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SELENIUM BALANCE AND STATUS IN THE FEMALE
DURING PHYSICAL AND SEXUAL MATURATION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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Age, gender, and fluctuations in reproductive hormones have been reported to affect selenium status. During puberty, when hormonal levels are changing, selenium intakes may be suboptimal. The goals of this study were: 1) to assess the relationship between selenium intakes and balance in females throughout puberty in an effort to determine the effect of intake on the existing selenium pool size; 2) to determine if selenium status changes in the female during physical and sexual maturation; and 3) to determine if selenium balance, net absorption, and status differ between adolescent females at threshold calcium intake (1500 mg/day) versus calcium intake below the current RDA for adolescent females (<1200 mg/day). Yearly selenium balances were performed in a metabolic unit over a 2-week period during 1993-95 in 26 healthy Caucasian females. Average pubertal stage was 3.6±0.8, 4.0±0.8, and 4.4±0.9 for 1993, 1994, and 1995, respectively. Selenium content of food, feces, and urine were determined to calculate individual balances and net selenium absorption \[\text{balance} = \left(\frac{\text{selenium intake} - \text{fecal selenium}}{\text{selenium intake}}\right) \times 100\%\]. Polyethylene glycol was used as a continuous fecal marker. Average daily balance was -2±18, +6±18, and -11±17 μg selenium for 1993, 1994, and 1995,
respectively. Overall average net absorption was 74±5%. Selenium status [serum and erythrocyte selenium and glutathione peroxidase] measurements were all within normal ranges. The results of this study suggest that: 1) selenium absorption may be greater among adolescent females than among female and male adults; 2) during earlier puberty, an increased daily intake of selenium, over the range of intakes studied, increased selenium balance among these adolescent subjects, until later puberty, when it was more difficult for them to maintain positive selenium balance; 3) an intake of approximately 100µg/day of selenium resulted in selenium balance in our population, suggesting that their habitual selenium intake was closer to 100 µg of selenium/day than to the current RDA (45µg/day); 4) rapid pubertal development at the intake of our subjects may adversely impact attaining positive selenium balance, compared to a more gradual rate of development; 5) selenium status may be compromised during peak linear growth; 6) higher estradiol concentrations reflect the progression of sexual maturation and may be associated with a compromised selenium status; and 7) the use of dietary recalls and records may not reflect actual selenium content of the diet. This information will contribute to the knowledge regarding dietary selenium recommendations for adolescent females. Since there has been limited knowledge regarding the relationship between selenium status and the developmental changes of females, this research will help fill a gap and further define the unique needs of adolescent females.
Dedicated to my Lord, Savior, and
source of strength, Jesus Christ, and
my lovely wife Lisa
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CHAPTER 1

INTRODUCTION

"Because of biological, social, and political factors, women are at unique risk for major nutrition-related diseases and conditions including cardiovascular disease, certain cancers, osteoporosis, diabetes, and weight-related problems." (The American Dietetic Association and The Canadian Dietetic Association 1995)

In the past, the unique health care needs of females have been ignored, overlooked, or assumed to be that of their male counterparts. Even nutritional guidelines like the Recommended Dietary Allowances (RDAs) have been largely based on studies done on male subjects. The 1989 RDAs included recommended selenium intakes for infants, children, and adults (National Academy of Sciences 1989); however, studies using adult male subjects were used as the basis for these recommendations (Yang et al. 1987). These recommendations may not be appropriate since gender-related differences in selenium status have been reported (Bratakos et al. 1990, Capel and Smallwood 1983, Igarashi et al. 1984, Pinto and Bartley 1969). Estrogen status also appears to influence selenium status (Cha et al. 1987).
1991, Chang et al. 1994, McAdam et al. 1994). While the mechanism has not been determined, partitioning of selenium may largely be influenced by the changing estrogen status during the female life cycle. In addition, selenium status has been shown to differ between adolescents and younger children (Smith et al. 1994).

There is limited knowledge regarding the relationship between selenium status and the developmental changes of females. The unique needs of adolescent females should not be overlooked, considering the generally poor dietary habits of this population and the possible reproductive events to follow.

The goals of this study were: 1) to assess the relationship between selenium intakes and balance in females throughout puberty in an effort to determine the effect of intake on the existing selenium pool size; 2) to determine if selenium status changes in the female during physical and sexual maturation; and 3) to determine if selenium balance, net absorption, and status differ between adolescent females at threshold calcium intake (1500 mg/day) versus calcium intake below the current RDA for adolescent females (<1200 mg/day). The research hypotheses were: 1) selenium intake necessary to maintain existing selenium pool size will increase in the female with progression of sexual maturation; 2) selenium status, as assessed through measurements of serum and erythrocyte selenium and glutathione peroxidase, will decline in the female with progression of adolescence; and 3) selenium balance, net absorption, and status will not differ between
adolescent females at threshold intake or those consuming less than the recommended amount of calcium. Experiments were designed to answer the following questions: 1) Are selenium balance and status affected by sexual maturation in the female?; and 2) Does threshold calcium intake affect selenium balance and status in the adolescent female?

A selenium balance study was used in the project. The selenium status of the adolescent female subjects was assessed through measurements of serum and erythrocyte selenium and glutathione peroxidase. A possible limitation of the study was that maturation during puberty or calcium supplementation would not have had an effect on selenium status. In that event, the relationship between selenium intake and selenium balance during adolescence would have become the focus.

It was anticipated that the results of this balance study would determine the effect of selenium intakes on the existing pool of selenium and the concurrent selenium status of the study group. This information would fill a gap in the existing body of selenium nutrition knowledge by providing a basis for dietary selenium recommendations for adolescent females. This research would subsequently clarify the nutritional recommendations for selenium intakes among this population, which was expected to be greater than the current recommendations (45µg/day). The rapid growth associated with adolescence was hypothesized to decrease the existing pool size of selenium, resulting in a decline in selenium status.
CHAPTER 2

REVIEW OF LITERATURE

Selenium-General Information and History

Selenium, element 34 on the periodic table, has a molecular weight of 78.96 and exists in oxidation states -2, 0, +2, +4, and +6; however, +2 is not commonly found in nature (Diplock 1987, Krehl 1970). This metal has been shown to have functional roles in several mammalian selenoproteins and is usually associated with protein in animal tissues (Burk and Hill 1993). Consequently, muscle meats, organ meats (especially liver and kidney), and seafood are dependable sources of the mineral (Gibson 1990, World Health Organization 1987). Grains and seeds, however, can vary in their selenium content, depending upon the selenium content of the soils on which they were grown (Gibson 1990, World Health Organization 1987). Fruits and vegetables are usually poor sources of the mineral, and drinking water does not provide substantial quantities of dietary selenium (World Health Organization 1987).
This trace mineral was first identified as an element by Berzelius in 1817 (Krehl 1970, Reddy and Massaro 1983). In the thirteenth century, however, Marco Polo observed that there were seleniferous plants in the mountainous region of western China that, if eaten, would cause the hoofs of the animals to drop off (Krehl 1970). Similar observations were recorded in the late nineteenth and early twentieth centuries by an army surgeon, Dr. T. C. Madison, in the Nebraska territory and by General Custer in Nebraska, the Dakotas, and other western territories (Krehl 1970). After being identified in 1957 as part of "factor 3" for the prevention of liver necrosis in rats (Schwarz and Foltz 1957), several experiments followed which led to the discoveries that selenium would alleviate diseases like exudative diathesis and pancreatic fibrosis in chicks, muscular dystrophy (white muscle disease) in farm animals (sheep, cattle, foals, chickens, pullets), hepatosis diaetetica in pigs, and reproductive disorders in livestock (Krehl 1970, Schwarz 1961, Thompson and Scott 1969, Underwood 1977). An association was documented in 1979 between low selenium status and a cardiomyopathy, Keshan Disease, primarily affecting children and women of childbearing age which illustrated the essentiality of selenium in humans (Keshan Research Group 1979).
**Selenium-Biological Function**

Selenium has two fates in metabolism: 1) incorporation into selenoproteins (Burk and Hill 1993) and 2) methylation and excretion (Bopp et al. 1982). This review will focus on selenium's biological function in selenoproteins.

The trace element selenium has been shown to have functional roles in several mammalian selenoproteins; in fact, nearly all selenium in animal tissues is associated with protein (Burk and Hill 1993). Nine selenoproteins have been identified (Burk and Hill 1994, Larsen and Berry 1995) and include cellular or classical glutathione peroxidase (Rotruck et al. 1973), plasma or extracellular glutathione peroxidase (Takahashi et al. 1987), phospholipid hydroperoxide glutathione peroxidase (Ursini et al. 1985), gastrointestinal glutathione peroxidase (Chu et al. 1993), selenoprotein P (Burk and Hill 1994), type 1 iodothyronine deiodinase (Berry et al. 1991), type 3 iodothyronine deiodinase (St. Germain et al. 1994), sperm mitochondrial capsule selenoprotein (Calvin 1978), and selenoprotein W (Vendeland et al. 1993).

Selenoproteins are proteins that contain the selenocysteine form of selenium (Burk & Hill 1993). This selenoamino acid may account for up to eighty percent of the body's selenium (Hawkes et al. 1985). Selenocysteine synthesis uses inorganic selenium in a transfer RNA (tRNA)-mediated process (Sunde 1990). Its biosynthesis occurs on a specific tRNA (Bock et al. 1991), which is very common in the animal kingdom (Lee et al. 1990). Insertion into the nascent polypeptide

In addition to the aforementioned selenoproteins found in eukaryotic species, selenocysteine occurs in several bacterial enzymes, including glycine reductase, formate dehydrogenases, and hydrogenases (Stadtman 1990). Several factors are required for the incorporation of selenocysteine in Escherichia coli. The genes that code for these factors include selC, selA, selD, and selB (Forchhammer 1989, Forchhammer 1990). The selC gene codes for tRNA^{UCA} which accepts serine and inserts selenocysteine by recognizing the UGA codon in a cotranslational process (Leinfelder et al. 1988). The products of the selA and selD genes, selenocysteine synthase and selenophosphate synthetase respectively, convert the serine residue attached to the tRNA to selenocysteine by catalyzing the replacement of the side-chain oxygen in serine by selenium (Berry et al. 1995, Burk and Hill 1993, Forchhammer and Bock 1991, Leinfelder et al. 1989). And finally, SELB (the product of selB) is needed after selenocysteyl-tRNA biosynthesis (Leinfelder 1989). It is a translation factor involved in selenoprotein biosynthesis (Forchhammer 1989, Forchhammer 1990), that is, an elongation factor (Burk and Hill 1993). For the
UGA codon not to specify termination, a specific stem-loop structure in the region of the codon is necessary (Burk and Hill 1993, Heider et al. 1992).

Less is known about the above process in eucaryotic cells. As with procaryotes, a unique tRNA that has the anticodon for UGA is utilized (Burk and Hill 1993). Three factors are necessary for the incorporation of the selenoamino acid selenocysteine into protein: sec-tRNA\textsuperscript{Sec}\textsuperscript{Sec}, the stem loop, and UGA. Unlike procaryotes, the stem loops necessary for the eucaryotic process are not proximate to the UGA codon, rather they are separated by hundreds of bases (Burk and Hill 1993). The identification of the selenocysteine insertion sequence (SECIS) element was elucidated by the structural and functional analysis of type 1 deiodinase mRNA by Berry et al. (1991). SECIS is the stem-loop structure in the 3' untranslated region of the mRNAs encoding the glutathione peroxidases, selenoprotein P, and types 1 and 3 iodothyronine deiodinase (Larsen and Berry 1995).

THE SELENOPROTEINS

Nine selenoproteins have been identified (Burk and Hill 1994, Larsen and Berry 1995) and include cellular or classical glutathione peroxidase (Rotruck et al. 1973), plasma or extracellular glutathione peroxidase (Takahashi et al. 1987), phospholipid hydroperoxide glutathione peroxidase (Ursini et al. 1985), gastrointestinal glutathione peroxidase (Chu et al. 1993), selenoprotein P (Burk-Sel.P), type 1 iodothyronine deiodinase (Berry et al. 1991), type 3 iodothyronine deiodinase (St. Germain et al. 1994), sperm mitochondrial capsule selenoprotein
Glutathione peroxidases

The majority of selenium research has been related to its antioxidant role in the enzyme glutathione peroxidase. Four glutathione peroxidase enzymes have been identified, including cellular or classical glutathione peroxidase (Rotruck et al. 1973), plasma or extracellular glutathione peroxidase (Takahashi et al. 1987), phospholipid hydroperoxide glutathione peroxidase (Ursini et al. 1985), and gastrointestinal glutathione peroxidase (Chu et al. 1993). While each of the aforementioned enzymes are distinct, in general, glutathione peroxidase rids the body of peroxides that can damage cells and tissues by catalyzing their reduction to water. Selenium, as part of glutathione peroxidase, has interdependent roles with vitamin E, iron (as catalase), and zinc and copper (as superoxide dismutase) as one of the antioxidant nutrients (Groff et al. 1995).

Cellular or classical glutathione peroxidase

Selenium was discovered to be a vital component of cellular or classical glutathione peroxidase by Rotruck et al. (1973). It is found in virtually all cells, but there is a wide variation of its activity among different species and tissues (Burk and Hill 1993). Approximately 70 percent of cellular glutathione peroxidase is found in the cytosol of cells, while the remainder resides in the mitochondrial
matrix of the cell (Groff et al. 1995). Cellular glutathione peroxidase, using the tripeptide glutathione (glycine, cysteine, and glutamate) as the reducing substrate, can reduce hydrogen peroxide and free organic hydroperoxides to water (Burk and Hill 1993, Groff et al. 1995).

Structurally, the active enzyme has four identical 22.0 kDa subunits each containing one selenocysteine residue, and therefore it contains 4 g atoms of selenium per mol of protein (Burk and Hill 1993, Yeh et al. 1995). Forstrom et al. (1978) reported that the selenoamino acid selenocysteine was the selenium moiety in glutathione peroxidase. This finding was confirmed upon the elucidation of the sequences of rat and bovine glutathione peroxidase (Condell and Tappel 1982, Epp et al. 1983). The structure of the mouse glutathione peroxidase, a 201 amino polypeptide, was elucidated in 1986 (Chambers et al. 1986). Selenocysteine was found to be at the active site of the enzyme and encoded by TGA, the termination codon (Chambers et al. 1986). In turn, the cDNA coding the human glutathione peroxidase enzyme confirmed this finding (Mullenbach et al. 1987). The nucleotide sequences for glutathione peroxidase elucidated in several species and tissues indicate that the enzyme is highly conserved in higher animals (Sunde 1990).

In mammalian species, cellular glutathione peroxidase may represent a storehouse for selenium that can be used for other purposes (Burk and Gregory 1982, Sunde 1990, Yang et al. 1989). In a selenium deficiency, the activity of this
enzyme is affected. Saedi et al. (1988) reported that less mRNA for hepatic glutathione peroxidase production is made with decreased selenium availability, diminishing the enzyme activity. A rapid increase in mRNA levels accompanied by a gradual increase in enzyme activity is noted with selenium supplementation to control levels (Saedi et al. 1988). As the only known biochemical function of selenium for many years, measurement of this enzyme has been utilized to assess selenium nutritional status (Yeh et al. 1995).

**Plasma or extracellular glutathione peroxidase**

Plasma or extracellular glutathione peroxidase, a glycoprotein, was purified and characterized from human plasma in 1987 (Takahashi et al. 1987). Takahashi and Cohen (1986) first reported its distinction from cellular glutathione peroxidase in 1986. Besides plasma, this enzyme is found in human milk (Bhattacharya et al. 1988).

Extracellular glutathione peroxidase appears to be synthesized in the lung and kidney (Chu et al. 1992). While extracellular glutathione peroxidase is a separate gene product, it shares the same sequence identity as cellular glutathione peroxidase (Takahashi et al. 1990). Like cellular glutathione peroxidase, the extracellular enzyme consists of four identical subunits (23.0 kDa), each containing one selenocysteine residue (Burk and Hill 1993, Yeh et al. 1995).

Extracellular glutathione peroxidase assessment can serve as an index of selenium nutritional status (Burk and Hill 1993). Like cellular glutathione
peroxidase, its activity would be diminished during a selenium deficiency. This enzyme may function other than as a glutathione peroxidase, since the concentration of its reducing substrate, glutathione, is low in extracellular fluids (Burk and Hill 1993). Continued research in this area will further clarify its role.

Phospholipid hydroperoxide glutathione peroxidase

After purifying it from pig heart, Ursini et al. (1985) characterized phospholipid hydroperoxide glutathione peroxidase (previously peroxidation-inhibiting protein) as a 23.0 kDa monomer, unlike the aforementioned glutathione peroxidases which consist of four identical subunits containing one selenocysteine per subunit (Yeh et al 1995). Zhang et al. (1989) studied the tissue distribution of this enzyme in rats of different age groups and compared the results with other glutathione peroxidases. The results suggested a tissue- and age-specific expression of the different peroxidases. Zhang et al. (1989) reported that phospholipid hydroperoxide is much more conserved (more constantly expressed) in different age groups than other glutathione peroxidases. In addition to being studied in rats, Maiorino et al (1991) recently identified phospholipid hydroperoxide as the 18 kDa selenoprotein expressed in human tumor cell lines.

The role of phospholipid hydroperoxide glutathione peroxidase is to reduce fatty acid hydroperoxides that have been esterified to phospholipids (Ursini et al. 1985). It also can reduce the hydroperoxides of cholesterol and cholesterol ester in membranes and low density lipoproteins (Thomas et al. 1990a, Thomas et al. 12
Protecting against lipid peroxidation and playing a role in eicosanoid metabolism appear to be its primary biological roles (Ursini and Bindoli 1987, Ursini et al. 1987).

A selenium deficiency can also adversely affect phospholipid hydroperoxide glutathione peroxidase activity; however, in rodents, phospholipid glutathione peroxidase activity has been demonstrated to be more slowly depleted by selenium deficiency than cellular glutathione peroxidase (Weitzel et al. 1990). Thompson et al. (1995) recently reported that the phospholipid hydroperoxide glutathione peroxidase activities of liver, lung, thymus, heart, and testes were lower in selenium-deficient than selenium-supplemented rats. Cellular glutathione peroxidase activity of liver was also lower. The activity of phospholipid hydroperoxide glutathione peroxidase was more closely associated with growth than was the activity of cellular glutathione peroxidase, as evidenced by increased growth and normalized liver phospholipid hydroperoxide glutathione peroxidase activity in selenium-deficient rats after selenium supplementation by injection. This treatment produced only a very slight increase in cellular glutathione peroxidase activity of liver.

**Gastrointestinal glutathione peroxidase**

Gastrointestinal glutathione peroxidase was recently characterized (Chu et al. 1993). This selenium-dependent enzyme was characterized by expressing a gastrointestinal glutathione peroxidase cDNA isolated from human hepatoma HepG2
cells in human mammary carcinoma MCF-7 cells (Chu et al. 1993). This enzyme is a tetrameric protein localized in the cytosol. (Chu et al. 1993). Chu and Esworthy (1995) recently deduced the structure of the glutathione peroxidase 2 (hgph2) gene encoding gastrointestinal glutathione peroxidase. The gene structure confirms that the cellular and gastrointestinal glutathione peroxidase isoenzymes are the products of distinct genes (Chu and Esworthy 1995).

While the 22.0 kDa protein has substrates similar to those of cellular glutathione peroxidase (hydrogen peroxide, tert-butyl hydroperoxide, cumene hydroperoxide, and linoleic acid hydroperoxide), Chu et al. (1993) reported that gastrointestinal glutathione peroxidase had a relatively higher reactivity toward organic hydroperoxides when their enzyme activities were normalized against \( \text{H}_2\text{O}_2 \)-metabolizing activity.

In humans, gastrointestinal glutathione peroxidase mRNA was easily detected in liver and colon samples and occasionally in breast samples. However, human kidney, heart, lung, placenta, and uterus did not contain the mRNA (Chu et al. 1993). In the rat, this selenoprotein has only been detected in the gastrointestinal tract (Chu et al. 1993). Chu and Esworthy (1995) noted that gastrointestinal glutathione peroxidase mRNA was readily detected by Northern blotting techniques in all parts of the rat gastrointestinal tract, and the ileum had high expression relative to all other regions. Gastrointestinal glutathione peroxidase appears to be the major glutathione-dependent peroxidase activity in the rat
gastrointestinal tract (Chu et al. 1993). Chu et al. (1993) speculated that the selenoenzyme may have a significant role in protecting mammals from the toxicity of ingested lipid hydroperoxides.

Research assessing the effect of selenium deficiency on gastrointestinal glutathione peroxidase has not been reported. Szymanska and Siegers (1992) did report, however, that during selenium deficiency the selenium-dependent glutathione peroxidase activity of the rat gastrointestinal mucosa decreased. Their research may reflect the activity of the aforementioned enzyme, in view of its reported abundance in the gastrointestinal tract.

Selenoprotein P

Evidence for selenoprotein P was reported in the literature by Burk in 1973 (Burk 1973). His research showed rapid incorporation of $^{75}$Se into a plasma protein. Purification of this protein was unsuccessful; however, it was shown to be distinct from glutathione peroxidase (Hermann 1977). Subsequently, selenium-deficient rats were found to rapidly incorporate selenium into $^{75}$Se-P (selenoprotein P) preferentially over glutathione peroxidase (Burk and Gregory 1982). Motsenbocker and Tappel (1982) found that this protein contained selenocysteine. They reported that this selenoprotein was located in the plasma and suggested it be named selenoprotein P (Motsenbocker and Tappel 1982).

Selenoprotein P and plasma glutathione peroxidase are the only known plasma selenoproteins (Burk and Hill 1994). Selenoprotein P is the major form of
selenium in rat serum (Read et al. 1990). In addition to being purified from rats (Burk and Hill 1994, Read et al. 1990), it has also been purified from human plasma (Akesson et al. 1994). Human plasma contains approximately one tenth of the quantity of selenoprotein P found in rat plasma (Burk and Hill 1994). Human selenoprotein P cDNA was recently cloned from human liver and heart cDNA libraries and sequenced (Hill et al. 1993). A 69 percent identity of the nucleotide sequence and 72 percent identity of the deduced amino acid sequence was reported when comparing the open reading frame of the human cDNA with the rat cDNA (Hill et al. 1993). There is also high conservation between human and rat in two regions in the 3' untranslated portion (Hill et al. 1993). This glycoprotein with a molecular weight of 43.0 kDa (Burk and Hill 1994, Read et al. 1990) contains approximately 8 (7.5 ± 1) selenium atoms per molecule as selenocysteine (Burk and Hill 1994, Read et al. 1990). The cloned cDNA sequence predicts 10 residues (Burk and Hill 1994, Hill et al. 1991, Hill et al. 1993) suggesting that the protein in plasma is a modification of the predicted one (Burk and Hill 1994). The glycosylation of selenoprotein P appears to be N-linked without evidence of microheterogeneity (Burk and Hill 1994).

Selenoprotein P is synthesized and excreted by the liver and heart (Burk and Hill 1994, Hill et al. 1993). Expression of selenoprotein P has been demonstrated in liver, kidney, heart, lung, and testis by Northern analysis (Burk and Hill 1994). In human and rat cultured liver cells, selenium affects the expression of
selenoprotein P and cellular glutathione peroxidase differently (Hill et al. 1995). Recently it has been shown that several forms of selenoprotein P can be separated, based on SDS-PAGE migration as well as heparin sepharose affinity (Chittum et al. 1995). Gomez and Tappel (1989) reported that selenoprotein P has a receptor and that selenoprotein P-related proteins may be widespread in nature. Since that initial report, Wilson and Tappel (1993) suggested that numerous tissues possess receptors for selenoprotein P; however, Burk et al. (1991) did not support this finding with the exception of brain tissue.

The precise function of selenoprotein P is unknown; however, it has been hypothesized to have a functional role in transport and oxidant defense (Burk and Hill 1994, Gomez and Tappel 1989). Since selenium is covalently bound in the molecule and it has been found that many tissues express selenoprotein P, the likelihood of its role as a transport protein is questionable (Burk and Hill 1994). It may, however, serve to distribute selenium throughout the body (Burk and Hill 1994). The proposed function of oxidant defense has been supported by demonstrating a temporal association between selenoprotein P and antioxidant effect of selenium (Burk et al. 1991, Burk et al. 1995, Burk and Hill 1994). When Burk et al. (1995) examined the relationship of lipid peroxidation with diquat-induced liver necrosis in selenium-deficient rats, their results indicated that of the four selenoproteins studied (selenoprotein P, cellular glutathione peroxidase, extracellular glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidase),
selenoprotein P best correlated with protection against the aforementioned injury (Burk et al. 1995). Further research should elucidate its function(s).

Selenium deficiency can differentially affect the regulation of the selenoproteins (Burk and Hill 1994). A deficiency of this trace mineral causes a decrease in the plasma concentration of selenoprotein P to less than 10 percent of that in selenium-replete rats (Read et al. 1990). When the supply of selenium is limiting, selenoprotein P synthesis has priority over glutathione peroxidase synthesis (Burk and Hill 1994). Yang et al. (1989) fed rats varying degrees of selenium and found that as selenium was added to the diet, selenoprotein P concentration increased, followed by plasma and liver glutathione peroxidase activities. Since a selenium deficiency did not alter mRNA transcription rate, the regulation of selenoprotein P mRNA levels appears to be regulated through their degradation (Burk 1987, Burk and Hill 1994). Although the transport function of selenoprotein P has been debated (Burk and Hill 1994), during a selenium deficiency, plasma selenoprotein P may deliver selenium to the testes as a priority (Wilson and Tappel 1993).

Finally, selenoprotein P may be a valuable new biochemical marker of selenium status (Persson-Moschos et al. 1995). Persson-Moschos et al. (1995) described a radioimmunoassay using polyclonal antibodies to human selenoprotein P which may become particularly useful in clinical studies and assessment of individuals' selenium status.
Thyroid hormone deiodinases

Normal development, growth, and metabolism are all dependent on thyroid hormone, the sole source of which is the thyroid gland (Berry and Larson 1992). T3 is responsible for most, if not all, of the biological effects of thyroid hormone. When iodine is available in adequate quantities, the thyroid's main secretory product is T4 (Berry and Larson 1992). In general, the thyroid deiodinases function in the formation and regulation of active thyroid hormone (Larsen and Berry 1995). The deiodination of thyroxine (T4) to form tri-iodothyronine (T3) and the conversion of reverse (r) T3 to 3-3'-di-iodothyronine is catalyzed by iodothyronine deiodinase (Groff et al. 1995). While Types 1, 2, and 3 of this enzyme exist, only types 1 and 3 have been found to have the selenoamino acid selenocysteine in the active center (Larsen and Berry 1995).

Type I iodothyronine deiodinase

Recently, type I iodothyronine deiodinase, 27.8 kDa, was identified as a selenoenzyme (Behne et al. 1990). Behne et al. (1990) found that the deiodinase subunit contained one selenium atom per molecule and suggested that a highly reactive selenocysteine was the residue essential for the catalysis of 5'-deiodination. Berry et al. (1991) found that the mRNA for type I iodothyronine deiodinase, which is primarily found in liver and kidney, contained a UGA codon for selenocysteine. The selenoamino acid was necessary for maximal activity, providing the rationale why T4 to T3 conversion is impaired in selenium deficiency.
and identifying an essential role for selenium in thyroid hormone action (Berry et al. 1991). Unlike type I, type II iodothyronine deiodinase does not contain a selenocysteine or selenomethionine (Safran et al. 1991), suggesting that the type II enzyme is not a selenoprotein. Characterization of its mRNA will further support or refute whether or not this thyroid hormone deiodinase is a selenoprotein (Larsen and Berry 1995).

Selenium deficiency can affect the activity of type I iodothyronine deiodinase; in fact, in selenium deficient rats, plasma thyroxine levels increase secondary to the decrease in type 1 iodothyronine deiodinase activity (Beckett et al. 1987). T₃ levels also decrease during a selenium deficiency since conversion of T₄ to T₃ is impaired by the decreased deiodinase activity (Beckett et al. 1987, Berry et al. 1991).

Behne and Kyriakopoulos (1993) reported that the selenium requirement for normal concentrations of type I iodothyronine deiodinase is lower than that for glutathione peroxidase. Previous studies (Behne and Hofer-Bosse 1984, Behne et al. 1988) indicated that during periods of insufficient selenium intake specific tissues have priority over others as a regulatory mechanism. Brain, endocrine organs, and reproductive organs have priority over other organs and organ systems, and the selenium supply to other selenoproteins has priority over glutathione peroxidase (Behne and Kyriakopoulos 1993). In cell culture (LLC-PK1) experiments, it was observed by Oertel et al. (1993) that compared with glutathione
peroxidase, expression of type I iodothyronine deiodinase is observed at 10-fold-
lower concentrations of selenium in the growth medium. They concluded that
selenium deficiency may impede cell growth and prevent expression of the
selenoprotein, impairing thyroid hormone action in vivo (Oertel et al. 1993).
Selenium deficient animals, in fact, have been shown to be euthyroid (Burk 1983).

Type 3 iodothyronine deiodinase

Type 3 iodothyronine deiodinase has recently been shown to contain
selenocysteine (St. Germain et al. 1994). This enzyme is the major enzyme that
catalyzes inner-ring deiodination of T₄ or reverse T₃ (Larsen and Berry 1995).
While Type 1 can catalyze this reaction also, it prefers to catalyze the removal of
a phenolic or outer-ring iodine (Larsen and Berry 1995). Activity of this enzyme
is greatest in adult rat brain, skin, and placenta, and it is greatest in liver, muscle,
brain, and the central nervous system of fetal rats (Burrow et al. 1994). St.
Germain et al. (1994) recently cloned the cDNA for Xenopus laevis which revealed
the presence of an in-frame TGA codon encoding selenocysteine, as in type 1
iodothyronine deiodinase. However, in selenium-deficient animals, type 3 activity
is relatively unaffected (St. Germain et al. 1994). Further research is needed to
further clarify the role of selenium in type 3 iodothyronine deiodinase.

Sperm mitochondrial capsule selenoprotein

As early as 1964, it was suggested that the role of selenium in male
reproduction be investigated (McConnell and Burton 1981). In 1978, Calvin
identified and localized a selenium-containing polypeptide with a molecular weight of 17.0 kDa in rat spermatozoon within a keratinous fraction of the sperm (Calvin 1978). Calvin and Cooper later presented supporting information that showed the association of a cysteine-rich selenopolypeptide with the keratinous outer membrane of sperm mitochondria (Calvin and Cooper 1979, Combs and Combs 1986). Recently, the cDNA clones encoding the selenium-containing polypeptide of the keratinous mitochondrial capsule in mouse sperm were characterized (Kleene et al. 1990). The longest open reading frame encodes a polypeptide that is 143 amino acids in length and contains 21 percent cysteine (Kleene et al. 1990). This selenoprotein contains three selenocysteines in the first 35 amino acids of a total of 197 residues (Karimpour et al. 1992). The mouse protein resembles the size and amino acid composition of bull mitochondrial selenoprotein (Kleene et al. 1990). Pallini et al. (1979) found isolated bull sperm mitochondria to contain a 20.0 kDa cysteine-rich polypeptide. They also reported that this polypeptide appeared quite similar to the aforementioned selenoprotein identified in rat spermatozoon. Human semen has been demonstrated to contain several polypeptides ranging in molecular weight from 16.0-100.0 kDa (McConnell and Burton 1981). Basically, selenium is necessary for the flagella integrity of the sperm (Calvin 1978, McConnell and Burton 1981), is associated with a specific keratin with a fibrous fraction of the sperm tail (McConnell and Burton 1981), and is localized in the midpiece portion of the spermatozoan (Brown and Burk 1973).
During sexual maturation, the selenium content of male gonads has been reported to increase (Bedwal and Bahuguna 1994). Several studies have shown that selenium is required for normal spermatogenesis in the rat, mouse, boar, and bull (Combs and Combs 1986, Watanabe and Endo 1991). Pond et al. (1983) found that the accumulated selenium in ram and bull testes is incorporated into developing spermatozoa. Limited research has been done in this area using human subjects. It has been shown that serum selenium decreases during male sexual maturation (Marano et al. 1991). Marano et al. (1991) suggested an involvement of sexual hormones in regulating serum selenium levels; however, these results may reflect the onset of spermatogenesis during puberty with inadequate dietary intake of the nutrient, causing the decrease in selenium level. The selenium content of human spermatozoa has been shown to be less than that of dog and bull spermatozoa (McConnell and Burton 1981). McConnell and Burton (1981) observed a rat testicular selenoprotein (15.0 kDa) which traveled with the maturing spermatozoa to the epididymis. In their human experiments, they observed an abundant protein with a similar molecular weight, 13.0 kDa (McConnell and Burton 1981). Xu et al. (1983) determined the selenium concentration of blood and seminal plasma and related it to sperm density, motility, morphology, and semen volume. A positive correlation was observed between selenium concentration in seminal plasma and sperm density in normospermic men, but not among the oligospermic men, that is, those with a deficient amount of spermatozoa in their seminal fluid.
Selenium deficiency affects the synthesis of sperm mitochondrial capsule selenoprotein in a negative manner. This trace mineral deficiency may result in sterility due to reduced sperm motility (from midpiece breakage), infertility, impaired spermatogenesis, and/or aspermatogenesis in rats, mice, boars, and bulls (Combs and Combs 1986, Watanabe and Endo 1981). Other mammalian species may be affected in a similar manner.

**Selenoprotein W**

Selenoprotein W was recently purified from rat muscle (Vendeland et al. 1993) and its cDNA subsequently cloned (Beilstein et al. 1995, Yeh et al. 1995). Previous research had suggested that this protein was a selenoprotein (Beilstein et al. 1981). As with the other selenoproteins, the selenium in Selenoprotein W is present as selenocysteine (Beilstein et al. 1995, Vendeland et al. 1993). Its concentration has been found to be approximately 0.92 g atoms of selenium per g mol of protein (Vendeland et al. 1993).

After purification involving ammonium sulfate fractionation, Sephadex G-50 gel filtration, cation exchange chromatography on CM-Sephadex, and reverse phase high pressure liquid chromatography using a C-18 Vydac column, four proteins were separated (Vendeland et al. 1993). Their masses ranged from 9.6-9.9 kDa (Vendeland et al. 1993). The major amino acids in the protein were found to be glutamate, glycine, lysine, leucine, and valine (Vendeland et al. 1993). In addition, the cDNA for selenoprotein W has recently been cloned (Beilstein et al. 1993).
1995, Yeh et al. 1995). Beilstein et al. (1995) reported that the clones produced 670 bases of cDNA sequence including 50 bases of the 5'-untranslated region, 267 bases of coding sequence, and 370 bases of the 3' untranslated region (Beilstein et al. 1995). This work confirmed the known peptide sequence and identified the residues necessary to complete the peptide sequence (Beilstein et al. 1995). Similar to sperm mitochondrial coil selenoprotein (Karimpour et al. 1992), selenoprotein W has a proline near the selenocysteine residues (Yeh et al. 1995). A stem loop is also present in the sequence (Beilstein et al. 1995).

The function of Selenoprotein W is currently unknown; however, Vendeland et al. (1993) speculated that this selenoprotein may be an antioxidant, in view of the presence of selenium in its structure. Yeh et al. (1995) subsequent to the research by Vendeland et al. (1993) determined the compartmentation and tissue distribution of Selenoprotein W. This selenoprotein is primarily cytosolic; however, small amounts are associated with membranes (Yeh et al. 1995). Rats have been shown to have Selenoprotein W in muscle, brain, testis, spleen, heart, lung, and kidney; however, it has not been detected in liver (Vendeland et al. 1995, Yeh et al. 1995). Similarly, sheep distribute the selenoprotein in muscle, heart, spleen, lung, and kidney. They also have a very small quantity in liver (Gu et al. 1995-abstract, Yeh et al. 1995). Rabbits and cattle also have this polypeptide in muscle tissue (Yeh et al. 1995). Recent work with other species, including guinea
pigs, indicates that selenoprotein W is present in many tissues and suggests species differences on its tissue distribution (Yeh et al. 1995).

Tissue levels of selenoprotein W can be affected by selenium status (Yeh et al. 1995). Selenium deficiency reduced skeletal muscle mRNA to approximately 25 percent of levels of rats receiving supplemented diets (Vendeland et al. 1995). A graded response to dietary selenium was not observed among rats receiving at least 0.1 ppm selenium for 4-6 weeks (Vendeland et al. 1995). After nine weeks of selenium supplementation (3 ppm selenium), the selenoprotein W content of muscle became saturated (72 ng/mg protein) after its gradual increase over time (Gu et al. 1995). It is speculated that selenoprotein W may play a role in muscle metabolism (Yeh et al. 1995). Current research is inadequate to determine its exact function in metabolism.

SUMMARY

Selenoproteins are proteins that contain the selenocysteine form of selenium (Burk & Hill 1993). Selenium's incorporation into selenoproteins is one of its primary biological functions. Of the nine selenoproteins currently known (Burk and Hill 1994, Larsen and Berry 1995), cellular or classical glutathione peroxidase and plasma or extracellular glutathione peroxidase are the prime functional parameters used for the assessment of selenium status (Burk and Hill 1993, Yeh et al. 1995).

Among the selenoproteins that have been identified, there exists a wide variation in their physiological roles. The glutathione peroxidases rid the body of
peroxides that can damage cells and tissues by catalyzing their reduction to water. Each of the four glutathione peroxidase enzymes currently known are distinct and contribute uniquely to metabolism. Like the glutathione peroxidases, selenoprotein P appears to play a role in oxidant defense; however, the precise function of this selenoprotein is unknown (Burk and Hill 1994). In addition to selenoprotein P, selenoprotein W may play a role in oxidant defense (Vendeland et al. 1993). It may also be involved with muscle metabolism (Yeh et al. 1995); however, its precise function is also unknown. The thyroid deiodinases function in the formation and regulation of active thyroid hormone (Larsen and Berry 1995). Unlike type 2, types 1 and 3 iodothyronine deiodinase are selenoproteins (Larsen and Berry 1995). Sperm mitochondrial capsule selenoprotein is vital to the integrity of sperm flagella as part of a keratin in the spermatozoan midpiece portion (Brown and Burk 1973, Calvin 1978). As molecular biological and basic research continue, additional selenoproteins will undoubtedly be discovered.

**Selenium-Metabolism**

As previously discussed, selenium has two fates in metabolism: 1) incorporation into selenoproteins (Burk and Hill 1993) and 2) methylation and excretion (Bopp et al. 1982). This discussion will focus not only on the methylation and excretion of selenium but also its absorption and transport.
Selenium compounds are freely absorbed in the gastrointestinal tract, in fact, absorption does not appear to be limited by any physiological control mechanisms (Robinson et al. 1978, Stewart et al. 1978). Primary absorption occurs in the duodenum, with less absorption occurring in the jejunum and ileum. Virtually no absorption of selenium occurs in the stomach (Groff et al. 1995). The respiratory tract and skin can absorb selenium; however, quantitative data in humans has not been published (Diplock 1987).

The dietary form of selenium can influence the intestinal absorption of the mineral (Groff et al. 1995). Organic selenium compounds are usually the naturally-occurring forms of selenium found in foods. Selenomethionine, selenocystine, selenocysteine, and selenium-methyl selenomethionine are the usual compounds and are thought to represent selenium analogs of sulfur-containing amino acids which become incorporated into plant proteins (Groff et al. 1995). Sodium selenite is generally used to supplement animal feeds where levels of the mineral are low in natural feeds, making this form a potentially-important dietary form of selenium in addition to the aforementioned organic forms (Groff et al. 1995).

Various selenium compounds have been shown to absorb differently across the intestinal tract (Thomson and Robinson 1986, Vendeland et al. 1992). In the rat, selenomethionine absorption was significantly greater than selenate or selenite (Vendeland et al. 1992). In human subjects, McAdam et al. (1985) also found that selenomethionine was more effectively absorbed than selenite. The organic
Selenoamino acids have been estimated to be absorbed with an efficiency of 50-80%, while absorption from selenites has an efficiency of 44-70%. Selenomethionine is viewed to have superior absorption compared to selenocysteine, while selenates are thought to be superior to selenites (Thomson and Robinson 1986). In fact, the rate of selenate absorption, which occurs by carrier mediated transport, has been shown to be more rapid than that of selenite, which occurs through simple diffusion (Wolfram et al. 1985).

Several dietary compounds have been shown to augment the absorption of selenium, including ascorbic acid, vitamin A, vitamin E, and reduced glutathione in the intestinal lumen (Combs and Combs 1986). Unlike these factors, chelation and precipitation of selenium by heavy metals and phytates hinder selenium absorption (Burk and Hill 1993, Forbes and Erdman 1983).

In the blood, selenium binds to alpha and beta globulins (Groff et al. 1995). For example, lipoproteins have been shown to contain and transport selenium including the alpha-2 globulin, very low density lipoprotein (VLDL), and the beta globulin, low density lipoprotein (LDL) (Groff et al. 1995). In addition, selenoprotein P has been hypothesized to have a functional role in selenium transport (Burk and Hill 1994, Gomez and Tappel 1989). As previously discussed, selenoprotein P is synthesized and excreted by the liver and heart (Burk and Hill 1994, Hill et al. 1993). Expression of selenoprotein P has been demonstrated in liver, kidney, heart, lung, and testis by Northern analysis (Burk and Hill 1994).
It has been shown that selenoprotein P has a receptor and that selenoprotein P-related proteins may be widespread in nature (Gomez and Tappel 1989). Since their initial report, Wilson and Tappel (1993) suggested that numerous tissues possess receptors for selenoprotein P; however, Burk et al. (1991) did not support this finding with the exception of brain tissue. Since selenium is covalently bound in the molecule and it has been found that many tissues express selenoprotein P, the likelihood of its role as a transport protein is questionable (Burk and Hill 1994). It may, however, serve to distribute selenium throughout the body (Burk and Hill 1994).

Absorbed selenium becomes incorporated into a wide variety of selenoproteins (Groff et al. 1995), in fact, nearly all selenium in animal tissues is associated with protein (Burk and Hill 1993). As previously discussed, nine selenoproteins have been identified (Burk and Hill 1994, Larsen and Berry 1995) and include cellular or classical glutathione peroxidase (Rotruck et al. 1973), plasma or extracellular glutathione peroxidase (Takahashi et al. 1987), phospholipid hydroperoxide glutathione peroxidase (Ursini et al. 1985), gastrointestinal glutathione peroxidase (Chu et al. 1993), selenoprotein P (Burk and Hill 1994), type 1 iodothyronine deiodinase (Berry et al. 1991), type 3 iodothyronine deiodinase (St. Germain et al. 1994), sperm mitochondrial capsule selenoprotein (Calvin 1978), and selenoprotein W (Vendeland et al. 1993). Selenoamino acids not incorporated into proteins are catabolized to liberate the selenium for excretion.
This is accomplished by a lyase, for example, selenocysteine beta-lyase hydrolyzes selenocysteine into alanine and hydrogen selenide (Esaki et al. 1981, Groff et al. 1995). The selenide not utilized by the body is methylated to dimethyl selenide, dimethyl diselenide, or trimethyl selenonium ion and excreted (Burk 1991).

Urinary and fecal selenium excretion comprise the primary avenues of disposal for this trace mineral. Selenium is excreted from large pools that have a slow turnover with approximately 80% excreted via urine, while 20% is via feces (Veillon et al. 1990). Lower values have been reported, however. Urinary and fecal excretion has been reported to account for 50-67% and 40-50% of total selenium excreted, respectively (Groff et al. 1995). Selenium can also be lost by the skin and lungs with excessive intakes of selenium (Groff et al. 1995). Fan and Kizer (1990) documented this loss via the breath. The volatile compound, dimethylselenide, is eliminated upon exhalation and is distinguished by its garlicky odor (Groff et al. 1995).

Humans appear to regulate selenium excretion mechanisms over a wide variation in intakes to remain in selenium balance (Levander 1987). There does not appear to be a homeostatic control mechanism for absorption of selenium; however, humans appear to adjust their selenium excretion for maintenance of selenium status (Vendeland et al. 1992). Robinson et al. (1985) compared the diet of individuals from North America and New Zealand. It was reported that individuals from South Dakota excrete 120 μg of the 150 μg of selenium that pass
through their kidneys daily, while individuals from New Zealand only excrete 15 \( \mu g \) of the 50 \( \mu g \) of selenium that pass through their renal system (Robinson et al. 1985). In other words, it appears that selenium is excreted to a lower degree when dietary intake of the mineral is lower.

Finally, the current literature related to selenium metabolism indicates that the body's selenium can be classified into two biochemical pools: 1) the selenite-exchangeable metabolic pool, and 2) the selenomethionine-containing compounds (Janghorbani et al. 1990a). The selenite-exchangeable metabolic pool contains intermediate forms of selenium and end products like glutathione peroxidase, selenoprotein P, and dimethyl selenide (Janghorbani et al. 1990a). It appears that the selenomethionine-containing compounds in the second pool may act as storage, similar to ferritin or hemosiderin, and the selenite-exchangeable metabolic pool is responsible for the functional manifestations of selenium (Janghorbani et al. 1990a).

A recent study using a rat model explored the quantitative relationship between the size of the selenite-exchangeable metabolic pool and total body or liver selenium in rats of varying age and past selenium intake (Janghorbani et al. 1990b). This research highlighted the potential use of single time point stable isotope label administration for selenium status assessment, since it is minimally invasive and is potentially applicable to all human populations (Janghorbani et al. 1990b). While rat and human measurements will undoubtedly be different, the general properties should be the same (Janghorbani et al. 1990b). For example, compared to skeletal
muscle, liver exhibits a faster rate of selenium turnover, contributing a larger amount of selenium to the exchangeable metabolic pool (Janghorbani et al. 1989, Patterson et al. 1989).

In addition to the aforementioned rat study, Janghorbani et al. (1990a) recently carried out a series of human metabolic experiments designed to explore the possible utility of an in vivo stable-isotope dilution method for the assessment of body selenium status and reported that this may be a suitable method for selenium status assessment over a wide range of usual intakes (Janghorbani et al. 1990a). This method of assessment, which considers the concept of body pool size or of the exchangeable pool of selenium, has not been given extensive attention secondary to the lack of practical approaches for safe and noninvasive administration of nonradioactive (stable) isotope tracers (Janghorbani et al. 1990a). While this method is safe, its relationship to functional parameters of selenium status and its expense deserve consideration. Other assessment methods (glutathione peroxidase activities in blood components, selenium concentration of tissues or body fluids) are primarily useful in populations with low habitual intakes of selenium, rather than those under conditions of wide ranges of habitual selenium intakes (Janghorbani et al. 1990a). Glutathione peroxidase activities are often saturated below the usual intake of many populations, and selenium concentrations can be highly variable.
As previously summarized, Janghorbani et al. (1990a) summarized that based upon what is currently known about selenium metabolism, there are two biochemical pools of selenium and that the size of the selenite-exchangeable metabolic pool may be related to the adaptation to different habitual selenium intakes (Janghorbani et al. 1990a). Advantages of this approach include: 1) the size of the selenite-exchangeable metabolic pool can be measured accurately; 2) the size of the selenite-exchangeable metabolic pool does not appear to be dependent on physiologic variables that influence turnover of proteins, which is a limitation of using an enzyme marker as an index of trace element status; and, 3) the size of the selenite-exchangeable metabolic pool reflects the size of the pool present in the whole body, unlike plasma selenium concentrations which may not be related to intracellular selenium concentrations (Janghorbani et al. 1990a). Based upon our current understanding of selenium metabolism, this may be a suitable method of selenium assessment; however, research is still needed in pool size assessment using an in vivo isotope-dilution technique.

**Selenium-Requirements**

In 1989, the first recommended dietary allowance (RDA) was established for the trace mineral selenium (National Research Council 1989). The National Research Council (1980) had previously recommended a safe and adequate range of selenium intake for adults of 50-200 μg/day. The requirement for selenium in
the diets of humans was confirmed when an association between low selenium status and Keshan disease was documented (Keshan Disease Research Group 1979). Prevention and treatment of this cardiomyopathy, which is common to selenium poor areas of China, with selenite further suggested the essentiality of selenium (Chen et al. 1980). Keshan Disease primarily affects children <15 years of age and women of childbearing age.

The experimental induction of deficiency or toxicity in human subjects is the ideal method to determine nutritional requirements; however, this method is not ethical (Mertz 1995). Consequently, the factorial method, extrapolation from animal experiments, and balance studies are the three methods that can be employed in determining nutrient needs (Mertz 1995). In the past, balance studies have been used extensively in the calculation of trace mineral requirements (Mertz 1987). Several selenium balance studies have been conducted in humans (Bunker et al. 1988, Levander and Morris 1984, Levander et al. 1981, Robinson et al. 1973, Stewart et al. 1978).

However, since humans appear to regulate selenium excretion mechanisms over a wide variation in intakes to remain in balance, the balance technique was not used in determining the selenium requirements for humans (Levander 1987). The 1989 RDA was determined by examination of selenium intakes of Chinese individuals in areas with and without a deficiency of the trace mineral, that is, the Keshan province of China where Keshan disease was prevalent and neighboring
provinces without endemic proportions of the disease (Ge and Yang 1993, National Research Council 1989). In 1988, Yang et al. (1988), using dietary surveys in the aforementioned populations, reported that the minimum selenium requirements for adult Chinese men and women were 19 μg/day and 13 μg/day, respectively.

In addition to using dietary surveys for estimating selenium requirements of Chinese individuals, Yang et al. (1987) observed the plasma glutathione peroxidase activities of men with low selenium status receiving a daily dietary intake of approximately 10 μg Se plus selenomethionine supplementation prescribed in graded doses. After five months of treatment, plasma glutathione peroxidase activity plateaued similarly among the treatments groups receiving at least 30 μg of supplemental selenium per day (Yang et al. 1987). Thus, 40 μg of selenium was determined to be the adult physiological requirement of selenium for Chinese males (Yang et al. 1987).

To formulate the RDA for selenium, the estimated physiological requirement for Chinese males was adjusted for weight, since the subjects studied were approximately 60 kg and the reference North American adult weighs 79 kg and 63 kg for males and females, respectively (National Research Council 1989). In addition, selenium requirements appear to be related to body weight, specifically lean body mass (Levander and Morris 1984, National Research Council 1989). After weight calculations, adjustments were made for individual variation in selenium requirements by using a safety factor of 1.3, chosen arbitrarily by an
assumed coefficient of variation of 15% (National Research Council 1989). Thus, a recommended intake of 0.87 μg/kg/day for adults was calculated. Considering the weight of the reference North American, the RDA was rounded to 70 μg/day and 55 μg/day for males and females, respectively (National Research Council 1989). Subsequently, Alfthan et al. (1991) reported that a daily intake of 100 μg of selenium saturated the glutathione peroxidase activities of both plasma and erythrocytes. Requirements for the elderly have been assumed to be that of their younger-adult counterparts since research on the requirements for this group is lacking (National Research Council 1989).

Besides publishing RDAs for adults and the elderly, the National Research Council (1989) formulated recommendations for other stages of the life span, including pregnancy, lactation, infancy, childhood, and adolescence. Hormone levels have been suggested to affect circulating levels of selenium and glutathione peroxidase since, regardless of selenium intake, plasma selenium, plasma glutathione peroxidase, and erythrocyte glutathione peroxidase fell below control values during pregnancy (Smith and Picciano 1986). The RDAs for selenium during pregnancy is an additional 10 μg/day of the trace mineral (National Research Council 1989). This recommendation was based upon a study that examined selenium retention during pregnancy (Swanson et al. 1983). The subjects, consuming approximately 150 μg (140-163 μg) of selenium daily, were found to retain 10 μg/day and 23 μg/day of selenium during the second and fourth quarters.
of pregnancy, respectively (Swanson et al. 1983). In view of the aforementioned high levels of selenium intake (compared to the usual selenium intake of the subjects), the positive selenium balances observed in both pregnant and nonpregnant subjects in the study may not indicate true retention of the mineral (National Research Council 1989, Swanson et al. 1983). The reason for this is, regardless of reproductive status, a positive balance would indicate that the intake and/or the bioavailability of the element is greater than the habitual intake and that the pool size is increasing (Mertz 1987). Thus, assuming an 80% absorption rate reported by Levander (1983) and an average selenium accretion of 5.0-6.5 μg/day accretion of selenium, the aforementioned RDA for pregnancy was established. During lactation, an additional 20 μg/day of dietary selenium is recommended (National Research Council 1989). The committee recommended this increase after considering the average selenium concentration of human milk (15-20 μg/liter) in North American women (Mannan and Picciano 1987) and the typical secretion of milk (750 ml per day) (National Research Council 1989). As with pregnancy, an 80% absorption rate of selenium was assumed to occur during lactation which adjusted the predicted 13 μg/day loss to the recommended additional 20 μg/day of selenium (National Research Council 1989).

For younger individuals, selenium research is limited. Thus, for infants, children, and adolescents, the RDAs were extrapolated from the adult values based on weight and a subjective factor added to allow for growth (National Research
Recommended intakes for infant 0-6 months of age are 10 µg/day, while infants 6-12 months should consume 15 µg/day (National Research Council 1989). Children 1-3 years, 4-6 years, and 7-10 years are recommended to consume 20 µg/day, 20 µg/day, and 30 µg/day, respectively (National Research Council 1989). The National Research Council (1989) recommends an intake of 40 µg/day for adolescent males 11-14 years old and an intake of 50 µg/day for adolescent males 15-18 years old. For adolescent females, an intake of 45 µg/day for those 11-14 years old and an intake of 50 µg/day for those 15-18 years old has been recommended (National Research Council 1989). Further research in infants, children, and adolescents should refine these recommendations.

**Selenium-Deficiency**

Selenium was identified in 1957 as part of "factor 3" for the prevention of liver necrosis in rats (Schwarz and Foltz 1957). After this breakthrough, several experiments followed which led to the discoveries that selenium would alleviate diseases like exudative diathesis and pancreatic fibrosis in chicks, muscular dystrophy (white muscle disease) in farm animals (sheep, cattle, foals, chickens, pullets), hepatosis diaetetica in pigs, and reproductive disorders in livestock (Krehl 1970, Schwarz 1961, Thompson and Scott 1969, Underwood 1977). In animals, simultaneous deficiencies of both selenium and vitamin E lead to the aforementioned diseases and can be corrected or thwarted with supplementation of
either nutrient (National Research Council 1983). However, in rats fed a selenium-deficient diet for two generations, a pure selenium deficiency has been demonstrated in spite of sufficient vitamin E in the diet (McCoy and Weswig 1969).

In humans, an association was documented in 1979 between low selenium status and a cardiomyopathy, Keshan Disease, illustrating the essentiality of selenium (Keshan Research Group 1979). However, deficiencies of selenium are rare in the United States. In fact, the primary group of individuals that have developed a selenium deficiency have been those receiving total parenteral nutrition (TPN) for extended periods of time without selenium supplementation (Brown et al. 1986, Kien and Ganther 1983, Lockitch et al. 1990, van Rij et al. 1979). Until recently, TPN was not routinely supplemented with this trace mineral.

Postoperative patients who received TPN for 10-40 days were studied (van Rij et al. 1979). The male and female patients ranged in age from 28-72 years. Their suboptimal plasma selenium levels ranged from 9-31 ng/mL; in fact, one patient (9 ng/mL) presented with an inability to walk after complaining of thigh muscle pain. This was successfully treated with daily selenium supplementation of 100 μg as selenomethionine (van Rij et al. 1979). Kein and Ganther (1983) published a case report about a 7-year-old male child that had received TPN for approximately 1.5 years and subsequently developed intermittent leg muscle pain and tenderness. The child developed white nail beds after 2 years of TPN and,
upon evaluation, was found to have very low serum and urinary selenium concentrations and very low serum and whole blood glutathione peroxidase activities (Kein and Ganther 1983). After almost three years of TPN, intravenous selenium supplementation (42 μg/day elemental inorganic selenium) was initiated which markedly improved the serum enzyme activities and resolved the fingernail bed abnormalities.

A 33-year-old Caucasian female who complained of arm and leg weakness after being on home TPN for four years was found to have remarkably low selenium concentrations and glutathione peroxidase activities (Brown et al. 1986). After daily selenious acid supplementation (400 μg) for six weeks, her symptoms were alleviated with a concomitant increase in plasma selenium from 5 ng/mL to 117 ng/mL (Brown et al. 1986). In 1990, Lockitch et al. (1990) described a 17-year-old female who died after a cardiac arrest secondary to septic shock. The patient had been on TPN for 17 months prior to her death; however, supplemental selenium was added to the infusion only seven months before her death when severe biochemical selenium deficiency was diagnosed (Lockitch et al. 1990). At autopsy, the diagnosis was cardiomyopathy due to prolonged selenium deficiency (Lockitch et al. 1990). Lockitch et al. (1990) noted that in long-standing selenium deficiency, sepsis may contribute the final insult to a damaged myocardium, triggering symptomatic cardiac failure and sudden death.

The most commonly found selenium deficiency, Keshan Disease, was
identified in 1935 in China (Keshan County, Heilongjiang Province) and has only been described in an area that runs from northeast to southwest China (Ge and Yang 1993). As previously discussed, the essentiality of selenium was illustrated when an association was documented in 1979 between low selenium status and this cardiomyopathy that primarily affects children less than 15-years-old and women of childbearing age (Chen et al. 1980, Keshan Research Group 1979). Keshan disease is typically presented by dilation of the heart and multifocal myocardial necrosis with fibrous replacement secondary to oxidant injury (Ge and Yang 1993). Chen et al. (1980) described four phases of Keshan disease, which is related to the selenium status of those affected, including acute, chronic, subacute, and latent. Acute Keshan disease presents with cardiogenic shock, pulmonary edema, and severe arrhythmia, while the chronic disease presents with severe enlargement of the heart and congestive heart failure. The subacute disease primarily occurs in children and presents with facial edema and gallop rhythm, and latent Keshan disease presents with a mild heart enlargement and normal heart function (Chen et al. 1980).

While factors other than selenium are probably related to Keshan disease, selenium supplementation has been successfully used to treat Keshan disease (Chen et al. 1980, Ge and Yang 1993, Keshan Research Group 1979). Chinese individuals living in one area (Mianing county, Sichuan Province) of the aforementioned selenium-poor belt were studied and found to have low selenium
concentrations in their blood, urine, and hair. Children supplemented with selenium had a significantly lower incidence of Keshan disease than those in the control (unsupplemented) group; in fact, children living in this county have since been supplemented with selenium (sodium selenite) on a weekly basis (Chen et al. 1980).

Keshan disease incidence appears to vary with seasons of the year. The occurrence of the disease peaks in winter or summer for northeastern China or southern China, respectively (Ge and Yang 1993). While the pattern of this phenomenon is currently unknown, changes in climate, selenium intakes, vitamin E status, or biological agents may explain the variations seen (Chen et al. 1980, Ge and Yang 1993).

Finally, Coxsackieviruses have been implicated as possible co-factors in the etiology of the selenium-responsive cardiomyopathy, Keshan disease (Beck et al. 1994a). Recent research has demonstrated that selenium deficiency in the mouse allows Coxackievirus B3 to cause significant heart damage, while selenium-adequate mice do not exhibit any disease pathology (Beck et al. 1994a). Coxsackievirus is usually benign, that is, amyocarditic (Beck et al. 1995). Recovered Coxsackievirus from the hearts of selenium-deficient mice with significant heart damage were found to have mutated to a virulent phenotype, as demonstrated by inoculation into selenium-adequate mice with resultant heart damage (Beck et al. 1994a). However, the myocarditic lesions occurred more quickly and were more severe in selenium-
deficient mice, compared to the selenium adequate mice (Beck et al. 1994b). These reports suggested that amyocarditic Coxsackievirus had mutated to a virulent phenotype, and Beck et al. (1995) subsequently reported that sequence analysis revealed six nucleotide changes between the virulent virus recovered from the selenium-deficient host and the avirulent input virus. It appears that a selenium deficiency drives changes in a viral genome, permitting an avirulent virus to acquire virulence due to genetic mutation (Beck et al. 1995).

Unlike this cardiac disorder (Keshan disease), Keshan-Beck disease, is a deficiency disease of selenium that affects the bones and is characterized by chondronecrosis with severe pain. Shortened digits/extremities and enlarged joints of the fingers, toes, and knees are common (Fan and Kizer 1990). Like Keshan disease, Keshan-Beck disease is common among children; however, in addition to occurring in China, this selenium deficiency disease also occurs in North Korea and Eastern Siberia (Fan and Kizer 1990).

**Selenium-Toxicity**

Selenium toxicity was first recorded several hundred years ago. As previously discussed, in the thirteenth century, Marco Polo observed selenium toxicity and that there were seleniferous plants in the mountainous region of western China that, if eaten, would cause the hoofs of the animals to drop off (Krehl 1970). Similar observations were recorded in the late nineteenth and early
twentieth centuries by an army surgeon, Dr. T. C. Madison, in the Nebraska territory and by General Custer in Nebraska, the Dakotas, and other western territories (Krehl 1970). Since then, the most widespread occurrence of selenium toxicity occurred in China between 1961 and 1964; however, selenium toxicity is not unknown to the United States (Fan and Kizer 1990, Yang et al. 1983).

As Custer and Madison observed, there are regions of the United States that have high concentrations of selenium in the soil. In spite of this natural phenomenon, ingestion of hydrogen selenide, selenium oxychloride, selenium dioxide, or selenium hexaflouride from industrial accidents and overdoses with selenium supplementation have been the primary origins of toxicity cases in the United States (Fan and Kizer 1990). An improperly manufactured dietary selenium supplement containing 27.3 mg selenium/tablet is the hallmark case of intoxication in the United States (Helzlsouer et al. 1985). Thirteen individuals were affected by this manufacturing problem (Helzlsouer et al. 1985). Anorexia, abdominal pain, diarrhea, fatigue, irritability, depression, emaciation, pulmonary edema, hemorrhage, liver and kidney necrosis, garlic/sour milk breath odor, neurologic deterioration, blindness, ataxia, respiratory distress, dental caries, nail and hair changes, and loss of hair and nails are toxicity symptoms (Fan and Kizer 1990, Helzlsouer et al. 1985, Jensen et al. 1984).

The aforementioned Chinese intoxication epidemic occurred in Enshi County in the early 1960s (1961-1964) (Yang et al. 1983). Large quantities of selenium
were ingested by this population when a drought damaged an entire rice crop, which forced the residents to eat corn and vegetables grown on Enshi Country soil that contains high concentrations of selenium (Yang et al. 1983). Yang et al. (1983) reported that residents presented with classic symptoms including changes in skin (red, swollen, and blistered), hair and nails (brittle, discolored, and eventual loss), and nervous system function. The most severe cases of intoxication involved changes in nervous system function and included peripheral anesthesia, convulsions, paralysis, and motor disturbance (Yang et al. 1983). While the mechanism of selenium toxicity has not been defined, there may be an adaptive mechanism for individuals exposed chronically to high levels of selenium. For example, individuals living in Enshi County with adequate concentrations of selenium have blood levels of 0.095 μg/mL, while those living in the aforementioned area had levels of 3.2 μg/mL during the epidemic (Yang et al. 1983).

**Selenium-Assessment of Status**

The total selenium content of the human body varies (Gibson 1990). Adult values for total selenium content in the United States and New Zealand are 15 mg and 3-6 mg, respectively (Schroeder 1970, Steward 1978). Estimation of dietary selenium intakes, measurement of selenium levels in blood, tissues, or excreta, and determination of glutathione peroxidase activity in various blood components are the common techniques used for assessing selenium status (National Academy of
Cellular or classical glutathione peroxidase and plasma or extracellular glutathione peroxidase are the prime functional parameters used for the assessment of selenium status (Burk and Hill 1993, Yeh et al. 1995).

Since the selenium content of foods can vary based upon the selenium content of soil in which the food was grown, dietary selenium intakes are difficult to measure (National Academy of Sciences 1989). Thus, use of dietary recalls and records in conjunction with food composition tables may not reflect actual selenium content of the diet; however, they can provide intake patterns of the individual and highlight foods that usually are good sources of selenium.

Measurement of selenium levels in tissues or excreta is also used for selenium assessment. While the largest pool of selenium is contained in liver for humans, assessment of this tissue is not practical. Other specimens used for selenium assessment methods include serum, plasma, whole blood, erythrocyte, platelet, urinary, hair, and toenail selenium measurements (Gibson 1990). The most common measures of selenium status are plasma/serum and erythrocyte selenium which approximate short- and long-term selenium status, respectively (Levander et al. 1985). Use of platelet selenium concentrations in human studies is limited, since a large amount of blood is necessary to obtain an adequate amount of platelets for analysis. (Gibson 1990). Plasma/serum selenium is influenced by short-term changes in dietary intakes, thus approximating acute selenium status (Levander et al. 1981). Daily urinary excretion, the primary excretory route for
selenium, is related to plasma selenium and correlated with recent dietary intake, unless intake is very low (Gibson 1990, Robinson et al. 1978). Urinary excretion is not, however, commonly used for assessment of selenium status.

Selenoprotein P may be a valuable new biochemical marker of selenium status (Persson-Moschos et al. 1995). As previously discussed, selenoprotein P is the major form of selenium in rat serum (Read et al. 1990). In addition to being purified from rats (Burk and Hill 1994, Read et al. 1990), it has also been purified from human plasma (Akesson et al. 1994). Human plasma contains approximately one tenth of the quantity of selenoprotein P found in rat plasma (Burk and Hill 1994). Persson-Moschos et al. (1995) described a radioimmunoassay using polyclonal antibodies to human selenoprotein P which may become particularly useful in clinical studies and assessment of individuals' selenium status. Hill et al. (1996) reported that the concentration selenoprotein P was an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects. In fact, it appears to be as sensitive as other indices in common use (Hill et al. 1996).

While hair selenium can be an index of chronic selenium status, the use of antidandruff shampoos containing selenium can influence this measurement (Gibson 1990). Since selenium is incorporated into toenails as they grow, they may be an index of very long-term selenium status, reflecting selenium intake from approximately 6-12 months prior to sample collection (Longnecker et al. 1993). Like hair, toenail selenium may be influenced by personal hygiene products and
invalidate its use for selenium status measurement. However, a recent review did report that the toenail selenium assay may be a valid selenium assessment tool (Nutrition Reviews 1991). Longnecker et al. (1991) investigated whether high dietary selenium intake was associated with adverse effects and reported that dietary selenium and the concentration of selenium in whole blood, serum, urine, and nails were all correlated. Because of the highly variable concentration of selenium in different samples of the same food, toenail and blood selenium concentrations may be more accurate in assessing dietary intake than dietary data are (Longnecker et al. 1993). Longnecker et al. (1993) reported that toenail selenium concentrations may be used in lieu of blood for measurement of long-term selenium intake since it appears to provide a time-integrated measure of dietary selenium intake. Further research in this area is necessary.

Of the nine selenoproteins currently known (Burk and Hill 1994, Larsen and Berry 1995), cellular or classical glutathione peroxidase and plasma or extracellular glutathione peroxidase are the prime functional parameters used for the assessment of selenium status (Burk and Hill 1993, Yeh et al. 1995). Both cellular and extracellular glutathione peroxidase activity diminish during a selenium deficiency. In addition to glutathione peroxidase, type I iodothyronine deiodinase has been considered for selenium status measurement. Behne and Kyriakopoulos (1993) reported that the selenium requirement necessary to maintain normal concentrations of type I iodothyronine deiodinase is lower than that for glutathione peroxidase.
Previous studies (Behne and Hofer-Bosse 1984, Behne et al. 1988) indicated that during periods of insufficient selenium intake specific tissues have priority over others as a regulatory mechanism (Behne and Kyriakopoulos 1993). In cell culture (LLC-PK1) experiments, it was observed by Oertel et al. (1993) that, compared with glutathione peroxidase, expression of type I iodothyronine deiodinase is observed at 10-fold-lower concentrations of selenium in the growth medium. Since glutathione peroxidase levels decrease prior to type I iodothyronine deiodinase levels, glutathione peroxidase is the functional parameter of choice for assessment of selenium status.

Finally, as previously discussed, pool size assessment literature is limited. However, an in vivo isotope-dilution technique for the measurement of pool size may be a suitable method for selenium status assessment over a wide range of usual intakes (Janghorbani et al. 1990a). Further research should clarify the usefulness of this assessment method.

**Selenium—Gender and Life Cycle Effects**

Both human and animal studies have reported differences in selenium status between genders. The strongest evidence for gender-related differences have been observed in several animal studies. Liver glutathione peroxidase activities have been shown to be up to 80% higher in female rats compared to males (Capel and Smallwood 1983, Igarashi et al. 1984, Pinto and Bartley 1969). These differences
may be related to liver sex hormone metabolism. Liver glutathione peroxidase activity increases in female rats after sexual maturity (Pinto and Bartley 1969). The effect of sexual maturation on selenium status in humans has not been studied extensively. Marano et al. (1991) compared the degree of sexual maturation to serum selenium in a population of Italian children. Considerable differences were found in the two sexes at the end of maturation. Males showed a significant decrease in serum selenium; however, serum selenium remained relatively constant in females.

As previously discussed, Calvin (1978) identified and localized a selenium-containing polypeptide with a molecular weight of 17.0 kDa in rat spermatozoon within a keratinous fraction of the sperm. This structural selenoprotein, sperm mitochondrial capsule, is vital to the integrity of sperm flagella as part of a keratin in the spermatozoan midpiece portion (Brown and Burk 1973, Calvin 1978). During sexual maturation, the selenium content of male gonads has been reported to increase (Bedwal and Bahuguna 1994). In fact, several studies have shown that selenium is required for normal spermatogenesis in the rat, mouse, boar, and bull (Combs and Combs 1986, Watanabe and Endo 1991). Pond et al. (1983) found that the accumulated selenium in ram and bull testes is incorporated into developing spermatozoa. Wallace et al. (1987) summarized that fragile and malformed mitochondrial capsules lead to reduced stability of the sperm tail in selenium-deficient animals. It appears that hormones responsible for spermatogenesis
regulate testis selenium levels to ensure the spermatozoa of an adequate selenium supply, and that this supply has priority over that supplied to other tissues (Behne et al. 1987).

Results from our lab indicate that healthy adolescents and adolescents with phenylketonuria (PKU) had lower plasma selenium levels than healthy pre- and grade-school children (Smith et al. 1994). These results suggest that selenium intakes may not be adequate to meet the needs of rapidly growing adolescents (Smith et al. 1994).

Previous reports suggested that hormone levels influenced circulating levels of selenium and glutathione peroxidase (Smith and Picciano 1986). Smith and Picciano (1986) found that regardless of selenium intake, plasma selenium, plasma glutathione peroxidase, and RBC glutathione peroxidase fell below control values during pregnancy. It also has been reported that plasma selenium and glutathione peroxidase activities parallel fluctuations in estradiol concentrations during the rat estrus cycle (Cha et al. 1991). A recent human study also described a fluctuation in selenium status, as measured by plasma selenium and glutathione peroxidase, with the fluctuation of hormones, including estrogen, during the menstrual cycle (McAdam et al. 1994). In a recent cross-sectional study, three generations were studied to assess the effect of life cycle differences on selenium status (Chang et al. 1994). Despite no difference in selenium intakes among the groups, post-menopausal women were shown to have lower selenium status than their peri-
menopausal daughters but similar status compared to their younger granddaughters. Estrogen status may be responsible for these differences (Chang et al. 1994).

**Balance Studies-General Information**

Nutrient balance studies measure the intake and output of a specific nutrient. They have been used in formulating nutrient requirements and for the assessment of nutrients available for absorption (Mertz 1987). In the past, balance studies have been used extensively in the calculation of trace mineral requirements (Mertz 1987). While the experimental induction of deficiency or toxicity in human subjects is the ideal method to determine nutritional requirements, this is not ethical (Mertz 1995). Mertz outlined in his recent review that the factorial method, extrapolation from animal experiments, and balance studies are the three methods that can be employed in determining nutrient needs (Mertz 1995). However, "a balance study does not determine 'the' requirement for a mineral element, but the intake required to maintain the existing pool size" (Mertz 1987). Thus, a zero balance demonstrates that the intake during the study is equal to the habitual intake and bioavailability of the nutrient and is the amount necessary to maintain the existing pool size (Mertz 1987). A positive balance would indicate that the intake and/or the bioavailability of the element is greater than the habitual intake and that the pool size is increasing, and a negative balance would indicate the opposite (Mertz 1987). The results of balance studies can be used as a basis of dietary recommendations if the
status of the nutrient being studied has been assessed by an independent method (Mertz 1987). While the dietary recommendations can not be applied to populations with differing nutritional status, the amount of a specific nutrient can be determined for a relatively homogeneous population (Mertz 1995).

Like other experimental methods, balance studies have limitations. Balance studies are expensive to conduct and time consuming (Gibson 1990). Without precise measurements of food, stool, urine, and other excreta, they also can be grossly inaccurate (Gibson 1990, Mertz 1987). However, with appropriate nutritional evaluation of a homogenous, healthy population, an estimate of the habitual dietary intake using the balance method can be used as a basis of dietary recommendations (Mertz 1987 and 1995).

**Balance Studies-Selenium**

Several selenium balance studies done on adults have been published (Bunker et al. 1988, Levander and Morris 1984, Levander et al. 1981, Robinson et al. 1973, Stewart et al. 1978); however, balance studies in adolescents have not been reported. In 1973, Robinson et al. (1973) reported the trace mineral balance of four New Zealand women (19-21 years) who participated in a balance study of the metabolic effects of meal frequency in 1966-67. Brilliant blue and/or chromic oxide were used as fecal markers (Robinson et al. 1973). The selenium intake ranged from 18-26 μg/day and was less than the intake of selenium among United
States residents (Robinson et al. 1973). Robinson et al. (1973) reported that the selenium balance was possibly in equilibrium; however, this study was limited by its ability to accurately predict the selenium intakes and outputs primarily due to the methods utilized and susceptibility of contamination (Robinson et al. 1973). This study utilized diet, urine, and fecal samples from a previous balance study conducted in 1966-67, increasing the likelihood of sample contamination. Accuracy of the selenium measurements was complicated by the numerous steps in the diaminonaphthalene fluorometric assay. In addition, selenium could not be determined in the samples that utilized chromic oxide as a stool marker (Robinson et al. 1973).

Stewart et al. (1978) documented the pattern of selenium intake and excretion of four apparently healthy, New Zealand adult females (22-34 years) consuming an ad libitum diet, assessed their utilization of selenium in food, and estimated whole-body selenium in the same population. Total selenium intake and selenium output (urinary and fecal) were measured to calculate selenium balance (Stewart et al. 1978). Brilliant blue was used as a fecal marker (Stewart et al. 1978). Absorption, excretion, and turnover of \(^{79}\text{Selenium}\)selenomethionine or \(^{79}\text{Selenium}\)selenite was used to calculate whole-body selenium. The true intestinal absorption of selenium (mean 79%), calculated using absorption of the tracer \(^{79}\text{Selenium}\) and assuming that the tracer is partitioned for excretion between urine and feces in the same proportions as is absorbed food selenium, was observed to
be higher than the apparent selenium absorption (mean 55%) ([(food selenium-fecal selenium)/food selenium] \times 100\%) (Stewart et al. 1978). Whole-body selenium was estimated to be 6.1 mg, about one-third that of United States residents (Stewart et al. 1978). Plasma selenium measurements, usually one-third of those found in Americans, averaged 58 $\mu$g/L with a range of 48-76 $\mu$g/L (Stewart et al. 1978). Stewart et al. (1978) reported that these apparently, healthy New Zealand adult females required about 24 $\mu$g of selenium to maintain selenium balance and were estimated to need a minimum of 20 $\mu$g of selenium/day for health maintenance.

Levander et al. (1981) conducted a selenium balance study to determine if depletion/repletion studies were a feasible method of investigating selenium balance in humans and thereby estimating human selenium requirements. Another goal of the study was to measure the bioavailability of selenium in tuna fish and high-selenium wheat (Levander et al. 1981). This comparison, however, was hindered because of technical difficulties. Six, apparently healthy, non-smoking 24-33-year-old North American men participated in the 70-day total confinement metabolic study (Levander et al. 1981). During the 45-day depletion period, a formula diet containing 33-36 $\mu$g selenium/day was fed to the subjects (Levander et al. 1981). The repletion period (25 days) diet consisted of the depletion period diet plus 200 $\mu$g selenium/day as high-selenium wheat or tuna (Levander et al. 1981). Polyethylene glycol, 2.0 g/day, was used as a continuous fecal marker (Levander et al. 1981). Selenium balance was -21 $\mu$g/day after 12 days of adaptation to the
depletion diet. Selenium balance was +65 µg/day during the first 12 days of repletion and +25 µg/day thereafter (Levander et al. 1981). From this research, Levander et al. (1981) estimated North American men to need a dietary selenium intake of approximately 70 µg/day to replace losses and maintain selenium body stores.

Levander and Morris (1984) examined selenium intake and balance in free-living North American adults on self-selected diets over a 12-month period. Their investigation was part of a dietary intake and nutritional status investigation of free-living individuals over extended periods of time (Kim et al. 1983, Mertz and Kelsay 1983). At four times during the year, corresponding to the four seasons, dietary selenium intake and balance were measured in 27 free-living adults (12 males, 15 females) consuming self-selected diets (Levander and Morris 1984). The apparently healthy, nonvegetarian population ranged in age from 19-50 years (Levander and Morris 1984). Brilliant blue dye was used as a fecal marker (Levander and Morris 1984). They found that adult males and females needed 80 µg and 57 µg of selenium/day, respectively, to maintain selenium balance. About 1 µg of dietary selenium per kg body weight per day was needed by this population to stay in balance and was a function of lean body mass and historical selenium intake (Levander and Morris 1984). Their results indicated that substantially higher dietary selenium intakes were necessary to maintain balance in North American
adults compared to that needed for adult female New Zealanders (Levander and Morris 1984, Stewart et al. 1978).

Bunker et al. (1988) carried out balance studies in 24 apparently healthy elderly people (69-85 years; 11 males and 13 females) and 20 housebound elderly people with chronic disease (69-85 years; 7 males and 13 females) eating self-selected diets in their own homes. The five-day metabolic studies involved collection of duplicate diets, urine, and feces into plastic containers to avoid contamination; however, use of a fecal marker was not documented (Bunker et al. 1988). The healthy subjects had slightly lower whole-blood and erythrocyte selenium values than those published for younger adults, while their whole blood, erythrocyte, and plasma selenium concentrations were significantly higher than their housebound counterparts. No difference in whole-blood glutathione peroxidase activity was observed between the two groups (Bunker et al. 1988). The healthy subjects were found to be in positive selenium balance, while the housebound subjects were found to be in equilibrium (Bunker et al. 1988).

Female Development During Adolescence

Puberty is derived from a Latin word which means "to grow hairy" (Sigelman and Shaffer 1995). It can be defined as "the period or age at which a person is capable of sexual reproduction of offspring" (Random House 1975). Puberty has also been defined as "a rapid change to physical maturation involving
hormonal and bodily changes that occur primarily during early adolescence" (Santrock 1993). Plant (1994) highlights that puberty is a period of transition and development. This developmental period, which involves rapid growth, is marked by a cascade of events that includes ovarian cyclicity (Plant 1994).

Overall, there are three growth periods for humans: 1) early growth, before the age of two, which has a relatively fast velocity; 2) a relatively constant growth rate during the preschool and primary grades; and finally, 3) the pubertal growth spurt, which starts at approximately age 10 in females (Dunger and Preece 1987). During the 5-7 years of puberty, a child gains about 20% of adult height and 50% of weight, much of which occurs during an 18-24 month period of rapid growth (Mahan and Escott-Stump 1996). Marshall and Tanner (1970) highlighted the sequence of events for sexual development. While individuals vary, female sexual maturation can be summarized as follows: 1) height spurt, 9.5-14.5 years; 2) menarche (first menstruation), 10-16.5 years; 3) breast development, 8-18 years; and, pubic hair development, 11-14 years (Marshall and Tanner 1970).

The aforementioned sexual development is marked by a cascade of events that includes ovarian cyclicity (Plant 1994). The size of the ovary increases in a rectilinear (bounded by straight lines) fashion from infancy to adulthood, and the prepubertal increase in this organ is secondary to age-related increases in the number and size of antral follicles and in the quantity of medullary stroma (Plant 1994). However, the catalyst for ovarian steroidogenesis during puberty remains
elusive (Bruot et al. 1986). The first clinical signs of puberty (labial hair and initiation of breast enlargement) appear between the ages of 8-10 years when serum estradiol concentrations (collected in the morning) begin to increase to values seen among adult females during the early follicular phase of the menstrual cycle (Bidlingmaier et al. 1973, Jenner et al. 1972, Winter and Faiman 1973).

Unlike the male system, the female system functions periodically and is known as the ovarian cycle (Litwack 1993). While the cycling process begins at puberty, regular cycles with ovulation do not become established until several years following the first menstrual cycle; in fact, ovulation usually occurs for the first time six months after menarche (Plant 1994). However, follicular growth and steroidogenesis (without ovulation) progress with puberty and are evidenced by secondary sexual characteristics and menarche (Plant 1994).

The 28-day female cycle begins when gonadotropin releasing hormone is released (day 1) in a rhythmic fashion through the coordination of the central nervous system and the hypothalamus (Litwack 1993). In turn, the gonadotropes, luteinizing hormone and follicle stimulating hormone are released, affecting the ovarian follicle, ovulation (day 14) and the corpus luteum (Litwack 1993). Estradiol (estrogen) and progesterone participate in the process by uterine endometrium receptors which causes thickening and vascularization of the uterine wall in preparation for implantation of a fertilized egg (Litwack 1993). With the demise of the corpus luteum, estradiol and progesterone levels markedly decrease.
which prohibits maintenance of the thickened endometrial wall. Menstruation occurs and is followed by another cycle with a new developing follicle (Litwack 1993).

During puberty, the aforementioned rapid growth and development are accompanied by changes in nutritional requirements. This is not surprising, in view of the rapid rate of growth and virtual doubling of body mass (Dunger and Preece 1987). While the exact requirement for most nutrients during adolescence is unclear, normal growth and development and good health is the goal (Dunger and Preece 1987). Nutrient recommendations are often interpolated from studies using adults or children (Mahan and Escott-Stump 1996b).

Adolescents often show wide variations in energy expenditure, and current recommendations represent average requirements (Dunger and Preece 1987). Growth rate and activity level must be considered (Mahan and Escott-Stump 1996b). Protein requirements for adolescents should be based upon sex, age, nutritional status, and protein quality and are usually 15-20% percent of energy requirements (Dunger and Preece 1987, Mahan and Escott-Stump 1996b). While dietary proteins provide essential amino acids for growth, they will only be made available for growth if total energy needs are first met (Dunger and Preece 1987). When possible, protein requirements should be related to changes in lean body mass (Dunger and Preece 1987).
Unlike the macronutrients, less research has been published related to adolescent vitamin and mineral needs. While the roles of many vitamins and minerals in growth and development are recognized, research is limited. In fact, recommendations are generally interpolated from those for children or adults, including selenium recommendations.
CHAPTER 3

MATERIALS AND METHODS

Design: A balance study was conducted to assess the selenium balance and hormonal status of 26 healthy Caucasian females. The study population was recruited as part of a 4-year calcium study which included a balance study. The study was approved by the Human Subjects Committee of The Ohio State University, Columbus, Ohio.

Subjects: Healthy Caucasian females (n=26) in pubertal stage 2 (approximate age 9-11 years) were enrolled into the balance study. Subjects were recruited from local school districts. Basic data on subject characteristics, a food record, anthropometry, and pubertal stage were obtained. Subjects satisfying the inclusion criteria (pubertal stage, appropriate calcium intake, and potential for compliance) were randomly assigned into a placebo or treatment group for the longitudinal calcium study (n=300). Thirteen of the subjects in the balance portion of the study were in the placebo group, and thirteen were in the calcium supplement group.
**Selenium balance study:** The subjects in the balance study had similar body composition measurements at the onset of the study. To preserve subject number, individuals who terminated their participation in the study were replaced with another participant from the longitudinal calcium study. The replacement subject had an identical calcium treatment regimen and was selected for similarity of body composition.

The balance studies were performed in the metabolic ward of the Clinical Research Center at The Ohio State University Medical Center. Selenium balance was evaluated at 24, 36, and 48 months of the study during 1993, 1994, and 1995, respectively. Individual balances were performed over a 2-week period. A constant daily diet, appropriate for age, sex, and body size containing approximately 100 μg selenium/day, was consumed by each subject throughout the balance study. Aliquots of duplicate stored food samples were homogenized and stored for future analysis. Subjects were equilibrated to the diet during the first week. Samples were collected during the second week. Samples included:

1. **Urine.** 24-hour urine collection, collected in preservative-free plastic containers.

2. **Feces.** An adapted bed commode was used. Polyethylene glycol (PEG-Carbowax 3350, Fisher Scientific), 1.5 g/day, was used as a continuous fecal marker.
3. Unconsumed Food. This food was stored, analyzed for selenium content, and subtracted from the daily intake of selenium.

4. Blood. Blood was drawn on days 7 and 14 into polypropylene syringes fitted with stainless steel needles and dispensed into non-heparinized vacutainers appropriate for trace element analyses. If blood was not available during the balance period, blood from the longitudinal visit closest to the balance study was used for a baseline value.

Selenium content of food, feces, and urine was determined to calculate balance. Selenium status was assessed through measurements of serum and erythrocyte selenium and glutathione peroxidase.

Measurement of food, fecal, urinary, serum, and erythrocyte selenium was performed using gas chromatography with an electron capture detector (McCarthy et al. 1981, Smith et al. 1982). Samples and selenium standards (0.5 ppm selenium selenite solution and bovine liver) were measured into glass weighing vials. To digest the organic and non-mineral portions of the samples, 1.5 milliliters of nitric acid containing 30 grams of magnesium nitrate hexahydrate per 100 milliliters was added to each sample and gradually heated to 500°C.

Digestion was followed by complexing and extraction of the selenium. Trace mineral analysis-grade 36% hydrochloric acid (1.5 milliliters) was added to the vials of cooled ash and subsequently heated to 90°C until the ash was dissolved. To the cooled vials 2.5 milliliters of 1% hydroxylamine sulfate, 1% EDTA, 15%
urea solution was added. A 0.5% weight for volume complexing solution of the chloride salt of 4-nitro-o-phenylene-diamine dissolved in 1 N. hydrochloric acid solution (100 microliters) was added, followed by heating to 45°C for 30 minutes. After cooling, one milliliter of toluene was added for selenium extraction. After mixing, a portion of the organic phase containing the o-diamine derivative 5-nitropiazselenol was pipetted into a screw cap vial with a Teflon® cap liner for subsequent gas chromatographic analysis.

Samples were analyzed for their selenium content using a gas chromatograph (Varian Model 3300 with electron capture detector, Sugarland, Texas) with a Durabond Megabor® DB 225 column (J & W Scientific, Folsom, California). The gas chromatograph utilized nitrogen as its carrier gas (30 milliliters/minute) and operated at the following temperatures: column, 190°C; injector, 220°C; and detector, 300°C. The data were integrated using the Star Integrator Workstation®. See Appendix A for a flow sheet summary of the complete laboratory procedure.

Glutathione peroxidase activity of serum and erythrocytes was determined using hydrogen peroxide by the method of Paglia and Valentine (Paglia and Valentine 1967). Into optically-matched quartz cuvettes, 500 microliters of diluted sample or KH buffer (blank), 200 microliters of glutathione reductase solution (1000 units/milliliter), 50 microliters of 40 mM glutathione, and 210 microliters of distilled, deionized water were pipetted. After incubation in a water bath at 37°C for 10 minutes, 10 microliters of NADPH solution, 20 microliters of sodium azide,
and 0.011 M hydrogen peroxide were added to cuvettes. After mixing, disappearance of NADPH was measured at 340 nanometers in a spectrophotometer (Gilford Response UV-VIS Spectrophotometer, Oberlin, Ohio) at 37°C for 5 minutes as an index of enzyme activity. Glutathione peroxidase was expressed in μmol NADPH/gram of protein or hemoglobin. Protein content of serum was measured by the Lowry method (Lowry et al. 1951) using Folin reagent, and the hemoglobin content of erythrocytes was measured using Drabkins solution in a spectrophotometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, Illinois). Appendices B and C summarize the glutathione peroxidase and protein/hemoglobin procedures, respectively.

Demographic data was collected on each subject at 24, 36, and 48 months. Age, height, weight, pubertal stage, and menstrual cycle information was obtained from hospital records logged during the subject’s admission to The Ohio State University Medical Center. Age was recorded in years and months. Each was rounded to the nearest month. Height was measured to the nearest centimeter and recorded in the medical record. Stature (standing height) was measured using a stadiometer, that is, a non-stretchable tape attached to a vertical, flat surface (wall), with a right-angle headboard. Weight was measured to the nearest tenth of a kilogram and recorded. A beam scale with nondetachable weights was used. It was checked for accuracy 2-3 times per year. Pubertal stage was recorded from the medical record form completed by the patient and research associate or
attending physician (see Appendix D). A menstrual cycle record sheet was completed by the nurse and patient during the study period (See Appendix D). It included information related to menarche and information related to previous or current menstrual cycle, as appropriate.

Additional information was obtained from the longitudinal calcium study data base. This data included body composition measurements obtained from dual-energy X-ray absorptiometry (DEXA), three-day dietary diary records (Nutritionist III, Version 8.5 for Macintosh, Hearst Corporation: San Bruno, California), urinary creatinine measurements, and estradiol concentration. These data were assessed within 6 months of the balance study and were not assessed during the balance portion of the study.

Data analysis: Statistical analyses were done using SPSS for Windows Version 6.1 (SPSS® Inc.:Chicago, Illinois) and Data Desk Professional Version 4.1 (Data Description Inc.:Ithaca, New York). Selenium balance, selenium status, and demographic data were summarized using univariate descriptive statistics with respect to their central tendency and variability of scatter. Correlations were assessed between pubertal, anthropometric, balance, and selenium status variables. Strong, moderate, and weak correlations were defined as ±0.81 - 1.00, ±0.51 - 0.80, and -0.50 - +0.50, respectively (Devore and Peck 1986). A repeated measures analysis of variance was conducted to assess changes among the years for all variables over the three year period of this study. If the repeated measures
analysis of variance reported a significant difference between means, Tukey's HSD (Tukey's Honestly Significant Difference) post hoc test, was conducted. Linear regression was used to assess the relationships between: 1) daily selenium intake and daily selenium balance; 2) daily selenium intake/kg lean tissue and daily selenium balance; 3) daily selenium balance and average pubertal stage; and, 4) absolute change in average pubertal stage and selenium balance. Absolute change in pubertal stage was used as an index of pubertal change rate and was calculated by subtracting a previous pubertal stage \{(breast pubertal stage + pubic hair pubertal stage)/2\} from the attained average pubertal stage. Linear regression was also used to assess the relationship between estradiol concentration and daily selenium balance, net absorption of selenium, and selenium status assessment parameters at 24 and 36 months. A step wise multiple regression analysis was conducted to assess variables useful in predicting selenium balance and other variables. Two-tailed t-tests, including Levene's Test for Equality of Variances after stratification by the noted characteristic, were used to assess the differences between: 1) baseline (day 7) and day 14 selenium status measurements at 36 and 48 months; 2) pre- and post-menarchial subjects for growth, maturation, selenium balance, net absorption of selenium, selenium status variables, daily intake of selenium during the study, and usual selenium intake; 3) subjects with menses during the balance period and those without for selenium balance, net absorption of selenium, selenium status variables, daily selenium intake during the study, and
usual selenium intake; 4) 1993 and 1994 estradiol concentrations; 5) selenium content of study diets by chemical analysis and selenium content of study diets by computer data analysis or published food table analysis; 6) calcium treatment and placebo groups for selenium balance, net absorption of selenium, selenium status variables, daily selenium intake during the study, and usual selenium intake. \( P < 0.05 \) was considered significant.
CHAPTER 4

RESULTS AND DISCUSSION

The increases in age and sexual maturation/pubertal stage are illustrated in Table 1.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Age (Years)(^a)</th>
<th>Breast Development(^a)</th>
<th>Pubic Hair Development(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>12.22±0.63, n=26</td>
<td>3.33±0.66, n=26</td>
<td>3.85±0.88, n=26</td>
</tr>
<tr>
<td>1994</td>
<td>13.13±0.71, n=26</td>
<td>3.50±0.69, n=26</td>
<td>4.50±0.57, n=26</td>
</tr>
<tr>
<td>1995</td>
<td>14.07±0.70, n=26</td>
<td>3.87±0.70, n=26</td>
<td>5.00±0.62, n=26</td>
</tr>
</tbody>
</table>

Table 1: Age and pubertal development (mean±SD).
\(^a\)p<0.001, 1995 > 1994 > 1993.

Likewise, growth and anthropometric parameters, including height, weight, and measures of body composition, increased with age (Tables 2 and 3).
<table>
<thead>
<tr>
<th>Study Year</th>
<th>Height (cm)*</th>
<th>Weight (kg)*</th>
<th>Body Mass Index (kg/m²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>154.1 ± 6.2</td>
<td>48.4 ± 8.1</td>
<td>20.4 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
<tr>
<td>1994</td>
<td>159.2 ± 6.3</td>
<td>52.9 ± 6.5</td>
<td>20.9 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
<tr>
<td>1995</td>
<td>162.1 ± 6.4</td>
<td>56.2 ± 6.6</td>
<td>21.4 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
</tbody>
</table>

Table 2: Anthropometric and body mass index measurements (mean±SD).
*p < 0.001, 1995 > 1994 > 1993.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>% Fat Tissue*</th>
<th>Fat Tissue (kg)*</th>
<th>Lean Tissue (kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>23.5 ± 8.1</td>
<td>10.2 ± 5.6</td>
<td>32.7 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
<tr>
<td>1994</td>
<td>25.7 ± 9.6</td>
<td>13.2 ± 6.4</td>
<td>36.1 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
<tr>
<td>1995</td>
<td>26.7 ± 7.0</td>
<td>13.9 ± 5.8</td>
<td>38.0 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
<td>n=26</td>
</tr>
</tbody>
</table>

Table 3: Body Composition Measurements by DEXA (mean±SD).
*p < 0.001, 1995 > 1994 > 1993.

The height, weight, body mass index, percent fat tissue, fat tissue, and lean tissue measurements were significantly different (p < 0.001) from each other among years.
as measured by repeated measures analysis of variance and appeared to increase in a linear fashion with progression of the study.

The results of this study were not unexpected and indicated that as age and pubertal stage progressed, the body composition of the adolescent female subjects changed. Prior to puberty, the distribution of fat and muscle tissues are similar in males and females, 15% and 19%, respectively (Mahan and Escott-Stump 1996b). During the physical and sexual development associated with puberty, however, females gain half as much lean tissue compared to their male counterparts. In adulthood, the average body fat proportions for males and females are 15% and 22%, respectively (Mahan and Escott-Stump 1996b).

Daily selenium balance was measured as daily selenium intake minus daily selenium excretion (daily fecal selenium plus daily urinary selenium). Daily fecal selenium was calculated on daily fecal collections corrected for PEG (Figure 1).

\[
\text{Daily Fecal Selenium (\(\mu g/day\))} = \frac{\text{fecal selenium (\(\mu g/g\ stool\))}}{\text{PEG recovered (g/g stool)}} \times 1.5 \text{ g PEG/day}
\]

Figure 1. Equation for calculating daily fecal selenium corrected for PEG.

Selenium balance did not differ significantly among years, while selenium intake (p < 0.001), urinary selenium excretion (p < 0.001), and fecal selenium excretion (0 < 0.05) did, as measured by repeated measures analysis of variance (Table 4).
Table 4: Selenium balance (mean±SD).

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Selenium Intake&lt;sup&gt;a&lt;/sup&gt; (µg/day)</th>
<th>Urinary Selenium&lt;sup&gt;a&lt;/sup&gt; (µg/day)</th>
<th>Fecal Selenium&lt;sup&gt;b&lt;/sup&gt; (µg/day)</th>
<th>Selenium Balance (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>107±4 n=26</td>
<td>78±11 n=26</td>
<td>32±10 n=26</td>
<td>-2±18 n=26</td>
</tr>
<tr>
<td>1994</td>
<td>104±14 n=26</td>
<td>73±14 n=26</td>
<td>25±4 n=26</td>
<td>6±18 n=26</td>
</tr>
<tr>
<td>1995</td>
<td>137±7 n=26</td>
<td>112±21 n=26</td>
<td>36±8 n=26</td>
<td>-11±17 n=26</td>
</tr>
</tbody>
</table>

Daily selenium intake was significantly greater in 1995 compared to 1993 and 1994, which were not significantly different from each other. Daily urinary excretion of selenium likewise was significantly greater in 1995, compared to the previous years of the study. Daily urinary excretion of selenium was not significantly different in 1993 and 1994. Daily fecal selenium excretion in 1994 was significantly less than excretion in 1993 and 1995. The 1993 and 1995 daily fecal excretions of selenium were not significantly different from each other.

It was hypothesized that the selenium intake necessary to maintain existing selenium pool size would increase in the female with progression of sexual maturation. With an increased daily intake of selenium during the 48 month portion of the study, daily selenium balance did not differ significantly from the
previous years ($p > 0.05$). In view of the increased daily intake and no difference in daily balance, it may seem that the increased intake was necessary to maintain or increase the selenium pool. However, daily urinary excretion of selenium significantly increased ($p < 0.0001$) with the increase in selenium intake. In addition, since the study diet, on average, contained at least twice the recommended intake for selenium, it is not possible to assess if balance would have been achieved on an intake different from the range of intake consumed by our subjects during each study period.

For each year of the study, the relationship between daily selenium balance was plotted against daily selenium intake and daily selenium intake/kg lean tissue. Average pubertal stage was also plotted against these two variables for each year. In addition, balance data from 1993-1995 were pooled to illustrate the relationships between daily selenium intake, daily selenium intake/kg lean tissue, or average pubertal stage and daily selenium intake among our subjects within the range of intakes evaluated during the three years of the study.

To illustrate the relationship between daily selenium intake and daily selenium balance during the individual years of the study, scatter plots were plotted. In 1993, as daily selenium intake increased, daily selenium balance tended to increase but was not statistically significant ($p > 0.05$); however, a quadratic relationship was found to be statistically significant ($p < 0.05$). In 1994, however, as daily selenium intake increased, daily selenium balance also increased ($p < 0.05$, 75
r=0.5704). A moderate correlation was noted between the two variables. Finally, in 1995, as daily selenium intake increased, daily selenium balance tended to decrease, but was not significant (p > 0.05). These data appear to suggest that, in early puberty, as daily selenium intake increases, daily selenium balance will also increase. As these subjects matured, however, daily balance did not increase with an increasing intake, suggesting that balance was more difficult to maintain.

Balance data from 1993-1995 were pooled to illustrate the relationship between daily selenium intake and daily selenium balance among our subjects within the range of intakes evaluated during the three years of the study. A scatter plot of daily selenium intake versus daily selenium balance was plotted (Figure 2). Linear regression was utilized to illustrate the relationship between daily selenium intake and daily selenium balance. A quadratic relationship was found that could account for approximately 13% of the variability seen in selenium balance among our subjects by knowing daily selenium intake (p < 0.005, R² = 0.1277). For our subjects to maintain selenium balance within the range of intakes studied, as Figure 2 illustrates, it appears that selenium is retained up to a certain intake (approximately 110-115 µg/day). After that intake, it appears that a mechanism facilitates a greater excretion of the trace mineral to stay in balance. While the range of selenium intakes during the study was undoubtedly safe, this may be a protective mechanism to avoid excessive selenium. A similar pattern may also be observed at higher intakes to avoid toxicity.
Figure 2. Scatter plot of daily selenium intake versus daily selenium balance.
Scatter plots were plotted to illustrate the relationship between daily selenium intake/kg lean tissue and daily selenium balance during the individual years of the study. In 1993, as daily selenium intake/kg lean tissue increased, daily selenium balance significantly decreased and was moderately correlated with daily selenium intake/kg lean tissue ($p < 0.005, r = -0.5924$). Likewise, in 1994, as daily selenium intake/kg lean tissue increased, daily selenium balance significantly decreased ($p < 0.005, r = -0.5572$). A moderate correlation was noted between the two variables. Finally, in 1995, as daily selenium intake increased, daily selenium balance tended to increase, but was not significant ($p > 0.05$). These data suggest that during earlier stages of puberty (1993 and 1994), as the daily intake of selenium/kg lean tissue increased, daily selenium balance significantly decreased. However, as puberty progressed the amount of selenium intake/kg lean tissue was not as influential on daily selenium balance.

Balance data from 1993-1995 were pooled to illustrate the relationship between daily selenium intake and daily selenium intake/kg lean tissue among our subjects within the range of intakes evaluated during the three years of the study. A scatter plot of daily selenium intake versus daily selenium balance was plotted (Figure 3). Linear regression was utilized to illustrate the relationship between daily selenium intake/kg lean tissue and daily selenium balance. A quadratic relationship accounted for approximately 6% of variability seen in daily selenium balance but only approached significant levels with $p$-values of 0.0620 and 0.0517.
Figure 3. Scatter plot of daily selenium intake versus selenium intake/kg lean tissue.
for selenium intake/kg lean tissue and selenium intake/kg lean tissue², respectively (Figure 3).

While the relationship was not statistically significant, as predicted by Levander and Morris (1984), the amount of lean tissue appears to influence selenium balance. As Figure 3 illustrates, for our subjects to maintain selenium balance, it appears that selenium is retained up to a certain intake per kg lean tissue (approximately 325 µg/kg/day). After that intake, it appears that a mechanism facilitates a greater excretion of the trace mineral to stay in balance. As previously suggested, this may be a protective mechanism, especially with excessive intakes of selenium.

To illustrate the relationship between average pubertal stage and daily selenium balance during the individual years of the study, scatter plots were plotted. In 1993, as pubertal stage increased, daily selenium balance tended to decrease but was not statistically significant (p > 0.05). In 1994, as average pubertal stage increased, daily selenium balance tended to increase but was not significant (p > 0.05). Finally, in 1995, as average pubertal stage increased, daily selenium balance tended to increase; however, this relationship was not significant. A quadratic model, however, illustrated that as average pubertal stage increases, daily selenium balance also increases until approximately average pubertal stage 4.5 and then decreases (p < 0.05, R² = 0.2436). These data suggest that during early puberty, daily selenium balance decreased, while daily selenium balance decreased
with advancing maturity. These data suggest that balance was more difficult to maintain for these adolescent subjects as the rapid growth associated with puberty ensued. During the 1993 portion of the study, a majority of the subjects were 12-years-old, the age of the peak height spurt in adolescent females (Marshall and Tanner 1970).

Balance data from 1993-1995 were pooled to illustrate the relationship between daily selenium intake and average pubertal stage among our subjects within the range of intakes evaluated during the three years of the study. A scatter plot of average pubertal stage versus daily selenium balance was plotted. Linear regression was utilized to illustrate the relationship between average pubertal stage and daily selenium balance. The relationship was not significant (p>0.05, r=0.1966); however, as pubertal stage increased, daily selenium balance tended to increase, within the range of intakes studied over the three years. These data may suggest that during early puberty, daily balance was more difficult to maintain for these adolescent subjects.

Rate of pubertal development, rather than a specific stage of development, could impact selenium status. Consequently, scatter plots were plotted, and linear regression was used to assess the relationship between absolute change in average pubertal stage and daily selenium balance. Absolute change in average pubertal stage was used as an index of pubertal change rate and was calculated by
subtracting a previous pubertal stage \((\text{breast pubertal stage} + \text{pubic hair pubertal stage})/2\) from the attained average pubertal stage.

The plot of absolute change in average pubertal stage from 1993-1994 versus daily selenium balance in 1994 suggests that these variables were moderately correlated and that as the absolute change in average pubertal stage increases, selenium balance decreases \((p < 0.05, r = -0.5435)\). This suggests that as rate of puberty increases, as measured by absolute change in average pubertal stage, the amount of daily selenium intake necessary to maintain positive daily selenium was not achieved. The plots illustrating the rate of development between 1994-1995 and the during the overall study, 1993-1995, as assessed by linear regression, did not report significance; however, with a greater change in average pubertal stage, the balance tended to increase, especially during the overall study. Since puberty involves rapid growth and development, including ovarian cycling in females (Plant 1994), these data suggest that rapid development, as measured by absolute change in average pubertal stage, at the intake of our subjects, may adversely impact attaining positive selenium balance, compared to a more gradual rate. Compared to their faster developing counterparts, those developing at a slower rate may be able to compensate for growth by drawing on selenium reserves like classical or cellular glutathione peroxidase (Burk and Gregory 1982, Sunde 1990, Yang et al. 1989).
Net selenium absorption was measured using selenium intake and fecal selenium excretion (Figure 4).

\[
\text{Net Absorption} = \left( \frac{\text{selenium intake} - \text{fecal selenium excretion}}{\text{selenium intake}} \right) \times 100\%
\]

Figure 4: Equation for calculating net selenium absorption.

Net selenium absorption did not differ significantly among the years of the study \((p > 0.05)\). For the 26 subjects measured, net absorption was 71±10\%, 75±6\%, and 74±6\% for 1993, 1994, and 1995, respectively. Overall average net absorption of selenium over the three years of the study for the adolescent females measured was 74±5\%. Since stable isotopes were not used in this study, these values probably underestimated selenium absorption, as reexcretion of previously absorbed selenium back into the gastrointestinal tract was not measured (Veillon et al. 1990).

Compared to other selenium balance studies, the net absorption of selenium was higher among our subjects (74±5\%) (Bunker et al. 1988, Levander and Morris 1984, Levander et al. 1981, Stewart et al. 1978). Stewart et al. (1978) documented the pattern of selenium intake and excretion of four apparently healthy, New Zealand adult females (22-34 years) consuming an ad libitum diet, assessed their utilization of selenium in food, and estimated whole-body selenium in the same population. Net or "apparent selenium absorption" was reported to be 55±5\%
Levander et al. (1981) conducted a selenium balance study in six, apparently healthy, non-smoking 24-33-year-old North American men to determine if depletion/repletion studies could be used to investigate selenium balance in humans, thereby estimating human selenium requirements. This study, which is a basis for selenium requirements in males, found a net selenium absorption of $62 \pm 6\%$ (Levander et al. 1981).

Levander and Morris (1984) examined selenium intake and balance in 27 free-living North American adults (12 males, 15 females) on self-selected diets over a 12-month period. The apparently healthy, nonvegetarian population ranged in age from 19-50 years (Levander and Morris 1984). The male and female subjects had net selenium absorptions of $61 \pm 2\%$ and $68 \pm 2\%$, respectively (Levander and Morris 1984). Bunker et al. (1988) carried out balance studies in 24 apparently healthy elderly people (69-85 years; 11 males and 13 females) and 20 housebound elderly people with chronic disease (69-85 years; 7 males and 13 females) eating self-selected diets in their own homes. The healthy elderly subjects were found to have a net selenium absorption of $57\%$, while the housebound elderly subjects were found to have a net selenium absorption of $50\%$ (Bunker et al. 1988).

Compared to the results of these adult balance studies, the higher absorption of selenium seen among our adolescent female subjects may be due to an increased need for selenium during the adolescent growth spurt. In view of the similar absorption measured by Levander et al. (1984) in his North American adult female
subjects (68±2%), the absorption of these adolescent subjects may reflect the onset of ovarian cycling in these young women.

Selenium absorption does not appear to be limited by any physiologic or a homeostatic control mechanism; however, humans appear to adjust their selenium excretion for maintenance of selenium status (Robinson et al. 1978, Stewart et al. 1978, Vendeland et al. 1992). Urinary and fecal selenium excretion comprise the primary avenues of disposal for this trace mineral, and it appears that selenium is excreted to a lower degree with lower dietary intakes of the mineral (Robinson et al. 1985).

The proportion of selenium excreted in the urine was compared to total excretion by calculating percent selenium excretion by urinary means (Figure 5).

\[
\% \text{ selenium excretion in urine} = \frac{\text{Urinary selenium}}{\text{Fecal selenium} + \text{Urinary selenium}} \times 100\%
\]

Figure 5: Equation for calculating percent selenium excretion by urinary means.

The overall percent of selenium excreted by the urinary route was 74±5% for the 3 years of the study. While there were no differences among the 3 years, of the total selenium excreted, the urinary excretion of selenium was 71±5%, 74±4%, and 76±6% for 1993, 1994, and 1995, respectively.
As summarized above, the overall percent of selenium excreted by the urinary route for the adolescent subjects was 74±5%. Veillon et al. (1990) reported that approximately 80% of excreted selenium is via urine, while 20% is via feces. Lower values have been reported, however. Urinary and fecal excretion has been reported to account for 50-67% and 40-50% of total selenium excreted, respectively (Groff et al. 1995). Several selenium balance studies have reported less than 80% selenium excretion by urinary means (Bunker et al. 1988, Levander and Morris 1984, Levander et al. 1981, Stewart et al. 1978).

When Stewart et al. (1978) studied four apparently healthy, New Zealand adult females (22-34 years) consuming an ad libitum diet containing about 24 μg of selenium, the urinary excretion of the trace mineral, compared to the total selenium excreted, was found to be 55%. While in the selenium balance study conducted in six, apparently healthy, non-smoking 24-33-year-old North American men, Levander et al. (1981) reported that his male subjects excreted 57-67% of selenium by the urinary route. Levander et al. (1981) noted that the dietary intake changed 7-fold during the study; however, the urinary output (expressed as a fraction of the total output) only changed by 4%.

Levander and Morris (1984) studied apparently healthy, free-living, nonvegetarian North American male and female adults (19-50 years) and found that the male subjects excreted 59% of their total selenium losses in urine, while the female subjects excreted 63% by the same means. In the elderly balance study,
Bunker et al. (1988) found that the apparently healthy elderly subjects (69-85 years) excreted 50% of total selenium excreted in the urine and the housebound elderly subjects with chronic disease (69-85 years) excreted 48%.

The higher percentage of selenium excreted by urinary means, compared to other balance studies, appears secondary to an increased intake of selenium of the adolescent subjects. Since the kidney is the primary route of excretion for this trace mineral, this increased excretion in urine may be a protective mechanism to avoid excessive selenium.

In addition to hypothesizing that the selenium intake necessary to maintain existing selenium pool size would increase in the female with progression of sexual maturation, it was also hypothesized that selenium status, as assessed through measurements of serum and erythrocyte selenium and glutathione peroxidase, would decline in the female with progression of adolescence. The rapid growth associated with adolescence was hypothesized to decrease the existing pool size of selenium, resulting in a decline in selenium status.

Selenium status assessment measurements are summarized in Tables 5 and 6. Baseline serum and erythrocyte selenium measurements did not differ from follow-up measurements within study years; however, baseline serum measurements were shown to be significantly different by repeated measures analysis of variance.
<table>
<thead>
<tr>
<th>Study Year</th>
<th>Serum Selenium, Baseline (Day 7) (ng/mL)</th>
<th>Serum Selenium, Follow-up (Day 14) (ng/mL)</th>
<th>Erythrocyte Selenium, Baseline (Day 7) (ng/mL)</th>
<th>Erythrocyte Selenium, Follow-up (Day 14) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>99±25 n=24</td>
<td>N/A</td>
<td>180±41 n=18</td>
<td>N/A</td>
</tr>
<tr>
<td>1994</td>
<td>111±18 n=26</td>
<td>107±14 n=26</td>
<td>186±24 n=26</td>
<td>197±30 n=26</td>
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<tr>
<td>1995</td>
<td>110±14 n=26</td>
<td>114±12 n=26</td>
<td>201±40 n=26</td>
<td>201±36 n=26</td>
</tr>
</tbody>
</table>

Table 5: Serum and erythrocyte selenium measurements (mean±SD).

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Serum GPx, Baseline (Day 7) (U/g protein)</th>
<th>Serum GPx, Follow-up (Day 14) (U/g protein)</th>
<th>Erythrocyte GPx, Baseline (Day 7) (U/g hemoglobin)</th>
<th>Erythrocyte GPx, Follow-up (Day 14) (U/g hemoglobin)</th>
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</thead>
<tbody>
<tr>
<td>1993</td>
<td>2.62±1.57 n=12</td>
<td>N/A</td>
<td>15.8±5.2 n=19</td>
<td>N/A</td>
</tr>
<tr>
<td>1994</td>
<td>3.01±1.51 n=25</td>
<td>3.48±0.99 n=25</td>
<td>23.8±7.1c n=26</td>
<td>19.4±5.6c n=26</td>
</tr>
<tr>
<td>1995</td>
<td>3.34±0.79b n=26</td>
<td>3.85±0.99b n=26</td>
<td>16.2±5.3 n=26</td>
<td>16.6±5.0 n=26</td>
</tr>
</tbody>
</table>

Table 6: Serum and erythrocyte glutathione peroxidase (GPx) activities (mean±SD).

*p<0.05, 1994 > 1993, 1995.

*p<0.05, follow-up serum GPx activity > baseline activity.

*p<0.05, follow-up erythrocyte GPx activity < baseline activity.
However, the post hoc test did not confirm that the mean serum selenium concentration measured in 1993 was significantly lower than the 1994 and 1995 concentrations. Pearson correlation coefficients for serum selenium and parameters measuring growth and development (age, height, weight, body mass index, % fat tissue, fat tissue mass, lean tissue mass, pubertal stage, presence of menses, and onset of menses) were assessed. Since serum selenium concentrations reflect recent selenium intake, selenium intake during the study was also assessed. These results revealed that baseline serum selenium concentration measurements were weakly correlated with only weight ($p < 0.01$, $r=0.3040$), lean tissue ($p < 0.05$, $r=0.2281$), breast pubertal stage ($p < 0.05$, $r=0.2373$), pubic hair pubertal stage ($p < 0.05$, $r=0.2497$), and presence of menses ($p < 0.05$, $r=0.2407$). Follow-up serum selenium concentration was not significantly correlated with any of these variables.

Unlike the serum selenium measurements, the erythrocyte selenium measurements did not differ significantly between years. Pearson correlation coefficients assessing the relationship between erythrocyte selenium and aforementioned parameters measuring growth and development revealed that baseline erythrocyte selenium concentration measurements were weakly correlated with body mass index ($p < 0.05$, $r=0.2868$), fat tissue percent ($p < 0.01$, $r=0.3294$), and fat tissue ($p < 0.01$, $r=0.3141$), while follow-up erythrocyte selenium concentrations were weakly correlated with body mass index ($p < 0.05$, $r=0.3343$) and selenium balance ($p < 0.05$, $r=-0.3232$). In addition, since
erythrocyte selenium concentrations reflect long-term selenium intake, usual selenium intake was also assessed. These two variables were not significantly correlated. Serum glutathione peroxidase measurements did not differ (p > 0.05) among study years, while erythrocyte glutathione peroxidase was significantly greater in 1994 than in the 1993 and 1995 study years. During 1994, the baseline erythrocyte glutathione peroxidase activity was greater than the follow-up activity (p < 0.05). During 1995, the follow-up serum glutathione peroxidase activity was greater than baseline activity (p < 0.05). Only baseline measurements were available for 1993. Considering the variables assessed with the serum selenium concentrations, Pearson correlation coefficients revealed that follow-up serum glutathione peroxidase activities were weakly correlated with only height (p < 0.05, r = 0.3291) and lean tissue (p < 0.05, r = 0.2951). Baseline erythrocyte glutathione peroxidase activities, considering the variables examined with erythrocyte selenium, were weakly correlated with only average pubertal stage (p < 0.05, r = 0.2743). Serum glutathione peroxidase measurements did not differ (p > 0.05) among study years, while erythrocyte glutathione peroxidase differed significantly among study years. During 1994, the baseline erythrocyte glutathione peroxidase activity was greater than the follow-up activity (p < 0.05). While this phenomenon has not been reported in the literature, the difference between the three years of the study and within 1994 for erythrocyte glutathione peroxidase activities may be secondary
to a different type of vacuutainer being used (glass versus plastic) during the baseline blood draw of 1994. During 1995, the follow-up serum glutathione peroxidase activity was greater than baseline activity ($p<0.05$), which appears secondary to an increased intake of the mineral during the study period.

Although weak, the correlation coefficients appear to support the fact that selenium status does not decline during adolescence. Weak, positive correlations were found between measurements of growth, body composition, and pubertal stage and selenium assessment parameters, indicating that with growth and development of our population, selenium status improved.

Subjects were stratified by menarche for each year of the study to assess differences between growth, maturation, selenium balance, net absorption of selenium, selenium status variables, daily intake of selenium, and usual intake of selenium between the groups. As expected, during 1993 the average pubertal stage was greater ($p<0.005$) among the post-menarchial subjects ($n=8$) compared to those who were pre-menarchial ($n=18$). Breast pubertal stage ($p<0.005$) and pubic hair pubertal stage ($p<0.01$) were also greater among the post-menarchial subjects. Lean tissue mass was also greater ($p<0.05$) among the post-menarchial subjects. Baseline erythrocyte glutathione peroxidase was greater ($p<0.05$) among pre-menarchial subjects compared to their post-menarchial counterparts and were 17.8 ± 5.1 and 12.3 ± 3.4 U/g hemoglobin, respectively. The other variables assessed were not significantly different at 24 months.
At 36 months (1994), none of the growth, maturation, selenium balance, net absorption of selenium, and selenium status variables, and selenium intake variables differed between the post-menarchial subjects (n=19) compared to those who were not (n=7). At 48 months, however, the post-menarchial subjects (n=21) compared to the pre-menarchial subjects (n=4) had greater average pubertal stage (p<0.01), breast pubertal stage (p<0.05), and pubic hair pubertal stage (p<0.01), as expected. Daily selenium balance was greater (p<0.05) among post-menarchial subjects compared to the pre-menarchial subjects with balances of -8±16 and -27±16, respectively. The other variables, including selenium intake during the study or usual selenium intake, were not significantly different in 1995 between the groups.

Considering these data related to menarche, it appears that the lower concentration of erythrocyte selenium seen among those past menarche in 1993 may be due to the rapid growth and development associated with puberty in these 12-year-old females. Peak linear growth (the height spurt) occurs at approximately age 12 in females (Marshall and Tanner 1970). The greater proportion of lean tissue seen among these females (post menarche) may suggest that selenium is being used for synthesis of other selenoproteins, or it may reflect the blood loss associated with menses, contributing to the lower erythrocyte selenium concentrations observed. In mammalian species, cellular glutathione peroxidase may represent a storehouse for selenium that can be used for other purposes (Burk and Gregory 1982, Sunde
1990, Yang et al. 1989). The increased daily selenium balance seen among subjects post-menarche, compared to those who were not, may indicate that the habitual intake of selenium among the post menarche subjects was greater. However, it may also indicate that at the same level of intake during the study, the pre-menarchial subjects, compared to their post-menarchial counterparts, required a greater intake of selenium to maintain positive selenium balance for growth.

Post-menarchial subjects were stratified by presence of menses during the balance period for each year of the study to assess differences in selenium balance, net absorption of selenium, and selenium status variables. At 24 months, none of the subjects were menstruating during the balance period. At 36 months, of those menstruating (n=9), compared to those who were not (n=10), none of variables differed between the groups. At 48 months, follow-up serum selenium concentration was significantly greater among the subjects having their menstrual period (n=4), compared to those not having their menstrual period (n=17), and had concentrations of 123±10 ng/mL and 110±11 ng/mL, respectively. None of the other variables assessed were different between the groups. First, the increase in follow-up serum selenium concentration seen among those menstruating may reflect the small group size of the subjects having their period and be due to chance. The differences could not be explained by differences in usual intake of selenium or daily intake of selenium during the study since selenium intakes did not differ between the two groups.
As previously discussed, a recent human study described a fluctuation in selenium status with the fluctuation of hormones, including estradiol, during the menstrual cycle (McAdam et al. 1994). Since the erythrocyte concentrations did not decrease during the menstrual cycle, when estradiol concentration is decreased, it is unlikely that menstruation is causing the differences seen here. However, this difference may be secondary to these fluctuations. Yet, without simultaneously assessing estradiol and selenium concentrations, the difference seen here cannot be attributed to estradiol or other hormones.

To assess the influence of estradiol on the selenium status of our subjects, estradiol concentrations were examined (Table 7).

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Estradiol Concentration (pg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>23.25±22.52</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
</tr>
<tr>
<td>1994</td>
<td>46.04±45.75</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
</tr>
</tbody>
</table>

Table 7: Estradiol Concentrations (mean±SD).
*p < 0.005, 1994 > 1993.
The concentrations reported in Table 7 were not obtained during the balance portion of the study, as previously discussed. Rather, estradiol concentrations were assessed in 1993 and 1994 within 6 months of the balance study. While these measurements cannot accurately reflect actual estradiol concentrations at the time of the balance study, they may be useful for identifying stage of maturation.

As expected, the values for estradiol significantly increased (p < 0.005), in our population of adolescent females with progression of the study. However, the concentrations were not as great as values seen in adult perimenopausal females. In adult perimenopausal females, during the follicular phase, the estradiol concentration ranges from 10-200 pg/mL, while during the mid-phase, it ranges from 100-400 pg/mL. During the luteal phase, estradiol concentrations range from 10-250 pg/mL. After menopause concentrations of estradiol are < 20 pg/mL (The Ohio State University Clinical Laboratories, Columbus, Ohio).

In 1993, linear regression showed that daily selenium balance, net absorption of selenium, and selenium status parameters were not significantly related to estradiol concentrations. In 1994, only erythrocyte glutathione peroxidase was significantly related (p < 0.05) to estradiol concentrations and indicated that as estradiol concentration increased, erythrocyte selenium decreased. The higher estrogen concentrations seen in 1994 reflect the progression of sexual maturation seen, compared to 1993. This decrease in erythrocyte selenium concentrations, as previously suggested, may reflect the blood loss associated with menses or may be
due to the greater proportion of lean tissue seen among more mature females. As previously discussed, in mammalian species, cellular glutathione peroxidase may represent a storehouse for selenium that can be used for other purposes (Burk and Gregory 1982, Sunde 1990, Yang et al. 1989). Further work is needed to draw firm conclusions regarding the role of estradiol and other hormones on selenium status in adolescent females.

Urinary creatinine measurements were obtained, in view of its relationship to changes in lean body mass (Matkovic et al. 1995). Data are summarized in Table 8. No significant differences were found between the years, and no significant correlations were found between urinary creatinine measurements and selenium balance and status parameters.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Urinary Creatinine (mg/dL)</th>
<th>Urinary Creatinine (mg/24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>114±43</td>
<td>739±311</td>
</tr>
<tr>
<td></td>
<td>n=24</td>
<td>n=24</td>
</tr>
<tr>
<td>1994</td>
<td>145±75</td>
<td>1146±543</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=26</td>
</tr>
<tr>
<td>1995</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 8: Urinary creatinine measurements (mean±SD).
Selenium concentrations of the daily balance study diets were found to be significantly different from analyses by computer data base and a recently published selenium content of food table by Mahan and Escott-Stump (1996a) in their selenium concentrations for foods section of their clinical nutrition text. The results from the two-tailed t-tests, used to assess the differences between the normally-distributed values obtained from chemical analysis of the daily diets and either computer data base analysis or published food table values, are shown in Table 9.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>[Selenium] of Diet by Chemical Analysis (µg)</th>
<th>Data Base [Selenium] Values (µg)*</th>
<th>Food Table [Selenium] Values (µg)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>109±14, n=15</td>
<td>164c</td>
<td>131c</td>
</tr>
<tr>
<td>1994</td>
<td>101±14, n=10</td>
<td>118d</td>
<td>129d</td>
</tr>
<tr>
<td>1995</td>
<td>142±19, n=12</td>
<td>145</td>
<td>131e</td>
</tr>
</tbody>
</table>

Table 9: Selenium content of daily study diet by chemical analysis compared to computer data base analysis and published values (mean±SD).
*Nutritionist III, Version 8.5 for Macintosh, Hearst Corporation.
*Mahan and Escott-Stump 1996a
*p < 0.001, chemical analysis < data base, food table.
*P < 0.005, chemical analysis < data base, food table.
*p < 0.05, chemical analysis > food table.
Use of reference data bases, like computer data bases and published selenium concentrations of foods in tables, for the assessment of usual selenium intakes and status has been cautioned since selenium concentrations of foods vary widely with geographical region (Levander 1991). Selenium concentrations of the balance study diets determined by chemical analysis were found to be significantly different from computer analyzed and table-analyzed, except for 1995 when compared to computer analysis. The computer and food table values tended to over estimate intakes during the first two years of the study, compared to 1995 when the intakes were underestimated by food table analysis or more accurately estimated by computer analysis. This difference undoubtedly reflects the selenium content of the soil upon which the foods for animal feeds and/or food production were grown. These data support the fact that the use of dietary recalls and records may not reflect actual selenium content of the diet; however, dietary recalls and records can provide intake patterns of the individual and highlight foods that usually are good sources of selenium.

Multiple regression analysis found that 11% of the variability seen in baseline serum selenium concentrations could be predicted by knowing baseline erythrocyte selenium concentration (p < 0.05). Thirty-four percent of the variability seen in baseline erythrocyte selenium concentrations could be predicted by knowing the subjects' body mass index (p < 0.01), stage of breast development (p < 0.05), daily intake of selenium (p < 0.05), and baseline serum selenium concentration
(p<0.01) according to the multiple regression analysis. Finally, multiple regression analysis found that 55\% of the variability seen in baseline erythrocyte glutathione peroxidase activities could be predicted by knowing the subjects' fecal selenium excretion (p<0.0001), body weight (p<0.0001), and net absorption of selenium (p<0.0001).

Usual dietary intakes did not differ among the years by repeated measures analysis of variance for kilocalories, protein, or selenium intakes (Table 10). Usual dietary selenium intake was weakly correlated with dietary protein intake (p<0.001, r=0.4444).

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Caloric Intake (kcal)</th>
<th>Protein Intake (g)</th>
<th>Selenium Intake (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>1770±339 n=26</td>
<td>64.1±15.9 n=26</td>
<td>48±32 n=26</td>
</tr>
<tr>
<td>1994</td>
<td>1841±356 n=26</td>
<td>70.0±15.4 n=26</td>
<td>52±29 n=26</td>
</tr>
<tr>
<td>1995</td>
<td>1934±495 n=23</td>
<td>71.3±21.3 n=23</td>
<td>65±30 n=22</td>
</tr>
</tbody>
</table>

Table 10: Usual dietary intake measurements (mean±SD).

The subjects were stratified by calcium treatment group to assess the effect of calcium intake at threshold intake (1500 mg/day) versus calcium intake below the current RDA for adolescent females (< 1200 mg/day) on selenium balance, net
absorption of selenium, selenium status variables, daily selenium intake during the study, and usual selenium intake. In view of newer recommendations for adolescent female calcium intakes (1500 mg/day), the effect of increased calcium intakes on selenium metabolism is useful information since new calcium recommendations may alter the adolescent pattern of eating and affect other nutrient intakes. Net absorption of selenium was of particular concern. While calcium has not been reported to affect selenium metabolism, chelation and precipitation of selenium by heavy metals and phytates have been shown to hinder selenium absorption (Burk and Hill 1993, Forbes and Erdman 1983).

Considering each year of the study, daily selenium balance and net absorption of selenium were not significantly different between the groups. In fact, considering the selenium status variables, only two-tailed t-test results for follow-up erythrocyte selenium concentrations (36) were significantly greater (p<0.01) among subjects with threshold calcium intakes, compared to their lower calcium intake counterparts. The values were 213±35 ng/mL and 182±11 ng/mL for the adequate and substandard calcium groups, respectively. Baseline values were not significantly different.

Erythrocyte selenium concentrations reflect long-term selenium intakes. While baseline erythrocyte selenium concentrations were not different, the difference seen in follow-up erythrocyte selenium concentrations may reflect greater habitual intakes of selenium among subjects in the calcium treatment group.
(threshold intake). However, selenium intakes from dietary records were not significantly different between the calcium treatment groups.

In conclusion, the results of this study appear to suggest that: 1) selenium absorption may be greater among adolescent females than among female and male adults due to an increased need for the mineral or onset of ovarian cycling; 2) during earlier puberty, an increased daily intake of selenium, over the range of intakes studied, increased selenium balance among these adolescent subjects, until later puberty, when it was more difficult for them to maintain positive selenium balance; 3) an intake of approximately 100\(\mu\)g/day of selenium resulted in selenium balance in our population of adolescent females, suggesting that the habitual intake of selenium among these females was closer to 100 \(\mu\)g of selenium/day than to the current RDA (45\(\mu\)g/day); 4) rapid pubertal development at the intake of our subjects may adversely impact attaining positive selenium balance, compared to a more gradual rate of development; 5) selenium status may be compromised during peak linear growth, suggesting that selenium is being used for synthesis of other selenoproteins or is being lost secondary to blood loss associated with menses; 6) higher estradiol concentrations reflect the progression of sexual maturation and may be associated with a compromised selenium status; and 7) the use of dietary recalls and records may not reflect actual selenium content of the diet. This information will contribute to the knowledge regarding dietary selenium recommendations for adolescent females. Since there has been limited knowledge regarding the
relationship between selenium status and the developmental changes of females, this research will help fill a gap and further define the unique needs of adolescent females. Considering the generally poor dietary habits of this population and the possible reproductive events to follow, further research is needed to clarify selenium dietary recommendations. Research related to selenium balance in older adolescent females (15-18 years) and free-living balance studies among 11-18-year-old females would be beneficial. Studying a wider range of intakes than reported here would be valuable in predicting the selenium intakes necessary for balance. Of particular interest would be detailed monitoring of selenium status parameters and their relationship to hormonal changes throughout puberty.
CHAPTER 5

SUMMARY

In the past, the unique health care needs of females have been ignored, overlooked, or assumed to be that of their male counterparts. Even the 1989 Recommended Dietary Allowances (RDAs) have been largely based on studies done on male subjects. Since age, gender, and fluctuations in reproductive hormones have been reported to affect selenium status, selenium intakes may be suboptimal during puberty, in view of the changing hormonal levels. Therefore, the goals of this study were: 1) to assess the relationship between selenium intakes and balance in females throughout puberty in an effort to determine the effect of intake on the existing selenium pool size; 2) to determine if selenium status changes in the female during physical and sexual maturation; and 3) to determine if selenium balance, net absorption, and status differ between adolescent females at threshold calcium intake (1500 mg/day) versus calcium intake below the current RDA for adolescent females (<1200 mg/day). Yearly selenium balances were performed in a metabolic unit over a 2-week period during 1993-95 in 26 healthy Caucasian females. Average pubertal stage was 3.6±0.8, 4.0±0.8, and 4.4±0.9 for 1993, 1994, and 1995,
respectively. After diet equilibration (approximately 100 μg/day) during week 1, selenium content of food, feces, and urine were determined to calculate individual balances and net selenium absorption \{[(\text{selenium intake} - \text{fecal selenium})/\text{selenium intake}] \times 100\% \}. Polyethylene glycol was used as a continuous fecal marker. Average daily balance was $-2\pm 18$, $+6\pm 18$, and $-11\pm 17$ μg selenium for 1993, 1994, and 1995, respectively. Overall average net absorption of selenium over the three years of the study for adolescent females measured was $74\pm 5\%$. Selenium status [serum and erythrocyte selenium and glutathione peroxidase] measurements were all within normal ranges. The results of this study appear to suggest: 1) selenium absorption may be greater among adolescent females than among female and male adults due to an increased need for the mineral or onset of ovarian cycling; 2) during earlier puberty, an increased daily intake of selenium, over the range of intakes studied, increased selenium balance among these adolescent subjects, until later puberty, when it was more difficult for them to maintain positive selenium balance; 3) an intake of approximately 100μg/day of selenium resulted in selenium balance in our population of adolescent females, suggesting that the habitual intake of selenium among these females was closer to 100 μg of selenium/day than to the current RDA (45μg/day); 4) rapid pubertal development at the intake of our subjects may adversely impact attaining positive selenium balance, compared to a more gradual rate of development; 5) selenium status may be compromised during peak linear growth, suggesting that selenium is being used
for synthesis of other selenoproteins or is being lost secondary to blood loss associated with menses; 6) higher estradiol concentrations reflect the progression of sexual maturation and may be associated with a compromised selenium status; and 7) the use of dietary recalls and records may not reflect actual selenium content of the diet. This information will contribute to the knowledge regarding dietary selenium recommendations for adolescent females. Since there has been limited knowledge regarding the relationship between selenium status and the developmental changes of females, this research will help fill a gap and further define the unique needs of adolescent females. Considering the generally poor dietary habits of this population and the possible reproductive events to follow, further research is needed to clarify selenium dietary recommendations. Research related to selenium balance in older adolescent females (15-18 years) and free-living balance studies among 11-18-year-old females would be beneficial. Studying a wider range of intakes than reported here would be valuable in predicting the selenium intakes necessary for balance. Of particular interest would be detailed monitoring of selenium status parameters and their relationship to hormonal changes throughout puberty.


APPENDIX A, METHODS FOR SELENIUM ANALYSIS
Selenium Analysis for Serum, Red Blood Cells, Milk, and Urine
(McCarthy et al. 1981, Smith et al. 1982)

Digestion

1. Add 10-500 microliters of sample, 50 microliters of 0.5 ppm selenium standard solution, or 20 milligrams of reference standard (bovine liver) to 7 milliliter Pyrex weighing vial.

2. Add 1.5 milliliters of nitric acid containing 30 grams of magnesium nitrate hexahydrate/100 milliliters of acid.

3. Place in modular dry-bath for 60 minutes at 105°C, followed by 30 minutes at 115°C, and 2 or more hours at 130°C.

4. Remove vials from modular dry-bath. Sample will look thick (almost dry).

5. Place on a hot plate on high setting for 1 or more hours. Watch carefully that no sample spits out.

6. Remove from hot plate when fumes of nitrogen oxide cease and the sample looks like a yellowish-white ash.

7. Place vials in muffle furnace at 500°C for 60 minutes.

8. Allow vials to cool in muffle furnace several hours before opening door and removing.

Complexing and Extraction

1. Add 1.5 milliliters of 36% hydrochloric acid (for trace mineral analysis) to vials of cooled ash.

2. Lightly cap vials and place in modular dry-bath at 90°C for 15-20 minutes, until ash is completely dissolved.

3. Cool to room temperature.

4. Add 2.5 milliliters of 1% hydroxylamine sulfate, 1% EDTA, 15% urea solution to each vial. Mix and allow to stand for 10 minutes.
5. Add 100 microliters of complexing solution (chloride salt of 4NPD in 1 N hydrochloric acid at 0.5% w/v).

6. Place vials in modular dry-bath at 45°C for 30 minutes.

7. Cool to room temperature.

8. Add 1 milliliter of toluene and mix for 5 seconds.

9. Remove approximately 0.5 milliliters of the toluene layer (organic phase) with a Pasteur pipet or pipettor and place in screw cap vial with Teflon cap liner for subsequent gas chromatographic analysis.

**Gas Chromatography**

1. Instrument: Varian 3300 Gas Chromatograph with Electron Capture Detector (ECD) and Make-Gas attachment (Sugarland, Texas).


3. Carrier Gas: Nitrogen (56-60 psi)

4. Temperatures of operation:  
   - Column: 190°C  
   - Injector: 220°C  
   - Detector: 300°C

5. Software: Star Integrator
Selenium Analysis for Stool and Solid Food
(McCarthy et al. 1981, Smith et al. 1982)

Digestion

1. Add 0.5 grams of sample, 50 microliters of 0.5 ppm selenium standard solution, or 20 milligrams of reference standard (bovine liver) to 7 milliliter Pyrex weighing vial.

2. Add 1.5 milliliters of nitric acid containing 30 grams of magnesium nitrate hexahydrate/100 milliliters of acid.

3. Place in modular dry-bath for 30 minutes at 45°C. Swirl after 15 minutes and 30 minutes of heating.

4. Increase temperature to 90°C and heat for 30 minutes. Swirl after 15 minutes and 30 minutes of heating.

5. Continue heating in modular dry-bath for 60 minutes at 105°C, followed by 30 minutes at 115°C, and 2 or more hours at 130°C. Swirl after 15 minutes of heating.

6. Remove vials from modular dry-bath. Sample will look thick (almost dry).

7. Place on a hot plate on high setting for 1 or more hours. Watch carefully that no sample spits out.

8. Remove from hot plate when fumes of nitrogen oxide cease and the sample looks like a yellowish-white ash.

9. Place vials in muffle furnace at 500°C for 60 minutes.

10. Allow vials to cool in muffle furnace several hours before opening door and removing.

Complexing and Extraction

1. Add 1.5 milliliters of 36% hydrochloric acid (for trace mineral analysis) to vials of cooled ash.
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3. Cool to room temperature.

4. Add 2.5 milliliters of 1% hydroxylamine sulfate, 1% EDTA, 15% urea solution to each vial. Mix and allow to stand for 10 minutes.

5. Add 100 microliters of complexing solution (chloride salt of 4NPD in 1 N hydrochloric acid at 0.5% w/v).

6. Place vials in modular dry-bath at 45°C for 30 minutes.

7. Cool to room temperature.

8. Add 1 milliliter of toluene and mix for 5 seconds.

9. Remove approximately 0.5 milliliters of the toluene layer (organic phase) with a Pasteur pipet or pipettor and place in screw cap vial with Teflon cap liner for subsequent gas chromatographic analysis.

**Gas Chromatography**

1. Instrument: Varian 3300 Gas Chromatograph with Electron Capture Detector (ECD) and Make-Gas attachment (Sugarland, Texas).


3. Carrier Gas: Nitrogen (56-60 psi)

4. Temperatures of operation:
   
<table>
<thead>
<tr>
<th>Component</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>190°C</td>
</tr>
<tr>
<td>Injector</td>
<td>220°C</td>
</tr>
<tr>
<td>Detector</td>
<td>300°C</td>
</tr>
</tbody>
</table>

5. Software: Star Integrator
APPENDIX B, METHODS FOR GLUTATHIONE PEROXIDASE ACTIVITY ANALYSIS
Glutathione Peroxidase Activity Analysis
for Red Blood Cells (RBC) and Serum
(Paglia and Valentine 1967)

Sample Preparation

<table>
<thead>
<tr>
<th>RBC (microliters)</th>
<th>KH Buffer (microliters)</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>980</td>
<td>50</td>
</tr>
<tr>
<td>12 (50x dilution)</td>
<td>1080</td>
<td>500</td>
</tr>
<tr>
<td>60 (50x dilution)</td>
<td>1140</td>
<td>1000</td>
</tr>
<tr>
<td>20 (50x dilution)</td>
<td>980</td>
<td>2500</td>
</tr>
</tbody>
</table>

Table 11. Red blood cell preparation for glutathione peroxidase activity assessment.

<table>
<thead>
<tr>
<th>Serum (microliters)</th>
<th>KH Buffer (microliters)</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td>120</td>
<td>1080</td>
<td>10</td>
</tr>
<tr>
<td>48</td>
<td>1152</td>
<td>25</td>
</tr>
<tr>
<td>600 (25x dilution)</td>
<td>600</td>
<td>50</td>
</tr>
<tr>
<td>120 (10x dilution)</td>
<td>480</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 12. Serum preparation for glutathione peroxidase activity assessment.
Running Samples

1. Set up Gilford Response UV-VIS Spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio).
   a. Wavelength = 340
   b. Scan length = 5 minutes
   c. Temperature = 37°C

2. Add the following to the optically-matched quartz cuvettes and incubate at 37°C for 10 minutes:
   a. 200 microliters of glutathione reductase solution
   b. 50 microliters of glutathione solution
   c. 500 microliters of sample or 500 microliters of KH buffer (blank)
   d. 210 microliters of deionized, distilled water

3. Add 10 microliters of NADPH solution to the cuvette and mix.

4. Add 20 microliters of sodium azide solution and wipe sides of cuvettes with kim wipe.

5. Add 10 microliters of hydrogen peroxide solution, mix quickly, and run on spectrophotometer.

Results

Glutathione peroxidase results are expressed in relation to protein or hemoglobin concentration. See Appendix C for procedures.
APPENDIX C, METHODS FOR TOTAL PROTEIN AND TOTAL HEMOGLOBIN ANALYSIS
Total Protein
(Lowry et al. 1951)

Standards

<table>
<thead>
<tr>
<th>Volume of Standard (microliters)</th>
<th>Volume of DI Water (microliters)</th>
<th>[Protein]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1200</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>1150</td>
<td>25</td>
</tr>
<tr>
<td>100</td>
<td>1100</td>
<td>50</td>
</tr>
<tr>
<td>200</td>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>700</td>
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<td>800</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>1000</td>
<td>200</td>
<td>500</td>
</tr>
</tbody>
</table>

Table 13. Preparation of standards for total protein analysis.

Procedure

1. Label tubes.

2. Add sample and deionized, distilled water to make 1200 microliters in tube.

3. Add 6.0 milliliters of alkaline copper reagent to each tube and mix. Let stand 10 minutes.

4. Add 300 microliters of Folin reagent to each tube and mix. Let stand 30 minutes.

5. Read on spectrophotometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, Illinois) at 500 nm.
Total Hemoglobin

Standards

<table>
<thead>
<tr>
<th>Hemoglobin Standard Solution</th>
<th>Drabkin’s Solution</th>
<th>Hemoglobin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 milliliters</td>
<td>6.0 milliliters</td>
<td>0.0 g/100 milliliters</td>
</tr>
<tr>
<td>2.0 milliliters</td>
<td>4.0 milliliters</td>
<td>6.0 g/100 milliliters</td>
</tr>
<tr>
<td>4.0 milliliters</td>
<td>2.0 milliliters</td>
<td>12.0 g/100 milliliters</td>
</tr>
<tr>
<td>6.0 milliliters</td>
<td>0.0 milliliters</td>
<td>18.0 g/100 milliliters</td>
</tr>
</tbody>
</table>

Table 14. Preparation of standards for total hemoglobin analysis.

Procedure

1. Label test tubes.
2. Add 5.0 milliliters of Drabkin’s solution to each test tube.
3. Add 10 microliters of red blood cells, rinsing pipet capillary tube 3-4 times with the reagent in the test tube to assure complete blood transfer. Vortex well.
4. At 540 nm, zero spectrophotometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, Illinois) with blank, then measure absorbance of each unknown.
5. Measure the absorbance of each working standard at 540 nm and construct a standard curve plotting the absorbance of the standards vs. hemoglobin concentration.
6. Determine total hemoglobin concentration (g/100 milliliters) of unknown directly from calibration curve.
APPENDIX D, MEDICAL RECORD FORMS
Figure 6. Stages in breast development form.
Figure 7. Stages in development of pubic hair form.
MEDICAL CAMP RECORD SHEET

NAME____________________________________

Have you started your periods? _______

First day of last period? _______

Duration of periods? _______

Please fill out this record sheet every day. This information is very important for the study. Answer this question:

Are you having your period?

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<th>Date</th>
<th>YES</th>
<th>NO</th>
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Figure 8. Menstrual cycle record form.