se depleted rats as □. There were no significant differences between means for controls and Se depleted rats at any time point, as determined by paired *t*-test ($p \leq 0.05$). Male and female weanling rats were fed a Se sufficient diet (0.1 mg Se/kg diet) for 6 weeks after which one group in each sex continued on the control diet and one group was switched to a Se deficient diet (0.035 mg Se/kg diet).

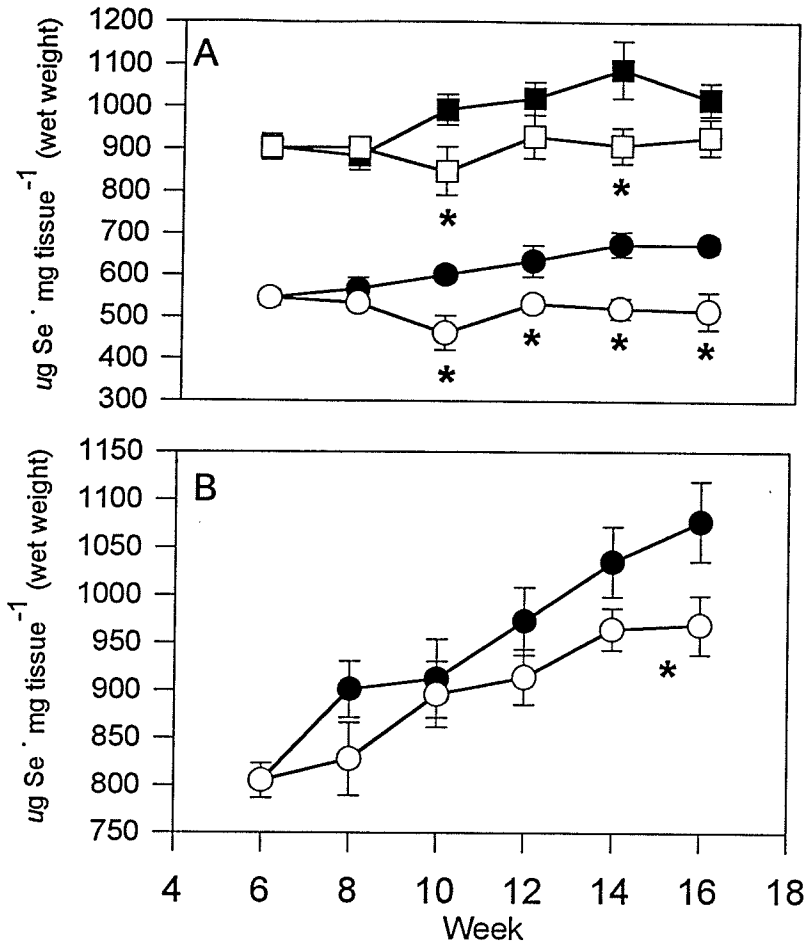

Figure 2. Tissue Se concentration of liver (A) and kidney (B) from rats fed either a control diet or a Se deficient diet. In (A), data points represent mean±SEM, where n=10 at week 6 and n=5 at weeks 8 to 16. Means for male controls are shown as ●, male Se depleted rats as ○, female controls as ■, and female Se depleted rats as □. In (B), data were not found to differ significantly between males and females, as determined by ANOVA ($p > 0.05$), and the data have been pooled for both sexes. Data points represent mean±SEM, where n=20 at week 6 and n=10 at weeks 8 to 16. Means for control rats are shown as ●, and for Se depleted rats as ○. Significant differences between means for controls and Se depleted rats at each week are indicated by (*), as determined by paired *t*-test ($p \leq 0.05$). Male and female weanling rats were fed a Se sufficient diet (0.1 mg Se/kg diet) for 6 weeks after which one group in each sex continued on the control diet and one group was switched to a Se deficient diet (0.035 mg Se/kg diet).

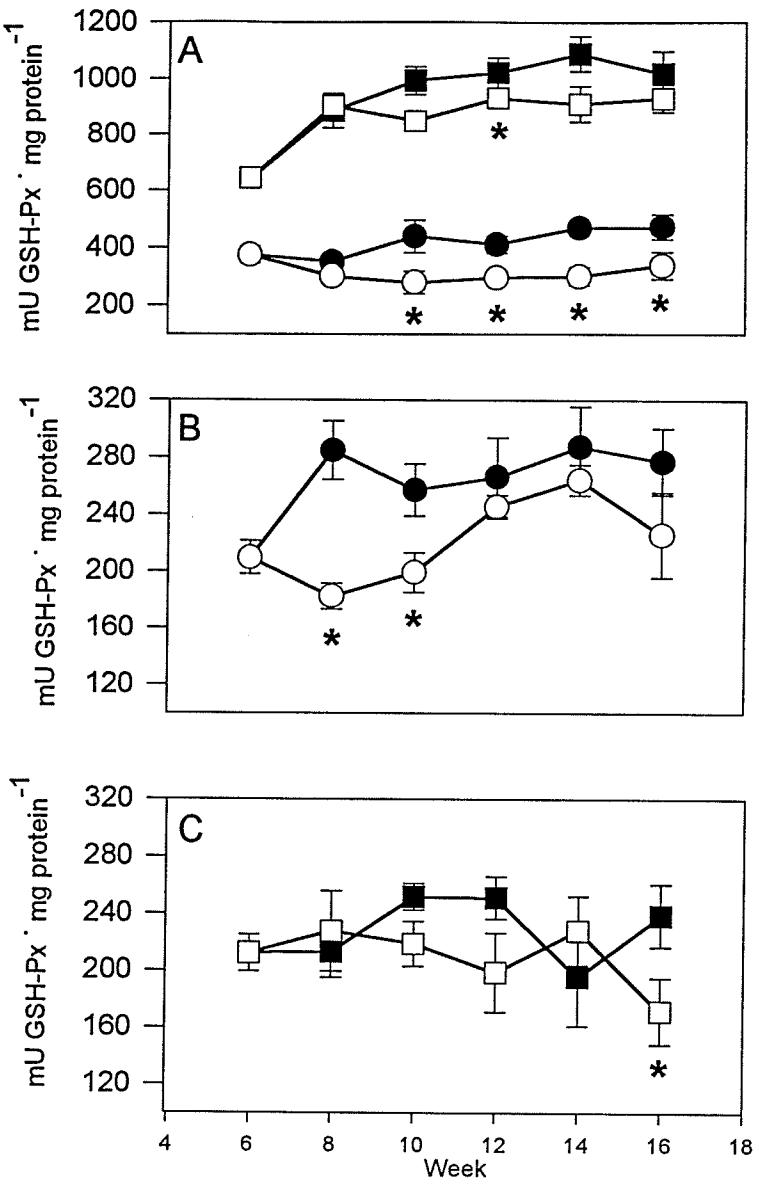

Figure 3. GSH-Px activity of male and female liver (A), male kidney (B), and female kidney (C) from rats fed either a control diet or a Se deficient diet. Data points represent mean±SEM, where n=10 at week 6 and n=5 at weeks 8 to 16. Means for male controls are shown as ●, male Se depleted rats as ○, female controls as ■, and female Se depleted rats as □. Significant differences between means for controls and Se depleted rats at each week are indicated by (*), as determined by paired *t*-test ($p \leq 0.05$). Male and female weanling rats were fed a Se sufficient diet (0.1 mg Se/kg diet) for 6 weeks after which one group in each sex continued on the control diet and one group was switched to a Se deficient diet (0.035 mg Se/kg diet).

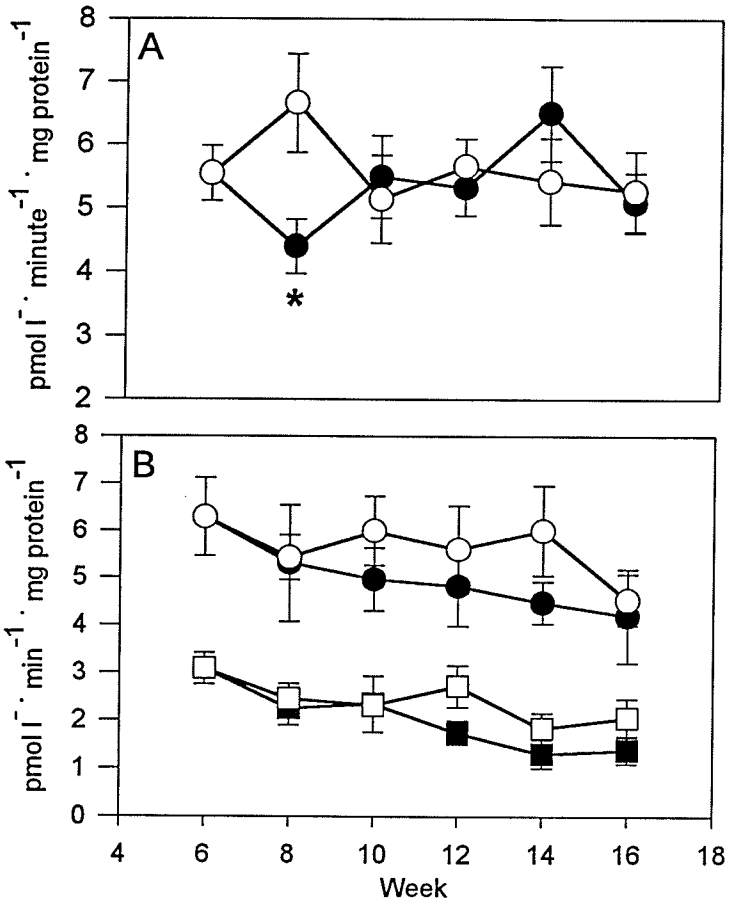

Figure 4. DI-I activity of liver (A) and kidney (B) from rats fed either a control diet or a diet deficient in Se. In (A), data were not found to differ significantly between males and females, as determined by ANOVA ($p>0.05$), and the data have been pooled for both sexes. Data points represent mean$\pm$SEM, where n=20 at week 6 and n=10 at weeks 8 to 16. Means for control rats are shown as ●, and for Se depleted rats as ○. In (B), data points represent mean$\pm$SEM, where n=10 at week 6 and n=5 at weeks 8 to 16. Means for male controls are shown as ●, male Se depleted rats as ○, female controls as ■, and female Se depleted rats as □. Significant differences between means for controls and Se depleted rats did not occur at each individual study week, as determined by paired *t*-test ($p\geq0.05$). Male and female weanling rats were fed a Se sufficient diet (0.1 mg Se/kg diet) for 6 weeks after which one group in each sex continued on the control diet and one group was switched to a Se deficient diet (0.035 mg Se/kg diet).

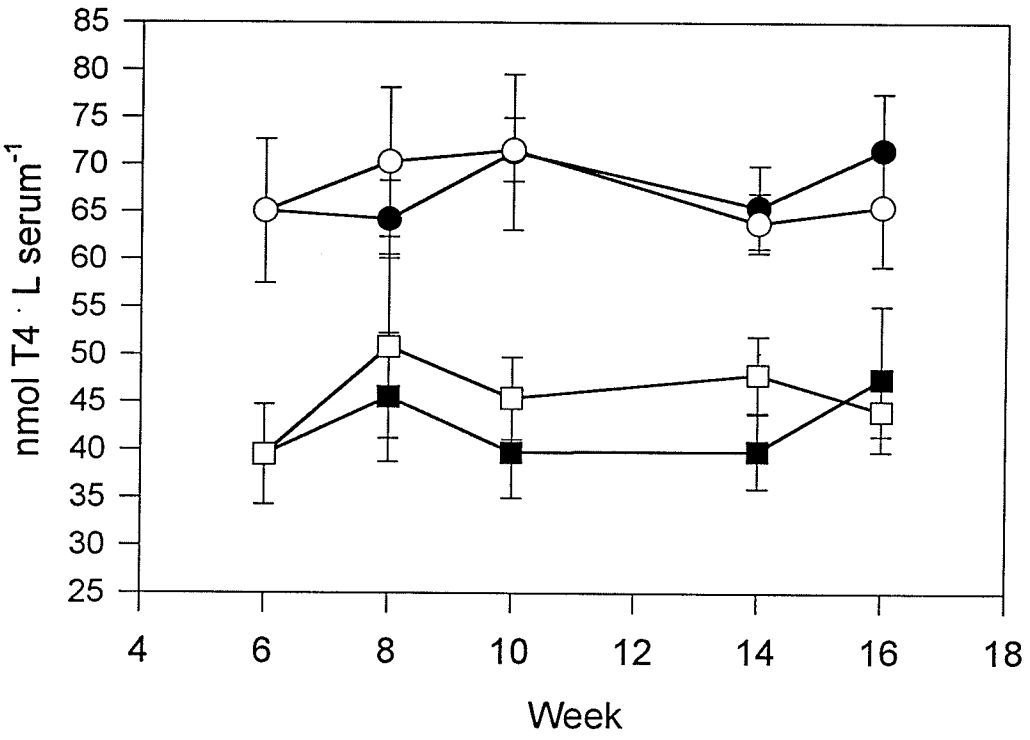

Figure 5. Serum T4 concentration from rats fed either a control diet or a diet deficient in Se. Data points represent mean±SEM, where n=8 at week 6 and n=4 at weeks 8 to 16. Means for male controls are shown as ●, male Se depleted rats as ○, female controls as ■, and female Se depleted rats as □. Significant differences between means for controls and Se depleted rats did not occur at each individual study week, as determined by paired *t*-test ($p \geq 0.05$). Male and female weanling rats were fed a Se sufficient diet (0.1 mg Se/kg diet) for 6 weeks after which one group in each sex continued on the control diet and one group was switched to a Se deficient diet (0.035 mg Se/kg diet).

DISCUSSION

Comparison at Baseline: Though significant work has been carried out on Se metabolism, most often only males have been studied and comparisons between sexes are scant. Baseline comparisons between male and female rats fed a Se sufficient diet for 6 weeks revealed some sex-specific characteristics of Se metabolism (Table 1). The higher hepatic Se concentration observed in females appears to be reflected in the higher GSH-Px activity present; both Se concentration and GSH-Px activity were 1.7x greater in livers of females. Other researchers have also observed that females had almost twice the GSH-Px activity in liver than males (L'Abbé et al 1991, Thompson et al 1995). These data suggest a greater physiological need for GSH-Px activity in the female liver as compared to the liver of males. Both tissue Se and GSH-Px activity in kidney were similar between sexes when rats were fed a Se adequate diet, and this was also observed by Thompson et al (1995). No differences between sexes were revealed in this study with respect to GSH-Px activity in erythrocytes or thyroid.

In previous studies, comparisons have also been made of Se content and GSH-Px activity between tissues. When Se distribution in female rats was analyzed (Behne and Wolters 1983), tissue Se concentration was observed to be highest in kidney. This was not found to be true in the females of this study where liver had the highest Se content. Rat strain and age differences may play a role in the difference between studies. Both studies did agree, however, that GSH-Px activity was significantly greater in liver than in kidney and, in the present study, this finding was consistent in males. This suggests that a greater percentage of Se in liver is present in the form of GSH-Px, while in kidney

a larger amount of the Se present is in the form of other Se containing compounds. A previous comparison found a similar relationship when the percentage of tissue Se bound to GSH-Px was calculated (Behne and Wolters 1983).

The present study has also revealed that while hepatic DI-I activity did not differ between sexes, activity in kidney was 2x greater in males than in females. A comparison of DI-I activity between liver and kidney was made in male rats (DePalo et al 1994) and slightly higher values were found in kidney, as shown in the males of this study. This comparison does not hold true for females where liver and kidney had similar activities, suggesting that females have a lesser capacity for DI-I activity, when considering liver and kidney alone. Serum T4 levels for control rats tended to be higher in males than in females ($p=0.06$) and this would be consistent with a greater need for DI-I activity in males.

Comparison of Response to Depletion: Several responses of tissue Se content and GSH-Px activity to Se depletion were also sex-specific and tissue-specific phenomena (Figures 2 and 3). Although baseline hepatic tissue Se and GSH-Px activity were lower in males, these parameters decreased by a greater percentage in males than in females during Se depletion. This emphasizes the importance of hepatic GSH-Px in females, and suggests that, as a source of Se during depletion, it is not as labile in females as it is in males. In a previous study using male rats, kidney was shown to retain Se more readily than liver during Se depletion (Behne and Höfer-Bosse 1984). The present study revealed that Se depleted females respond differently as, unlike males, their liver and kidney had similar Se retention capabilities. Se concentration of kidney in both control

and Se depleted rats increased significantly over the course of the study, though to a lesser extent in Se depleted rats. This suggests that kidney has a strong, active affinity for the acquisition of Se, even when the element is inadequate in the diet.

Thyroidal GSH-Px did not respond to Se depletion. Thyroid has been shown to be more resistant to Se depletion than other tissues (Beech et al 1995, Behne and Kyriakopoulos 1993, Vadhanavikit and Ganther 1993). The retention of GSH-Px in the thyroid is probably related to the function of GSH-Px in neutralizing H_2O_2, produced by the thyroid for use in thyroid hormone synthesis, and its reactive byproducts (Combs et al 1975). Golstein et al (1988) have also suggested that, as a result of this function, thyroidal GSH-Px activity levels may play an important role in the regulation of thyroid hormone synthesis, and the resulting concentrations in circulation.

DI-I activity in liver and kidney did not decrease in response to Se depletion. Hepatic DI-I activity in Se depleted rats increased significantly over that of controls at week 8 but then stabilized near control levels for the remaining weeks of the study (Figure 4A). DI-I activity in kidney of Se depleted rats actually increased in comparison to controls (Figure 4B). Although significant differences were not found when Se depleted rats were compared to controls at each individual week, when examined over the entire depletion phase, a significant increase in renal DI-I activity was observed in both the male and female Se depleted groups (Table 2). Increased DI-I activity in liver and thyroid (Behne and Kyriakopoulos 1993) and in thyroid alone (Beech et al 1995) during Se depletion has previously been reported but most often DI-I activity, at least in liver and kidney, has decreased in response to Se depletion (Beckett et al 1989, DePalo

et al 1994). The increase in kidney DI-I activity found in this study was consistent with the increased Se content of that tissue during Se depletion.

Differences in serum T4 levels between Se depleted and control groups were not found to be statistically significant due to the high standard deviations and low sample number (n=4). The trends presented by the data (Figure 5) do suggest, however, that the response to Se depletion was an initial increase in T4, followed by a decrease. If circulating T4 concentration is influenced by the amount of peripheral DI-I activity, then the decrease in serum T4, beginning at week 10 in males and week 16 in females (Figure 5), may have resulted from the increased DI-I activity seen in kidney at week 10 in males and at week 12 in females (Figure 4B). Although the pattern of T4 response seemed to follow that of the kidney DI-I activity response during Se depletion, the significant increase in liver DI-I activity at week 8 (Figure 4A) did not cause an apparent subsequent decrease in T4. Considering this, the difference in T4 response between diet groups may have been artifact, or were influenced by some other factor, such as DI-I activity in the thyroid, not analyzed in this study. In light of the small changes in serum T4 concentration, it is unlikely that there would have been an effect of diet on circulating T3 concentration in this study, which is generally more stable than T4.

Previous studies of Se depletion have produced severely decreased GSH-Px activities, significant decreases in DI-I activities and circulating T3 concentration, and significant increases in circulating T4 (Arthur et al 1990, Beckett et al 1987, 1989). These were short term studies (4-6 weeks) that used weanling rats fed a diet with an extremely low Se content (<0.005 mg Se/kg diet). In different studies that used a

longer period of Se depletion, only slightly increased T4 levels (Meinhold et al 1992) or no change in plasma T4 (Meinhold et al 1993) occurred, despite having greatly decreased hepatic DI-I activity. Also in the long term studies, there was either only a slight increase (Meinhold et al 1992) or no change in plasma T3 (Meinhold et al 1993). Vadhanavikit and Ganther (1993) experimented with varying levels of Se in the diet, over a 20 week treatment period, and found that while Se content and GSH-Px decreased with each decreasing level of Se used, severe reductions in these parameters and changes in hepatic DI-I activity and circulating T4 that occurred at 0.01 mg Se/kg diet did not occur at 0.05 mg Se/kg diet. It seems that the threshold for dietary Se, below which severe responses occur, lies between these two dietary levels. The level of dietary Se used in the present study (0.035 mg Se/kg diet) falls between these two dietary Se concentrations, and is moderately low relative to the extremely low levels used in the previously cited studies. As a consequence, the results of this study were not as dramatic and indicate the ability to adapt to a prolonged period of a moderately low intake of Se. Also in the present study, the Se stores of rats fed the control diet for 6 weeks appear to be able to reasonably maintain selenoenzyme activities, probably through increased retention and redistribution of Se within the body.

It is likely that homeostatic mechanisms, brought about by adaptation to dietary Se deficiency during long term exposure to inadequate dietary Se to maintain normal thyroid hormone levels, are present, though their method of action is not clear. It has been postulated that reduced clearance of T3 from circulation, also due to decreased DI-I activity in peripheral tissues, would lend to the maintenance of T3 levels (Beckett et al

1992), but this does not explain how T4 levels could be maintained. A reasonable suggestion is that this is allowed to occur through maintained DI-I activity and by increased direct synthesis of T3 by the thyroid. Both mechanisms would result in decreased T4 and increased T3 secreted by the thyroid, thus offsetting the changes caused by decreased peripheral DI-I activity. Previous long term studies did show maintenance of thyroidal DI-I activity (Vadhanavikit and Ganther 1993, Meinhold et al 1992).

The data presented in this study suggest that the female rat has a distinctly greater physiological need for hepatic GSH-Px activity, as evidenced not only by a significantly greater activity per mg protein but also by a smaller decrease in activity during Se depletion. Due to the many sex-specific responses of tissues in rat to Se depletion, attention should be paid to gender when using GSH-Px activity and tissue Se content to determine Se status. The Se content of kidney in Se depleted groups continued to increase throughout the depletion period, and renal DI-I activity also increased in response to Se depletion, thus confirming that tissues not only have different retention factors for Se when the element is depleted from the diet (Behne and Höfer-Bosse 1984) but that redistribution of Se to priority tissues and priority enzymes does occur. There appears to be a margin of inadequate dietary Se concentration within which physiological adaptations to Se metabolism occur in order to maintain biological functions of priority, such as thyroid hormone metabolism.

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IV. EXPERIMENT 2

VARYING CONCENTRATIONS OF DIETARY IODINE AND SELENIUM INTERACT IN THYROID HORMONE METABOLISM

INTRODUCTION

Adequate amounts of both I and Se are required for optimal thyroid hormone (TH) metabolism. As a structural component of TH, I is a primary requirement for TH synthesis. Thyroxine (T4) is produced solely by the thyroid and, though it is the most abundant TH form, is relatively biologically inactive. Triiodothyronine (T3), the biologically active TH form, is present in comparatively small concentrations, most of which is produced by the deiodination of T4 in peripheral tissues. Low availability of I to the thyroid decreases TH synthesis dramatically. TH production is controlled by thyroid stimulating hormone (TSH), secreted by the pituitary gland in response to circulating levels of TH and by thyroidal autoregulatory mechanisms in response to I availability.

Se plays a secondary role in the control of TH metabolsim. The deiodinating enzyme which produces most of the circulating T3, type I iodothyronine 5'-deiodinase (DI-I) is a selenoenzyme with most of the activity occurring in liver, kidney, and thyroid. Deiodination of T4 is also catalyzed by type II 5'-deiodinase (DI-II) which produces T3 primarily for local use, and occurs in the central nervous system, pituitary gland, and brown adipose tissue. The deiodinase enzymes and their activities have been reviewed in detail (Körhle 1993). Se may have another indirect role in the control of TH synthesis

in the form of another selenoenzyme, Se dependent glutathione peroxidase (GSH-Px). GSH-Px is involved in the cell antioxidant system and within the thyroid it is thought to be the main antioxidant system in the neutralization of the cytotoxic H_2O_2 and its oxidative byproducts (Combs et al 1975). H_2O_2 is produced by the thyroid as a cofactor in TH synthesis (Dumont 1971).

Observations of the complex relationship between I and Se in TH metabolism have raised questions pertaining to the interaction effects of varying levels of dietary I and Se. It has been suggested that Se deficiency can further compound the adverse effects of an I deficiency; the increased plasma TSH and thyroid weights seen with I deficiency were further increased by concurrent Se deficiency (Arthur et al 1992). However, there is other evidence which suggests that Se deficiency has a moderating effect on the clinical parameters associated with low I availability, where the decreased serum T4 and T3 and increased TSH and thyroid weight seen in hypothyroid rats were reversed by Se deficiency (Golstein et al 1988). It has also been suggested that supplementation with Se when both I and Se are deficient, may cause a rapid increase in thyroidal GSH-Px, neutralizing the H_2O_2 produced and thus decreasing TH production (Corvilain et al 1993). Alternatively, the restoration of DI-I activity after Se supplementation would increase the deiodination of T4 to T3 and T3 to T2, where the increased catabolism of TH would result in loss of I from the system, exacerbating the hypothyroid condition (Golstein et al 1988). Contempré et al (1993) have also demonstrated that a high iodine dose given to rats deficient in both I and Se produced greater thyroid tissue damage, and later showed that this damage was irreversible

(Contempré et al 1995), than when a high I dose was given to animals that were previously only I deficient.

Thus there is a demonstrated need to clarify both the effects of combined I and Se deficiency and the effects of imbalanced intakes of the two elements on TH metabolism, which may result when supplementation with one mineral is provided when both are deficient. The present study was designed to investigate these interaction effects.

METHODS

Animals and Diets: The protocol for this study was approved by the Health Protection Branch Animal Care Committee of Health Canada, Ottawa, ON. Male weanling Sprague-Dawley rats (Charles River Canada, St. Constant, PQ, Canada) were fed an AIN-93G diet with modified Se and I contents in a 3 x 3 factorial design with diets containing a combination of low, normal or high concentrations of I and Se. The basal diet contained <0.03 mg I/kg diet (by analysis), and 0.2 and 1.0 mg I/kg diet were added in the form of potassium iodate to prepare the normal and high I diets respectively. The basal diet also contained <0.05 mg Se/kg diet (by analysis), and 0.18 and 1.0 mg Se/kg diet were added in the form of sodium selenate to prepare the normal and high Se diets respectively. Rats were housed individually in stainless steel cages and given free access to food and distilled water for 6 weeks. The diet groups consuming the normal Se diet contained 6 rats and all other diet groups contained 7 rats. Food consumption and body weights were recorded weekly. Food source was removed the

afternoon prior to necropsy. Necropsy method was via exsanguination from the abdominal aorta following anesthetization with 5% isofluorane (500 mL/minute oxygen).

Tissue Iodine Analysis: Diet and liver iodine concentration were determined using acid digestion followed by colorimetric analysis according to a modified method of Fischer et al (1986). Concentrated nitric acid (10 mL) was added to Kjeldahl flasks containing sample (approximately 1.0 g) and left overnight at room temperature. Sulfuric (5.5 mL) and perchloric (20.0 mL) concentrated acids were added and flasks were then heated at medium temperature for 30 minutes, with cold-fingers inserted. Nitric and perchloric acids were then distilled off at high heat and the cooled digests were made up to 100 g with distilled, demineralized water. Test tubes containing 1.0 mL digest and 300 μL each of 10% NaCl, arsenic reagent (6 g arsenic trioxide, 400 mL 1.25N NaOH, 112 mL concentrated sulfuric acid, diluted to 2L with H_2O), and ceric reagent (20 g ceric ammonium sulfate, 112 mL sulfuric acid, diluted to 2 L with H_2O) were incubated at 37°C for 30 minutes and iodine determined on a Hewlett Packard diode array spectrophotometer (8452A, Mandel Scientific Company, Ltd., Guelph, ON) at 410 nm. Standards were prepared from a stock solution of 1.0 mg/mL iodic acid (0.1 - 0.6 μg/mL). A standard reference material (Non-fat skim milk powder, #1549, National Bureau of Standards, Washington, DC) was used as quality control.

GSH-Px Activity Analysis: GSH-Px activity was determined in erythrocytes, liver, kidney and thyroid. The method has previously been described in detail (L'Abbé et al 1991). Homogenized tissues were diluted with glutathione reagent as such: liver and kidney 1:10, thyroid 1:6. Hemoglobin was analyzed using Drabkins reagent (Sigma

Diagnostics, #525-2) in an automated assay at 30°C with a 415/450 nm filter. The assay was standardized with human lyophilized hemoglobin, using 0.36 and 1.09 μg/L as low and high standards. Total protein content of tissues was determined using an Abbott QuickStart® Total Protein kit (#LN 5A13-22, Abbott Laboratories, Mississauga, ON, Canada) following manufacturer's instructions. Low and high total protein standards of 2 and 8 mg/mL were used (Agent®, Abbott Laboratories, Mississauga, ON, Canada). Homogenates from the GSH-Px analysis, after further dilution with glutathione reagent (liver, 1:4; kidney, 1:5; thyroid, 1:6), were used for protein analysis. Results were expressed as mU GSH-Px/mg hemoglobin or /mg protein, where one U of activity catalyzed the oxidation of 1.0 mmol of reduced NADPH/minute.

DI-I Activity Analysis: DI-I activity was determined in liver and kidney. Full details of the procedure have been described elsewhere (Hotz et al 1996). The method used ^{125}I-reverse T3 as substrate and centrifuge filter units containing 1 g Dowex-50W resin to separate the released ^{125}I. Counts in the total filtrate were measured on an Auto-Gamma 5000 series gamma counter system (#5530, Packard Instrument Company, Downers Grove, IL). Total protein was determined on the same homogenate used for the DI-I assay, by the procedure described for GSH-Px activity analysis. Results were expressed as pmol I^- released $\cdot$ minute^{-1} $\cdot$ mg protein^{-1}.

Thyroid Hormone Analysis: Serum T4 was determined using a commercial T4 reagent kit (#445995, Beckman Instruments, Inc., Brea, CA) on a Beckman SYNCHRON CX System. T4 in serum competes with a T4 conjugate for T4-specific antibody where the amount of T4 conjugate not binding with antibody determines the rate of substrate

hydrolysis (0-nitrophenyl -β-D-galactopyranoside to 0-nitrophenol), as determined by absorbance change. Serum T3 was determined using a commercial T-Uptake reagent kit (#445999) using the same system as for T4 analysis. T4 conjugate binds to unoccupied sites of thyroxine binding protein in the sample serum. Thyroxine binding protein is also a carrier for T3. The amount of conjugate binding to the protein reduces the rate of substrate hyrolysis, as determined by the same reaction mechanism described for T4 analysis. TSH was analyzed in serum using a commercial RIA kit (#RPA 554, Amersham International, Amersham, UK) according to the manufacturers instructions.

Statistical Analysis: The significant effects due to diet treatments were determined by two-way ANOVA ($p \leq 0.05$) and significant differences between group means were determined by Least Squared Differences ($p \leq 0.05$). For the analysis of the effect of dietary I, where dietary Se had no significant effect on the response of a parameter, the data for all Se groups were pooled. Where dietary Se level had a significant effect on the response of a parameter, Least Squared Differences was determined between the group means from the normal Se intake groups only. The analysis for the effects of dietary Se are presented in a similar manner as described for the effects of dietary I. Where a significant interaction occured between I and Se, data were reported in figures for all treatment combinations. Statistical analysis was performed using the software package CSS: Statistica (StatSoft, Inc., Tulsa, OK).

RESULTS

Low intake of dietary I produced some of the typical effects of hypothyroidism

in rats **(Table 1)**. Plasma T4 concentration was significantly decreased ($p<0.000001$) while thyroid weight and serum TSH were significantly increased ($p<0.000001$ and $p<0.003$ respectively). Plasma T3 was not affected by any of the diet treatments. Thyroidal GSH-Px activity increased significantly with low I intake ($p=0.02$). None of these parameters were modified by high I intake as compared to normal I intake. Hepatic I content, however, reflected the level of I intake where low intake decreased and high intake increased liver I concentration as compared to controls.

The response of selenoenzyme activities to different levels of dietary Se were examined **(Table 2)**. While GSH-Px activity of liver, kidney, and erythrocytes was decreased approximately 2-fold with low Se intake, increased activity with the high Se diet occurred in kidney and erythrocytes, but not liver when compared to controls. Thyroidal GSH-Px activity was unaffected by the amount of Se in the diet. DI-I activity was less responsive to dietary Se levels than GSH-Px activity. While no changes were seen in the liver, kidney DI-I activity was decreased with the low Se diet. Also, the high Se diet caused a significant increase (19%, data not shown) in blood glucose concentration of the animals ($p=0.004$).

Some parameters of thyroid hormone metabolism demonstrated significant interactive responses to varying levels of Se and I. Plasma T4 **(Figure 1)**, was decreased by low dietary I when Se intake was normal or high. Low Se intake, however, altered the response of plasma T4 to dietary I concentration; at normal I intake, low dietary Se increased plasma T4 over that of controls and the combination of low Se and low I intake produced a plasma T4 concentration similar to that of controls. Low Se intake did not

have the effect of increasing plasma T4 when I intake was high.

Thyroidal GSH-Px activity was modified interactively by dietary I and Se concentration **(Figure 2)**. Activity did not differ from controls when both I and Se were consumed at either normal or high dietary levels. However, low dietary I increased thyroidal GSH-Px activity at each dietary level of Se and the greatest increase occurred when Se intake was high. Within the low Se groups, dietary I affected activity at each level where low I increased and high I decreased the activity of GSH-Px.

Table 1

Effects of Dietary Iodine[1]

	LI	NI	HI[2]
Liver I, ng/g[3]	28 ± 12^{A}	52 ± 29^{B}	67 ± 15^{C}
Plasma T4, nmol/L[4]	26 ± 6^{A}	48 ± 10^{B}	42 ± 9^{B}
Plasma T3, %uptake[4]	45 ± 2^{A}	45 ± 1^{A}	44 ± 1^{A}
Serum TSH, ng/mL[4]	1.21 ± 0.57^{A}	0.73 ± 0.10^{B}	0.78 ± 0.15^{B}
Thyroid Weight, mg[3]	26 ± 5^{B}	15 ± 2^{A}	16 ± 4^{A}
GSH-Px, mU/mg protein			
Thyroid[4]	30 ± 8^{B}	19 ± 5^{A}	17 ± 8^{A}
Liver[3]	500 ± 80^{A}	434 ± 37^{A}	489 ± 58^{A}
Kidney[3]	322 ± 35^{A}	335 ± 30^{A}	334 ± 32^{A}

[1]Comparison of the effects of dietary I concentration on various parameters of thyroid hormone metabolism. Results are presented as mean$\pm$SD and superscript letters indicate significant differences between means as determined by Least Squared Differences test ($p \leq 0.05$).

[2]LI=low I diet (basal, 0.05 mg I/kg diet), NI=normal I diet (basal + 0.2 mg I/kg diet), and HI=high I diet (basal + 1.0 mg I/kg diet).

[3]Dietary Se had no significant effect as determined by 2-way ANOVA ($p > 0.05$) and data were pooled for all dietary Se level groups; n=20.

[4]Dietary Se had a significant effect, as determined by 2-way ANOVA ($p \leq 0.05$) and data represent results from the normal Se intake groups only; n=6.

Table 2

Effects of Dietary Selenium[1]

	LSe	NSe	HSe [2]
DI-I, pmol $I^- \cdot minute^{-1} \cdot mg\ protein^{-1}$			
Liver[3]	3.5 ± 1.2^A	3.8 ± 0.9^A	3.5 ± 1.3^A
Kidney[3]	4.6 ± 0.7^A	5.7 ± 1.2^B	5.2 ± 1.3^{AB}
GSH-Px, mU/mg protein			
Liver[3]	207 ± 34^A	473 ± 64^B	466 ± 83^B
Kidney[3]	161 ± 26^A	330 ± 31^B	407 ± 45^C
Thyroid[4]	17 ± 4^A	19 ± 5^A	22 ± 5^A
GSH-Px, mU/mg hemoglobin			
Erythrocyte[3]	128 ± 29^A	249 ± 21^B	413 ± 73^C
Plasma T4, nmol/L[4]	61 ± 13^A	48 ± 10^A	49 ± 10^A
Plasma T3, %uptake[3]	45 ± 1.6^A	44 ± 1.4^A	45 ± 1.1^A
TSH, ng/mL[4]	0.83 ± 0.13^A	0.73 ± 0.10^A	1.00 ± 0.27^B

[1]Comparison of the effect of dietary Se level on selenoenzyme activity and circulating T4 and TSH concentrations. Results are presented as mean±SD and superscript letters indicate significant difference between means, as determined by Least Squared Differences test ($p \leq 0.05$).

[2]LSe=low Se diet (basal, 0.035 mg Se/kg diet), NSe=normal Se diet (basal + 0.18 mg Se/kg diet), and HSe=high Se diet (basal + 1.0 mg Se/kg diet).

[3]Dietary I had no significant effect, as determined by 2-way ANOVA ($p > 0.05$) and data were pooled for all dietary I level groups; for LSe and HSe, n=21, and for NSe, n=18.

[4]Dietary I had a significant effect, as determined by 2-way ANOVA ($p \leq 0.05$) and data represent results from the normal Se diet groups only; for LSe and HSe, n=7, and for NSe, n=6.

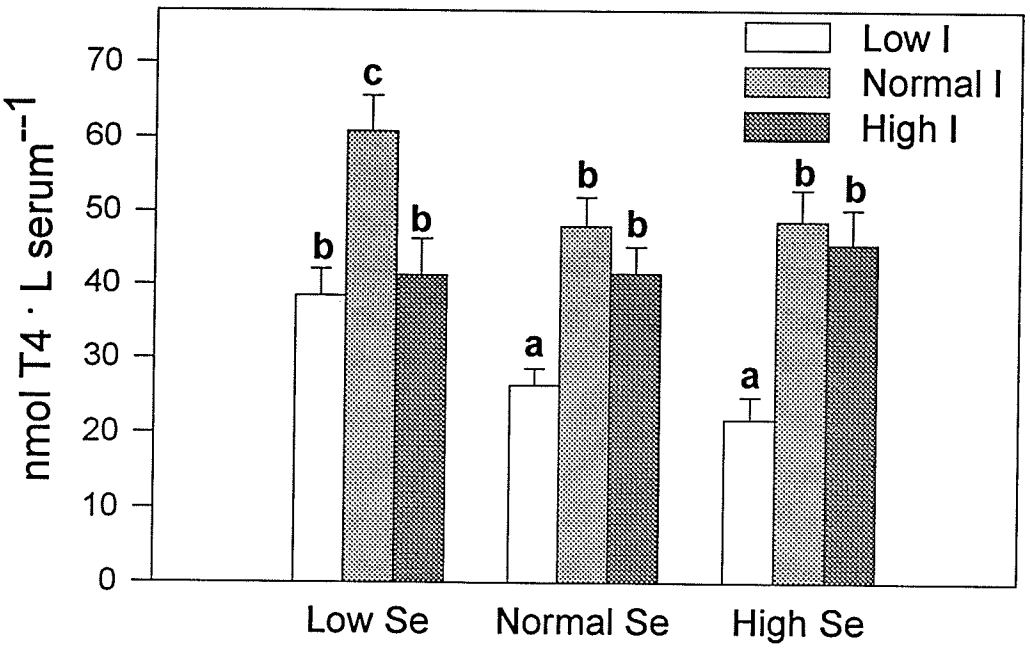

Figure 1. The response of plasma T4 in rats to various levels of dietary I and Se. Bars represent mean±SEM; for low and high Se groups, n=7 and for normal Se groups, n=6. The low, normal, and high diets contained <0.03 mg I/kg diet (basal), basal + 0.2 mg I/kg diet, and basal + 1.0 mg I/kg diet respectively and <0.05 mg Se/kg diet (basal), basal + 0.18 mg Se/kg diet, and basal + 1.0 mg Se/kg diet respectively. Significant differences between means were determined by a Least Squared Differences test ($p \leq 0.05$) and are indicated by the lowercase letters.

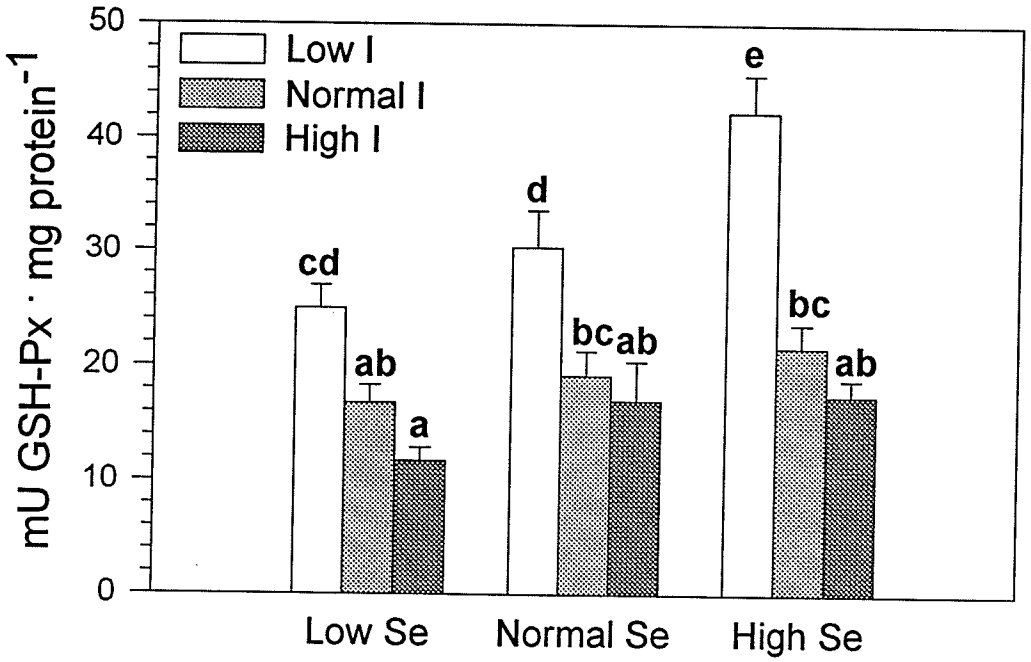

Figure 2. The response of thyroidal GSH-Px activity in rats to various levels of dietary I and Se. Columns represent mean±SEM; for low and high Se groups, n=7 and for the normal Se groups, n=6. The low, normal, and high diets contained <0.03 mg I/kg diet (basal), basal + 0.2 mg I/kg diet, and basal + 1.0 mg I/kg diet respectively and <0.05 mg Se/kg diet (basal), basal + 0.18 mg Se/kg diet, and basal + 1.0 mg Se/kg diet respectively. Significant differences between means were determined by a Least Squared Differences test ($p \leq 0.05$) and are indicated by the lowercase letters.

DISCUSSION

The response of the various parameters of TH metabolism to low dietary I intake were indicative of the hypothyroid state; reduced plasma T4 concentration, increased secretion of TSH, and increased thyroid weight compared to controls were demonstrated (Table 1). In previous studies, the response of circulating T3 concentration to low dietary I has been variable. In some studies, I deficiency in rats decreased the T3 concentration (Golstein et al 1988, Beckett et al 1990) while in others T3 has remained unchanged (Abrams and Larsen 1973, Beckett et al 1993, and Meinhold et al 1993), or even slightly increased (Meinhold et al 1992). In the present study, plasma T3 was unchanged from control levels. Possible reasons for the differences in results may relate to the mechanisms within the thyroid which function to maintain normal circulating T3 concentration when I is deficient. Direct synthesis of T3 has been shown to increase when I availability is low (Abrams and Larsen 1973) and thyroidal DI-I activity has been shown to increase in the presence of TSH (Erickson et al 1982), thus increasing the amount of T3 derived from T4 deiodination. Both of these mechanisms are believed to operate in the rat to maintain normal T3 levels and to utilize I more efficiently when its availability is low (Laurberg 1984). Differences in the I content of the treatment diets and the length of the depletion period may account for the variable response of T3 in response to I deficiency. The maintenance of normal circulating T3 concentration is not likely to be attributed to increased DI-I activity in peripheral tissues as peripheral DI-I activity has not previously (Beckett et al 1993, Janssen et al 1994) or presently been shown to increase during I deficiency.

Low dietary I significantly increased the activity of thyroidal GSH-Px and this may be attributed to TSH stimulation, which also occured in this study, or to the thyroidal autoregulatory response to low I. The requirement for GSH-Px activity in the thyroid would conceivably be greater with low I status. TSH stimulates thyroidal H_2O_2 production (Corvilain et al 1991) and, with less I available for oxidation, excess H_2O_2 would be generated, thus increasing the need for neutralization mechanisms such as the GSH-Px antioxidant system. This response appears to be specific to the thyroid as GSH-Px activity in liver and kidney were unaffected by the level of I in the diet.

Varying concentrations of dietary Se differentially affected selenoenzyme activity in the different tissues analyzed. Tissue GSH-Px activity of rats is typically reduced by consumption of low Se diets and in this study, reduction occured in liver, kidney, and erythrocytes, but not thyroid. In rats with high Se intake, the large, significant increase in GSH-Px activity in kidney and erythrocytes compared to controls was unexpected. This seems to indicate that the amount of GSH-Px activity observed at the control level of Se (basal + 0.18 mg /kg diet) was suboptimal in those tissues.

The activity of GSH-Px in the thyroid was not dependent on the concentration of dietary Se; neither low Se nor high Se altered activity from that found in controls. Thyroid has previously been shown to have a higher Se retention factor (Behne et al 1988) and to be less susceptible to loss of GSH-Px activity (Beech et al 1995, Behne and Kyriakopoulos 1993, Vadhanavikit et al 1993) than tissues such as liver and kidney. The need for homeostatic control of thyroidal GSH-Px activity may be indicative of its important function in the thyroid in the neutralization of H_2O_2 in order to prevent

cytoxicity. The increased thyroidal GSH-Px activity seen with low I intake and elevated serum TSH does, however, seem to be limited by the amount of dietary Se as activity increased with increasing Se intake.

Compared to previous studies, which have observed large decreases in DI-I activity (>60%) in peripheral tissues using the more severely deficient diet (<0.005 mg Se/kg diet) (Arthur 1990, Beckett 1989, 1992, Beech et al 1995), the moderately Se deficient diet used in this study (<0.05 mg Se/kg diet) produced limited effects on DI-I activity. While hepatic DI-I activity was unaffected by the Se deficient diet, renal activity was decreased by 19% in this study. Reduced DI-I activity has typically resulted in elevated levels of circulating T4 (Arthur et al 1990, Beckett et al 1989, 1992). T4 was elevated in the low Se group by 19% and, although this difference did not reach statistical significance ($p=0.06$), the increase was of similar magnitude as the decrease in renal DI-I activity.

The high Se diet caused a significant elevation in blood glucose concentration as compared to controls (7.0 vs 8.7 mmol/L, $p<0.00001$). A similar finding was demonstrated previously in Se supplemented mice compared to Se deficient mice, and this study also indicated that the turnover of glucose was increased in Se deficient mice, as determined by $^{14}CO_2$ exhalation (Wendel and Otter 1987). Further intermediary metabolism enzyme analyses need to be carried out to determine the specific role of Se in glucose metabolism.

Various combinations of I and Se intake levels produced interactive effects on plasma T4 and thyroidal GSH-Px activity. While low dietary I alone decreased plasma

T4, and low dietary Se alone tended to increase plasma T4, the combination of low dietary I and Se resulted in plasma T4 concentration similar to that of controls (Figure 1). This appears as a compensatory effect of combined I and Se deficiency. It has previously been hypothesized that compensation in the level of T4 by Se deficiency would be protective for a developing fetus, as the amount of maternal T4 it receives is crucial for normal neurological development (Dumont et al 1994). This does not, however, discount the possible role of concurrent I and Se deficiency in the myxedematous type cretinism caused by thyroid degeneration prenatally or in infancy, and attributed to the lack of thyroidal GSH-Px activity with TSH stimulated H_2O_2 production (Dumont et al 1994). Although thyroidal GSH-Px activity with dual deficiency in the present study is similar to that of the controls, it does appear to be limited by the amount of available Se and may not be sufficient to neutralize the expected increase in H_2O_2.

Combined I and Se deficiency did not further increase serum TSH concentration or thyroid weight above that found with I deficiency alone (data not shown). These results support those found in previous studies (Beckett et al 1990, Golstein et al 1988, Meinhold et al 1992) where Se deficiency either alleviated or had no effect on the parameters of hypothyroidism, and counter those found by others (Arthur et al 1992, Beckett et al 1993). This variation may result from differences in dietary Se and I concentrations used and the length of the treatment period and demonstrate the sensitivities of these systems to mineral availability.

Animals consuming the high I diets tended to have decreased plasma T4 and this

has previously been attributed to the thyroidal autoregulatory response to high I availability (Ingbar 1984). However, also of concern are the effects of high I intake when Se intake is low and the possible thyroidal damage that can occur. Irreversible damage of the thyroid gland caused by I supplementation to animals deficient in both I and Se has recently been demonstrated (Contempré et al 1995). It was proposed that excess I in the thyroid, through a series of molecular reactions, would react with H_2O_2 to form free radicals, thus causing subsequent tissue damage. The low thyroidal GSH-Px activity, and hence antioxidant capability, seen in the present study when diets contained high I and low Se, supports this hypothesis (Figure 2). Although Se deficiency with normal I did not decrease thyroidal GSH-Px activity, high I intake seemed to either exhaust or inhibit the activity, thus increasing the potential for tissue damage. This finding may suggest that caution be used in I supplementation when Se is also deficient, especially in infants and children whose thyroids are still developing, and that further investigation of this interaction is needed.

Another issue of concern centers around the possible harmful effects of Se supplementation when I and Se are both deficient, as demonstrated previously in rats (Golstein et al 1988) and humans (Contempré et al 1991). Golstein et al (1988) suggested that T4 synthesis could be regulated by the activity of thyroidal GSH-Px. Increased activity, thought to result from Se supplementation, would neutralize H_2O_2 in thyrocytes and decrease TH synthesis, as H_2O_2 was previously shown to be the limiting factor in thyroglobulin iodination (Corvilain et al 1991). This hypothesis is not supported by the data of the present study as thyroidal GSH-Px activity was responsive to neither

low nor high Se intake. Se supplementation in an I deficient, pregnant woman may, according to hypothesis, prevent thyroid degeneration and myxedematous type cretinism of the fetus, but would, however, pose the danger of decreasing maternal T4 below the amount that would be produced by concurrent I and Se deficiency, thus putting the fetus at risk of neurological damage (Dumont et al 1994).

Several aspects concerning the effects of I and Se in deficient or supplemented states on thyroid hormone metabolism have been elucidated by this study. GSH-Px activity in the thyroid was found to be regulated to a greater degree by I status, than by Se status. In addition, this study supports the possible danger of I supplementation when Se is deficient, as it could result in increased thyroid tissue necrosis, therefore caution may be needed when supplementing Se deficient infants or children with I. Concurrent I and Se deficiency appears to maintain T4 at control levels but this dual deficiency could still be clinically identified by elevated circulating TSH. Se deficiency concurrent with I deficiency did not appear to further antagonize TH status, but did instead produce a control level of circulating T4, which could be protective in pregnant women against neurological damage to the fetus. In the case of pregnant women and other adults, iodine supplementation would still be the preferred treatment.

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V. SUMMARY

The objectives of this study were to determine the effects of dietary selenium deficiency on thyroid hormone metabolism of rats, and relate those effects to other indices of selenium status, and to determine the interactions of dietary iodine and selenium on thyroid hormone metabolism when both elements are deficient and when their intakes are imbalanced.

The data presented in the first experimental study revealed a number of differences in selenium metabolism between male and female rats when fed a control diet and when fed a selenium deficient diet. Females seem to have a greater physiological need for GSH-Px activity in the liver; not only were females shown to have a significantly higher hepatic GSH-Px activity than males with normal selenium intake, but this activity was also found to be more resistant to selenium depletion in females than in males. DI-I activity in kidney was found to be two times greater in males than in females. Due to the finding of many sex-specific responses in parameters of rat selenium metabolism, caution must be used when assessing selenium status as the differences due to gender can be as large as those attributed to selenium intake.

The kidney has presented some interesting aspects of selenium metabolism. In the first experiment, kidney continued to accumulate selenium even when it was depleted from the diet. Also, DI-I in the kidney responded to selenium depletion by increasing its activity. In the second experiment, kidney, as well as erythrocytes, showed an increased GSH-Px activity with high selenium intake, as compared to normal selenium intake. This suggests that the optimum level of dietary selenium in these male rats was

greater than the normal amount of selenium, according to the AIN-93G diet formulation. The metabolism of selenium in the kidney appears to be more specific than it is in the liver and this may be attributed either to the biological importance of an as yet unknown selenoenzyme, or perhaps to the role of the kidney in increasing selenium retention during its depletion. At the very least, this data supports the theory that redistribution of Se to priority tissues, and to priority enzymes, does occur.

Both studies demonstrated that various parameters of thyroid hormone metabolism are quite resistant to selenium depletion at the basal level of selenium provided by these diets (<0.05 mg selenium/kg diet). In the first experiment, not only did selenium depletion fail to decrease DI-I activity, but in kidney it was actually increased. In the second experiment, DI-I activity was slightly decreased in the kidney while no change occurred in hepatic DI-I activity. The difference in response of kidney DI-I activity to the low selenium diet between the two experiments is likely attributable to 1) the ability of the animals in experiment 1 to deposit selenium stores during the first 6 weeks of the study when they were fed the control diet, 2) the ability of these same animals to adapt to selenium depletion during the long term course of the study, and 3) to the lack of selenium stores in the weanling rats and the increased need for selenium during their high growth period.

Circulating T4 concentration responded differently to low selenium intake in the two studies. In the first experiment, serum T4 showed no significant response to selenium depletion, as compared to controls. In the second experiment, plasma T4 increased in response to low selenium intake, and this increase may be attributed to the

decreased DI-I activity observed in the kidney. The contribution of peripheral DI-I activity to the circulating amount of T4 may be questionable though, as in experiment 1 the large, significant decrease in hepatic DI-I activity in response to selenium depletion did not appear to affect the amount of circulating T4 observed. It is possible that the thyroid itself has a more important role in regulating circulating thyroid hormone concentration than the peripheral DI-I activities and this could occur either through its own DI-I activity, or by thyroid autoregulatory mechanisms.

Dietary iodine and selenium produced interactive effects on circulating T4 in the second study. The opposing effects of iodine and selenium deficiency resulted in the restoration of plasma T4 to control levels when the elements were concurrently deficient. The effect of selenium in normalizing T4 levels is thought to be protective in a pregnant woman against neurological damage to the fetus, which can result from the low supply of maternal T4 to the fetus when iodine alone is deficient.

Both studies also concluded that thyroidal GSH-Px activity is resistant to selenium depletion. The second study indicated that this activity is regulated primarily by iodine as opposed to selenium. Thyroidal GSH-Px activity was significantly increased by low iodine intake probably by either TSH stimulation, which was also shown to increase with iodine deficiency, or through autoregulatory mechanisms of the thyroid responding to low iodine intake. However, the ability of low iodine to increase this activity was enhanced by increasing availability of dietary selenium. It thus appears that thyroidal GSH-Px activity was therefore limited by selenium availability and the potential for high amounts H_2O_2, also produced in higher amounts in the thyroid in response to iodine deficiency,

to cause damage to the thyroid tissue seems likely. Additionally, a high amount of iodine in the thyoid may also have toxic effects, and if GSH-Px activity is compromised during severe selenium deficiency, thyroid tissue damage may also ensue.

In summary, the body's ability to compensate for selenium deficiency appears to be substantial in those parameters related to thyroid hormone metabolism. Due to the interactive effects of selenium and iodine on thyroid hormone metabolism demonstrated in the present study, and on previous knowledge of such interactions, the erradication of both mineral deficiencies through a gradual increase in intake, such as through food fortification, as opposed to high dose supplementation, would be recommended.