EFFECTS OF IODINE AND SELENIUM DEPLETION ON GROWTH AND BONE QUALITY OF RATS

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in partial fulfillment of
the requirement for
the degree of
DOCTOR OF PHILOSOPHY
July, 2005

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my advisor Dr. Barbara Stoecker for her intelligent supervision, constructive guidance, relentless patience, and encouragement in the completion of this project. I also appreciate the professional help and encouragement over the last four years of the committee members, Brenda J. Smith, Dr. Larry Claypool, and Dr. Bahram H. Arjmandi.

I would also like to acknowledge Dr. Edralin Lucas and Dr. Sin-Hee Kim for their friendship, their wise advice and their professional help. Thanks to my colleagues and friends: Victoria Lehloenya, Archana Ellath, Amani Soliman, Dr. Doyu Soung, Djibril Traore, Jarrod King and Anagha Patade for their help. I thank the faculty, staff, and students of the Department of Nutritional Sciences at Oklahoma State University for their contribution to the success of this project.

My special thanks go to my husband, Alpha Kabine Kaba and my two sons, Cheick Abdoul Gadiri Kaba, and Ibrahima Kaba for their patience, moral support, and understanding throughout this long and difficult ordeal. Thanks to my mother, Hadja Fatoumata Kaba for giving me the opportunity to reach this achievement.

I would like to dedicate this work to the memory of my father, Elhadj Mamady Toure.

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LIST OF ABBREVIATIONS

AIN = American Institute of Nutrition
ALP = Alkaline phosphatase
BMA = Bone mineral area
BMC = Bone mineral content
BMD = Bone mineral density
Ca = Calcium
CDC = Centers for Disease Control
COMP = Cartilage oligomeric matrix protein
ConnD = Connectivity density
CTx = C-telopeptide of collagen cross-link
DA = Degree of anisotropy
DEXA = Dual energy X-ray absorptiometry
DPD = Deoxypyridinoline
EDTA = Ethylenediaminetetraacetic acid
EIA = Enzyme-immuno assay
FE = Finite element analysis
FRAP = Ferric reducing ability of plasma
GH = Growth hormone
GSG = Oxidized glutathione
GSH = Glutathione
GSH-Px = Glutathione peroxidase
GSHR = Glutathione reductase
I = Iodine
IACUC = Institutional Animal Care and Use Committee
ICCIDD = International Council for the Control of Iodine Deficiency Disorders
ICTP = Cross-linked C-telopeptide of type I collagen
IDD = Iodine deficiency disorders
IGF-1 = Insulin like growth factor 1
IGFBP-3 = Insulin like growth factor binding protein 3
Ihh = Indian hedgehog
IL-1 = Interleukin-1
IL-6 = Interleukin-6
IL-8 = Interleukin-8
IRMA = Immunoradiametric assay
KBD = Kashin-Beck disease
MDA = Malondialdehyde
Mg = Magnesium
MMWR = Morbidity and Mortality Weekly Report
MTX = Methotrexate

$NADP^+$ = Oxidized nicotinamine adenine dinucleotide
NADPH = Reduced nicotinamide adenine dinucleotide
NF-kß = Nuclear factor kappa ß
NTx = N-telopeptide of collagen cross-link
OA = Osteoarthritis
OC = Osteocalcin
OPG = Osteoprotegerin
P = Phosphorus
PHV = Peak height velocity
PTH = Parathyroid hormone
PTHrP = Parathyroid hormone related peptide
RA = Rheumatoid arthritis
RANK = Receptor activator of nuclear factor kappa
RANKL = Receptor activator of nuclear factor kappa ß ligand
RIA = Radioimmunoassay
RNS = Reactive nitrogen species
ROS = Reactive oxygen species
RTH = Resistance to thyroid hormone
SAC = School-age children
SD = Standard deviation
Se = Selenium
SERMs = Selective estrogen receptor modulators
SMA = Second moment of area
SMI = Structural model index
T_3 = Triiodothyronine
T_4 = Thyroxin
TBARS = Thiobarbituric acid reactive substances
TbN = Trabecular number
TbSp = Trabecular separation
TbTh = Trabecular thickness
TNF-α = Tumor necrosis factor alpha
TRAP = Tartrate resistant acid phosphatase
TR-α = Thyroid hormone receptor α
TSH = Thyroid stimulating hormone
UL = Tolerable upper intake level
UNICEF = Uinted nation children fund
VOI = Volume of interest
WHO = World Health Organization
YKL-40 = Human cartilage glycoprotein 39

CHAPTER I

INTRODUCTION

According to the Canadian Institute of Health Research and the Institute of Musculoskeletal Health and Arthritis, more than 400 million people around the world suffer from crippling, chronic pain of joint disease, osteoporosis, spine diseases and musculoskeletal trauma, and this number is predicted to increase to 570 million people by the year 2020 (1). Epidemiological studies and studies with animal models have associated the deficiency of iodine (I) and selenium (Se) with a type of osteoarthritis (OA) occurring in children in the first or second decade of life (2-5). Even though an inverse relationship between osteoarthritis and bone density or osteoporosis has been documented (6-8), local bone loss near affected joints and reduced BMD in non-articular bones is well recognized in both OA and rheumatoid arthritis (RA) (8-11).

Arthritis consists of conditions that affect the joints and surrounding tissues. The most common forms of arthritis are OA and RA (12). Approximately 40 million Americans are suffering from arthritis, and this number will increase to 59.4 million by the year 2020 (13). The Centers for Disease Control in its morbidity and mortality weekly report (14) noted 21% of US adults suffer from arthritis with blacks having similar prevalence to that of white people. Arthritis does not affect only the elderly,

even though the risk increases with age. Approximately 300,000 children in the United States suffer from some form of arthritis or rheumatic diseases. There are 8.4 million young adults between the ages of 18-44 who have arthritis and millions of others are reported to be at risk for developing it (15).

Osteoporosis is a metabolic bone disease characterized by low bone mineral density and microarchitectural deterioration of bone leading to its fragility and subsequent fracture (16). Each year in the United State, osteoporosis leads to a million and half fractures, mostly of the hip, spine and the wrist (17), and 12 - 20% of patients with hip fracture die within a year after fracture, usually from complications such as pneumonia, and blood clots in the lung, which are related to the fracture or the surgery to repair the fracture (12). The estimated national direct expenditures (hospitals and nursing homes) for treatment of osteoporosis and associated fractures was $17 billion in 2001 ($47 million each day) and the cost is rising (17).

While we experience this alarming situation, several factors have been implicated in the etiology of bone and articular diseases. Iodine and selenium deficiencies have been associated with osteoarthritis (2-3, 18) and osteoarthritis leads to bone loss (9, 11, 19). Unfortunately, iodine and selenium deficiencies are still major public health problems in many parts of the developing world.

The best known role of iodine in mammalian systems is for the synthesis of thyroid hormones which regulate multiple physiological processes including bone development, growth, and maintenance (20). Despite great strides made in human nutrition in the previous decades, deficiencies of iodine and selenium still exist in many countries. The World Health Organization estimates that 740 million people suffer from

iodine deficiency disorders (IDD) globally comprising 13% of the world population. An additional 30% are classified as at risk (21). Iodized salt has alleviated IDD in many parts of the world, but it is estimated that in countries with IDD, 1.6 billion people still do not have access to iodized salt (21).

Selenium is an essential trace element for humans. Its appearance in the food supply is closely related to geologic factors affecting soil selenium (22). Deficiency symptoms for selenium are linked to its normal uses in the body. It is an essential cofactor for glutathione peroxidases, enzymes that protect tissues from oxidative damage (23). Another vital role of selenium is in the conversion of the thyroid hormone thyroxine (T_4) to triiodothyronine (T_3), as a component of the selenoprotein 5'iodothyronine deiodinase, the enzyme responsible for this conversion (24). During selenium-deficient conditions, iodine can be held in the T_4 fraction by selenium deficiency's effects (25).

Selenium adequacy is required for normal thyroid function (24). In addition to the activation and the homeostasis of thyroid hormones, selenium may protect thyroid cells against oxidative damage, and thus improve thyroid function (25). Kashin-Beck disease (*Osteoarthritis deformans endemica*) is an endemic osteoarthropathy that affects the bone and joints of its sufferers, with a typical onset in the first or second decade of life (26). Selenium and iodine deficiencies have been associated with the disease (2, 5).

The mechanism whereby iodine and selenium may affect bones and joints is not clear. However, thyroid hormone (T_3) is believed to have an important role in the development and maintenance of both endochondral and intramembranous bone (27). Selenium is required for thyroid hormone metabolism. In addition, selenium may protect

bone and cartilage cells against oxidative damage (28). Despite the studies showing effects of iodine and selenium on bone, there is limited information on the effects of these trace elements on characteristics of bone such as density, microarchitecture, and strength during growth. There is also insufficient data on gender difference in bone response to these trace elements with respect to the above-mentioned characteristics.

Objectives:

The objectives of this study were

1. To investigate the effects of iodine and/or selenium depletion on growth and bone quality of growing male and female rats by assessing indicators of growth, bone density, microarchitecture, strength, and selected biochemical markers of bone metabolism.

2. To investigate gender differences in bone response to iodine and/or selenium depletion.

Hypotheses

To accomplish the above-cited objectives the following null hypotheses were proposed:

$H0_1$: Iodine and/or selenium depletion will not significantly reduce growth and bone density of growing rats.

$H0_2$: Iodine and/or selenium depletion will not negatively affect biochemical markers of

bone metabolism and oxidative status of growing rats.

$H0_3$: Iodine and/or selenium depletion will not significantly deteriorate the microarchitecture of growing rat bone.

$H0_4$: Iodine and/or selenium depletion will not impair biomechanical properties of the bone of growing rats

$H0_5$: Male and female rat bones will not be differently affected by iodine and/or selenium depletion.

Study Significance

Iodine and selenium deficiencies have been associated with retarded growth, osteopenia and osteoarthritis in growing individuals (2-3). Since osteoarthritis has been shown to cause bone loss, it is possible that iodine and selenium deficiency-related osteoarthritis may lead to increased bone fracture risk in the affected growing individuals.

Treatment options for both arthritis and osteoporosis are not without limitations and serious adverse side effects, and alternative ways of prevention and treatment are being investigated. If this study is confirmed by more animal and controlled clinical human studies, it may serve as a basis for dietary recommendation for preventing or decreasing the incidence of iodine and selenium deficiency-related bone and articular disorders.

Organization of the Dissertation

There are different ways of approaching investigation of effects of iodine and selenium depletion on bone using an animal model. This dissertation reviews key literature on functions, food sources and requirements for iodine and selenium, as well as bone disorders such as osteoporosis and arthritis and their relation with iodine and selenium deficiency. Following the review of the literature and methodology section, is a chapter prepared as a journal article for submission to the Journal of Nutriton. In the study presented in the form of journal article, lactating dams were fed iodine and/or selenium depleted diets and growth, iodine and selenium status, bone strength and structure, biochemical markers of bone metabolism, and antioxidant status were assessed. These chapters are followed by a chapter containing summary and conclusions, as well as suggestions for future study. Means for the differents dietary groups are presented in **Appendices D** through **Q.** Tables associated with chapter IV present factor means. The dissertation is formatted according to the author guidelines for Journal of Nutrition.

CHAPTER II

REVIEW OF THE LITERATURE

Physiological Functions of Iodine and Selenium

Physiological Functions of Iodine

The best-known role of iodine in mammals is its use in the synthesis of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) (29). Thyroid hormones regulate a variety of physiological processes such as growth and development of many organs including the skeleton (30) metabolism rate, protein synthesis, and thermoregulation (31). Thyroid hormone (T_3) directly stimulates its cell nuclear receptors influencing expression of several genes including those that regulate bone growth and function (30).

An insufficient dietary supply of iodine results in a variety of disorders grouped under the general heading of iodine deficiency disorders (IDD). Among these are goiter, abortion, stillbirth, decreased cognitive function, increased infant mortality, cretinism (31), cardiac insufficiency and iodine-induced hyperthyroidism (32). Iodine deficiency may also contribute to Kashin-Beck disease (33), a severe type of osteoarthritis reported in children and adolescents in certain areas of China and Tibet (2).

Physiological Functions of Selenium

Selenium is an essential trace element for humans. It acts in the body in the form of different selenoproteins, eighteen of which have been identified (23). These selenoproteins include four different glutathione peroxidases (GSH-Px 1, 2, 3 and 4), which catalyze the reduction of peroxides that can cause cellular damage (23-24, 34), and three iodothyronine deiodinases (types I, II and III) that are required for thyroid hormone metabolism and homeostasis (24, 35). Other selenoproteins mentioned by Sunde (23) and the Food and Nutrition Board (35) include three thioredoxin reductases (1 through 3) involved in the reduction of intramolecular disulfide bonds and the regeneration of ascorbic acid from its oxidized metabolites, selenoprotein P which is also involved in oxidant defense, selenoprotein W involved in muscle metabolism, and selenophosphate synthetase having a role in cancer protection (23) and required for selenium metabolism (35).

In addition to the activation and homeostasis of thyroid hormone, selenium as part of key antioxidant enzymes may improve thyroid function by protecting its cells against oxidative damage. Iodine deficiency results in the hyperstimulation of the thyroid by thyroid stimulating hormone (TSH) and consequently in increased production of hydrogen peroxide (H_2O_2) within the thyroid cells. Selenium adequacy may prevent the accumulation of H_2O_2 and thus thyroid cell destruction and thyroid failure (25). It is believed that selenium may protect bone and cartilage cells against oxidative damage in a similar manner (23).

Food Sources of Iodine and Selenium

The use of iodized salt has been the most effective means for control of iodine deficiency. Sea fish and other marine foods are frequently regarded as the most important natural sources of dietary iodine. Even in inland areas, fish remains the highest natural iodine food source (36). Milk and crops from iodine sufficient geographical areas may also be good sources of iodine.

The appearance of selenium in the food supply is related to the selenium content of the soil where the foods are grown (22). In regions with low selenium soil, deficiencies arise if the diet is confined to foodstuffs grown in that region.

Selenium is associated with protein in animal tissues. Selenium deficiency can be worsened by protein energy malnutrition (PEM). PEM has a dual impact on selenium status because selenium is often bound to the amino acid methionine in the consumed protein. In addition, low methionine intake forces the body to use seleno-methionine complexes in the manufacture of body proteins making the selenium unavailable until the protein is degraded by the body (23). Consequently meats (muscle meats and organ meats), and seafood are dependable dietary sources of the mineral (34). However, the selenium contents of grains and seeds vary depending on the content of the soil in which they were grown. Fruits, vegetables and drinking water do not provide substantial amounts of selenium (34). In general, beef, white bread (made of high selenium wheat), pork, chicken and eggs are believed to account for about half of the selenium in diets of people in the United States (37).

Requirements for Iodine in Humans

Several international groups have made recommendations for iodine intakeswhich are fairly similar. International Council for the Control of Iodine Deficiency Disorders (ICCIDD), WHO, and UNICEF recommend the following daily amounts: age 0-7 years, 90 µg; age 7-12 years, 120 µg; older than 12 years, 150 µg; and pregnant and lactating women,

200 µg (38)

A recent report by the Food and Nutrition Board, Institute of Medicine, National Academy of Sciences, USA, offers similar recommendations. It calculates an "Estimated Average Requirement" and from that derives an RDA (Recommended Daily Allowance). However, occasionally sufficient data are not available and instead an Adequate Intake (AI) is stated which may be set higher than the RDA would be, for safety. The recommendations are as follows: the AI for infants 0-6 months is 110 µg iodine and 7-12 months, 130 µg; the RDA's are: 1-8 year old, 90 µg; 9-13 years, 120 µg; 14 and older, 150 µg; pregnancy, 220 µg; lactation, 290 µg. The Food and Nutrition Board also sets the tolerable upper intake levels (UL) at 200 µg /day for children 1-3 years old, 300 µg/day for children ages 4-8, 600 µg/day for ages 9-13, 900 µg/day for ages 14-18 and 1100 µg/day for adults (39).

Requirements for Selenium in Humans

The recommended daily allowances are set for selenium for adolescent and adult men and women at 55 μg (35). The RDA for pregnant women is 60 μg/day. For lactating women it is 70 μg/day. The UL for adolescents and adults is 400 μg regardless of pregnancy or lactating state.

The adequate intake for infants from 0 to 6 months is 15 μg/day of selenium (2.1 μg/kg). The adequate intake for infant aged 7-12 months is 20 μg/day of selenium (2.2 μg/kg). The RDAs for children are 20 μg/day for ages 1-3 and 30 μg /day for 4-8 years. For children aged 9 to 13 years, it is 40 μg /day of selenium. For children aged 14 to 18 years, the RDA is 55 μg /day of selenium for both girls and boys (35). The tolerable upper intake level (UL) for 0 to 6 month is 45 μg /day. This value increases progressively to 280 μg/day for 13 year-old children (35).

Indicators of Iodine and Selenium Status

Indicators of Iodine Status

The pituitary gland responds to low levels of circulating thyroid hormones by increasing the secretion of its hormone, thyroid stimulating hormone (TSH), which drives the thyroid gland to enlarge, to increase iodine uptake from the blood and to produce more hormones. Precisely assessed thyroid size is one of the most sensitive indicators of community iodine nutrition (40). It is usually determined by palpation or by ultrasonography, the latter being more precise.

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition in remote areas in the community (40). Most iodine absorbed in the body eventually appears in the urine; therefore urinary iodine is a good marker of very recent dietary intake. Urinary iodine values may not be reliable in areas where substantial amounts of goitrogens such as thiocyanate are ingested from the staple food, because goitrogens prevent the uptake of iodine by the thyroid gland and the subsequent thyroid hormone synthesis (29). In this case urinary iodine may be normal but plasma TSH will be increased due to the lack of enough thyroid hormone feedback to the anterior pituitary (29).

Plasma level of thyroid stimulating hormone (TSH) is the most sensitive functional indicator of iodine status (29, 41). When dietary supplies of iodine are limited, stimulation of thyroid gland by increased plasma TSH may be enough to maintain circulating thyroid hormone levels. It is only when the deficiency is severe that thyroid hormone levels begin to decline (29). A blood spot of TSH in neonates is a valuable indicator of iodine nutrition (31).

Thyroglobulin is the most abundant protein of the thyroid, providing the matrix for thyroid hormones synthesis. Normally, small amounts are secreted or leak from the thyroid into the circulation (42). When the thyroid is swollen or injured, larger amounts of thyroglobulin are released into the blood in response to thyroid stimulating hormone (42). Iodine deficiency-related thyroid hyperplasia is associated with increased serum thyroglobulin. In this case, serum thyroglobulin reflects iodine nutrition over months or years (40).

Determining serum concentrations of the thyroid hormones T_4 and T_3 is usually not recommended for monitoring iodine nutrition because these tests are more cumbersome, more expensive, and less sensitive as indicators at the community level (40). The prevalence indicators of IDD and criteria for a significant public health problem are presented in **Table I** (adapted from (31))

Table I
Prevalence indicators of IDD and criteria for a significant public health problem

Variables	Normal	Mild	Moderate	Severe
Prevalence of goiter in school-age children (SAC) (%)	<5	5-19.9	20-29.9	>30
Frequency of thyroid volume in SAC >97th percentile by ultrasound (%)	<5	5-19.9	20-29.9	>30
Median urinary iodine in SAC and adults (μg/L)	100-200	50-99	20-49	<20
Frequency of neonatal TSH >5 μU/ml in whole blood (%)	<3	3-19.9	20-39.9	>40

Indicators of Selenium Status

Assessment of selenium status can be done through a variety of means, including measurement of specific selenoproteins. Estimation of dietary selenium intake, measurement of selenium concentration in blood, tissues, excreta, and determination of glutathione peroxidase activity in various blood components are the common techniques used for assessing selenium status (34). Based on the observation of Keshan disease in

China where there is severe selenium deficiency, the occurrence of the disease in a population indicates that the population is selenium deficient (35). Cellular and plasma glutathione peroxidases are the functional parameters commonly used for the assessment of long and short-term selenium status, respectively (29, 34). However, plasma selenoprotein P concentrations appear to be more affected by selenium deficiency than glutathione peroxidase activity (35).

Selenium in toenails reflects selenium intake from approximately 6 to 12 months before sample collection (43). Selenium concentrations in hair are also considered as indicators of long-term selenium status. However, the use of hair selenium as an indicator of status is limited because contamination of hair by selenium-containing shampoo may affect the selenium content of this tissue (35).

Impact of Iodine and Selenium Deficiencies on Bone Health

Kashin-Beck disease, a severe type of osteoarthritis, has been associated with the deficiency of iodine (3) and selenium (2, 4, 44). Kashin-Beck disease (Osteoarthritis deformans endemica) is a degenerative, disabling endemic osteoarticular condition that affects the bone and joints of its sufferers, with a typical onset in the first or second decade of life (2, 26). Kashin-Beck disease was first identified in 1849 by a Russian doctor, Nikolai Ivanovich Kashin, but its cause is still unknown. In Tibet, the risk factors seem to include selenium deficiency in the soil (2), fungal contamination of barley (the staple grain) (45), organic matter (fluvic acid) in the water (2), and iodine deficiency (2, 3, 46).

Kashin-Beck disease has been reported in certain areas of Tibet, northern China, Mongolia, Siberia, and North Korea (2, 33). In China, 30 million people live in areas where the disease is endemic, and at least 2 to 3 million people are estimated to be affected (2).

Initial symptoms of Kashin-Beck disease in pre-adolescents and adolescents include stiffness, swelling, and pain in the interphalangeal joints of the fingers. Levander reports that the disease is reversible at this point (26). As the disease progresses, generalized osteoarthritis occur in the elbows, knees, and ankles, with locking of joints often occurring in many cases by the third decade (26, 47). Impaired bone development as a result of degeneration and necrosis of the bone's epiphyseal growth plate has been suggested by Ge and Yang (48). The joint and articular cartilage changes are the source of its alternate name in China, Dagujie disease or "enlarged joint" disease (26). While selenium deficiency is accepted as a cause of the disease, all selenium deficient areas do not exhibit the disease, implicating other factors as necessary for full development of true Kashin-Beck disease. Kashin-Beck disease has been suggested by Suetens and colleagues (49) to result from oxidative damage to cartilage and bone cells when associated with decreased antioxidant defense.

Selenium deficient and fluvic acid supplemented mice, considered by Yang and colleagues (50) to be an animal model of Kashin Beck-disease, had irregular bone formation and substantial reduction in the number of lysine residues in type I collagen from bone and type II collagen from cartilage. A lower melting point of type I collagen from bone, and lower breaking force of bone were also found in the animals. In a study aiming to understand the role of selenium deficiency in the etiology of Kashin-Beck

disease, Sasaki and colleagues (51) observed decreased femur ash weight, and a decrease in the sulfotransferase activity (involved in glucosaminoglycan synthesis) in 3 to 11 month selenium-deficient rats.

Suetens and colleagues (52) also suggest a second mechanism whereby normal stimulation of bone remodeling by thyroid hormones may be blocked by certain mycotoxins in the fungal contaminated grain. Chasseur and colleagues (45) did not observe a decrease in the prevalence of Kahsin-Beck disease by iodine supplementation when a fungal species (*Alternaria sp.*) was present, and thus suggested a competitive binding of a mycotoxin to a thyroid hormone receptor in bone cells. Fluvic acid, an environmental contaminant involved in the etiology of Kashin-Beck disease, has been shown to covalently bind with iodine (53), suggesting that fluvic acid may interfere with iodine bioavailability.

Arthritis

Arthritis refers to conditions affecting the joint and surrounding tissues. The most common forms of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA) (12).

Osteoarthritis is a degenerative joint disease involving the hips, knees, neck, lower back, or hands that is prevalent in many parts of the world. It results in pain, lameness, and disability (54). OA usually develops in response to mechanical trauma from repetitive motion, from excess body weight, from heavy physical work or from high intensity sport performance (54, 55). The trauma thins and degrades the cartilage that cushions the ends of the bones. The bones then rub together, causing a grating sensation.

Reduced joint flexibility as well as swelling due to development of bony spurs (osteophytes) or an effusion caused by synovial fluid accumulation are also observed in OA (54) This form of arthritis affects 12.1% of U.S. adults or 20.7 million people (12). Osteoarthritis was the second most common diagnosis, after chronic heart disease, leading to social security disability payments due to long-term absence from work (56).

In osteoarthritis the synovium has been shown to be inflamed with concomitantly increased production of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin 8 (IL-8) (57). These cytokines induce the production of metalloproteinases that contribute to cartilage destruction (54, 57). Risk factors include age, trauma, occupation, exercise, gender and ethnicity, genetics, obesity, and diet (54).

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease that involves peripheral joints in the hands, wrists, elbows, shoulders, knees, feet, and ankles. It is characterized by a non-specific, symmetrical inflammation of peripheral joints, resulting in a progressive destruction of articular and periarticular joints and structures (58) as well as local bone loss (59). Symptoms include pain, swelling, stiffness, deformity, and reduced mobility and function (12). Agglutination tests are used to detect antibodies to altered gamma-globulins (rheumatoid factor) and immunoglobulin M (IgM) rheumatoid factor is found in about 70% of RA cases (58). Osteoporosis is well recognized in RA. RA is associated with local and systemic bone loss with involvement of cytokines such as the RANK/RANKL system and TNF-α, which promotes local and systemic osteoporosis (10, 60).

The causes of arthritis including osteoarthritis and rheumatoid arthritis have not been completely elucidated. Reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radical and hypochlorous acid, as well as reactive nitrogen species (RNS) such as nitric oxide and peroxynitrite are believed to contribute significantly to tissue injury and the resulting inflammation in RA (61). Cytokines such as IL-1, IL-6, TNF-α and cyclo-oxygenases are also implicated in the etiology of inflammatory joint disease, and diseases related to the bone loss, including OA and RA (62). Prevention and treatment measures for RA include the use of non-steroidal anti-inflammatory drugs (54), niacinamide (63), estrogen and 17-β estradiol (64), methotrexate (65), and cyclo-oxygenase-2 selective inhibitors (54).

Osteoporosis

Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and subsequent fracture, especially of the hip, spine and wrist, although any bone can be affected (66). Osteoporosis is a major public health problem for an estimated 44 million Americans, or 55 percent of the people 50 years of age and older. In the U.S, 10 million people are estimated to already have the disease and almost 34 million more are estimated to have low bone mass, placing them at increased risk for osteoporosis (17). Of the 10 million Americans estimated to have osteoporosis, 80% are women and 20% are men. Osteoporosis causes more than 1.5 million fractures annually, including: approximately

700,000 vertebral fractures, over 300,000 hip fractures, 250,000 wrist fractures, and 300,000 fractures at other sites (17).

Risk factors for osteoporosis include, gender, age, family history, body size, ethnicity, hormone levels, inactivity, smoking, alcohol consumption, certain medications such as glucocorticoids and aluminum-containing antacids, hyperthyroidism, sex hormone deficiency, genetic factors, hyperparathyroidism, multiple myelanoma, transplantation, chronic kidney, lung, and intestinal diseases, and inadequate intake of calcium and vitamin D (67).

Prevention and treatment options for osteoporosis include calcium and vitamin D supplementation, changes in diet and life style behaviors, exercise, estrogen replacement therapy, SERMs (selective estrogen receptor modulators) such as raloxifen and tibolone (68); bisphosphonates, calcitonin, and teriparatide (68).

Relationship between Arthritis and Osteoporosis

Even though some studies have shown an inverse association between osteoporosis and osteoarthritis (7), the occurrence of osteoporosis in osteoarthritic patients is well documented. Hip OA was not associated with increased bone density in the femoral head or the neck of the femur in middle-age men (69). Higher trabecular number accompanied with thinning and fenestration in dense cancellous bone areas of proximal tibia was observed in patients with OA (9), suggesting the trabecular bone in this region to be osteoporotic. In the elderly, knee osteoarthritis was associated with increased risk of vertebral and non-vertebral fractures independent of bone density and

postural stability (19). A lower modulus of elasticity was observed in the femoral neck of patients with OA compared to osteoporotic patients (70). In OA, decreased mechanical strength of subchondral bone due to immature collagen fibers, decreases in proline cross-links, and reduced mineralization has been reported by Bailey and colleagues (11). A hallmark of osteoarthritis is the degeneration of joint cartilage. The volume of tibia knee cartilage in older adults was positively associated with total body bone mineral density in men and women independent of age, BMI, tibia bone area, and physical activity (71). Forslind and colleagues (72) found a significant positive association between reduced bone mass and radiological joint damage in women with recent RA at baseline and after 2 years, and they suggested a common mechanism for the development of bone loss and joint destruction.

Indicators of Bone Quality

Bone quality is defined as a set of characratics influencing bone strength (72). These characteristics include the structural properties (geometry and microarchitecture) and the material properties (collagen and mineral), which are affected by turnover (72).

Bone quality may be determined by several factors, including its properties that affect its strength. The geometry of bone consists of the size and shape of bone. The size of bone is a determinant of bone strength. Reduced bone mineral content and smaller vertebral bone were seen in women with spinal fracture (74). The structural arrangement of bone (microarchitecture) is also strongly related to bone strength (75)

Collagen content and structure also affect bone quality. There is a reduced concentration of cross-links in bones from patients with osteoporosis (72). Collagen has smaller influence on the stiffness of bone, but improves bone toughness through intramolecular cross-links (73). Collagen fiber orientation explained 71% of variation in bone tensile strength in a linear regression analysis (76). Bone is formed by the production of a protein framework that hardens when calcium and phosphorus are deposited on it. Bone strength partly depends on this mineral deposition (76). Apart from bone mineral content, the perfection and the maturity of mineral crystals are also important determinants of bone strength (76).

Bone Mineral Density

Bone mineral density refers to the amount of minerals in a three-dimensional volume of bone. However, bone mineral density is also measured by dual energy X-ray absorptiometry (DEXA) based on a two-dimensional area. There is a strong correlation between fracture risk and low bone mass. The WHO has developed diagnostic categories that compare a person's bone density with the peak value for a healthy young adult using a T-score (66). A normal bone is indicated when bone mineral density or bone mineral content is within 1 standard deviation (SD) (+1 SD or -1 SD) of the young adult mean value. A low bone density (osteopenia) is indicated by a bone mineral density or bone mineral content of 1 to 2.5 SD below the young adult mean (-1 to -2.5 SD). Osteoporosis is defined by a bone density or bone mineral content of 2.5 SD or more below the young adult mean (>-2.5 SD). Severe osteoporosis is said to exist when bone mineral density or

bone mineral content is more than 2.5 SD below the young adult mean and there have been one or more fractures due to osteoporosis (66).

Bone Microarchitecture

Bone mass is not the only property that affects bone strength. Bone microarchitecture is also a determining aspect of bone strength and an essential component affecting the assessment of bone mechanical properties (75). Bone microarchitectural parameters such as trabecular thickness (TbTh,) trabecular number (TbN) , trabecular separation (TbSp), connectivity of the trabeculae, as well as width and porosity of the cortical bone seem to be determinants of bone fragility independent of bone density (77). Trabecular number and thickness decrease in aging (74). Silva and Gibson (74) developed an aged model of human vertebral trabecular bone by concurrently reducing the trabecular thickness and trabecular number of a young model with intact values. The reduction of trabecular thickess and number of the bone led to a decreased modulus of elasticity and strength of the model. When the bone mass of the aged model was restored by increasing the trabecular thickness, but not the trabecular number, the strength increased by 60%, but was still only 37% of its intact value, indicating that that a full recovery of bone strength requires a regeneration of the lost trabecular number (74).

The structural model index (SMI) is a 3-D bone structural parameter that quantifies the plate versus rod characteristics of trabecular bone (78, 79). An SMI of zero (0) pertains to a purely plate-shaped bone, and a value of 3 indicates a purely cylindrical rod-like structure, and values between designate mixtures of plate and rod forms (79).

Human tibial cancellous bone changes with aging from plate-like to rod-like, indicating a deterioration of the structure of bone with aging (78).

Microarchitectural deteriorations such as decreased trabecular connectivity have been related to increased possibility of fracture and one of the positive effects of parathyroid hormone (PTH) on bone is the restoration of moderate lost trabecular connectivity (80). Even though connectivity is believed to be important in the biomechanics of bone in osteoporosis (80), there is not much evidence to support this hypothesis in healthy bone. Kabel et al. observed an inverse association of connectivity with bone stiffness (81). Connectivity seems to be inversely associated with elastic properties of cancellous bone of people with no known bone disorders.

Degree of anisotropy (DA) refers to the extent to which a material has different properties in different directions (82, 83). Poor bones seem to have higher DA values. An analysis of porous hydroxyapatites with an anisotropic characteristic intended for the bone-graft market found the specimens to possess lower compressive moduli than isotropic specimens with the same apparent densities (84). Similarly, Chappard and colleagues (83) found higher DA values in the bone of subjects with vertebral fracture than in control subjects. Furthermore, an improvement in the structural properties of the vertebra (L1 and L2) of dogs following alendronate treatment was accompanied with a decreased degree of anisotropy in the bone specimens (85).

Bone Biomechanical Properties

Biomechanical properties of bone are those properties of bone that are associated with elastic and inelastic reactions when a force is applied. They also involve the relationship

between stress and strain (86). Examples of biomechanical properties of bone include all kinds of strength (compressive strength, tensile strength, and shear strength) and strain, modulus of elasticity (stiffness), hardness (86), and fatigue life (fracture of bone under repetitive stress) (86, 87). Bone strength depends on bone matrix volume, bone microarchitecture, and the degree of mineralization of bone (88). The more the cancellous bone is mineralized, the higher its stiffness. Young human bone is less mineralized than mature bone (88). Ciarelli and colleagues (89) suggest that both low and high mineralization may be detrimental to bone mechanical properties, with low mineralization levels causing reduced stiffness and strength and high mineralization leading to reduced fracture toughness due to increased brittleness.

Bone mechanical properties can be determined using three or four point bending techniques and fatigue tests for long bones (82, 87, 90). The compressive tests are more appropriate for small and cubic samples of trabecular bone (88).

There is not much information about the effect of iodine and selenium on the biomechanical properties of bone in growing individuals. However, retarded growth and lower breaking force of the tibia have been observed in selenium-depleted mice compared to the controls (50). Growth retardation and osteopenia were seen in second generation selenium-deficient male rats (4). Methamizol-induced hypothyroidism during postnatal development leads to decreased bone length and biomechanical competence (measured as Vickers microhardness) of the femurs and humeri in birds (20).

Biochemical Markers of Bone Metabolism

Bone density determination is valuable for evaluation of patients at risk for osteoporosis, but it does not give any information about the rate of bone turnover, therefore, supplementing bone density information with measurement of markers of bone turnover may enhance the prediction of fracture risk. Bone markers indirectly measure bone cell activities (79). Biochemical markers of bone metabolism are byproducts that are released into the blood stream and urine during the process of bone remodeling, which involves bone resorption and bone formation (91).

Serum and urine tests can detect these markers and provide information about the rate of bone resorption and formation. Bone formation can be evaluated using serum non-specific alkaline phosphatase (ALP), bone-specific alkaline phosphatase (B-ALP), osteocalcin, carboxyterminal propeptide of type I collagen (PICP), and aminoterminal propeptide of type I collagen (PINP) (91). Indicators of bone resorption such as cross-linked C-telopeptide of type I collagen, tartrate resistant acid phosphatase (TRAP), N-telopeptide of collagen cross-links (NTx), and C-telopeptide of collagen cross-links (CTx) can be determined in serum. Other bone resorption markers such as hydroxyproline, free and total pyridinoline, free and total deoxipyridinoline as well as NTx and CTx can be assessed in urine (91).

Bone specific alkaline phosphatase is an osteoblast product that is believed to be an essential enzyme for bone mineralization (91). Both bone specific and tissue non-specific alkaline phosphatase can promote mineralization by hydrolyzing a variety of phosphate compounds to make inorganic phosphate available for bone mineralization

(92). It has also been suggested that alkaline phosphatase may destroy inhibitors of mineral crystal growth and behave like a calcium binding protein (93).

Osteocalcin (bone gla-protein) is a peptide synthesized and secreted by osteoblasts during bone formation. It is mostly incorporated into bone matrix with some escaping into the blood; therefore, osteocalcin is accepted as a marker of bone formation. However, osteocalcin is also released from bone to the circulation during bone resorption. Therefore osteocalcin is more a marker of bone turnover than of bone formation (91).

Amino-teminal and carboxyterminal propeptide of type I collagen direct the assembly of the collagen triple helix and are separated from the newly formed collagen molecules and released into the circulation (94). Therefore, their concentration in serum may be an index of bone formation. However these byproducts of collagen synthses are also produced by other type I collagen-containing tissues such as the skin (94). Serum N-terminal and C-terminal propeptide of type I collage are less useful than ALP and OC as indicators of bone formation (94)

TRAP (tartrate resistant acid phosphatase, also known as type-5 acid phosphatase) is an iron-containing protein produced in different tissues with acid phosphatase activity and is one of the most abundant enzymes in osteoclasts (95). Serum TRAP is used as a biochemical marker of osteoclastic activity and bone resorption (96). However, it lacks specificity because other cells that are not related to bone such as erythrocytes and platelets also release TRAP into serum (96).

NTx and CTx are degradation products of type I collagen, mainly produced by cathepsin K. Pyridinoline, deoxypyridinoline, and cross-linked C-telopeptide of type I collagen (ICTP) are also degradation products produced by matrix metalloproteases (97).

Pyridinoline and deoxypyridinoline are the two cross-links present in the mature form of type I collagen. Urine levels of pyridinoline and deoxypyridinoline correlate with the breakdown of collagen released from bone matrix by the osteoclasts (98). This cross-linking structure, which is unique to collagen and elastin molecules, creates bonds between polypeptide chains in collagen fibrils to enhance stability. Pyridinoline and deoxypyridinoline cross-links can be excreted free or still bound to the peptide chains and either form can be measured. Deoxypyridinoline is the more abundant cross-link in bone collagen and is generally the one measured (98).

Factors that Influence Bone Quality

There are several factors that may influence bone quality. Those factors include gender (99), age, family history, ethnicity, hormone levels, nutrition (17), the use of some drugs, and some chronic diseases. However, the discussion in this study is limited to how bone quality is affected by gender, growth hormone levels, and selected dietary factors.

Gender

Women are at more risk for poor bone status because they have lighter bone, thinner bones and lose bone rapidly after menopause (12). Even though osteoporosis is more common in old women than men, Baxter-Jones and colleagues (99) found the sex difference in adolescents' bone mineral content (BMC) to be debatable as the difference is generally explained by anthropometric difference. These authors found no difference between boys and girls aged 8-19 years old with respect to BMC in the spine. The higher

BMC in males despite a significance of $P<0.05$, they considered to be less than the measurement error. Jarvinen et al. (100) observed a lower responsiveness to mechanical loading and higher bone density in female than male rats at puberty, and they suggested that estrogen causes deposition of an extra stock of minerals in female bone at this age and that this extra condensation of female bone seems to persist in adulthood in rats. Similarly, a longitudinal study of Canadian children showed a higher cross-sectional area (an index of bending strength) of the femoral neck in boys before peak height velocity (PHV), but for girls, the cross-sectional area was higher after PHV (101). After menopause, there is a greater decrease in trabecular volumetric bone mineral density in women than men at central sites but they are similar at peripheral sites (102).

Growth Hormone (GH)/Insulin-Like Growth Factor 1 (IGF-1)

Normal growth and development in young mammals depends on many factors including growth hormone (GH), thyroid hormones and nutritional status. The growth promoting effect of GH is believed to be mediated in part by IGF-1 (103). Growth hormone causes the liver (and to a less extent other tissues) to produce several small peptides called somatomedins (at least four somatomedins have been isolated), that in turn have potent effects of increasing all aspects of bone growth (104). The most important of these is somatomedin C, also called IGF-1, which in turn regulates GH secretion through a feedback stimulation of somatostatin (103). Both growth hormone and IGF-1 influence bone growth and increase bone mineral content and bone mineral density (105). The African pygmies have a congenital inability to synthesize enough IGF-1 and have reduced amounts of plasma IGF-1, while their plasma GH is either

normal or high (104). This might account for the short stature of these people. The bioavailability and bioactivity of IGF-1 is modulated by IGF-binding protein-3 (IGFBP-3), a GH dependent glycoprotein and the main carrier for IGF-1 in blood (103).

Yanovski and colleagues (105) investigated bone status and the levels of IGF-1 and IGF binding proteins in white and African American girls and found BMC, BMD and free IGF-1 to be higher in African American than white American girls, while IGF-binding protein-3 was similar or lower in African American girls. In the study, free IGF-1 was positively correlated with BMC and BMD in both groups.

Nutrition

Several nutritional and dietary factors have been shown to influence bone health. Some of the minerals that influence bone quality include calcium (67), phosphorus (106), magnesium, zinc (107), iron (108), copper (109), manganese (110), iodine and selenium (3-5, 18). Some of the vitamins associated with bone status include vitamin K (111, 112), vitamin D, vitamin C (106), and vitamin E (113).

In OA, cartilage degeneration is associated with inflammation of the synovial membrane and the release of reactive oxygen species (114). Therefore, vitamins and minerals as well as some food-derived phytochemicals exhibiting antioxidant properties are believed to have important roles in the prevention and treatment of the disease.

Vitamin C is necessary for collagen cross-linking, and bony defects are recognized as a part of the scurvy syndrome due to a weakening of the collagenous structure of bone (107). Vitamin C along with other antioxidants may protect bone against oxidative stress from smoking. High intake of vitamins C and E (but not β-carotene and selenium) decreased the odds ratio of hip fracture in current smokers (115).

Bone abnormalities associated with scurvy are believed to be caused by an impairment of hydroxylases that catalyse vitamin C dependent hydroxylation of prolyl and lysyl residues, important steps in collagen synthesis prior to the crosslinking of collagen molecules by the copper-dependent lysyl oxidase (116).

Lathyrism is a crippling disease originating from the consumption of some plant species (e.g. *lathyrus sativus*) containing toxic compounds (117). Defects in the synthesis of collagen due to inhibition of lysyl oxidase, a copper dependent enzyme, have been suggested as a cause of bone deformity accompanying the disease (118). Vitamin C administration reduced serum cytokines and improved collagen structure in rats with experimentally induced lathryrism (118).

Vitamin E and 17β estradiol are believed to accumulate in plasma membranes and to decrease membrane fluidity leading to protection against lipid peroxidation (119). Vitamin E in physiological doses prevented chondrocyte lipid peroxidation and cartilage matrix protein degeneration in rabbit chondrocytes treated with hydrogen peroxide and lipopolysaccharides (113). Vitamin E treatment decreased thiobarbituric acid reactants (TBARS) release from cultured bovine articular chondrocytes exposed to hydrogen peroxide (119). The combination of selenium as sodium selenite with vitamin E and vitamin C completely restored the structural alteration of long bone tissues in an osteoporotic rabbit model, while a complete restoration was not possible using the combination of only the two vitamins (120). Some dietary factors such as the consumption of food containing phytochemicals with antioxidant properties or having estrogen-modulating activities such as soy protein

and soy isoflavones (121-125) have been shown to improve bone quality.

Prevention and Treatment of Osteoporosis and Osteoarthritis

Prevention and treatment options for osteoporosis consist of changes in diet and lifestyle, as well as the use of drugs and hormones that act by inhibiting osteoclast-mediated bone resorption. To date, most of the drug and hormone options have some serious health-damaging side effects. The treatment and prevention options include calcium and vitamin D, changes in diet and life style behaviors, exercise, and medications such as estrogen, raloxifene (a selective estrogen receptor modulator or SERM), bisphosphonates (such as alendronate, residronate, and zoledronate), calcitonin, and teriparatide, (68, 126). Both calcium and vitamin D are commonly used in the treatment of osteoporosis. Although neither calcium nor vitamin D alone has been shown to prevent osteoporosis in postmenopausal women, the combination is beneficial (68). Estrogen reduces the incidence of fracture (126). In animal models, estrogen deficiency leads to an increase in osteoclast formation and bone loss. Inhibition of osteoclastogenesis is the major means by which estrogen prevents bone loss through diminishing the production of interleukin-1, interleukin-6, and TNF-α (127). However, estrogen increases the incidence of coronary artery diseases, stroke, breast cancer, and thromboembolic phenomena (126). Raloxifene, a SERM, which has been shown to prevent bone loss and fracture in postmenopausal women, inhibits growth of uterine tissues and decreases the incidence of breast cancer, but still increases the incidence of thromboembolic events (128). Calcitonin, via its receptor, inhibits osteoclastic bone resorption. However, calcitonin downregulates calcitonin receptor, and this may reduce

its effectiveness (129). Bisphosphonates act by inactivating osteoclasts to increase bone mineral density and prevent fracture, whereas long-term treatment with bisphosphonates causes microdamage accumulation and increased susceptibility to fracture in dogs (126).

The agents that have been used in some clinical trials as new or alternative drugs for the treatment of osteoporosis include androgens, growth hormone, insulin-like growth factor-1, and strontium ranelate. The drugs that are being developed to inhibit bone resorption include the OPG/RANKL/RANK system and cathepsin K inhibitors, vitronectin receptor antagonists, cytokines and growth factors. New drugs to promote bone formation include the commonly used lipid-lowering statins and parathyroid hormone (PTH) (Teriparatide 1-34) (68). Parathyroid hormone is approved for the treatment of osteoporosis in men who are at high risk of fracture (17).

Other new drugs that are under investigation to treat bone-resorption diseases include the inhibitor of avB_3 integrin, an adhesion receptor that mediates attachment of osteoclasts to bone surface, and osteoprotegerin (OPG) (126). A single dose of OPG for two to three months to postmenopausal women inhibited both differentiation and activation of osteoclasts, and inhibited bone resorption in the women (130).

Prevention and treatment options for arthritis include exercise, diet, and the use drugs with analgesic and/or anti-inflammatory properties. The drugs used include paracetamol (54), estrogen (64), methotrexate (MTX), glucocorticoids (12), non-steroidal anti-inflammatory drugs (NSAIDs), and cyclo-oxygenase-2 (cox-2) selective inhibitors (54).

Methotrexate (MTX), a folate antagonist, is used in the treatment of RA but treatment is often discontinued because of side effects due to folate antagonism (65).

Whittle and Hughes propose that folic acid supplements be prescribed routinely to all patients receiving MTX. They recommend a dosing schedule of 5 mg of oral folic acid given on the morning following the day of MTX.

Glucocorticoids may cause a secondary osteoporosis that can be alleviated by bisphosphonate treatments (68), but these have their own adverse effects with long term treatment as mentioned earlier in this section. Cox-2 selective inhibitors are believed to increase the risk for myocardial infarction (54).

Most of the treatment options for both arthritis and osteoporosis have serious side effects (54). Therefore scientists are still looking for safe and efficient alternatives.

The effects of iodine and/or selenium on bone are being investigated for the same reason of finding efficient, safe and sustainable solutions for bone diseases. Potential links between iodine and/or selenium deficiencies and bone health have not been extensively studied. The following section presents some of the existing literature on the effects of iodine and/or selenium on bone.

Effects of Iodine and Selenium on Bone

Effects of Iodine on Bone

The best known role of iodine in mammals is for the synthesis of thyroid hormones. Before puberty, thyroid hormones may be the major hormone required for normal maturation of bone (131). Thyroid hormone (T_3) is an important determinant of postnatal somatic growth and skeletal development and a primary regulator of bone and mineral metabolism in the adult (132-133). Untreated childhood hypothyroidism leads to

profound growth retardation and delayed bone maturation. Linear growth is almost completely stopped, but can be resumed quickly by replacement of thyroid hormones to produce a rapid catch-up growth (131). In contrast, whereas thyrotoxicosis in children is associated with increased bone turnover and an accelerated growth rate, the advanced skeletal maturation that also occurs may lead to short stature (134)

The mechanism whereby iodine and selenium may affect bone is not clear. However, delayed bone maturation has been observed in sheep fed iodine-deficient diets (135). Thyroid hormone (T_3) is believed to stimulate bone mineralization and cartilage matrix proteoglycan production (30). T_3 stimulates production of IGF-I in human osteoblasts (136) and plasma IGF-1 regulates bone growth and density in mice (137). Lower serum levels of IGF-1 and IGF-binding protein-3 were found in children living in an area of severe iodine deficiency compared to children living in an area with mild iodine deficiency (138). On the other hand, hyperthyroidism is believed to cause bone loss by mechanisms that involve uncoupling of osteoblast and osteoclast activities (30). This indicates that normal bone turnover requires a physiological euthyroid status. More studies involving patients with thyroid hormone resistance, cell culture studies, and molecular studies have shown that thyroid hormones have important effects on skeletal growth and development.

Studies of Bone in Patients with Thyroid Hormone Resistance. The study of resistance to thyroid hormone (RTH) offers confirmation of the importance of T_3 for skeletal growth and development. RTH is a condition caused by a mutation in thyroid hormone receptor β gene (T_3 receptor α is normal in all cases) that generates receptors which display impaired responses to T_3 (131). The condition is characterized by different

degrees of tissue hypothyroidism in the presence of increased free T_4, free T_3, and TSH (131). Short stature occurs in 16% of affected children with delayed bone maturation in 47% (134). However, it is also believed that 84% and 53% of the children respectively have normal growth and bone maturation. The reason for this variability is partly explained by some genetic evidence suggesting fundamental roles for both thyroid hormone receptor α and β during growth and skeletal development. This genetic evidence is elaborated in the following section.

Studies of Genetically Modified Animal Models. In TR-α (thyroid hormone receptor alpha) knockout mice, there is growth arrest and delayed bone maturation, with disorganization of epiphyseal growth plate chondrocytes, fewer hypertrophic cells in the growth plate, and delayed cartilage mineralization and bone formation (30, 139). The growth arrest and delayed bone maturation were reversed after T_3 replacement of 1μg/day for one week (139). In contrast, in T_3 receptor β knockout mice, there is disruption of the pituitary-thyroid feedback axis and profound hearing loss, but little effect on skeletal development and growth (140). Harvey and colleagues (30) agree and suggest that TR-α_1 knockout mice lack expression of TR-α_1, but TR-α_2, and TR-β are maintained and the skeletal development is normal. This suggests that TR-β is functional in bone in the absence of TR-α_1. The combination of TR-α_1 and β mutations to produce TR-α_1 and β double knockout mice resulted in growth retardation and delayed bone maturation (141). The TR-α_1 and β double knockout mice also have been shown to be growth hormone and IGF-1 deficient (30). Growth hormone (GH) replacement reverses some of the growth retardation but does not affect the defective ossification (142),

indicating that T_3 may exert direct effects on growth plate chondrocytes in addition to effects that may be mediated by GH and IGF-1 (142).

Thyroid Hormone in Cell Culture Studies. There is evidence documenting the expression of T_3 receptors in osteoblasts, chondrocytes, and osteoclasts indicating that direct T_3 effects on skeleton are important (30, 131). Cultured human fetal epiphyseal chondrocytes have been shown to possess specific nuclear binding sites for T_3 with appropriate binding affinities in the nanomolar range (143). These cells respond to T_3 *in vitro* with increased alkaline phosphatase activity after nine days of treatment. Similarly, treatment of rat epiphyseal chondrocytes with T_3 *in vitro* for 96 hours increased alkaline phosphatase activity and IGF-1 receptor mRNA expression, in addition to decreasing cell proliferation (144). Thyroid hormone receptors are expressed at sites of intramembranous and endochondral bone formation in a variety of species. Thyroid hormone receptor α_1, α_2, and β_1 mRNA and proteins are expressed in osteoblasts and osteocytes and in reserve and proliferative zones of epiphyseal growth plate chondrocytes (30). High affinity nuclear-binding sites for T_3 and thyroid hormone receptor mRNA and proteins have been identified in primary cultured osteoblasts, osteoblastic bone marrow stromal cells, and growth plate chondrocytes (30).

T_3 receptors in human osteoblasts and osteoclasts at sites of active bone remodeling have been demonstrated by immunohistochemistry (145). This may suggest that both osteoblast and osteoclast activities are affected by abnormalities in thyroid status leading to an imbalance in bone turnover. It has been suggested that rat, mouse, and human osteoblasts are primary T_3 target cells and that osteoclast responses may be secondary, or coupled to these primary T_3 actions on osteoblasts (131). T_3 has also been

shown to stimulate osteoclastic bone resorption, but this effect is believed to be mediated by T_3-responsive osteoblasts as osteoclasts probably do not express functional thyroid hormone receptors (30). T_3 may stimulate osteoclastic bone resorption through the stimulation of the production of IL-6 and IL-8, cytokines that have been implicated in osteoclast synthesis and function (146).

Studies using chondrocytes from different species showed that T_3 regulates chondrocyte proliferation and the organization of chondrocyte columns, promotes terminal hypertrophic differentiation and induces calcification of cartilage matrix (30). The induction of terminal hypertrophic chondrocyte differentiation by T_3 may be by promoting the expression of cyclin-dependent kinase inhibitors, which could stop cell cycle progression of proliferating chondrocytes to facilitate the onset of terminal differentiation (30). Williams and coworkers (131) suggest that T_3 may induce terminal hypertrophic chondrocyte differentiation by inhibiting the expression or the action of Indian hedgehog (Ihh) and Parathyroid/Parathyroid hormone related peptide (PTH/PTHrP) receptor both of which act as signaling molecules to maintain chondrocyte proliferation and delay hypertrophic differentiation, and both of which are considered as potential T_3 target genes in the epiphyseal growth plate (147). The prehypertrophic chondrocytes that are committed to the hypertrophic phenotypes express and secrete Ihh, which regulates the rate of hypertrophic differentiation (131). Ihh acts directly on cells within the adjacent perichondrium to stimulate the production and release of PTHrP. PTHrP is secreted and acts (via its receptor) on prehypertrophic uncommitted proliferative chondrocytes to maintain cells in a proliferative state and to delay or inhibit the onset of hypertrophy (131, 147).

The terminal differentiation of proliferating chondrocytes into hypertrophic cells is the primary event that characterizes endochondral ossification and linear growth. An increase in hypertrophic cell volume correlates with both the rate of long bone growth and the associated mineralization of cartilaginous matrix (131). Williams, Robson, and Shalet (131) proposed the following mechanism involving GH, IGF-1, and T_3 during endochondral ossification: Growth hormone stimulates differentiation of resting zone chondrocyte precursor cells, IGF-1 stimulates their subsequent clonal expansion, and T_3 terminates the clonal expansion and induces the terminal hypertrophic chondrocyte differentiation. As the epiphyseal growth plate is sealed by a mineralized bone marrow (148), it is also possible that T_3 plays an important role in promoting vascular invasion and osteoblast migration to facilitate new bone formation (30)

Hypothyroidism in rats causes growth retardation due to growth plate dysgenesis in which hypertrophic chondrocyte differentiation does not continue (30). There are also abnormalities in growth plate structure with abnormal proliferating chondrocyte columns and cartilage matrix, as well as impaired vascular invasion of the primary spongiosium (149).

Effect of Selenium on Bone

Selenium is required for T_3 synthesis and thyroid hormone homeostasis (24). Therefore it may indirectly protect bone through thyroid hormones. Physiological doses of selenium may also directly protect bone through its antioxidative properties. Selenium is an essential component of the enzyme glutathione peroxidase as well as thioredoxine reductases at the active center of which selenium catalyzes reduction of hydroperoxides

produced from oxidized species such as superoxide and lipoperoxides (150). Thus, it may protect bone and cartilage cells against oxidative damage (151). Dreher and colleagues (151) demonstrated a selenite-dependent GSH-Px mediated antioxidative defense of fetal human osteoblasts against hydrogen peroxide and reactive oxygen species. In the study it was shown that osteoblasts express an antioxidative system to protect themselves against H_2O_2 after bone resorption is mediated by osteoclasts. Therefore lack of GSH-Px may lead to impaired osteoblast function and could be involved in metabolic bone diseases.

Selenium in Cell Culture Studies. One of the proposed mechanisms for the protective effects of estrogen on bone is through the augmentation of oxidant defense to lower the concentration of reactive oxygen (ROS) species within bone cells (152). ROS in high concentrations not only damage many cell constituents, but also affect many signaling proteins such as TNF-α and NF-kβ which are essential for osteoclasts at levels considerably lower than those that cause injury (152). Cytokines such as IL-1 up-regulate their own activity by inducing the production of oxidants that may inhibit cytosolic enzymes. Selenium as an essential component of glutathione peroxidases and thioredoxin reductases helps keep cytosolic enzymes in their reduced form, therefore adequate selenium nutrition may protect bone by down-regulating cytokine signaling (63).

Selenium in Epidemiological Studies. Selenium concentrations are relatively low in the serum of patients with RA when compared with healthy controls. Plasma selenium levels were significantly (<0.001) lower in patients with RA than in normal, healthy controls (153). Oxidative stress as measured by the levels of lipid peroxidation products

such as hydroxiperoxides and thiobarbituric acid reactive substances (malonaldehyde also called malondialdehyde) are elevated in the synovial fluid and serum from patients with RA with a concomitant decrease of plasma antioxidants (vitamin E and β-carotene) (154). Kashin-Beck disease has been suggested to result from oxidative damage to cartilage and bone cells when associated with decreased antioxidant defense (52). Kamanli and colleagues (155) found lower concentrations of plasma GSH-Px, catalase, glutathione, β-carotene, and vitamin E in patients with RA as compared with the controls. In the same study, significantly higher concentrations of C-reactive protein, lipid peroxidation markers, and rheumatoid factor were found in patients with RA than in controls.

Selenium in Human Intervention Studies. Even though selenium seems to be important in the prevention and treatment of bone diseases in animal models, most of the human intervention studies with selenium have not shown beneficial effects of selenium supplementation in preventing bone diseases. A selenium-containing organic compound called PZ51, which has a GSHPx-like effect in catalyzing glutathione-dependent reduction of hydroperoxides, has anti-inflammatory activity (150). Ebselen A, another selenium containing compound has been shown to block IL-1 mediated proteoglycan degeneration, and to reduce prostaglandin E_2 (PGE_2) release from the cartilage culture of bovine nasal septa (156)

However, Peretz and coworkers (157) did not find any clinical benefits of selenium supplementation on 55 patients with moderate RA in a double blind, placebo-controlled trial. The authors suspected a placebo effect (that could be explained by an improvement in both groups of patients and controls) on the intervention trial. In addition, selenium supplementation with normal or slightly increased intakes of Se had

no effect on serum levels of somatotropin (growth hormone), IGF-1, and IGF binding proteins 1 and 3 in healthy women (158). Moreover, Moreno-Reyes and colleagues (2003) did not find any beneficial effect of selenium supplementation for 12 months on established Kashin-Beck disease in rural Tibetan children (5-15 year old) after iodine deficiency was corrected. Similar results had been found by Tarp et al. (159) in a long-term selenium supplementation of RA patients and controls with selenium. In Tarp's study, even after 26 weeks of treatment, patients with RA had granulocyte GSHPx activity significantly lower than those of controls, regardless of the nutritional selenium status. The unresponsiveness of granulocyte GSHPx to selenium supplementation has been suggested to explain the predominantly negative effect seen with selenium in patients with RA (150).

Selenium Studies in Animal Models. Selenium deficiency has been shown to decrease plasma osteocalcin, plasma IGF-1, bone mineral density and bone mineral content of growing male rats (4). Dietary vitamins (E, C, A, B_6, B_2) and selenium led to a significant increase in the expression of GSH-Px and Cu/Zn-superoxide dismutase in the articular cartilage and the synovium, and a significant decrease in incidence of mechanically-induced osteoarthritis in STR/1N mice (28). Ebselen, a novel organo-selenium coumpound that catalycally inactivates peroxides in vitro in a manner similar to that of GSH-Px, has been found to inhibit peroxide-dependent inflammation. It also inactivated leukotriene B_4 generated by pig leukocytes in vitro by isomerizing the eicosanoid to its biologically inactive 6-trans isomer (160). The authors also suggest that ebselen was an effective inhibitor of monoarthritis induced in mice with amidated glucose oxidase.

However, excess selenium caused reduced production of IGF-1 (somatomedin C) and growth retardation in young rats (161). Selenium treatment (3.3 mg sodium selenite per liter of drinking water for 35 days induced a significant reduction in circulating IGF-I and a significantly shorter tibia length in male rats (162). Both selenium deficiency and selenium toxicity for 12 to 14 weeks led to a decreased stiffness (modulus of elasticity determined by a tensile test) of the tibia and femur of weanling rats (75).

CHAPTER III

METHODS

The methodology section of this paper consists of presentation of an experimental design involving the dietary manipulation of male and female rats as well as the determination of the effect of iodine and/or selenium status. It also shows the assessment of growth, biochemical, physical, microarchitectural, and mechanical properties of the bone of rats and lipid peroxidation status of the rats. A statistical analysis of main outcome measures ends the section.

Animal Experiment and Study Design

Animal Feeding and Handling. Fourteen Sprague Dawley pregnant rats (120 g to 186 g) were ordered from Harlan Teklad, (Indianapolis, IN) and fed an adaptation diet (low iodine, low selenium) for the last 5 to 7 days of pregnancy and a week of lactation. Then the lactating dams were randomly assigned to four experimental diets: adequate selenium, adequate iodine (+Se+I); adequate selenium low iodine (+Se-I); low selenium, adequate iodine (-Se+I); low selenium, low iodine (-I -S) as indicated in **Table I.** The experimental design is illustrated in **Figure 1.** Dams were assigned to the experimental diets one week after delivery.

At three weeks of age, nine male and nine female pups per dietary treatment were randomly selected and continued on the diets of their respective mothers for seven more weeks. The pups were fed *ad libitum* with free access to reverse osmosis water. During the feeding experiment, two pups (one male and one female) died from the group consuming low iodine, adequate selenium diet. The male died of an unknown cause at the vivarium during the 6th week and the female died in our laboratory during the anesthesia during the 7th week at the end of the experiment. Thus the total number of pups remaining for the experiment was 70. The use of rats in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Oklahoma State University (see the approval form in **Appendix** A)

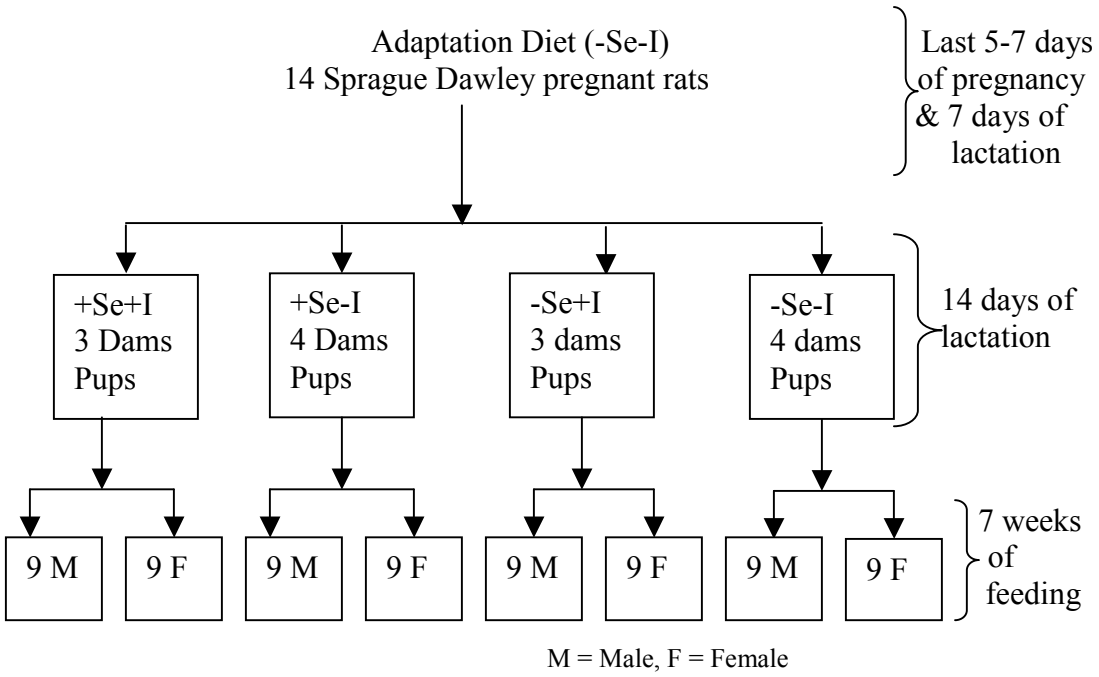

Figure 1: Experimental design

Preparation of the Experimental Diets. The experimental diets followed the recommendations of the American Institute of Nutrition (AIN) for growing rodents (163), (see **table II)** with some modifications. The diets were isocaloric and isonitrogenous. Because casein is contaminated with some selenium, torula yeast was used as protein source instead of casein while correcting for the amount of carbohydrate provided by torula yeast. Cornstarch was replaced with dextrose to make the diets more palatable for the rats. Minerals and vitamins were equivalent for the four different diets, except for iodine and selenium, which were either added or not added according to whether the diet was

Table II:
Composition of the experimental diets (g/kg diet)

Ingredients	+I +Se	+I -Se	-I +Se	-I -Se
Torula yeast	340	340	340	340
Dextrose	389.49	389.49	389.49	389.49
Sucrose	100	100	100	100
Soybean oil	70	70	70	70
Fiber (celufil)	50	50	50	50
Mineral mix +I+Se	35	-	-	-
Mineral mix +I-Se	-	35	-	-
Mineral mix -I +Se	-	-	35	-
Mineral mix -I-Se	-	-	-	35
Vitamin mix	10	10	10	10
L-cystine	3	3	3	3
Choline	2.5	2.5	2.5	2.5

I = Iodine. Se = Selenium. (+I+Se) = Adeaute iodine, adequate selenium. (+I-Se) = Adequate iodine, low selenium. (-I+Se) = Low iodine, adequate selenium. (-I-Se) = Low iodine, low selenium.

adequate or low in iodine and/or selenium. Potassium iodate and sodium selenate were used as sources of iodine and selenium respectively. The mineral mix was made in the Nutritional Sciences laboratory at Oklahoma State University following the recommendations of the AIN for growing rodents. The composition of the mineral mix is

presented in **Appendix B.** The vitamin mix formulated according to the recommendations of AIN (Cat # 40060) was ordered from Harlan Teklad (Madison, WI) and kept refrigerated until diets were mixed. The diets were mixed in batches of 5 kg in a mixer. Diets were kept refrigerated and pups continued to be fed daily the diets of their mothers for 7 weeks after weaning, after which they were necropsied.

Necropsy of the Pups. The day before the necropsy, the pups were put in individual metabolic cages and fasted overnight for a 12-hour urine collection. On the day of necropsy, the urine volume of the rats was measured and recorded. Then the rats were anesthetized with intraperitoneal injections of mixed ketamine (75mg/kg body weight) : xylazine (10mg/kg body weight), and weighed while they were sleeping. Blood was drawn from their abdominal artery before dissecting them for the collection of other tissues. Blood was first kept on ice and then centrifuged at 8,000 rpm for five minutes to separate serum that was stored at -20°C. Urine samples were also stored at –20°C. A portion of the liver was quickly collected, weighed and put in liquid nitrogen before being stored at -70°C. The other portion of the liver as well as the heart were weighed and kept at -20°C. The thyroid gland and spleen were weighed and discarded. The left leg (left femur and the left tibia together) were excised and kept in -20°C. The right femur and tibia were isolated, cleaned, and frozen at -20°C. The vertebral column was also excised and kept at -20°C.

Determination of Weight Gain, Organ Weight, and Body Lean and Fat Mass.

Weight gain was assessed by subtracting the weight of the rat recorded on the day of the start of the experimental diet from the weight recorded on the day of necropsy. Thyroid weight was determined by directly excising the thyroid gland and weighing it at

the time of the necropsy. Other organs such as liver, heart and spleen were also excised and weighed. The body lean and fat weights were assessed using DEXA. In this dissertation all organ weights and the lean and fat weights are expressed as percent of total body weight.

Biochemical Analyses:

To ascertain the iodine status of the rats, thyroid weight, serum thyroxin (T_4) and serum triiodothyronine (T_3) were assessed. Serum T_4 and T_3 were assessed in the pups using radioimmunoassay (RIA) kits (Diagnostic Products Corp., Inc, Los Angeles, CA) following the manufacturer's instruction. The test in both determinations involved the competition of a radioactive (^{125}I)-labeled thyroid hormone with the sample thyroid hormone for binding to an antibody site. After an incubation time for the reaction and the elimination of unbound compounds, the radioactivity of the complex (antibody-radioactive-labeled thyroid hormone) was determined using a gamma counter. The level of radioactivity, representing the amount of radioactive-labeled thyroid hormone bound to the antibody site was inversely proportional to the concentration of the thyroid hormones in the sample.

The selenium status of the rats was determined by measuring liver glutathione peroxidase activity by a spectrophotometric method described by Lawrence and Burk (164). The test is based on the measurement of GSH-Px activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione (GSG) produced upon reduction of hydroxyperoxide by GSH-Px, is recycled to its reduced form by glutathione reductase (GSHR) and by NADPH, which becomes oxidized to $NADP^+$. The oxidation of NADPH

to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the absorbance at 340 nm is directly proportional to GSHPx activity in the sample.

Liver thiobarbituric acid reactive substances (TBARS), mostly composed of malondialdehyde (MDA) were assessed as an indicator of lipid peroxidation. Liver MDA was assessed using the method described by Phelps and Harris (165) with modification pertaining to the preparation of the liver extract and the determination of protein concentrations in the liver extract. The supernatant of liver homogenate from liver samples stored at -70 C was prepared in the following way: liver sample was homogenized in 50mM phosphate buffer (PBS with 1 mM EDTA, 1 mM phenymethylsulfonylfluoride, and 1 μM pepstain A and trypsin inhibitor at 80 mg/L buffer PH = 7.4) using a homogenizer with cold teflon-glass and overhead stirrer (Wheaton Science Product Cat # 903475). The homogenate was centrifuged at 900 g at 4 ºC for 15 min. The supernatant was decanted, and recentrifuged at 12,000 g (11,400 rpm) at 4 ºC for an additional 15 min. The latter supernatant was aliquoted and stored at -80 ºC for TBARS analysis. Protein concentrations of the liver extracts were determined by the Bio-Rad Protein Assay (Bio-Rad Laboratory, Hercules, CA) using bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO) as the standard.

As an indicator of antioxidant status, serum ferric reducing ability of plasma (FRAP) was determined. Serum FRAP was assessed colorimetrically with a commercially available kit from Roche Diagnostics (Somerville, NJ) using a Cobas-Fara II Clinical Analyzer (Montclair, NJ).

As indicators of biochemical markers of bone metabolism we assessed serum osteocalcin, serum alkaline phosphatase (ALP), serum tartrate resistant acid phosphatase

(TRAP), urinary deoxypyridinoline (DPD), urinary calcium, urinary magnesium, and urinary phosphorus.

Serum osteocalcin was analyzed to assess bone formation using a rat osteocalcin immunoradiometric assay (IRMA) kit (Immunotopics, Inc, San Clemente, CA) following the manufacturer's instructions. Two different antibodies to rat osteocalcin are used in the assay. An affinity purified polyclonal goat antibody recognizing the C-terminal portion of the molecule was immobilized onto plastic beads for capture and another affinity purified polyclonal goat antibody recognizing the amino terminal portion of the molecule was radiolabeled for detection. A sample containing rat osteoclacin was incubated simultaneously with an antibody coated bead and the ^{125}I labeled antibody. Osteocalcin contained in the sample is immunologically bound by the immobilized antibody and the radiolabeled antibody to form a "sandwich" complex:
Bead/Anti-Rat Osteocalcin-Rat Osteocalcin-^{125}I Anti-Rat Osteocalcin. At the end of the incubation period, the bead is washed to remove any unbound labeled antibody and other components. The radioactivity bound to the beads is then measured in a gamma counter. The radioactivity of the antibody complex bound to the bead is directly proportional to the amount of rat osteocalcin in the sample.

Serum alkaline phosphatase as a non-specific indicator of bone formation was assessed using a colorimetric kit from Roche Diagnostics (Somerville, NJ). The test was performed on a Cobas-Fara II Clinical Analyzer (Montclair, NJ). The Roche reagent for alkaline phosphatase uses 4-notophenylphosphate as the orthophosphate monoester and 2-amino-2-methyl-1, 3-propanediol as the phosphate receptor and buffer. The 4-nitrophenylphosphate is colorless, but the resultant 4-nitrophenoxide ion has a strong

absorbance at 405 nm. The rate of increased absorbance at 405 nm is proportional to the enzyme (alkaline phosphatase) activity.

Serum TRAP was assessed as an indicator of bone resorption using a colorimetric kit from Roche Diagnostics System Inc (Nutley, NJ) and the Cobas-Fara II Clinical Analyzer (Roche, Montclair, NJ).

Urinary DPD was analyzed as an indicator of bone resorption using the Metra DPD EIA kit (Quidel Corporation, San Diego, CA) following the manufacturer's instruction and using a plate reader. The assay is a competitive enzyme immunoassay in a microtiter strip-well format using a monoclonal anti-DPD antibody coated on the strip to capture DPD. DPD in the sample competes with conjugated DPD-alkaline phosphatase for the antibody and the reaction is detected with a p-Nitrophenyl phosphate substrate using a plate reader at 405 nm. Metra DPD results were expressed based on urinary concentrations of creatinine. Urinary creatinine was assessed colorimetrically using a kit from Roche Diagnostics Inc. (Nutley, NJ) and the Cobas-Fara II clinical analyzer (Roche, Montclair, NJ).

Urinary Ca, Mg, and P were determined to know the amounts of the minerals lost in the urine. Urinary calcium and magnesium were assessed using flame atomic absorption spectrometry (Perkin Elmer, 5100PC).

Urinary phosphorus was assessed using a colorimetric kit from Roche Diagnostics (Nutley, NJ) and the Cobas-Fara II clinical analyzer (Roche, Montclair, NJ). During the test, phosphorus reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with a yellow color. The intensity of this color, measured at 340 nm, is proportional to the concentration of inorganic phosphorus in the sample.

Bone Measurement by Dual Energy X-ray Absorptiometry (DEXA)

Femoral, tibial, and vertebral (L_{3-5}) bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD), were assessed by dual energy X-ray absorptiometry (DEXA) with small animal high-resolution software (Hologic QDR4500A Elite, Waltham, MA). BMC is an estimate of the amount of mineral present in the bone, BMA estimates the two-dimensional area occupied by bone, and BMD is equal to BMC divided by BMA.

Bone Structure

The microarchitecture of the trabeculae of vertebra (L_3), and proximal tibia were analyzed using microcomputed tomography (μCT). Specimens of bone stored at -20°C were scanned using the μCT-40 (Scanco Medical AG, Zurich, Switzerland). The proximal tibia was scanned distally from the proximal growth plate. Contours were placed on a total of 125 consecutive tomographic slices (16.5 μm thickness per slice) beginning at 25 slices from the growth plate. Contours were also placed on 50 slices scanned from the midshaft region of the tibia to analyze cortical bone volume, thickness, and porosity.

The vertebra (L_3) was scanned from proximal to distal growth plate. Contours were placed on a volume of interest (VOI) beginning and ending at 10 slices (165 μm per slice) away from the growth plate in order to include in the VOI only the secondary spongiosa within the two growth plates. Bone morphometric parameters including trabecular bone relative volume (BV/TV), trabecular number (TbN), trabecular separation (TbSp), and trabecular thickness (TbTh), as well as the structural model index (SMI), connectivity density (ConnD) of the trabeculae, and the degree of anisotropy (DA) were obtained for L_3.

The 3-D images were also obtained for the analyzed bone specimens for visualization as needed.

Bone Biomechanical Tests

Bone Biomechanical Test Using 3-point Bending. The femur from the whole left leg that was kept at 20° C was separated from the tibia and cleaned to perform the 3-point bending test. The femur was first measured using an electronic digital cliper (Model No 14-648-17, VWR, Friendswood, Texas) to determine its length and its midshaft location as well as the anterior-to-posterior and lateral-to-lateral external diameters at the midshaft level. Then the femur was broken via a 3-point bending method using a texture analyzer (T.\ .XT2i® Texture Analyzer, Texture Technology Corp., Scarsdale, NY). In the 3-point bending test, the ends of the femur were supported by two fulcra (24 mm apart from each other) and the force was delivered to the midshaft by a crosshead moving at a constant speed. The femur was broken from the anterior to the exterior plane by applying a breaking force perpendicularly to the long axis of the bone with a crosshead speed of 5.0 mm/minute. The following parameters were obtained for the femur: the yield load (force) in gram, which was converted into N, ultimate load in N, and stiffness in N/mm. Next, the anterior-to-posterior and the lateral-to-lateral internal diameters of the femur at the level of midshaft were measured as well as the thickness of the cortical bone using the same caliper that was used to measure the length of the bone. Other biomechanical parameters such as the modulus of elasticity, yield force, ultimate force, yield stress and ultimate stress were calculated as described by Kiebzak and colleagues (90) and by Turner and Burr (82).

Briefly, the value of the second moment of area of the femur was first calculated using the following formula:

$$\text{SMA } (mm^4) = \frac{\Pi\,[(BD^3) - (bd^3)]}{64}$$

Where, B is the lateral-to-lateral external diameter in mm, D the anterior-to-posterior external diameter in mm, b the lateral-to-lateral internal diameter in mm, and d the anterior-to-posterior internal diameter of the femur at midshaft level in mm. Then this value of SMA was used in the calculation of yield stress, ultimate stress and the modulus of elasticity of the femur. The yield stress was calculated using the following formula:

$$\text{Yield Stress } (N/mm^2) = \frac{\text{Yield load x L x C}}{4 \text{ x SMA}}$$

Where, L is the distance between the two fulcra that support the bone and is equal to 24 mm, and C is equal to half of the anterior-to-posterior external diameter.

The ultimate stress was calculated using the following formula:

$$\text{Ultimate Stress } (N/mm^2) = \frac{\text{Ultimate load x L x C}}{4 \text{ x SMA}}$$

The value of the modulus of elasticity was calculated using the following formula:

$$\text{Modulus of Elasticity } (N/mm^2) = \frac{\text{Stiffness x } L^3}{48 \text{ x SMA}}$$

Bone Biomechanical Test Using Finite Element Analysis by Micro-CT. Finite element analysis uses specialized computer software and the micro-computed tomography (μCT) histomorphometric data to simulate compression of a region of interest of a material and determine the behavior of the material in response to the compression. The third

lumbar vertebra, L_3 was scanned from the proximal growth plate to the distal growth plate using μCT. A volume of interest (VOI) composed of secondary spongiosa of L_3 trabecular bone that was obtained for the determination of L_3 microarchitecture was subjected to a high friction compression test in the z direction. This allowed the determination of mechanical properties of the bone specimen such as average strain, total force, physiological force, stiffness, size independent stiffness, and average von Mises stress of the trabecular cores of L_3

Bone Ash Weight and Mineral Content Using Atomic Absorption Spectrometry

After the 3-point bending test, the left femur was ashed to determine the amounts of individual minerals in it using atomic absorption spectrometry. The concentrations of Ca, Mg, Zn, and Fe were determined using flame atomic absorption spectrometry (Perkin Elmer, 5100PC). The femurs were weighed, dried at 105 ºC for 24 hours, re-weighed, and placed in individual acid-washed crucibles. An acid digestion with concentrated nitric acid and hydrogen peroxide at 85 ºC was followed by a dry ashing in a muffle furnace. Bone was ashed in the muffle furnace at 375 ºC for 24 hours three times with intermittent acid digestions and dryings until white ash was obtained (166). After cooling, bone ash was weighed and dissolved in 12.2 ml of 5.2% nitric acid solution (11.6 mL water + 600 μL concentrate nitric acid). Then appropriate dilutions of the stock solution were done for Ca, Mg and Zn for the analysis, while the Fe was directly assessed in the stock solution. For Ca analysis the stock solution was diluted (1:2805) using 0.05% nitric acid containing 0.1% lanthanum. For Mg and Zn the stock solution was diluted (1:14) in 0.05% nitric acid solution.

Statistical Analyses

Data were analyzed using SAS (Statistical Analysis System) version 8 (SAS Institute Inc., Cary, NC). A split plot arrangement in a completely randomized design with sub sampling of the subunits (pups) and considering the main units (dams) as random factors along with Proc Mixed and Least Square Means were used to determine the main and interaction effects of the independent variable (iodine, selenium and sex) on the dependent variables (indicators of growth, iodine status, selenium status, bone quality, and oxidative status of the pups). Whenever an interaction effect was significant, slice analysis in Proc Mixed was used to determine the significance of differences between different levels of an independent variable at a given level of the other interacting independent variable with respect to the outcome variable. An effect was considered to be significant at $P< 0.05$.

CHAPTER IV

EFFECTS OF IODINE AND SELENIUM STATUS ON GROWTH AND BONE QUALITY OF GROWING RATS

Introduction

Iodine and selenium are essential trace elements in higher vertebrates. The deficiencies of these trace elements have been implicated in the etiology of a severe type of osteoarthritis, called Kashin-Beck disease, occurring in children and adolescents (1), (2). The mechanism by which iodine and selenium may affect bone and cartilage in children is not clear. However iodine is required for the synthesis of the thyroid hormones, thyroxin (T_4) and triiodothyronine (T_3). Thyroid hormone (T_3) is necessary for normal bone development and maturation in infants and children and for normal bone and mineral metabolism in adults (3). Untreated childhood hypothyroidism causes growth retardation that can be reversed by thyroid hormone replacement to produce a rapid catch up growth (4). Delayed bone maturation due to iodine deficiency has been observed in sheep (5). Thyroid hormones stimulate IGF-1 (insulin-like growth factor-1) production in humans (6), bony fish (7) and rats (8), and circulating IGF-1 regulates bone growth and density in mice (9). T_3 also stimulates bone mineralization and cartilage

matrix proteoglycan formation (10). Low serum levels of IGF-1 and IGF binding protein-3 (IGFBP-3) were found in pubertal children living in an area of severe iodine deficiency compared to children living in an area with mild iodine deficiency (11)

Fluvic acid, an environmental contaminant involved in the etiology of Kashin-Beck disease, has been shown to covalently bind with iodine, indicating that fluvic acid may interfere with the biovavailability of iodine (12). The mycotoxins produced by fungus of *alternaria* species, also implicated in the etiology of Kashin-Beck disease, have been suggested to compete for thyroid hormone (T_3) receptors (13). Thyroid hormone receptors are expressed in intramembranous and endochondral bone formation sites, in cultured osteoblasts and osteocytes, and in reserve and proliferative zones of growth plate chondrocytes (10).

The mechanism of action of selenium on bone and cartilage is not clear. Growth retardation, osteopenia, decreased serum alkaline phosphatase, and decreased IGF-1 were observed in second generation selenium-deficient male rats (14). Fluvic acid supplements and/or selenium deficiency impaired the structure of skeletal tissues in mice with characteristics similar to an early stage of osteoarthritis (15). These animals also had impaired collagen structure in both bone and cartilage and lower breaking force of the bone.

Selenium may protect bone and cartilage in several ways. Selenium is required for the activation and the homeostasis of thyroid hormones as an essential cofactor of iodothyronine deiodinases (16). Selenium as a part of antioxidant enzymes such as glutathione peroxidases (GSH-Pxs), thioredoxine reductases, and selenoprotein P may protect bone and cartilage cells against oxidative damage (17). Several selenoproteins

are expressed in fetal human osteoblasts where they contribute to cell differentiation and defense (17).

Iodine and selenium deficiencies have been associated with osteoarthritis, and osteoarthritis leads to bone loss. Some studies have shown an inverse association between osteoporosis and osteoarthritis (18-19). However, the occurrence of osteoporosis in osteoarthritic patients is well documented and several recent studies have demonstrated a positive relationship between osteoarthritis and bone loss (20-25).

Despite this link between osteoarthritis and bone loss, there is limited information about the effects of iodine and/or selenium on bone density, microarchitecture, and biomechanical properties during growth. There is also limited information about a possible gender difference in bone response to iodine and deficiency as most of the animal studies focused solely on male rats. It is also not clear whether the two trace elements may interact to affect bone. Because the deficiency of iodine and selenium is associated with osteoarthritis and arthritis is associated with bone loss, it is possible that Kashin-Beck disease-affected children will be at risk for bone fracture.

We hypothesized that iodine and selenium depletion would independently reduce growth, impair bone metabolism, decrease bone density, deteriorate bone microarchitecture, and decrease bone strength in both male and female rats and that the combined deficiencies would exacerbate these defects.

Methods

Animal Experiment and Study Design

Animal Feeding and Handling. Fourteen Sprague Dawley pregnant rats (120 g to 186 g) were ordered from Harlan Teklad, (Indianapolis, IN) and fed an adaptation diet (low iodine, low selenium) for the last 5 to 7 days of pregnancy and for a week of lactation. Then the lactating dams were randomly assigned to four experimental diets: Adequate selenium, adequate iodine (+Se+I); adequate selenium low iodine (+Se-I); low selenium, adequate iodine (-Se+I); and low selenium, low iodine (-I -S) as indicated in **Table I.** The experimental design is illustrated in **Figure 1.** Dams were fed the experimental diets for the last two weeks of lactation.

Nine male and nine female pups per dietary treatment were randomly selected (for a total of 72) and continued on the diets of their respective mothers for an additional 7 weeks. The pups were fed *ad libitum* with free access to reverse osmosis water. During the feeding experiment, two pups (one male and one female) died in the 6^{th} and 7^{th} weeks from the group consuming the low iodine, adequate selenium diet. The male rat died from an unknown cause in the 6^{th} week and the female died under the influence of anesthesia at the end of the experiment during the 7^{th} week. Thus the total number of pups remaining for the experiment was 70. The use of rats in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Oklahoma State University.

Preparation of the Experimental Diets. The experimental diets followed the recommendations of the American Institute of Nutrition (AIN) for growing rodents (26),

(see **Table I**) with some modifications. The diets were isocaloric and isonitrogenous. Because casein is contaminated with some selenium, torula yeast was used as a protein source instead of casein while correcting for the amount of carbohydrate provided by torula yeast. Cornstarch was replaced with dextrose to counteract the bitterness of torula yeast. Mineral and vitamin mixes were equivalent for the four different diets, except for iodine and selenium, which were either added or not added according to whether the diet was adequate or low in iodine and/or selenium. Potassium iodate and sodium selenate were used as sources of iodine and selenium respectively. The mineral mix was made in the Nutritional Sciences laboratory at Oklahoma State University. The vitamin mix was formulated according to the recommendations of AIN (Cat # 40060, Harlan Teklad, Madison, WI) and kept refrigerated until diets were mixed. The diets were mixed in batches of 5 kg in a mixer. Diets were kept refrigerated and pups continued to be fed daily the diets of their mothers for 7 weeks after weaning, after which they were necropsied.

Necropsy . The day before the necropsy, rats were fasted overnight in individual metabolic cages for a 12-hour urine collection. Then the rats were anesthetized with an intraperitoneal injection of mixed ketamine (75mg/kg body weight)/xylazine (10mg/kg body weight), and weighed while they were sleeping. Blood from the abdominal artery was chilled and then centrifuged for five minutes at low speed to separate serum that was stored at -20°C. Urine samples were also stored at –20°C. A portion of the liver was quickly collected, weighed and put in liquid nitrogen before being stored at -70°C. Tissues and bones were weighed and kept at -20°C.

Determination of Weight Gain, Organ weight, and Body Lean and Fat Mass.

Thyroid weight was determined directly by excising and weighing the thyroid gland at the time of necropsy. Organs such as liver, heart, and spleen also were excised and weighed. The body lean and fat weights were assessed using DEXA. In this dissertation all organ weights and the lean and fat weights are expressed as percent of total body weight.

Biochemical Analyses:

Serum thyroxin (T_4) and serum triiodothyronine (T_3) were measured using radio immunoassay (RIA) kits (Diagnostic Products Corp., Inc, Los Angeles, CA). Liver glutathione peroxidase activity was measured spectrophotometrically (27).

Liver thiobarbituric acid reactive substances (TBARS) measured as malondialdehyde (MDA) were assessed as an indicator of lipid peroxidation using the method of Phelps and Harris (1993) with modifications. Liver samples stored at -70 C were homogenized in 50 mM phosphate buffer (PBS with 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride, and 1 μM pepstain A and trypsin inhibitor at 80 mg/L buffer P^H = 7.4) using a cold teflon-glass homogenizer with overhead stirrer (Wheaton Science Products, Cat # 903475). The homogenate was centrifuged at 900 g at 4 ºC for 15 min. The supernatant was decanted, and recentrifuged at 12,000 g (11,400 rpm) at 4 ºC for an additional 15 min. The latter supernatant was aliquoted and stored at -80 ºC for TBARS analyses. Protein concentrations of the liver extracts were determined by the Bio-Rad Protein Assay (Bio-Rad Laboratory, Hercules, CA) using bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO) as the standard.

Serum ferric reducing ability of plasma (FRAP) was assessed colorimetrically with a kit from Roche Diagnostics (Somerville, NJ) using a Cobas-Fara II Clinical Analyzer (Montclair, NJ) as a measure of antioxidant status

As indicators of bone metabolism we assessed serum osteocalcin, serum alkaline phosphatase (ALP), serum tartrate resistant acid phosphatase (TRAP), urinary deoxypiridinoline (DPD), urinary calcium, urinary magnesium, and urinary phosphorus.

Serum osteocalcin was analyzed to assess bone formation using a rat osteocalcin immunoradiometric assay (IRMA) kit (Immunotopics, Inc, San Clemente, CA) following the manufacturers' instructions. Serum alkaline phosphatase, serum TRAP and urinary creatinine were assessed using colorimetric kits from Roche Diagnostics (Nutley, NJ) and Cobas-Fara II Clinical Analyzer (Montclair, NJ). Urinary DPD was analyzed as an indicator of bone resorption using the Metra DPD EIA kit (Quidel Corporation, San Diego, CA).

Urinary calcium and magnesium were determined using flame atomic absorption spectrometry (Perkin Elmer, 5100PC, Norwalk, CT). Urinary phosphorus was assessed using a colorimetric kit from Roche Diagnostics (Nutley, NJ) and the Cobas-Fara II clinical analyzer (Montclair, NJ).

Bone Measurement by Dual Energy X-ray Absorptiometry (DEXA)

Femoral, tibial, and vertebral (L_{3-5}) bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) were assessed by dual energy X-ray absorptiometry (DEXA) with small animal high-resolution software (Hologic QDR4500A Elite, Waltham, MA).

Bone Structure

The microarchitecture of the trabeculae of the third lumbar vertebra (L_3), and proximal tibia were analyzed using microcomputed tomography (μCT-40, Scanco Medical AG, Zurich, Switzerland). The proximal tibia was scanned in the distal direction from the growth plate. Contours were placed on a total of 125 consecutive tomographic slices (16.5 μm thickness per slice) beginning 25 slices from the growth plate. Trabecular bone volume fraction (BV/TV), trabecular number (TbN), trabecular thickness (TbTh), trabecular separation (TbSp), structural model index (SMI), connectivity density (ConnD), and degree of anisotropy (DA) were assessed. Contours were also placed on 50 slices scanned at the midshaft region of the tibia to analyze cortical bone volume, thickness, and porosity.

The vertebra (L_3) was scanned from proximal to distal growth plate. A countour was placed on a VOI beginning and ending 10 slices (165 μm) away from the growth plates in order to include in the VOI only the secondary spongiosa within the two growth plates. Bone morphometric parameters including trabecular BV/TV, TbN, TbSp, and TbTh, as well as the SMI, ConnD and degree of anisotropy were obtained for L_3.

Bone Biomechanical Tests

Bone Biomechanical Test Using 3-point Bending. An electronic Vernier caliper (Model No 14-648-17, VWR, Friendswood, Texas) was used to determine femur length and midshaft location as well as the anterior-to-posterior, and lateral-to-lateral external and internal diameters at the midshaft level. The femur was broken via a 3-point bending method (28) using a texture analyzer (T.\ .XT2i® Texture Analyzer, Texture Technology

Corp., Scarsdale, NY). Ends of the femur were supported by two fulcra (24 mm apart) and the force was delivered to the midshaft by a crosshead moving at a constant speed of 5.0 mm/minute. The yield load (force), ultimate load and stiffness were obtained. Other biomechanical parameters such as the modulus of elasticity, yield stress, and ultimate stress were calculated as described by Kiebzak et al. (28) and by Turner and Burr (29).

Bone Biomechanical Test Using Finite Element Analysis by micro-CT. The μCT histomorphometric data were used with finite element software to simulate compression of a region of interest and determine the behavior of the material in response to the compression. The trabecular bone sample used for the determination of L_3 microarchitecture was subjected to a high friction compression test in the z direction. This allowed the determination of mechanical properties of the bone specimen such as total force for compression, average strain, stiffness and size independent stiffness, and von Mises stresses.

Bone Ash Weight and Mineral Content Using Atomic Absorption Spectrometry

The concentrations of Ca, Mg, Zn, and Fe were determined using flame atomic absorption spectrometry (Perkin Elmer, 5100PC, Norwalk, CT). First femurs were weighed, dried at 105 ºC for 24 hours and ashed using repeated acid digestions with concentrated nitric acid and hydrogen peroxide at 85 ºC followed by dry ashing cycles in a muffle furnace. Bone ash was weighed and diluted to appropriate concentrations for analysis by flame atomic absorption spectrometry for Ca, Mg, Zn, and Fe using standard wavelengths.

Statistical Analyses

Data were analyzed using SAS (Statistical Analysis System) version 8 (SAS Institute Inc., Cary, NC). A split plot arrangement in a completely randomized design with sub sampling of the subunits (pups) and considering the main units (dams) as random factors was used along with Proc Mixed and Least Square Means (LSMEAS) to determine the main and interaction effects of the independent variable (iodine, selenium and sex) on the dependent variables (indicators of growth, iodine status, selenium status, bone quality, and antioxidant and lipid peroxidation status of the pups). We used slice analysis in the LSMEANS statement whenever an interaction effect was significant or close to significance. An effect was considered to be significant at $P< 0.05$.

Results

Weight Gain and Bone Density

The effects of iodine and selenium depletion on growth and indicators of iodine and selenium status are presented in **Table II**. Male rats had significantly higher weight gain than females for both selenium adequate ($P<0.0001$) and selenium deficient ($P<0.0001$) diets (**Figure 2**). Weight gain was significantly reduced by iodine deficiency in all rats ($P<0.008$) (**Table II**) and by selenium deficiency in males ($P<0.0001$). Selenium depletion did not affect the weight gain of females rats (**Figure 2**). Thyroid weight as a percentage of body weight was significantly increased by iodine depletion ($P<0.0001$), but not by selenium depletion.

Serum thyroxin (T_4) was significantly decreased by iodine deficiency ($P<0.0001$) and selenium depletion tended ($P<0.08$) to increase T4 (**Table II**). For serum triiodothyronine (T_3), there were no significant differences between males and females when selenium was adequate. But when selenium was deficient, females had higher serum T_3 than males. In males as well as in females, there were no significant differences between adequate selenium and deficient selenium with respect to serum T_3 (**Figure 2**). Selenium depletion markedly decreased hepatic glutathione peroxidase activity ($P<0.0001$) regardless of sex and iodine status of the rats (**Table II**).

The results for the analysis of tibial, femoral and vertebral BMA, BMC and BMD by DEXA are presented in **Table III** and **IV**. Iodine depletion independently reduced bone mineral area of the long bones (**Table III**). Although BMA displayed similar patterns in response to selenium depletion in all three bones, there was a significant independent reduction with selenium depletion only in the tibia. Selenium depletion decreased vertebral BMA in males ($P<0.05$) without affecting the BMA of vertebra in females. Bone mineral area was higher in males than females in both selenium adequacy ($P<0.0001$) and selenium deficiency ($P<0.0004$) in the vertebra. Selenium depletion decreased vertebral bone mineral area in males ($P<0.05$) without affecting the BMA of the vertebra for females (**Figure 3**).

In male rats, selenium depletion significantly decreased femoral ($P<0.05$), tibial ($P<0.02$) and vertebral ($P< 0.004$) bone mineral BMC ($P<0.02$) without affecting the BMC in females (**Figure 4**). When selenium was adequate, males had higher BMC than females in femur, tibia, and vertebra. The same pattern was observed in all the three

bone when selenium was deficient, but a significantly higher BMC in males than females in selenium deficiency was seen only in tibia.

Vertebral bone mineral density was significantly decreased by selenium depletion in males ($P<0.006$), and selenium depletion tended to decrease tibia bone mineral density in males ($P<0.09$) (**Figure 5**). Vertebral BMD was higher in males than females when Se was adequate ($P<0.04$).

Bone Growth, body Lean Mass, and Fat Mass

The lengths of the tibia and femur were measured using a digital caliper. Both iodine and selenium depletion significantly decreased tibia and femur lengths and males had longer tibia and femur than females ($P<0.0001$) (**Table V**). Mean femur cortical thickness as measured by the caliper was reduced by selenium deficiency ($P<0.04$).

There were no diet effects on whole body fat and lean mass as measured by DEXA. Whole body lean weight was higher in female than male rats, while whole body fat weight was higher in males than females ($P<0.04$) (**Table V**).

Organs (liver, spleen, and heart) were weighed at the time of necropsy. The weights of all the three organs as expressed in percentage of whole body weight were higher in female than male rats (**Table VI**). Both liver and spleen weights were decreased by iodine deficiency, and selenium depletion increased liver weight. There were no significant diet effects on heart weight.

Cancellous Bone Microarchitecture

The three-dimensional analysis of both proximal tibia and L_3 was performed using micro-computed tomography (μCT). The results for the assessment of the microarchitectural parameters for proximal tibia and the third lumbar vertebra (L_3) are in **Tables VII** and **VIII.**

Male rats had larger bone than females as evidenced by the significantly larger trabecular total volume (TV) in both tibia (**Table VII** and **VIII).** Iodine and selenium deficiency independently reduced the total trabecular volume in the tibia. Males had higher L_3 trabecular TV than females in both iodine adequacy ($P<0.002$) and iodine deficiency ($P<0.001$) (**Figure 6**). A significantly lower relative bone volume (BV/TV) was found in males than females in both tibia and L_3 (**Tables VII** and **VIII**). When iodine was adequate, the deficiency of selenium increased tibia ($P<0.04$) and vertebra ($P<0.03$) trabecular BV/TV, but when selenium was deficient, the adequacy of iodine tended to increase BV/TV in the tibia ($P<0.06$) (**Figure 6**).

Females had also higher tibial trabecular number ($P<0.0008$) and higher L_3 trabecular number ($P<0.0001$) than males (**Table VII** and **VIII).** In a pattern similar to that of BV/TV, tibia and L_3 TbN were increased in iodine adequate animals when selenium was deficient ($P<0.005$) and ($P<0.02$) respectively. When selenium was adequate, the depletion of iodine increased both Tibia and L_3 TbN. But when selenium was deficient the adequacy of iodine significantly increased tibia TbN ($P<0.02$) (**Figure 7**). There were no significant differences between iodine-adequate and iodine-deficient rats with respect to L_3 TbN when selenium was deficient.

Trabecular thickness (TbTh) was significantly higher in females than males for proximal tibia ($P<0.0001$) (**Table VII**). In selenium depleted rats, L_3 TbTh was higher in females than males ($P<0.004$), but when selenium was adequate, there were no significant differences between males and females in terms of L_3 TbTh (**Figure 8**).

The results from TbSp, ConnD, SMI, DA and BS/BV are presented in **Tables IX** and **X**. Tibial trabecular separation tended to be higher in males than in females ($P<0.06$) and L_3 trabecular separation was significantly higher in males than females ($P<0.0001$). Both tibia and L_3 trabecular separation were higher in iodine adequate animals when selenium was also adequate ($P<0.02$ and $P<0.002$ respectively), but when selenium was deficient, the adequacy of iodine decreased tibial TbSp ($P<0.02$) (**Figure 9**). L_3 trabecular separation was decreased in selenium adequate rats when iodine was deficient ($P<0.0009$) (**Figure 9**).

The connectivity density (ConnD) of both proximal tibia and L_3 trabecular bone was significantly higher in females than males ($P<0.0001$ and $P<0.0005$ respectively) (**Tables IX** and **X**). When iodine was adequate, the deficiency of selenium increased the connectivity for both tibia and L_3 ($P<0.02$ and $P<0.01$ respectively), but when selenium was deficient, the adequacy of iodine significantly increased ConnD in both tibia ($P<0.04$) and L3 ($P<0.005$) (**Figure 10**).

The structural model indices (SMI) of both tibia and L_3 trabecular bone were significantly lower in females than males which mean bone was more plate-like in females and more rod-like in males. When iodine was adequate, the deficiency of selenium decreased L_3 SMI ($P<0.03$) and tended to decrease tibial SMI ($P<0.08$). L_3 SMI

also tended to be decreased by selenium depletion in iodine adequate rats ($P<0.07$) (**Figure 11)**.

The degree of anisotropy was higher in males than females in the proximal tibia ($P<0.0001$) but higher in females than males in L_3 ($P<0.0002$). The mean degree of anisotropy in the proximal tibia was higher when iodine was adequate ($P<0.0001$) but diet did not have significant effects on DA in L_3 (**Tables IX** and **X**)

The ratio of bone surface to bone volume (BS/BV) was higher in males than females for tibia ($P<0.0001$), and L_3 BS/BV was higher in males than females when selenium was depleted ($P<0.0004$) (**Figure 12**).

Tibia Cortical Bone Microarchitecture

The 3-D analysis of 50 slices of tibial cortical bone presented in **Table XI** shows that males had higher cortical total volume ($P<0.0008$) and bone volume ($P<0.002$) than females when Se was adequate ($P<0.0001$) (**Table XI**). But when selenium was depleted, there were no significant sex differences with respect to tibial cortical TV and BV (**Figure 13**). Similarly, males rats had higher tibial cortical thickness than females when selenium was adequate ($P<0.04$). Tibial cortical porosity was decreased by iodine depletion in male rats ($P<0.003$) and when iodine was adequate, males had higher tibial cortical porosity than females ($P<0.03$) (**Figure 14**).

Biochemical Markers of Bone Metabolism

The measured bone formation markers were serum alkaline phosphatase and serum osteocalcin. Iodine and selenium depletion did not significantly affect serum

alkaline phosphatase. However, the values were higher in males than females ($P<0.03$) (**Table XII**). Serum osteocalcin levels were decreased by selenium depletion ($P<0.04$) and there were significantly higher values of serum osteocalcin in females than males ($P<0.02$) (**Table XII**).

As bone resorption markers, we assessed serum TRAP, urinary DPD, urinary Ca, Mg, and P (**Tables XII** and **XIII**). When iodine was adequate, serum TRAP was higher in males than females ($P<0.004$), but when iodine was deficient there were no significant differences between males and females in serum TRAP (**Figure 15**).

There were no significant effects of either diet or sex on urinary DPD and urinary Ca. However, urinary Mg ($P<0.007$), urinary P ($P<0.04$) (**Table XIII**) and urinary creatinine ($P<0.03$) (**APPENDIX M**) were higher in males than females. As markers of oxidative status we determined liver glutathione peroxidase (GSH-Px) activity, which was also an index of selenium status, serum FRAP, and liver TBARS as measured by malondialdehyde. Hepatic GSH-Px activity was decreased by selenium depletion ($P<0.0001$), but was not affected by iodine depletion (**Table II**). Serum FRAP concentrations were decreased by selenium depletion ($P<0.04$) (**Table XIII**) and there was significantly higher serum FRAP in females than males when iodine was deficient ($P<0.02$) (**Figure 15**). Serum FRAP was also increased by iodine depletion in females ($P<0.01$).

Hepatic TBARS as expressed in MDA levels in liver extract were increased by selenium depletion ($P<0.0001$), and iodine depletion ($P<0.03$); and males had higher levels of TBARS than females ($P<0.0001$) (**Table XIII**).

Bone Ash Weight and Mineral Content

There were no significant effects of iodine and/or selenium on femur wet weight, dry weight, and ash weight. However, femur wet weight and dry weight were both higher in males than females ($P< 0.02$), but femur ash weight as percent of either wet or dry weights was higher in females than males ($P<0.002$ and $P<0.03$ respectively) (**Table XIV**).

Femur concentrations of calcium, magnesium, zinc and iron as measured by flame atomic absorption spectrometry were not affected by iodine and/or sex of the animals. Selenium depletion increased femur Zn ($P<0.01$) and decreased femur Fe ($P<0.05$) without significantly affecting the bone concentrations of Ca and Mg (**Table XV**).

Biomechanical Analyses

Femur 3-Point Bending. Results for the 3-point bending test of femur are presented in **Table XVI**. Femur yield force (the load required to cause permanent damage to the femur at the level of midshaft) was significantly decreased by selenium depletion in iodine adequate rats ($P<0.009$) and was also reduced by iodine depletion in selenium adequate rats ($P<0.02$) (**Figure 16**). The ultimate force required to break the femur was decreased in iodine adequate rats when selenium was depleted ($P<0.002$) (**Figure 16**). When selenium was adequate, the depletion of iodine also decreased femur ultimate force ($P<0.0007$) (**Figure 16**). The stiffness of bone is defined as its rigidity (29). Iodine adequacy increased femur stiffness in female rats ($P<0.002$) without having any significant effects on the bone stiffness of males (**Figure 16**). When iodine was adequate, females had a tendency of higher femur stiffness than males ($P<0.07$). Femur

modulus of elasticity (the intrinsic stiffness or the stiffness corrected for the size of bone) was higher in females than males ($P<0.0006$) and selenium depletion tended to increase the values in all the animals ($P<0.06$) (**Table XVI**).

Yield stress and ultimate stress are the maximum amount of stress bone can sustain before having permanent damage and breaking respectively (29), therefore, they reflect the strength of bone (29). Femur yield stress ($P<0.008$) and ultimate stress ($P<0.02$) were both higher in females than males. There were no significant diet effects on femur yield stress and ultimate stress (**Table XVI)**

L_3 Finite Element Analysis (FE) by micro-CT. The results on the FE analysis of L_3 trabecular bone by micro-CT are presented in **Table XVII**. The FE analysis with high friction compression test in the z-direction of L_3 trabecular cores showed that the total force required to completely crush L_3 trabecular cores was higher in females than males ($P<0.0001$) and females had higher average strain than males ($P<0.0001$). The stiffness of L_3 trabecular cores tended to be higher in females than males ($P<0.09$). Size independent stiffness was higher in females than male rats ($P<0.0003$) (**Table XVII**), and the values were decreased in iodine adequate rats that were also selenium adequate ($P<0.03$) (F**igure 17**). When selenium was adequate, the adequacy of iodine significantly decreased L_3 size independent stiffness ($P<0.04$). The corrected average von Mises stress was higher in male than female rats (<0.0005) indicating a poorer bone quality in males.

Discussion

Growth, expressed by weight gain was significantly reduced by iodine depletion in all rats and by selenium depletion in male rats. The result of our study on weight gain with respect to selenium adequate and selenium deficient male groups is in agreement with those of Moreno-Reyes and colleagues (14) who observed a significant reduction of body weight in 74-day old, second-generation selenium-deficient male rats compared to control. This growth impairment due to selenium deficiency may be due to an insufficient pituitary local deiodination of T_4 to form T_3, which is needed for growth hormone synthesis (14). Moreno Reyes and colleagues found significantly decreased pituitary growth hormone and plasma IGF-I in second generation selenium-deficient male rats (14). A decreased growth due to iodine and/or selenium depletion in our study has been confirmed by decreased weight gain and decreased bone length.

In our study, mean thyroid weight was significantly increased by iodine deficiency as we expected. The thyroid weights and thyroxin levels in our control and iodine deficient males are similar to but higher than those found in our previous study (30) with weanling male rats fed the AIN diet for five weeks and the results found by Ruz et al. (31) with male rats fed the AIN diet for six weeks. However, thyroid weight was not affected by selenium deficiency. In addition, the combination of iodine and selenium deficiencies did not increase thyroid weight above the increase caused by iodine deficiency alone. This was strange because selenium deficiency is supposed to worsen the effect of iodine deficiency, as Se is needed for thyroid hormone metabolism. However, similar results were found by Ruz and colleagues (31).

Serum thyroxine level was significantly decreased by iodine deficiency, but there were no significant differences among iodine depleted and iodine adequate rats with respect to serum T_3. This might have been due to the considerable capacity of the body to conserve T_3. However, we suggest that a longer feeding of the animal would lead to decreased T_3 in iodine-deficient rats. There was a significant decrease in thyroid T_4 with T_3 being unaffected in iodine-depleted animals. This is consistent with results of studies considered by the Food and Nutrition Board (32), which indicates that iodine deficiency decreases serum T_4 concentrations while serum T_3 concentrations remain normal or increase.

Female rats had higher T_3 than males when selenium was deficient, probably due to some compensatory mechanisms in females we have yet not been able to identify. The significantly lower T_3 in males due to selenium depletion is consistent with a decreased weight gain in males due to selenium depletion. This might have contributed to the poorer bone quality of males compared to female rats. Probably, males are more sensitive to lack of Se necessary to produce iodothyronine deiodinases (Type I and type II) which are responsible for the conversion of T_4 to T_3 (16). There were no significant differences between adequate selenium and deficient selenium male rats with respect to serum T_3. The selenium deficient growing male rats of Moreno-Reyes and colleagues (14) did not experience a significant decrease in T_3 levels compared to control, but there was a trend toward decreased T_3 due to selenium depletion ($P = 0.06$), because they had a longer study period than we did.

Vertebral BMD was significantly lower in selenium depleted males and selenium depletion tended to decrease tibial bone mineral density. However, there were no

significant sex, diet or interaction effects on femoral bone mineral density. This was expected because the bone remodeling process is more active in the vertebra than the peripheral bones (33), and vertebra has more chancellors bone and, thus is more prone to bone loss than the tibia and femur.

Bone mineral area was significantly decreased by iodine depletion in femur and tibia of all animals with higher BMA in males than females. Selenium depletion also decreased BMA in the tibia of all the animals, in the vertebra of male rats and had a slight tendency (P=0.08) to decrease bone mineral area in the femur of male rats. This suggests that selenium may have more effects on the bone area of males than female rats.

Vertebral, tibial, and femoral bone mineral contents were all decreased by selenium depletion in male rats without any significant effects on the bone mineral content of female rats. This further confirms a possible greater demand of selenium for male rats than female rats, even though there were no sex effects on liver GSH-Px activity in our study. There are many other selenoproteins we did not assess including perm flagella seleoprotein, which is required for sperm motility and is suggested to play an important role in male fertility (34). Many other selenoproteins have been identified whose role have not been characterized.

The μCT analysis of trabecular bone structure showed that trabecular total volume was independently decreased by iodine and selenium depletion, and males had higher trabecular total volume in the tibia. This indicates that males had larger bones. Despite this larger bone in males, other microarchitectural parameters of trabecular bone such as TbN and TbTh were higher in females than males and TbSp was higher in the bone of males than females. In humans, trabecular number and thickness decrease in aging with

increased trabecular separation. (35). Since aging is associated with higher prevalence of bone disorders, it is probable that the bone quality of male growing rats is less than that of female growing rats in terms of microarchitecture.

The structural model index (SMI) of both L_3 and tibia trabeculae was significantly lower in females meaning that the structure of the bone for females was more plate-like. The SMI characterizes a 3-D bone structure composed of certain amounts of plates and rods (33). Ding and colleagues (33) as well as Lane et al. (36) define SMI as a value between 0 and 3, and suggested that the SMI value would be 0 in an ideal plate structure model, and 3 in an ideal cylindrical rod structure, independent of the physical dimensions of the structure. The authors suggest that human tibia cancellous bone changes with age from plate-like to rod-like. Negative values of SMI have been reported and are suggested to occur in very dense bones. In our study, most of the SMI values were within the range of 0 to 3, but some negative values were observed in five female rats fed adequate iodine, low selenium diet, and this contributed to the relatively lower mean SMI in this group of rats. In our study female rats had significantly lower L_3 SMI (more plate-like) than male rats indicating that female rats had better L_3 cancellous bone structure.

Cancellous bone connectivity density was higher in female than males. In contrast to the study of (37) who found an inverse relation between connectivity density and stiffness, both connectivity and stiffness were higher in female rats and we consider our female rats to have a better bone quality. Therefore, connectivity may be positively associated with elastic properties of the bone in growing rats.

Tibial cortical bone structure was also affected by sex and diet. Tibia cortical thickness as measured by micro-CT was higher in selenium adequate males that were also

iodine adequate**.** But this positive characteristic was offset by a concomitant increase in tibial cortical porosity in iodine adequate male rats, meaning that bigger bone size due to more rapid growth may deteriorate cortical bone structure. This is in part supported by high cortical bone volume and total volume in selenium adequate and iodine adequate males. The higher cortical porosity in iodine adequate male rats may be explained by the higher serum TRAP observed in these animals as compared to iodine adequate females.

Our data indicate that there is a significantly higher bone turnover in male than female rats. This was evidenced by higher serum ALP and TRAP activities in males than females. In addition, there were higher urinary excretions of P and Mg in males than females. Serum osteocalcin was higher in females and was decreased by selenium depletion. We do not know the mechanism by which selenium depletion may decrease serum osteocalcin levels. Moreno-Reyes and colleagues (14) also found decreased serum osteocalcin in selenium deficient male rats. There are 18 different selenoproteins identified in the mammalian system (34), and the functions of many of them have not yet been identified. The mechanism whereby selenium may affect serum osteocalcin levels and the reason for higher serum osteocalcin in females are not known and further investigation in this area is needed.

It has been suggested that estrogen increases the rate of bone mineralization in female rats at puberty compared to male rats (38). The mechanism of this high mineralization of female bone at this time of high circulating estrogen levels is not known. Because females had higher osteocalcin in this study and better bone status, it would be interesting to investigate whether this action of estrogen is mediated by osteocalcin.

An increase in liver MDA and a decrease in serum FRAP due to selenium-depletion may indicate a higher propensity of lipid peroxidation in selenium-deficient rats. MDA is a secondary product of lipid peroxidation, and its levels have been shown to be high in plasma of patients with rheumatoid arthritis including children with juvenile rheumatoid arthritis (39). The TBARS values obtained for our control rats are similar to the values found in seven-week old male (control) rats by Poon et al. (40) using a fluorescence method. Liver MDA was higher and serum FRAP was lower in males than females. Lipid peroxidation might have contributed to the poorer bone quality observed in male rats as male rats also had higher body fat and lower lean mass. Glutathione peroxidase activity and other selenoproteins with antioxidant properties such as thioredoxin reductases and selenoprotein P might have contributed to the value of serum FRAP in selenium adequate rats.

Iodine depletion also decreased liver TBARS. The best known role of iodine in the body is for the synthesis of thyroid hormone. However, substantial amounts of the body's iodine are non-hormonal and are concentrated in extrathyroidal tissues where their physiological functions are unknown. Iodine is rich in electrons and can act as an electron donor to hydrogen peroxide and peroxides. It can also bind to the double bonds of polyunsaturated fatty acids of cell membranes making them less sensitive to oxidation. Therefore iodine is believed to have antioxidant properties in tissues where it accumulates (41). For instance, the mammary gland concentrates iodine during pregnancy and lactation, and this concentration is suggested to prevent breast cancer (42). This probable antioxidant property of iodine might have contributed to the decreased liver TBARS values observed in iodine adequate rats.

Female rats had also higher femur ash weight than males. There were no significant sex effects on femur bone mineral content as measured by atomic absorption spectrometry. Because there was a higher femur ash weight in females than males, it is possible that some of the minerals we did not assess in the femur are higher in females. Inorganic phosphates are important components of bone and femur phosphorus concentration was not assessed in this study. Because urinary phosphorus excretion was higher in males than females, it would be interesting to assess the phosphorus concentrations of femur bone solution.

In this study, the fastest growing animals (iodine adequate rats and selenium adequate male rats) had the highest femoral breaking force (ultimate force). This may be due to their bigger bone size leading to a larger breaking cross-sectional area of the long bone. In addition, the higher cortical TV, BV, and thickness in selenium adequate males might have contributed to the higher breaking force. This bigger bone size in some male rats and other fast-growing rats might have also contributed to the relatively better bone quality with respect to bone area; mineral content and density by DEXA in comparison to bone microarchitecture, as DEXA analysis combines both cortical and cancellous bone.

The relatively better bone quality in single element-depleted or both iodine and selenium depleted rats compared to control rats and in female rats compared to males may be explained by their relatively slow growth rate. Normal growth requires a balanced bone reaorption and formation. Antiresorptive agents administrated to growing rats impair growth and lead to increased bone mass (43). This indicates that bone resorption is an important part of growth process. In addition, T_3 and estrogen might have had a synergistic or additive effects on females in slowing longitudinal growth by

inducing hypertrophy of the chondrocytes and accelerating the closure of the epiphyseal growth plate, which ends longitudinal growth in humans (10). Even though T_3 is believed to induce the differentiation of reserve chondrocytes to proliferative zone chondrocytes either directly (10, 44) or indirectly through GH and IGF-1 (4), its role in inducing hypertrophy in the chondrocytes to stop the clonal expansion of the cells is well recognized (10). We did not assess the estrogen levels of the rats. However, other research indicate that females have higher circulating estrogen than males at puberty (45) and our rats had reached the state of puberty by the time they were sacrificed. Estrogen (17 β-estradiol) supplementation of both males and females growing rats caused decreased body weight gain, tibial length, and width of growth plate, while the opposite effects were caused in overiectomized rats (46). This tempering effect of growth by estrogen was explained by a decrease in the width of hypertrophic zone of the growth plate chondrocytes and a predominant expression of estrogen receptor α and β in late proliferating and early hypertrophic chondrocytes in estrogen-supplemented rats.

Because iodine and selenium deficiencies have been associated with osteoarthritis, we assumed that iodine and/or selenium depletion would cause osteoarthritis and that osteoarthritis would lead to decreased bone quality in growing rats. Yet we did not assess any indices of osteoarthritis. This is an important limitation of this study because it makes it difficult to link our results with Kashin-Beck disease. However, some observations in our study are consistent with the characteristics of Kahin-Beck disease. The significant reduction of growth experienced by iodine and/or selenium-depleted animals in our study is a feature of Kashin-Beck disease (1) and we found a concomitant reduced growth and bone density in selenium depleted male rats. In

addition, Kashin-Beck disease has been suggested to result from oxidative damage (47) and we observed a significant reduction of circulating antioxidant in selenium deficient rats and a significant increase in lipid peroxidation in the liver of I and selenium depleted rats. Based on these observations, we suggest that iodine and selenium depletion studies may contribute to the understanding of the etiology of Kashin-Beck disease.

Conclusion

These results indicate that the degree of iodine and selenium depletion used in this study impaired iodine and selenium status in the animals, and this might have contributed to following observed effects on growth and bone quality of the growing rats. There was a substantial natural sex differences among the rats and iodine and selenium differentially affected the bone of males and female rats. Adequacy of both iodine and selenium caused a rapid growth with relatively impaired bone quality as measured by trabecular bone microarchitecture and stiffness. The combined deficiency of the two trace elements reduced growth and bone quality as measured by trabecular number and connectivity that could be improved by the adequacy of either iodine or selenium. Female rats had slower growth rate, better oxidative status and better biomarkers of bone turnover, higher bone ash weight, and higher bone strength. Mechanisms whereby iodine and selenium depletion affects bone quality require further investigations.

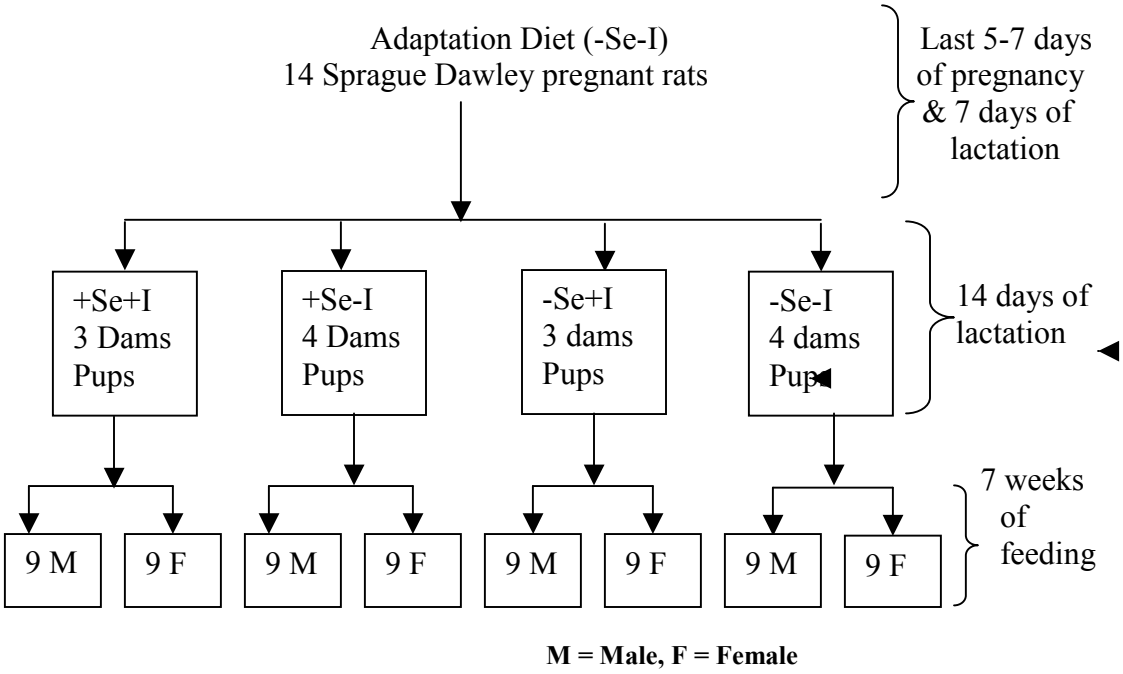

Figure 1: Experimental design

Table I

Composition of the experimental diets (g/kg diet)

Ingredients	+I +Se	+I -Se	-I +Se	-I -Se
Torula yeast	340	340	340	340
Dextrose	389.49	389.49	389.49	389.49
Sucrose	100	100	100	100
Soybean oil	70	70	70	70
Fiber (celufil)	50	50	50	50
Mineral mix +I+Se	35	-	-	-
Mineral mix +I-Se	-	35	-	-
Mineral mix -I +Se	-	-	35	-
Mineral mix -I-Se	-	-	-	35
Vitamin mix	10	10	10	10
L-cystine	3	3	3	3
Choline	2.5	2.5	2.5	2.5

I = Iodine. Se = Selenium. (+I+Se) = Adequate iodine, adequate selenium. (+I-Se) = Adequate iodine, low selenium. (-I+Se) = Low iodine, adequate selenium. (-I-Se) = Low iodine, low selenium.

Table II

Effects of sex and diet on weight gain, thyroid weight, serum thyroxin, serum triiodothyronine, and hepatic glutathione peroxidase activity

	Weight gain (g)	Thyroid weight (mg/100g BW)	Serum T_4 (µg/dl).	Serum T_3 (ng/dl).	Liver GSHPx Activity (mmole of NADPH oxidized/min/g)
Sex					
Males	191 ± 4	33 ± 3	2.2 ± 0.2	54.0 ± 2.3	8.22 ± 0.57
Females	134 ± 4	39 ± 3	2.4 ± 0.2	67.1 ± 2.2	7.43 ± 0.57
Diet					
+Se	175 ± 4	36 ± 3	2.0 ± 0.2	61.0 ± 2.60	14.20 ± 0.58
-Se	151 ± 4	37 ± 3	2.5 ± 0.2	60.0 ± 2.5	1.45 ± 0.56
+I	172 ± 4	7 ±3	3.9 ± 0.2	62.0 ± 2.60	7.79 ± 0.56
-I	153 ± 4	65 ±3	0.6 ± 0.2	59.1 ± 2.5	7.86 ± 0.58
Source of Variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	<0.008	<0.0001	<0.0001	0.45	0.93
Se	<0.002	0.80	<0.08	0.78	<0.0001
I*Se	0.43	0.91	0.18	0.30	0.49
Sex	<0.0001	0.15	0.53	<0.0001	0.33
I*Sex	0.87	0.30	0.18	0.11	0.76
Se*sex	<0.004	0.47	0.54	<0.05	0.56
I*Se*Sex	0.99	0.40	0.96	0.85	0.71

Values are least square means ± SEM. Effect is significant at $P<0.05$. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine. -I = Low iodine. BW = Body weight. T_4 = Thyroxin. T_3 =Triiodothyronine. GSHPx = Glutathione peroxidase.

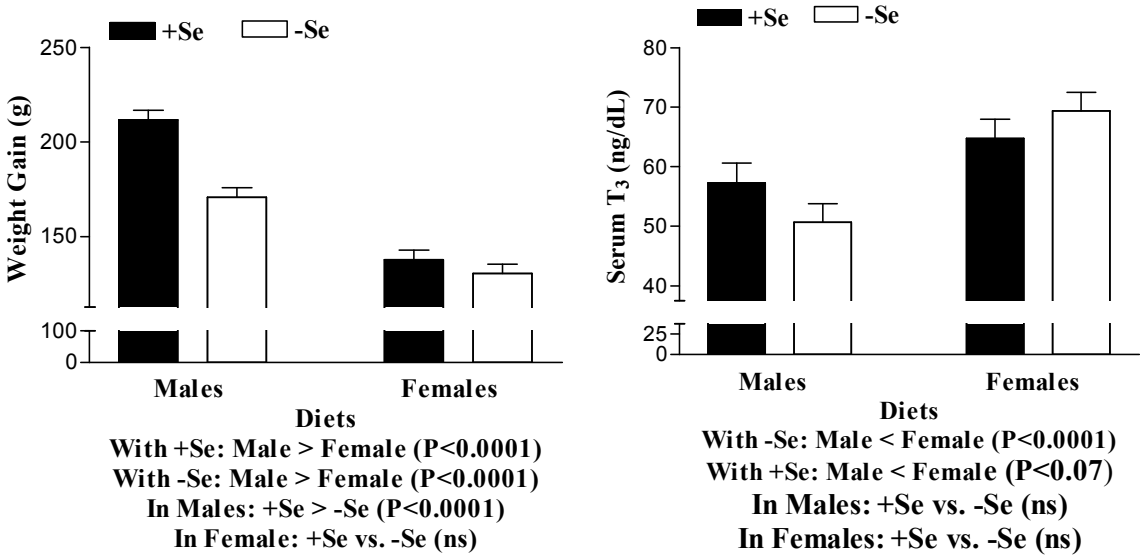

Figure 2: Interaction effects of selenium and sex on weight gain and serum triiodothyronine (T_3).

Table III

Effects of sex and diet on whole femur and whole tibia bone mineral area, bone mineral content, and bone mineral density by dual energy x-ray absorptiometry (DEXA)

	Femur BMA (cm^2)	Femur BMC (g)	Femur BMD (g/cm^2)	Tibia BMA (cm^2)	Tibia BMC (g)	Tibia BMD (g/cm^2)
Sex						
Males	1.58 ± 0.02	0.35 ± 0.01	0.222 ± 0.002	1.31 ± 0.02	0.217 ± 0.005	0.165 ± 0.002
Females	1.42 ± 0.02	0.32 ± 0.01	0.225 ± 0.002	1.15 ± 0.02	0.189 ± 0.005	0.164 ± 0.002
Diet						
+Se	1.53 ± 0.03	0.34 ± 0.01	0.224 ± 0.003	1.27 ± 0.02	0.211 ± 0.006	0.166 ± 0.002
-Se	1.47 ± 0.03	0.33 ± 0.01	0.221 ± 0.003	1.19 ± 0.02	0.195 ± 0.008	0.163 ± 0.002
+I	1.55 ± 0.03	0.35 ± 0.01	0.225 ± 0.002	1.27 ± 0.02	0.212 ± 0.006	0.166 ± 0.002
-I	1.45 ± 0.03	0.32 ± 0.01	0.220 ± 0.002	1.18 ± 0.02	0.19 4± 0.005	0.163 ± 0.002
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	<0.04	<0.06	0.19	<0.02	<0.06	0.42
Se	0.15	0.18	0.45	<0.04	<0.08	0.41
I*Se	0.57	0.53	0.68	0.37	0.44	0.77
Sex	<0.0001	<0.0006	0.59	<0.0001	<0.0001	0.65
I*Sex	0.12	0.30	0.82	0.74	0.67	0.65
Se*sex	0.08	<0.05	0.24	0.12	<0.03	<0.07
I*Se*Sex	0.85	0.97	0.84	0.20	0.39	0.95

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine. -I = Low iodine. BMA = Bone mineral area. BMC = Bone mineral content. BMD = Bone mineral density.

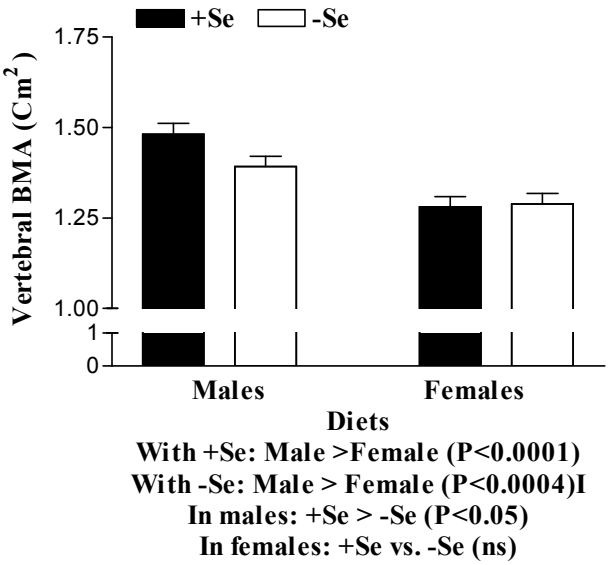

Figure 3: Interaction effects of selenium and sex on vertebral (L_{3-5}) bone mineral area (BMA)

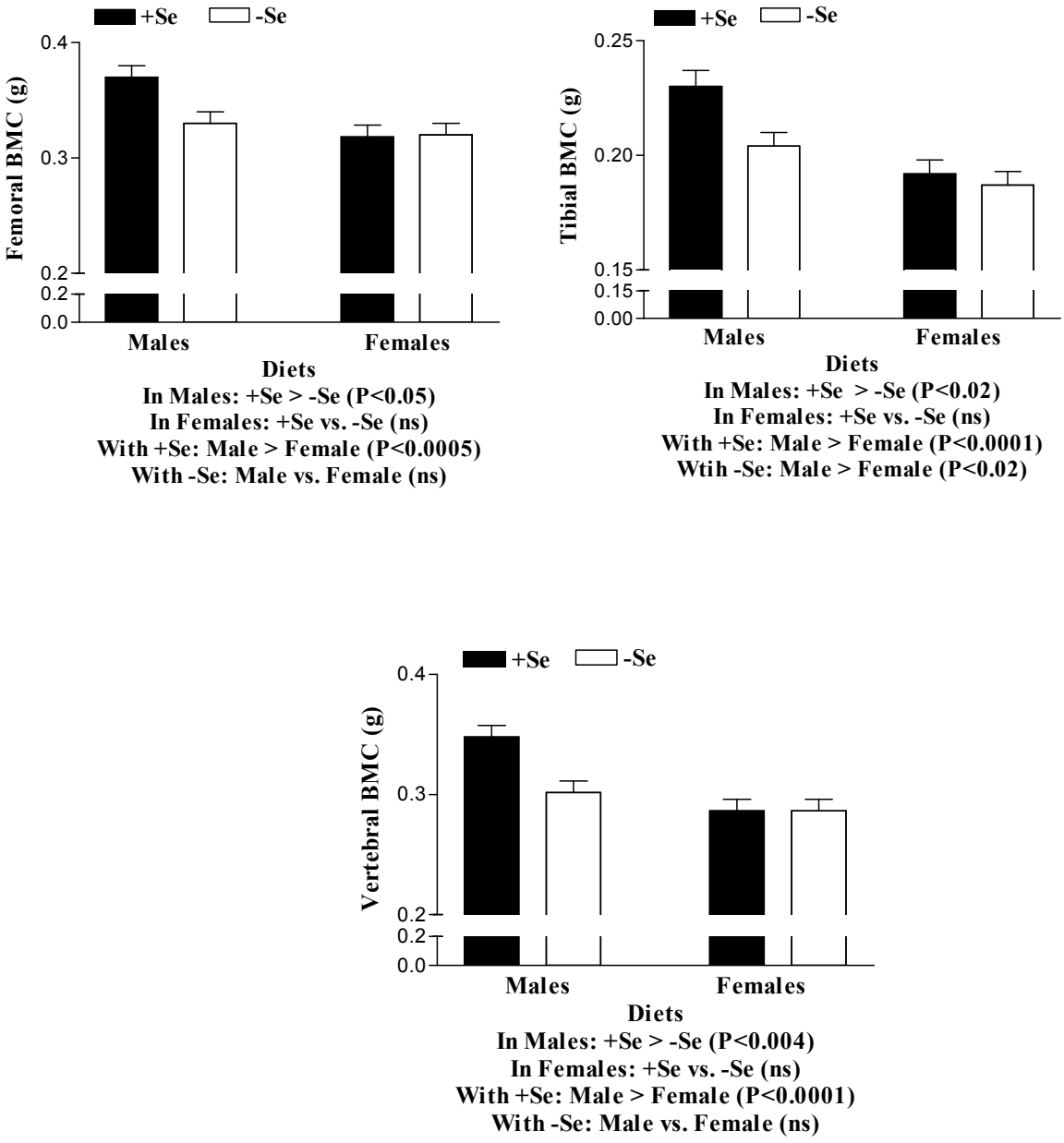

Figure 4: Interaction effects of selenium and sex on femoral, tibial, and vertebral bone mineral content (BMC)

Table IV

Effects of sex and diet on vertebral (L_{3-5}) bone mineral area, bone mineral content, and bone mineral density by dual energy x-ray absorptiometry (DEXA)

	Vertebral BMA (cm^2)	Vertebral BMC (g)	Vertebral BMD (g/cm^2)
Gender			
Males	1.44 ± 0.02	0.325 ± 0.007	0.226 ± 0.003
Females	1.29 ± 0.02	0.287 ± 0.007	0.223 ± 0.003
Diet			
+Se	1.38 ± 0.03	0.317 ± 0.008	0.229 ± 0.003
-Se	1.34 ± 0.03	0.294 ± 0.008	0.219 ± 0.003
+I	1.39 ± 0.03	0.317 ± 0.009	0.228 ± 0.003
-I	1.33 ± 0.02	0.294 ± 0.008	0.220 ± 0.003
Source of variation	*P-values*	*P-values*	*P-values*
I	0.12	0.08	0.14
Se	0.28	0.08	<0.07
I*Se	0.46	0.67	0.76
Sex	<0.0001	<0.0001	0.38
I*Sex	0.67	0.91	0.47
Se*Sex	<0.02	<0.002	<0.03
I*Se*Sex	0.94	0.99	0.90

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine. -I = Low iodine. BMA = Bone mineral area. BMC = Bone mineral content. BMD = Bone mineral density.

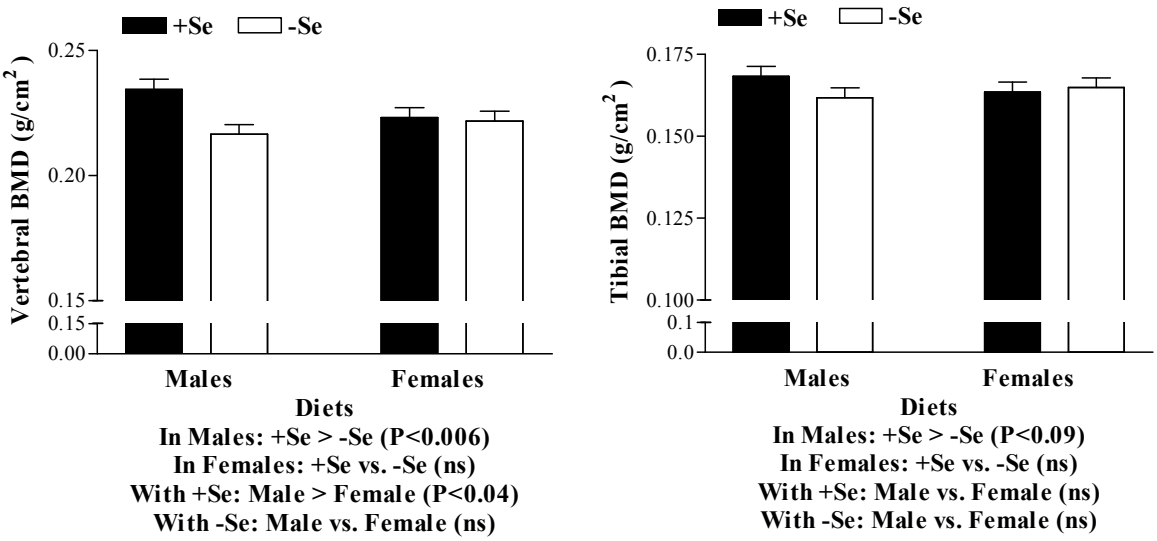

Figure 5: Interaction effects of selenium and sex on tibial and vertebral (L_{3-5}) bone mineral density (BMD)

Table V

Effects of sex and diet on tibial and femoral length, femur cortical thickness, and whole body lean and fat mass

	Tibia length (mm)	Femur length (mm)	Femur midshaft cortical thickness (mm)	Whole body lean mass (g/100g BW)	Whole body fat mass (g/100g BW)
Sex					
Males	37.40 ± 0.23	32.88 ± 0.19	0.66 ± 0.02	91.9 ± 0.3	8.1 ± 0.3
Females	35.80 ± 0.23	31.14 ± 0.19	0.68 ± 0.02	92.6 ± 0.3	7.4 ± 0.3
Diet					
+Se	37.09 ± 0.26	32.46 ± 0.23	0.71 ± 0.03	92.3 ± 0.3	7.7 ± 0.3
-Se	36.12 ± 0.25	31.57 ± 0.23	0.63± 0.03	92.2 ± 0.3	7.8 ± 0.3
+I	37.37 ± 0.26	32.77 ±0.24	0.70 ± 0.03	92.0 ± 0.3	8.0 ± 0.3
-I	35.83 ± 0.25	31.26 ± 0.22	0.64 ± 0.03	92.5 ± 0.3	7.5 ± 0.3
Source of variation		*P-values*	*P-values*	*P-values*	*P-values*
I	<0.002	<0.002	0.11	0.27	0.27
Se	<0.03	<0.03	<0.04	0.80	0.80
I*Se	0.49	0.12	0.41	0.36	0.36
Sex	<0.0001	<0.0001	0.69	<0.04	<0.04
I*Sex	0.16	0.43	0.97	0.71	0.71
Se*sex	0.09	0.42	0.92	0.47	0.47
I*Se*Sex	0.18	0.43	0.49	0.07	0.07

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -Se = Low selenium. +I = Adequate iodine. -I = low iodine.

Table VI

Effects of sex and diet on organ weight

	Liver weight (% BW)	Spleen weight (% BW)	Heart weight (% BW)
Sex			
Males	3.20 ± 0.05	0.235 ± 0.004	0.39 ± 0.01
Females	3.48 ± 0.05	0.283 ± 0.004	0.47 ± 0.01
Diet			
+Se	3.23 ± 0.05	0.264 ± 0.004	0.43 ± 0.01
-Se	3.45 ± 0.05	0.254 ± 0.004	0.43 ± 0.01
+I	3.43 ± 0.05	0.270 ± 0.004	0.43 ± 0.01
-I	3.24 ± 0.05	0.248 ± 0.004	0.43 ± 0.01
Source of variation	*P-values*	*P-values*	*P-values*
I	<0.02	<0.004	0.64
Se	<0.005	0.10	0.66
I*Se	<0.06	0.41	0.52
Sex	<0.0004	<0.0001	<0.0006
I*Sex	0.97	0.99	0.58
Se*sex	0.63	0.84	0.57
I*Se*Sex	0.54	0.71	0.83

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -Se = Low selenium. +I = Adequate iodine. -I = low iodine.

Table VII

Effects of sex and diet on proximal tibial trabecular total voulme, bone volume, bone volume fraction, trabecular number, and trabecular thickness

	Tibia TV (mm^3)	Tibia BV (mm^3)	Tibia BV/TV (1)	Tibia TbN (1/mm)	Tibia TbTh (mm)
Sex					
Males	24.27 ± 0.41	2.48 ± 0.12	0.102 ± 0.005	3.35 ± 0.10	0.053 ± 0.001
Females	18.47 ± 0.41	2.64 ± 0.12	0.143 ± 0.005	3.79 ± 0.10	0.058 ± 0.001
Diet					
+Se	22.35 ± 0.45	2.56 ± 0.14	0.118 ± 0.006	3.43 ± 0.11	0.055 ± 0.001
-Se	20.38 ± 0.44	2.56 ± 0.14	0.127 ± 0.006	3.71 ± 0.11	0.055 ± 0.001
+I	22.85 ± 0.45	2.75 ± 0.15	0.124 ± 0.006	3.59 ± 0.11	0.055 ± 0.001
-I	19.88 ± 0.44	2.37 ± 0.14	0.121 ± 0.006	3.55 ± 0.11	0.055 ± 0.001
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	<0.002	0.10	0.79	0.78	0.61
Se	<0.02	0.98	0.34	0.11	0.72
I*Se	0.35	<0.06	<0.03	<0.004	0.56
Sex	<0.0001	0.19	<0.0001	<0.0008	<0.0001
I*Sex	0.50	0.67	0.63	0.30	0.35
Se*sex	0.15	0.11	0.23	0.37	0.16
I*Se*Sex	0.46	0.73	0.83	0.70	0.71

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -S = Low selenium. +I = Adequate iodine. -I = Low iodine. TV = Total volume. BV= Bone volume. BV/TV = Bone volume fraction. TbN = Trabecular number. TbTh = Trabecular thickness.

Table VIII

Effects of sex and diet on L_3 total volume, bone volume, bone volume fraction, trabecular number, and trabecular thickness

	L_3 TV (mm^3)	L_3 BV (mm^3)	L_3 BV/TV (1)	L_3 TbN (1)	L_3 TbTh (mm)
Sex					
Males	11.56 ± 0.30	2.30 ± 0.10	0.197 ± 0.005	3.30 ± 0.03	0.069 ± 0.001
Females	9.90 ± 0.30	2.41 ± 0.10	0.244 ± 0.005	3.70 ± 0.03	0.071 ± 0.001
Diet					
+Se	11.23 ± 0.36	2.41 ± 0.12	0.216 ± 0.006	3.47 ± 0.03	0.070 ± 0.001
-Se	10.24 ± 0.35	2.30 ± 0.11	0.225 ± 0.006	3.52 ± 0.03	0.070 ± 0.001
+I	11.27 ± 0.36	2.44 ± 0.12	0.219 ± 0.006	3.47 ± 0.03	0.070 ± 0.001
-I	10.20 ± 0.34	2.26 ± 0.11	0.222 ± 0.006	3.53 ± 0.03	0.070 ± 0.001
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	<0.07	0.30	0.77	0.27	0.79
Se	0.09	0.52	0.27	0.33	0.92
I*Se	0.46	0.43	<0.03	<0.006	0.54
Sex	<0.0001	0.37	<0.0001	<0.0001	<0.04
I*Sex	0.81	0.54	0.77	0.19	0.82
Se*sex	0.10	<0.07	0.08	0.29	<0.02
I*Se*Sex	<0.03	0.12	0.97	0.87	0.69

Values are least square means ± SEM. Effect is significant at P<0.05. L_3 = Third lumbar vertebra. +Se = Adequate selenium. -Se = Low selenium. +I = Adequate iodine. -I = Low iodine. TV = Total tissue volume. BV = Bone volume. BV/TV = Bone volume fraction. TbNb = Trabecular number. TbTh = Trabecular thickness.

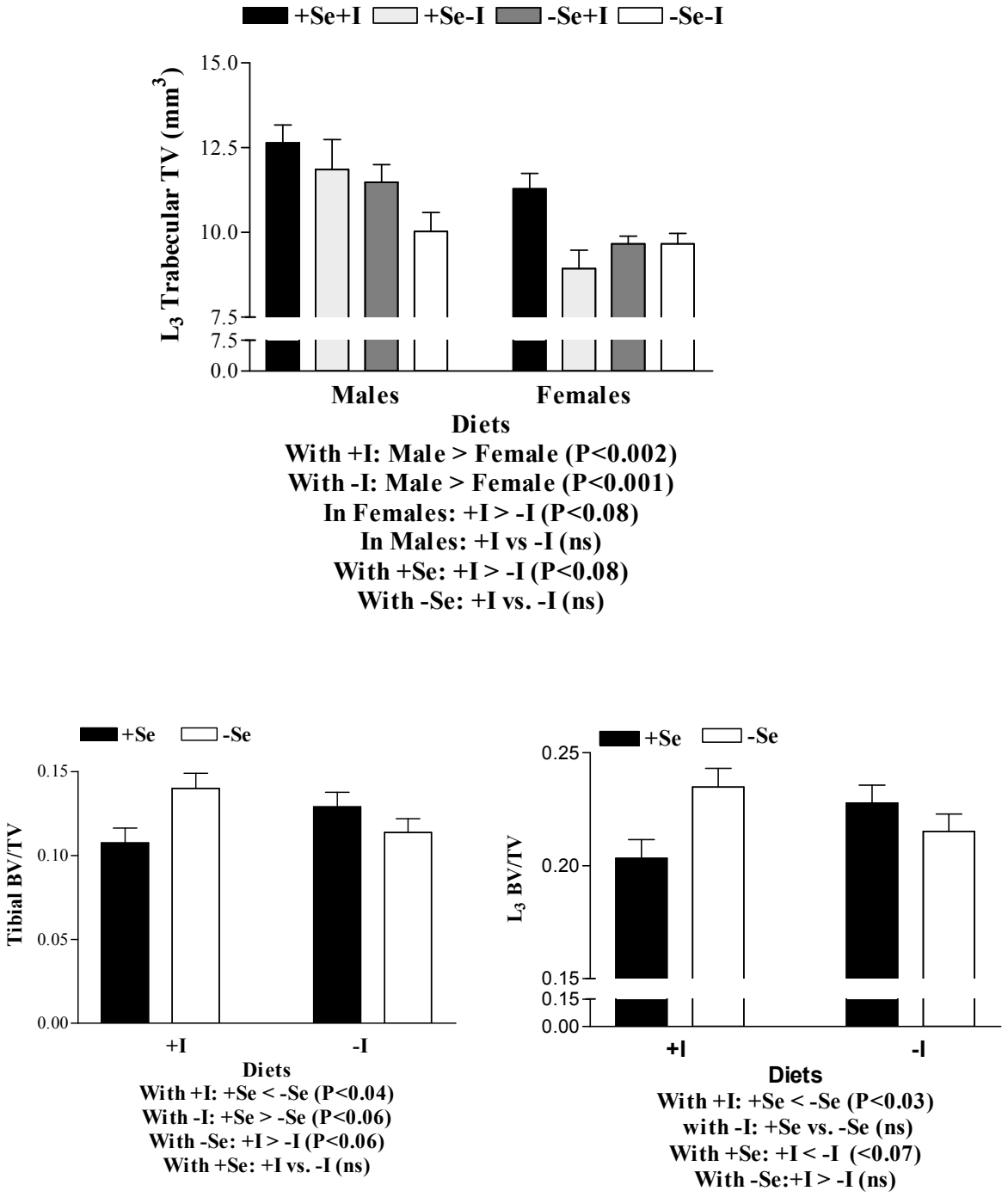

Figure 6: Interaction effects of iodine, seleniume, and sex on third lumbar vertebra (L_3) trabecular total volume and interaction effects of iodine and selenium on proximal tibial and L_3 trabecular bone volume fraction (BV/TV)

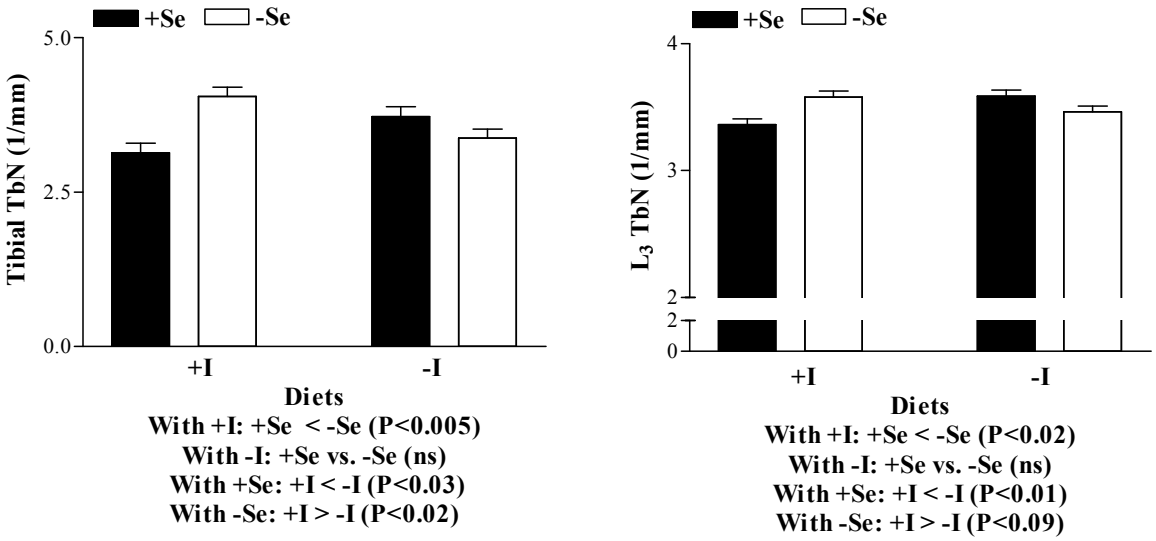

Figure 7: Interaction effects of iodine and selenium on proximal tibia and third lumbar vertebra (L_3) trabecular number (TbN).

Table IX

Effects of sex and diet on proximal tibia trabecular separation, connectivity density, structural model index, degree of anisotropy, and bone surface over bone volume

	Tibia TbSp (mm)	Tibia ConnD ($1/mm^3$)	Tibia SMI (1)	Tibia DA	Tibia BS/BV 1/mm
Gender					
Males	0.31 ± 0.01	48.74 ± 3.76	2.59 ± 0.04	2.33 ± 0.03	51.54 ± 0.68
Females	0.27 ± 0.01	78.32 ± 3.76	2.27 ± 0.04	2.14 ± 0.03	45.55 ± 0.68
Diet					
+Se	0.30 ± 0.01	59.00 ± 4.39	2.46 ± 0.05	2.27 ± 0.03	48.42 ± 0.77
-Se	0.28 ± 0.01	68.06 ± 4.23	2.39 ± 0.05	2.20 ± 0.03	48.67 ± 0.74
+I	0.29 ± 0.01	64.13 ± 4.38	2.41 ± 0.05	2.35 ± 0.03	48.94 ± 0.77
-I	0.30 ± 0.01	62.93 ± 4.24	2.45 ± 0.05	2.13 ± 0.03	48.45 ± 0.73
Source of variation	*P-values*	*P-values*	*P-values*	P-values	*P-values*
I	0.69	0.85	0.59	<0.0001	0.87
Se	0.32	0.17	0.34	<0.09	0.82
I*Se	<0.006	<0.01	<0.08	0.23	0.25
Sex	<0.06	<0.0001	<0.0001	<0.0001	<0.0001
I*Sex	0.48	0.91	0.80	0.44	0.78
Se*sex	0.68	0.39	0.70	0.39	0.23
I*Se*Sex	0.75	0.46	0.99	0.93	0.99

Values are least square means ± SEM. Effect is significant at P<0.05. +SI = Adequate selenium. -Se = Low selenium. +I = Adequate iodine. -I = Low iodine. TbSp = Trabecular separation. ConnD = Connectivity density. SMI = Structural model index. BS = Bone surface. BV = Bone volume.

Table X

Effects of sex and diet on L_3 trabecular separation, connectivity density structural model index, degree of anisotropy and bone surface over bone volume

	L_3 TbSp (mm)	L_3 ConnD ($1/mm^3$)	L_3 SMI (1)	L_3 DA (1)	L_3 BS/BV (1/mm)
Sex					
Males	0.301 ± 0.003	69.04 ± 1.63	1.12 ± 0.06	1.81 ± 0.02	34.40 ± 0.44
Females	0.264 ± 0.003	79.23 ± 1.63	0.48 ± 0.06	1.91 ± 0.02	32.03 ± 0.44
Diet					
+Se	0.284 ± 0.003	72.89 ± 1.65	0.93 ± 0.06	1.87 ± 0.02	33.36 ± 0.52
-Se	0.280 ± 0.003	75.39 ± 1.62	0.81 ± 0.05	1.85 ± 0.02	33.07 ± 0.52
+I	0.285 ± 0.003	75.75 ± 1.65	0.82 ± 0.07	1.87 ± 0.02	33.32 ± 0.54
-I	0.279 ± 0.003	72.53 ± 161	0.78 ± 0.07	1.85 ± 0.02	33.11 ±0.50
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	0.16	0.18	0.68	0.49	0.78
Se	0.37	0.30	0.18	0.39	0.70
I*Se	<0.0006	<0.005	<0.02	0.67	0.18
Sex	<0.0001	<0.0005	<0.0001	<0.0002	<0.0006
I*Sex	0.11	0.22	0.85	0.71	0.74
Se*sex	0.47	0.99	0.18	0.70	<0.03
I*Se*Sex	0.74	0.31	0.14	0.07	0.93

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -Se= = Low selenium. +I = Adequate iodine. -I = Low iodine. SMI = Structural model index. ConnD = Connectivity density. DA = Degree of anisotropy. Bs = Bone surface. BV = Bone volume.

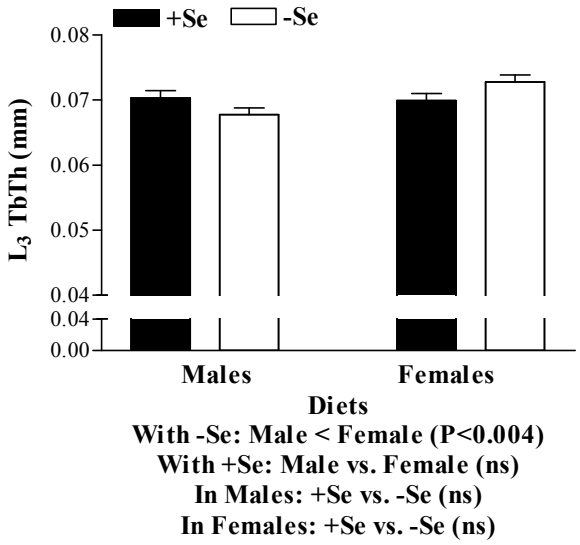

Figure 8: Interaction effects of selenium and sex on third lumbar vertebra (L_3) trabecular thickness (TbTh)

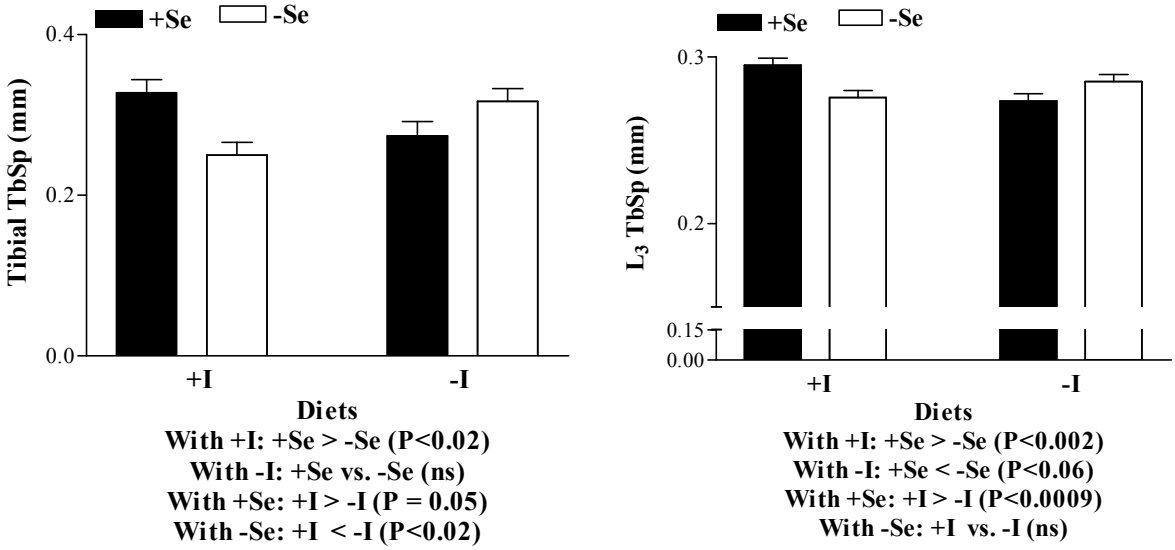

Figure 9: Interaction effects of iodine and selenium on trabecular separation (TbSp) of proximal tibia and third lumbar vertebra (L_3) trabecular bone

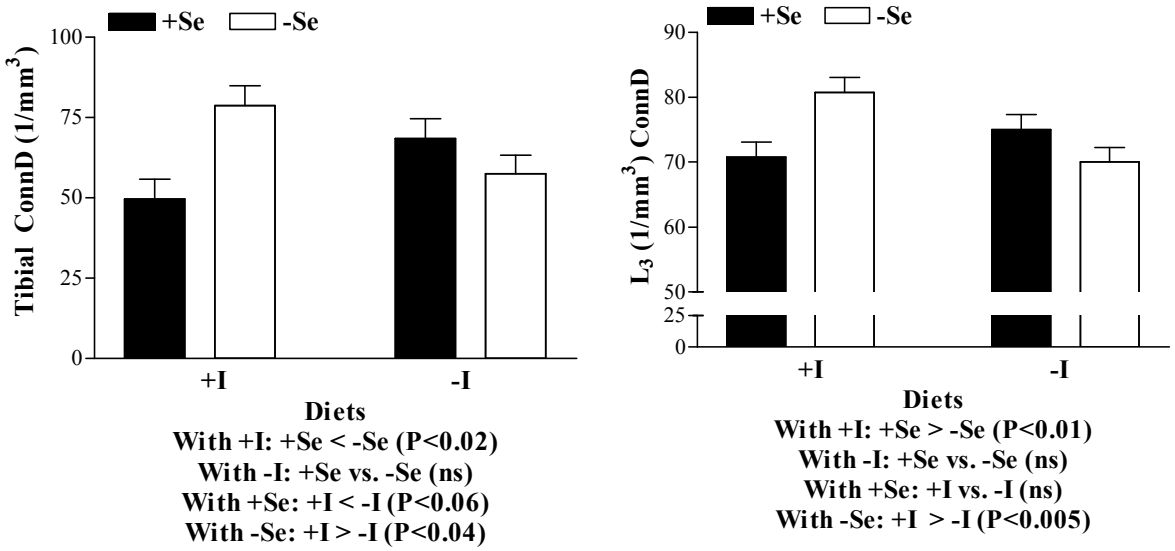

Figure 10: Interaction effects of iodine and selenium on connectivity density of proximal tibia and third lumbar vertebra (L3) trabecular bone

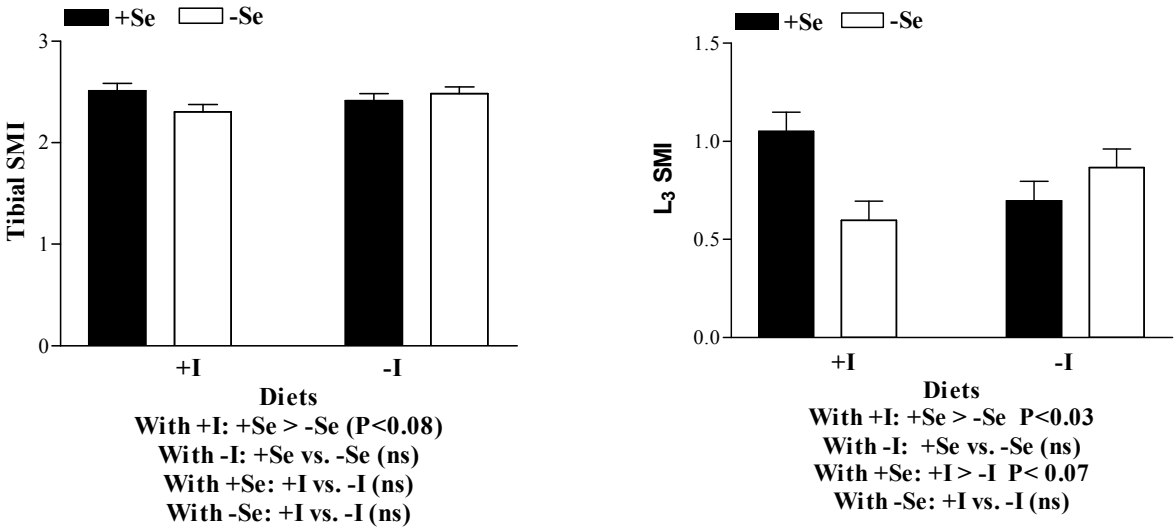

Figure 11: Interaction effects of iodine and selenium on structural model index (SMI) of proximal tibia and third lumbar vertebra (L_3) trabecular bone.

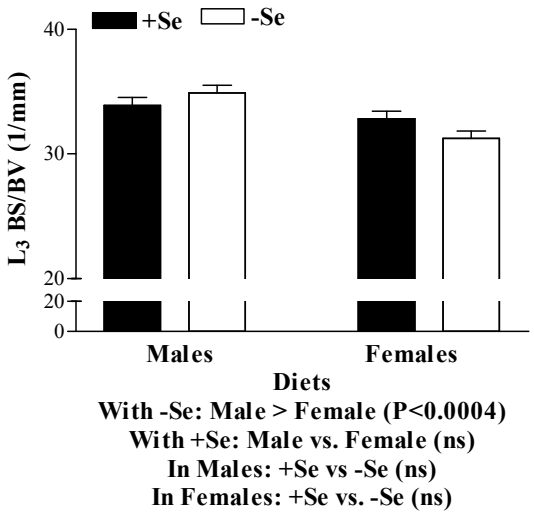

Figure 12: Interaction effects of selenium and sex on third lumbar vertebra trabecular bone surface over bone volume (BS/BV)

Table XI

Effects of sex and diet on tibial midshaft cortical total volume, bone volume, thickness, and porosity

	Tibial cortical TV (mm^3)	Tibial cortical BV (mm^3)	Tibial cortical bone thickness (mm)	Tibial cortical bone porosity (%)
Sex				
Males	2.49 ± 0.04	2.41 ± 0.04	0.491 ± 0.006	3.02 ± 0.11
Females	2.26 ± 0.04	2.20 ± 0.04	0.482 ± 0.006	2.90 ± 0.11
Diet				
+Se	2.41 ± 0.05	2.33 ± 0.05	0.485 ± 0.006	3.02 ± 0.12
-Se	2.35 ± 0.05	2.28 ± 0.04	0.488 ± 0.006	2.90 ± 0.11
+I	2.43 ± 0.05	2.35 ± 0.05	0.478 ± 0.006	3.16 ± 0.11
-I	2.32 ± 0.05	2.26 ± 0.04	0.495 ± 0.006	2.75 ± 0.11
Source of variation	*P-values*		*P-values*	*P-values*
I	0.15	0.19	<0.06	<0.04
Se	0.40	0.43	0.73	0.44
I*Se	0.11	0.12	0.24	0.46
Sex	<0.002	<0.002	0.31	0.43
I*Sex	0.99	0.87	0.82	<0.03
Se*sex	<0.06	<0.07	<0.04	0.87
I*Se*Sex	0.50	0.56	0.08	0.13

Values are least square means ± SEM. Effect is significant at $P<0.05$. +Se = Adequate selenium. –Se = low selenium. +I = Adequate iodine. –I = Low iodine. TV = Total volume. BV = bone volume. Porosity = (1-(BV/TV)) x 100.

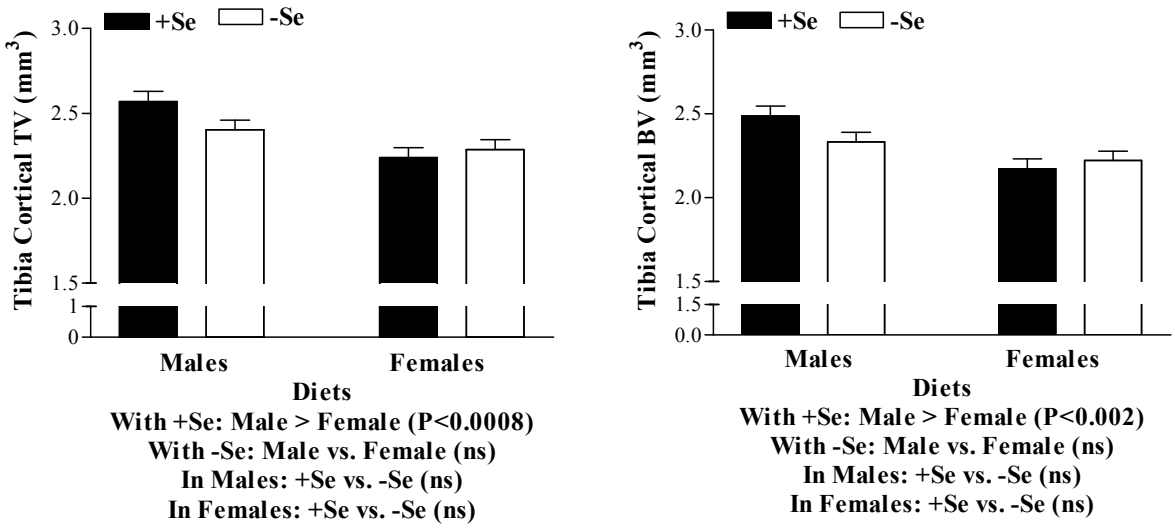

Figure 13: Interaction effects of selenium and sex on tibia midshaft cortical total volume (TV) and bone volume (BV)

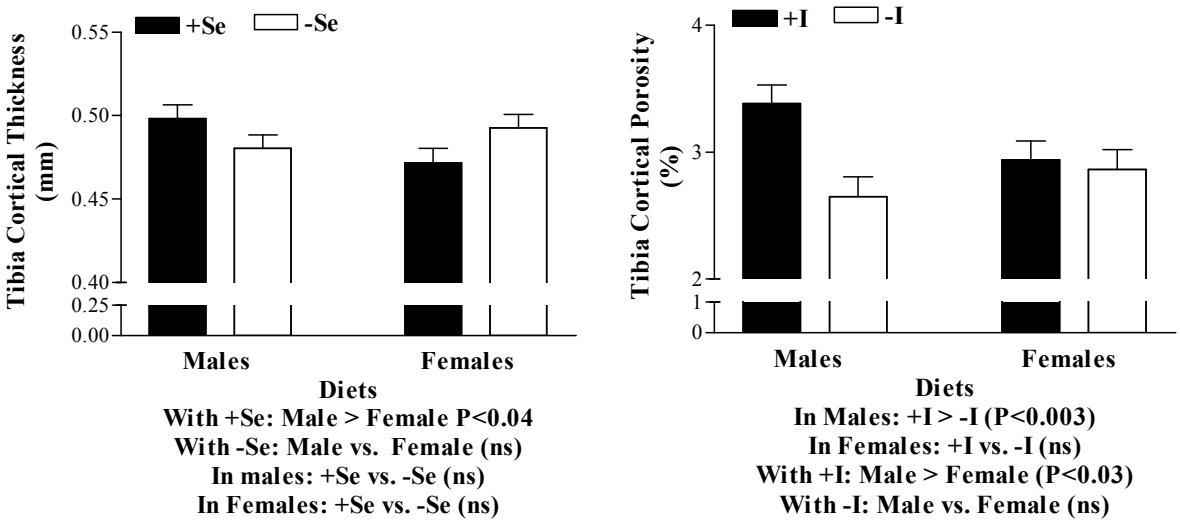

Figure 14: Interaction effects of selenium and sex on tibia midshaft cortical thickness and interaction effects of iodine and sex on tibia midshaft cortical porosity

Table XII

Effects of sex and diet on serum alkaline phosphatase, osteocalcin, tartrate resistant acid phosphatase, and urinary deoxypyridinoline

	Serum ALP (μmole/L)	Serum osteocalcin (ng/mL)	Serum TRAP (μmole/L)	Urinary DPD (nmole/mmole creatinine)
Sex				
Males	91.3 ± 7.3	31.3 ± 2.8	6.62 ± 0.37	168.0 ± 40.4
Females	71.8 ± 7.3	37.2 ± 2.8	5.86 ± 0.37	246.9 ± 40.9
Diet				
+Se	86.1 ± 9.2	41.0 ± 3.8	6.39 ± 0.47	198.0 ± 42.2
-Se	76.9 ± 9.0	27.5 ± 3.7	6.10 ± 0.46	216.9 ± 39.0
+I	88.6 ± 9.6	30.7 ± 4.0	6.38 ± 0.48	231.4 ± 39.6
-I	74.4 ± 8.6	37.8 ± 3.5	6.11 ± 0.44	183.5 ± 41.7
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*
I	0.30	0.21	0.69	0.41
Se	0.49	<0.04	0.67	0.74
I*Se	0.31	0.17	0.82	0.82
Sex	<0.03	<0.02	<0.04	0.18
I*Sex	0.27	0.21	<0.06	0.70
Se*sex	0.32	0.96	0.46	0.13
I*Se*Sex	0.21	0.23	0.59	0.49

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -Se = Low selenium. +I = adequate iodine. –I= Low Iodine. ALP = Alkaline phosphatase. TRAP = Tartrate resistant acid phosphatase. DPD = deoxypyridinoline.

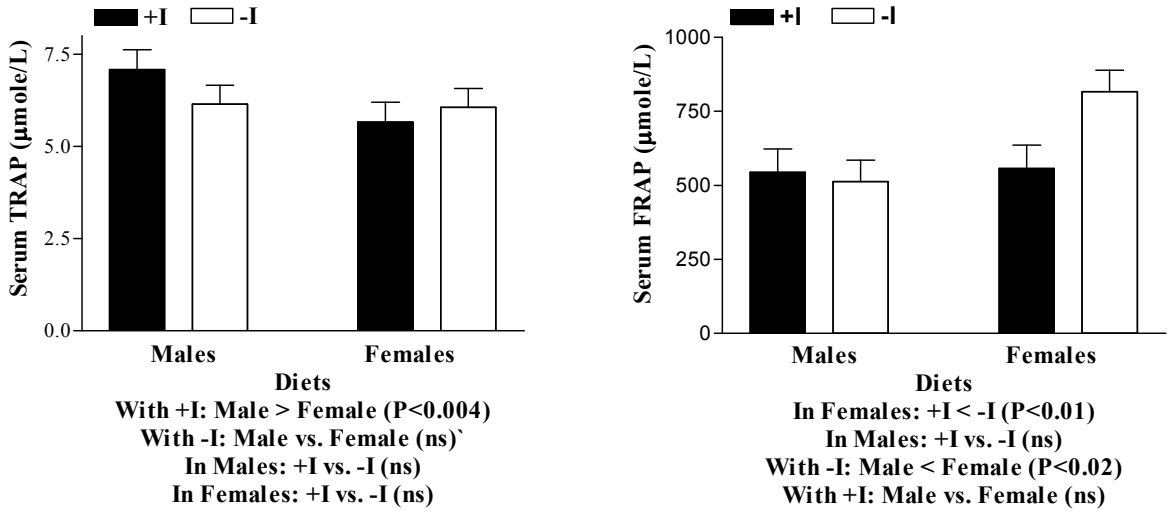

Figure 15: Interaction effect of iodine and sex on serum tartrate resistant acid phosphatase (TRAP) and serum ferric reducing ability of plasma (FRAP)

Table XIII

Effects of sex and diet on urinary Ca, urinary Mg, urinary P, serum FRAP and Liver thiobarbituric acid reactive substances

	Urinary Ca (mg/12 hours)	Urinary Mg (mg/12 hours)	Urinary P (mg/12 hours)	Serum FRAP (μmole/L)	Liver TBARS (nmole/mg protein)
Sex					
Males	0.85 ± 0.41	2.64 ± 0.21	7.74 ± 0.63	528.4 ± 53.5	0.291 ± 0.003
Females	1.25 ± 0.42	1.89 ± 0.21	5.82 ± 0.64	714.1 ± 53.6	0.267 ± 0.003
Diet					
+Se	1.28 ± 0.43	2.46 ± 0.24	7.52 ± 0.66	730.6 ± 61.0	0.267 ± 0.003
-Se	0.82 ± 0.41	2.07 ± 0.23	6.04 ± 0.61	511.9 ± 59.8	0.290 ± 0.003
+I	1.31 ± 0.43	2.59 ± 0.24	7.54 ± 0.62	550.6 ± 63.3	0.273 ± 0.003
-I	0.79 ± 0.42	1.94 ± 0.23	6.01 ± 0.65	691.9 ± 57.4	0.284 ± 0.003
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	0.40	0.09	0.09	0.13	<0.03
Se	0.46	0.28	0.11	<0.04	<0.0001
I*Se	0.40	0.27	<0.07	0.18	0.20
Sex	0.51	<0.007	<0.04	<0.02	<0.0001
I*Sex	0.29	0.35	0.78	<0.03	0.20
Se*sex	0.30	0.18	0.43	0.55	0.62
I*Se*Sex	0.37	0.69	0.92	0.67	0.06

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine. -I = low iodine. Ca = Calcium. Mg = Magnesium. P = Phosphorus. FRAP = Ferric reducing ability of plasma. TBARS = Thiobarbituric acid reactive substances.

Table XIV

Effects of sex and diet on femur wet weight, dry weight, and ash weight

	Femur wet weight (g)	Femur dry weight (g)	Femur ash weight (g)	Femur ash weight as percent of wet weight (%)	Femur ash weight as percent of dry weight (%)
Diet					
Males	0.69 ± 0.02	0.43 ± 0.01	0.26 ± 0.01	37.69 ± 0.60	61.03 ± 0.72
Females	0.63 ± 0.02	0.41 ± 0.01	0.27 ± 0.01	40.80 ± 0.60	63.32 ± 0.72
Diet					
+Se	0.68 ± 0.02	0.43 ± 0.01	0.27 ± 0.01	39.64 ± 0.61	62.27 ± 0.73
-Se	0.65 ± 0.02	0.41 ± 0.01	0.26 ± 0.01	38.85 ± 0.59	62.08 ± 0.71
+I	0.68 ± 0.02	0.43 ± 0.01	0.28 ± 0.01	39.46 ± 0.60	61.82 ± 0.71
-I	0.65 ± 0.02	0.40 ± 0.01	0.25 ± 0.01	39.03 ± 0.60	62.53 ± 0.73
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	0.20	<0.06	0.10	0.61	0.49
Se	0.38	0.20	0.22	0.36	0.85
I*Se	0.30	<0.08	0.38	0.67	0.10
Sex	<0.02	<0.02	0.41	<0.002	<0.03
I*Sex	0.32	0.47	0.41	0.29	0.91
Se*sex	0.39	<0.08	0.28	0.90	0.36
I*Se*Sex	0.56	0.58	0.61	0.83	0.75

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine. -I = low iodine.

Table XV

Effects of sex and diet on femur mineral content by atomic absorption spectrometry (AAS)

	Femur Ca (mg/g)	Femur Mg (mg/g)	Femur Zn (mg/g)	Femur Fe (mg/g)
Sex				
Males	188.8 ± 4.2	0.40 ± 0.09	0.292 ± 0.006	4.39 ± 0.14
Females	198.4 ± 4.2	0.27 ± 0.09	0.302 ± 0.006	4.16 ± 0.14
Diet				
+Se	193.7 ± 4.3	0.28 ± 0.09	0.284 ± 0.006	4.51 ± 0.15
-Se	193.6 ± 4.1	0.39 ± 0.09	0.310 ± 0.006	4.03 ± 0.15
+I	198.1 ± 4.2	0.42 ± 0.09	0.303 ±0.006	4.22 ± 0.15
-I	189.2 ± 4.2	0.26 ± 0.09	0.291 ± 0.006	4.32 ± 0.15
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*
I	0.15	0.23	0.14	0.65
Se	0.99	0.40	<0.01	<0.05
I*Se	0.61	0.42	0.23	0.10
Sex	0.12	0.33	0.21	0.27
I*Sex	0.57	0.37	0.29	0.19
Se*sex	0.43	0.35	0.19	0.30
I*Se*Sex	0.94	0.32	0.74	0.52

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. -Se = Low selenium, low iodine. +I = Adequate iodine. -I = Low iodine. Ca = Calcium. Mg = Magnesium. Zn = Zinc. Fe = Iron.

Table XVI

Effects of sex and diet on femur yield force, ultimate force, modulus of elasticity, yield stress, and ultimate stress by 3-point bending

	Yield force (N)	Ultimate force (N)	Stiffness (N/mm)	Modulus of elasticity (N/mm^2)	Yield stress (N/mm^2)	Ultimate stress (N/mm^2)
Sex						
Males	69.76 ± 1.89	78.30 ± 1.60	79.02 ± 2.55	3291.5 ± 141.9	113.2 ± 3.3	127.6 ± 3.4
Females	68.06 ± 1.89	75.14 ± 1.60	81.22 ± 2.55	4023.2 ± 141.9	126.3 ± 3.3	139.7 ± 3.4
Diet						
+Se	72.08 ± 1.91	79.41 ± 1.63	81.36 ± 2.62	3461.9 ± 144.1	119.2 ± 3.4	131.6 ± 3.5
-Se	65.74 ± 1.86	74.03 ± 1.58	78.88 ± 2.55	3852.8 ± 139.8	120.3 ± 3.3	135.7 ± 3.4
+I	71.54 ± 1.89	80.06 ± 1.58	85.52 ± 2.56	3768.9 ± 139.8	120.3 ± 3.3	134.9 ± 3.4
-I	66.28 ± 1.89	73.38 ± 1.63	74.73 ± 2.61	3545.8 ± 144.1	119.2 ± 3.4	132.4 ± 3.5
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*		
I	<0.07	<0.005	<0.02	0.27	0.82	0.60
Se	<0.03	<0.03	0.52	<0.06	0.82	0.40
I*Se	<0.07	<0.03	0.83	0.29	0.32	0.42
Sex	0.53	0.17	0.54	<0.0006	<0.008	<0.02
I*Sex	0.45	0.55	<0.05	0.28	0.74	0.99
Se*sex	0.70	0.45	0.48	0.72	0.99	0.78
I*Se*Sex	0.99	0.81	0.42	0.79	0.56	0.56

Values are least square means ± SEM. Effect is significant at P<0.05. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine.

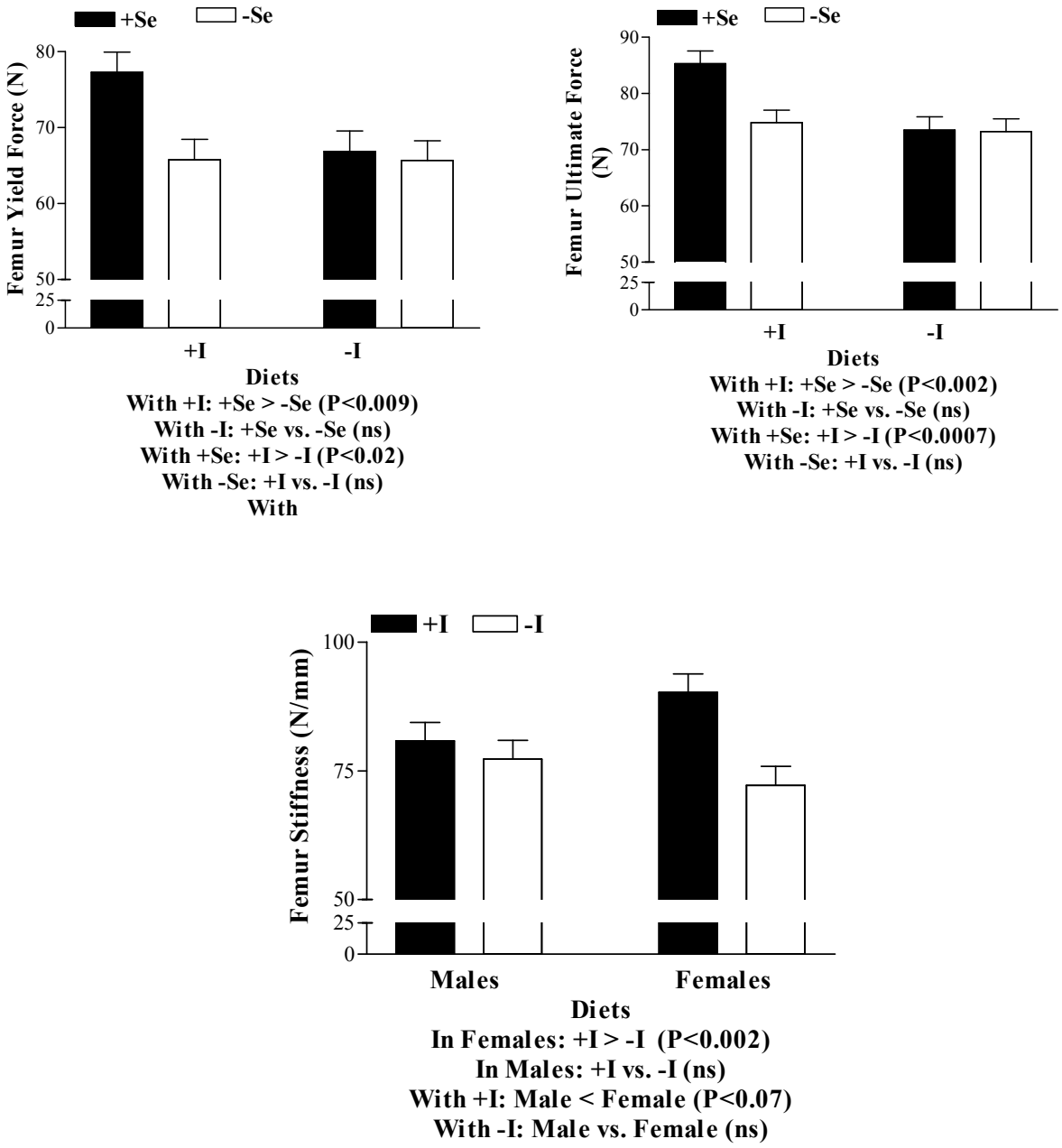

Figure 16: Interaction effects of iodine and selenium on the yield force and ultimate force for femur and interaction effects of iodine and selenium on femur stiffness

Table XVII

Effects of sex and diet on L_3 total force, physiological force, average strain, stiffness, size independent stiffness, and average von Mises stress by finite element analysis

	Total force (N)	Physiological force (N)	Average strain	Stiffness (N/m x 10^3)	Size independent Stiffness (N/m)	Average von Mises stress (MPa)
Sex						
Males	1245 ± 85	3.74 ± 0.25	0.183 ± 0.009	362073 ± 25065	374.4 ± 27.7	56.12 ± 1.97
Females	2016 ± 83	6.05 ± 0.25	0.262 ± 0.008	618366 ± 24591	674.2 ± 27.2	43.79 ± 1.94
Diet						
+Se	1567 ± 86	4.70 ± 0.26	0.217 ± 0.009	461823 ± 25428	493.5 ± 28.2	50.23 ± 1.99
-Se	1695 ± 82	5.08 ± 0.25	0.228 ± 0.008	518616 ± 24215	555.2 ± 27.0	49.68 ± 1.91
+I	1555 ± 82	4.67 ± 0.25	0.214 ± 0.008	445903 ± 24215	499.1 ± 27.3	50.23 ± 1.93
-I	1706 ± 86	5.12 ± 0.26	0.231± 0.009	534536 ± 25428	549.5 ± 28.0	49.68 ± 1.98
Source of variation	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I	0.21	0.21	0.17	0.24	0.23	0.84
Se	0.28	0.28	0.37	0.35	0.15	0.84
I*Se	<0.08	<0.08	<0.08	0.34	<0.05	<0.07
Sex	<0.0001	<0.0001	<0.0001	<0.09	<0.0003	<0.0005
I*Sex	0.72	0.72	0.87	0.84	0.84	0.66
Se*sex	0.30	0.30	0.80	0.72	0.27	0.48
I*Se*Sex	0.82	0.82	0.93	0.63	0.80	0.41

Values are least square means ± SEM. Effect is significant at $P<0.05$. +Se = Adequate selenium. –Se = Low selenium. +I = Adequate iodine.
L_3 = Third lumbar vertebra.

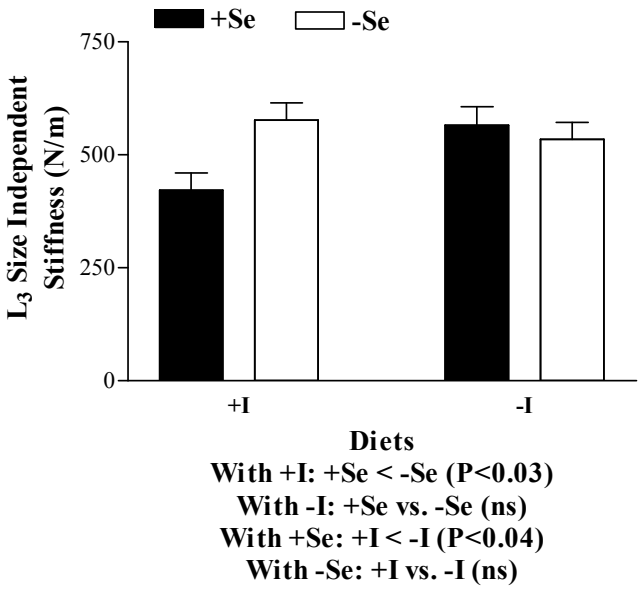

Figure 17: Interaction effects of iodine and selenium on L_3 size independent stiffness

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CHAPTER V

SUMMARY, CONCLUSIONS, AND SUGGESTIONS FOR FURTHER STUDIES

Summary

In this study we investigated the effects of iodine and/or selenium depletion on growth and bone quality of rats. To ascertain the iodine status of the rats we assessed thyroid weight, serum thyroxin, and triiodothyronine. Selenium status was determined by hepatic glutathione peroxidase activity.

Growth and Bone Density. Weight gain was reduced by iodine depletion in all the animals and by selenium depletion in male rats. Selenium depletion reduced growth, bone mineral area, content, and density in males without affecting the values in females. Thyroid hormone (T_3) concentrations were lower in males than females when selenium was depleted. Cortical thickness of the femur was reduced by selenium depletion in all the animals. Iodine and selenium depletion independently reduced BMA and bone length in tibia and femur and selenium depletion decreased vertebral BMA in male rats. Males had longer tibia and femurs than females. There were no significant differences between males and females with respect to bone mineral density as measured by DEXA.

Organ weights were higher in females than males and iodine depletion decreased both liver weight and spleen weight. Selenium depletion increased liver weight. There

were no diet effects on heart weight.

Microarchitecture. Female rats had better cancellous bone microarchitecture than male rats as evidenced by lower SMI, higher BV/TV, higher trabecular thickness, higher trabecular connectivity and lower trabecular separation than males. In general, the microarchtectural parameters of trabecular bone were impaired in iodine adequate rats when selenium was also adequate as shown by by lower BV/TV, TbN, TbTh, ConnD, and higher TbSp in iodine adequate rats when selenium was also adequate. However, when selenium was deficient the adequacy of iodine improved the trabecular bone quality as evidenced by increased trabecular number and connectivity by iodine adequacy in selenium depleted animals as compared with iodine and selenium depleted animals. In addition there was a tendency of improved bone volume fraction by the adequacy of iodine in selenium depleted rats as compared to both iodine and selenium depleted animals. The cortical bone volume and thickness were higher in males than females when selenium was adequate, but the adequacy of iodine increased cortical porosity in males. This was consistent with a higher circulating TRAP in iodine adequate male rats.

Biomechanical Properties. The 3-point bending of the femurs showed that selenium depletion decreased the breaking force (yield force and ultimate force) of femur in iodine adequate rats. Also when selenium was adequate, the depletion of iodine decreased the breaking force. In females, iodine depletion decreased femur stiffness without affecting the bone stiffness of male rats and the modulus of elasticity was higher in females than males. Bone strength (yield stress and ultimate stress) as measured by 3-point bending of the femur was also higher in female than male rats.

Finite element analysis of the trabecular cores of L_3 by μCT showed that force to entirely crush the bone and average strain were higher in females while von Mises stress was higher in males. Size independent stiffness of L_3 trabecular bone was decreased in selenium adequate rats when iodine was also adequate. This may partially be explained by growth rate.

Biochemical and Oxidative Status. Male rats had higher bone turnover, higher lipid peroxidation and lower femur ash weight than females. This was evidenced by a concomitant higher serum ALP and tartrate resistant acid phosphatase. Urinary excretion of P and Mg were also higher in male than female rats. Serum osteocalcin was decreased by selenium depletion, and osteocalcin concentrations were higher in females than males. There were no significant effects of iodine, selenium or sex on urinary Ca and urinary DPD.

Females had higher serum FRAP, lower TBARS, and higher femur ash weights than males. There were no significant sex effects on femur Ca, Mg, and Fe bone mineral content as measured by atomic absorption spectrometry. Selenium depletion increased femur Zn, but decreased femur Fe. Females had a slower growth rate, but a better bone quality as ascertained by less bone turnover, less lipid peroxidation, better bone microarchitecture, higher bone ash weight, higher bone volume fraction, and higher bone breaking and compressive strengths.

Conclusions

For this study we hypothesized:

1. Iodine and/or selenium depletion will not significantly reduce growth and bone density of growing rats. We were able to reject the first hypothesis because selenium depletion in male rats significantly reduced growth and bone density of growing rats as evidenced by reduced weight gain by iodine depletion in all rats and by selenium depletion in males. Decreased bone mineral area, content and density as well as decreased bone length and cortical thickness were also observed in iodine and/or selenium depleted animals.
2. Iodine and/or selenium depletion will not negatively affect biochemical markers of bone metabolism and oxidative status of growing rats. We were able to reject the second hypothesis because selenium depletion significantly decreased biomarkers of bone formation and impaired antioxidant status as shown by decreased serum osteocalcin and serum FRAP by selenium depletion. An independent increase of liver TBARS by iodine and selenium depletion was also observed. However, the other biomarkers of bone metabolism were not affected by either selenium or iodine depletion.
3. Iodine and/or selenium depletion will not significantly deteriorate the microarchitecture of growing rat bone. We were not able to reject the third hypothesis because the combined adequacy of iodine and selenium caused a deterioration of tibial and L_3 trabecular bone microarchitecture. However, when selenium was deficient, the adequacy of iodine improved the microarchitecture of the bone as compared to double depletion of iodine and selenium. This was shown by increased tibial trabecular number and an increase in both tibial and L_3 trabecular connectivity by iodine adequacy in selenium deficient rats. This indicates that combined deficiency of both iodine and selenium may be detrimental to bone.

4. Iodine and or selenium depletion will not impair biomechanical properties of the bone of growing rats. We were able to reject the fourth hypothesis because iodine and selenium depletion decreased femur bending force in all the animals as evidenced by decreased yield force and ultimate force by iodine and selenium depletion in selenium and iodine adequate rats respectively. In addition, iodine depletion decreased femur stiffness in female rats. However the combined adequacy of iodine and selenium reduced the compressive stiffness of L_3 trabecular cores.
5. Male and female rat bones will not be differently affected by iodine and/selenium depletion. We were able to reject the fifth hypothesis because selenium depletion had more effects on the bone of males, as evidenced by decreased weight gain, BMA, BMC and BMD by selenium depletion in males without affecting female rats. In general, iodine depletion affected both males and females, but there was a slight trend toward more effects of iodine depletion on females. This trend was supported by a significant decrease of femur stiffness in female by iodine depletion without affecting the bone thickness of male rats. This sex difference in response of bone to iodine and selenium depletion was accompanied by a slower growth rate, smaller bone size, lower bone turnover, better bone microarchitecture, less lipid peroxidation, and higher bone strength in female than male rats.

Suggestions for Further Studies

- Because lipid peroxidation was increased by both iodine and selenium depletion, and antioxidant status was impaired by selenium depletion, it would be interesting

to assess inflammatory markers such as IL1, IL-6, and TNF-α, and C-reactive protein to see whether increased lipid peroxidation and decreased antioxidant staus in selenium-deficient rats are accompanied by increased levels of these cytokines. Lipid peroxidation may lead to the generation of reactive oxygen species (ROS). ROS in high concentration damage bone cells and increase the production of inflammatory cytokines (79). These cytokines are necessary for osteoclast activity and increase their own activities by inducing the production of ROS (79)

- Because growth has been affected by both iodine and selenium, assessment of IGF-1, IGF-binding proteins, and growth hormone and their association with bone quality should be determined.
- We assumed that iodine and/or selenium depletion would cause osteoarthritis-like symptoms and that osteoarthtis would lead to impaired bone quality. But we did not directly assess any indices of osteoarthritis. Histomorphometric analysis of rat joint structure is needed as well as the assessment of biomarkers of osteoarthritis and rheumatoid arthritis. Diagnosis of osteoarthritis currently relies on clinical history and radiography in humans. However potential biomarkers of osteoarthritis have been proposed by Haq and colleagues (54). These markers include indices of cartilage destruction such as cartilage oligomeric matrix protein (COMP) and markers of synovial inflammation (C-reactive protein, hyaluronan, YKL-40, and metalloproteinases). Some of these potential markers for osteoarthritis will be assessed. Potential biomarkers of rheumathoid arthritis such as IL-1β, IL-6 and IL-8 (167) and rheumathoid factor IgM could also be determined in iodine and/or selenium-depleted growing rats.

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APPENDICES

APPENDIX A

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 12/12/2004

Date : Monday, January 21, 2002 Animal Care and Use From (ACUF) No: HE021

Proposal Title: EFFECT OF IODINE AND SELENIUM DEFICIENCIES ON BONE OF RATS

Principal Investigator:

Barbara Stoecker
425 HES
Stillwater, OK 74078

Reviewed and Processed as: Full Committee **Modification**

Approval Status Recommended by Reviewer(s) : Approved

Approved for the addition 2 pregnant rats and 16 pups.

Signatures :

Reagan Unruh for
Dr. Kent Olson, IACUC Chairperson

Monday, January 21, 2002
Date

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project or course must be submitted for review and approval by the IACUC, prior to initiating any changes in animal use. Modifications do not affect the original approval period. Modification approvals are valid for the duration of the protocol approval (see protocol expiration date). Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

APPENDIX B

Composition of the mineral mix

Mineral	g/kg mix
Essential mineral elements	
Calcium carbonate, anhydrous, 40.04% Ca	357.00
Potassium phosphate, monobasic, 22.76% P; 28.73% K	250.00
Sodium chloride, 39.34% Na; 60.66% Cl	74.00
Potassium sulfate, 44.87% K; 18.39% S	46.60
Potassium citrate, tri-potassium, monohydrate, 36.16% K	28.00
Magnesium oxide, 60.32% Mg	24.00
Ferric citrate, 16.5% Fe	6.06
Zinc carbonate, 52.14% Zn	1.65
Manganous carbonate, 47.79% Mn	0.63
Cupric carbonate, 57.47% Cu	0.30
∗Potassium iodate, 59.3% I	0.01
∗∗Sodium selenate, anhydrous, 41.79% Se	0.01025
Ammonium paramolybdate, 4 hydrate, 54.34% Mo	0.00795
Potentially beneficial mineral elements	
Sodium meta-silicate, 9 hydrate, 9.88% Si	1.45
Chromium potassium sulfate, 12 hydrate, 10.42% Cr	0.275
Boric acid, 17.5% B	0.0815
Sodium fluoride, 45.24% F	0.0635
Nickel carbonate, 45% Ni	0.0318
Lithium chloride 16.38% Li	0.0174
Ammonium vanadate, 43.55% V	0.0066
Powdered sucrose	209.806

∗The mineral mix for the diets depleted in I was prepared without adding potassium iodate.

∗∗The mineral mix for selenium depleted diets was prepared without adding sodium selenate. The mineral mix for the diet depleted in both iodine and selenium was prepared without adding neither potassium iodate nor sodium selenate.

APPENDIX C

Composition of the Vitamin Mix

Vitamin	g/kg mix
Nicotinic acid	3.000
Ca pentothenate	1.600
Pyridoxine-HCl	0.700
Thiamin-HCl	0.600
Riboflavin	0.600
Folic acid	0.200
D-Biotin	0.020
Vitamin B12 (cyanocobalamin in 0.1% manitol)	2.500
Vitamin E (all-rac-α-tocopheryl acetate) (500 IU/g)	15.00
Vitamin A (all trans Retinyl palmitate)(500,000 IU/g)	0.800
Vitamin D3 (cholecalciferol) (400,000 IU/g)	0.250
Vitamin K (phyloquinone)	0.075
Powdered sucrose	974.655

APPENDIX D

Effects of the diets on weight gain, thyroid weight, serum thyroid hormones, and liver glutathione peroxidase activity

Diet	Gender (n)	Weight Gain (g)	Thyroid Weight (g/100 g BW)	Serum T_4 (µg/dl).	Serum T_3 (ng/dl).	Liver GSH-PX Activity (mmole of NADPH oxidized/min/g)
+Se+I	Male (9)	223.86 ± 6.63	0.0060 ± 0.0004	3.642 ± 0.154	62.667 ± 3.468	14.774 ± 1.034
	Female (9)	149.18 ± 4.21	0.0071 ± 0.0012	3.328 ± 0.092	66.244 ± 4.193	12.966 ± 1.314
+Se-I	Male (8	197.06 ± 6.53	0.0562 ± 0.0060	0.393 ± 0.047	51.871 ± 1.947	14.888 ± 1.874
	Female (8)	126.12 ± 2.62	0.0730 ± 0.0068	0.720 ± 0.148	63.264 ± 2.185	14.178 ± 2.188
-Se+I	Male (9)	178.33 ± 3.23	0.0061 ± 0.0005	4.375 ± 0.486	52.529 ± 3.082	1.836 ± 0.443
	Female (9)	137.73 ± 2.40	0.0082 ± 0.0009	4.345 ± 0.298	66.553 ± 3.103	1.572 ± 0.246
-Se-I	Male (9)	163.60 ± 8.72	0.0644 ± 0.0089	0.401 ± 0.098	48.818 ± 4.759	1.374 ± 0.212
	Female (9)	122.84 ± 6.05	0.0681 ± 0.0104	0.963 ± 0.518	72.413 ± 6.410	1.005 ± 0.295
Source of Variation		*P-value*	*P-values*	*P-values*	*P-values*	*P-values*
I		<0.008	<0.0001	<0.0001	0.45	0.93
Se		<0.002	0.80	<0.08	0.78	<0.0001
I*Se		0.43	0.91	0.18	0.30	0.49
Sex		<0.0001	0.15	0.53	<0.0001	0.33
I*Sex		0.87	0.30	0.18	0.11	0.76
Se*sex		<0.004	0.47	0.54	<0.05	0.56
I*Se*Sex		0.99	0.40	0.96	0.85	0.71

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine.
+Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. BW = Body weight. T_4 = Thyroxin.
T_3 =Triiodothyronine.

APPENDIX E

Effects to the diets on tibia and femur bone mineral area, bone mineral content, and bone mineral density by dual energy x-ray absorptiometry

Diet	Gender (n)	Tibia BMA (cm^2)	Tibia BMC (g)	Tiba BMD (g/cm^2)	Femur BMA (cm^2)	Femur BMC (g)	Femur BMD (g/cm^2)
+Se+I	Male (9)	1.404 ± 0.037	0.238 ± 0.009	0.170 ± 0.003	1.681 ± 0.041	0.385 ± 0.015	0.228 ± 0.003
	Female (9)	1.245 ± 0.027	0.207 ± 0.006	0.166 ± 0.003	1.503 ± 0.037	0.341 ± 0.012	0.226 ±0.003
+Se-I	Male (8	1.317 ± 0.025	0.219 ± 0.007	0.166 ± 0.002	1.576 ± 0.073	0.349 ± 0.014	0.222 ± 0.004
	Female (8)	1.094 ± 0.023	0.177 ± 0.006	0.161 ± 0.002	1.344 ± 0.031	0.296 ± 0.008	0.220 ± 0.003
-Se+I	Male (9)	1.295 ± 0.022	0.210 ± 0.004	0.162 ± 0.001	1.542 ± 0.036	0.341 ± 0.008	0.221 ± 0.001
	Female (9)	1.148 ± 0.014	0.192 ± 0.004	0.167 ± 0.002	1.472 ± 0.034	0.332 ± 0.007	0.225 ± 0.003
-Se-I	Male (9)	1.211 ± 0.037	0.196 ± 0.009	0.163 ± 0.003	1.510 ± 0.043	0.327 ± 0.013	0.216 ± 0.004
	Female (9)	1.104 ± 0.048	0.181 ± 0.011	0.163 ± 0.004	1.343 ± 0.036	0.299 ± 0.013	0.222 ± 0.005
Source of Variation		P-values	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		<0.02	<0.06	0.42	<0.04	<0.06	0.19
Se		<0.03	<0.08	0.41	0.15	0.18	0.45
I*Se		0.37	0.44	0.77	0.57	0.53	0.68
Sex		<0.0001	<0.0001	0.65	<0.0001	<0.0006	0.57
I*Sex		0.74	0.67	0.65	0.12	0.30	0.82
Se*sex		0.12	<0.03	<0.07	<0.08	<0.05	0.24
I*Se*Sex		0.20	0.39	0.94	0.85	0.97	0.84

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. BMA = Bone mineral area. BMC = Bone mineral content. BMD = Bone mineral density.

APPENDIX F

Effects of the diets on vertebral bone mineral area, bone mineralcontent, and bone mineral density by dual energy x-ray absorptiometry

Diet	Gender (n)	Vertebral BMA (cm^2)	Vertebral BMC (g)	Vertebral BMD (g/cm^2)
+Se+I	Male (9)	1.531 ± 0.034	0.362 ± 0.012	0.236 ± 0.006
	Female (9)	1.319 ± 0.025	0.301 ± 0.009	0.228 ± 0.004
+Se-I	Male (8	1.423 ± 0.036	0.331 ± 0.012	0.232 ± 0.006
	Female (8)	1.242 ± 0.026	0.272 ± 0.009	0.219 ± 0.004
-Se+I	Male (9)	1.413 ± 0.024	0.310 ± 0.007	0.220 ± 0.005
	Female (9)	1.302 ± 0.016	0.297 ± 0.007	0.228 ± 0.004
-Se-I	Male (9)	1.367 ± 0.046	0.292 ± 0.013	0.213 ± 0.004
	Female (9)	1.271 ± 0.038	0.276 ± 0.014	0.216 ± 0.006
Source of Variation		*P-values*	*P-values*	*P-values*
I		0.12	<0.08	0.14
Se		0.28	<0.08	<0.07
I*Se		0.46	0.67	0.76
Sex		<0.0001	<0.0001	0.38
I*Sex		0.67	0.90	0.47
Se*sex		<0.02	<0.002	<0.03
I*Se*Sex		0.94	0.99	0.90

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium,low iodine. -Se+I = Low selenium, adequate iodine. -Se-I = Low selenium, low iodine. Vertebral = L_{3-5} = Lumbar 3, Lumbar 4, and Lumbar 5 together. BMA = Bone mineral area. BMC = Bone mineral content. BMD = Bone mineral density.

APPENDIX G

Effect of the diets on tibia trabecular total volume, bone volume, bone volume fraction, bone surface, bone surface over bone volume, and degree of anisotropy by micro computed tomography

Diet	Gender (n)	Tibia TV (mm^3)	Tibia BV (mm^3)	Tibia BV/TV	Tibia BS (mm^2)	Tibia BS/BV (1/mm)	Tibia DA (1)
+Se+I	Male (9)	27.481 ± 0.843	2.491 ± 0.115	0.091 ± 0.005	114.857 ± 5.204	51.518 ± 1.099	2.508 ± 0.033
	Female (9)	20.807 ± 1.008	2.546 ± 0.211	0.124 ± 0.010	107.353 ± 7.264	46.801 ± 1.268	2.318 ± 0.032
+Se-I	Male (8	23.688 ± 0.764	2.716 ± 0.233	0.115 ± 0.010	121.063 ± 9.519	50.028 ± 0.979	2.267 ± 0.062
	Female (8)	17.221 ± 0.460	2.538 ±0.161	0.147 ± 0.009	105.259 ± 6.454	45.036 ± 0.576	2.006 ± 0.146
-Se+I	Male (9)	23.653 ± 0.379	2.794 ± 0.137	0.118 ± 0.005	129.156 ± 5.328	51.513 ± 1.000	2.353 ± 0.022
	Female (9)	19.481 ± 0.304	3.138 ± 0.098	0.161 ± 0.005	129.642 ± 3.686	44.760 ± 0.079	2.228 ± 0.029
-Se-I	Male (9)		1.956 ± 0.232	0.087 ± 0.007	90.792 ± 9.622	53.175 ± 1.191	2.206 ± 0.038
	Female (9)	16.288 ± 0.561	2.296 ± 0.247	0.138 ± 0.012	95.206 ± 9.416	45.795 ± 1.059	2.023 ± 0.039
Source of Variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		<0.002	0.10	0.79	<0.05	0.87	<0.0001
Se		<0.02	0.97	0.34	0.98	0.82	<0.09
I*Se		0.35	<0.06	<0.03	<0.05	0.25	0.23
Sex		<0.0001	0.19	<0.0001	0.39	<0.0001	<0.0001
I*Sex		0.50	0.67	0.63	0.88	0.78	0.44
Se*sex		0.15	0.11	0.23	0.16	0.23	0.39
I*Se*Sex		0.46	0.73	0.83	0.60	0.99	0.93

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. -Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. SMI = Structural model index. TbTh = Trabecular thickness. ConnD = Connectivity density. BV/TV = Bone volume over total volume. BV = Bone volume. BS = Bone surface. DA = Degree of anisotropy.

APPENDIX H

Effect of the diets on tibia trabecular structural model index, number, thickness, and connectivity density by microcomputed tomography

Diet	Gender (n)	Tibia SMI (1)	Tibia TbN (1/mm)	Tibia TbTh (mm)	Tibia ConnD ($1/mm^3$)
+Se+I	Male (9)	2.662 ± 0.042	2.662 ± 0.042	0.053 ± 0.001	35.359 ± 3.608
	Female (9)	2.365 ± 0.081	3.262 ± 0.149	0.056 ± 0.001	63.809 ± 7.438
+Se-I	Male (8	2.540 ± 0.064	3.552 ± 0.237	0.054 ± 0.001	59.507 ± 8.135
	Female (8)	2.243 ± 0.054	3.927 ± 0.188	0.058 ± 0.001	80.277 ± 8.743
-Se+I	Male (9)	2.468 ± 0.040	3.859 ± 0.141	0.052 ± 0.001	63.830 ± 4.609
	Female (9)	2.139 ± 0.043	4.223 ± 0.151	0.058 ± 0.001	93.310 ± 3.034
-Se-I	Male (9)	2.666 ± 0.066	3.013 ± 0.180	0.051 ± 0.001	38.545 ± 4.826
	Female (9)	2.314 ± 0.081	3.735 ± 0.242	0.058 ± 0.001	75.988 ± 9.594
Source of Variation		*P-values*	*P-values*	*P-values*	*P-values*
I		0.59	0.78	0.61	0.85
Se		0.34	0.11	0.72	0.17
I*Se		<0.08	<0.004	0.56	<0.01
Sex		<0.0001	<0.001	<0.0001	<0.0001
I*Sex		0.80	0.30	0.35	0.91
Se*sex		0.70	0.37	0.16	0.39
I*Se*Sex		0.99	0.70	0.71	0.46

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. SMI = Structural model index. TbTh = Trabecular thickness. ConnD = Connectivity density. BV/TV = Bone volume over total volume. BV = Bone volume

APPENDIX I

Effect of the diets on tibia cortical total volume, bone volume, bone volume fraction, thickness, porosity, and surface by microcomputed tomography

Diet	Gender (n)	Tibia cortical TV ($mm^{3)}$	Tibia cortical BV (mm^3)	Tibia cortical BV/TV	Tibia cortical thickness (mm)	Tibia cortical bone porosity (%)	Tibia cortical bone surface (mm^2)
+Se+I	Male (9)	2.700 ± 0.091	2.603 ± 0.091	0.964 ± 0.003	0.487 ± 0.013	3.630 ± 0.319	3.507 ± 0.128
	Female (9)	2.334 ± 0.055	2.265 ± 0.056	0.971 ± 0.001	0.477 ± 0.008	2.943 ± 0.138	3.062 ± 0.076
+Se-I	Male (8	2.438 ± 0.096	2.374 ± 0.090	0.974 ± 0.002	0.512 ± 0.012	2.537 ± 0.252	3.112 ± 0.125
	Female (8)	2.209 ± 0.099	2.145 ± 0.097	0.971 ± 0.001	0.478 ± 0.013	2.961 ± 0.155	4.342 ± 1.546
-Se+I	Male (9)	2.378 ± 0.044	2.303 ± 0.042	0.969 ± 0.002	0.476 ± 0.005	3.137 ± 0.172	3.116 ± 0.057
	Female (9)	2.304 ± 0.031	2.237 ± 0.032	0.971 ± 0.002	0.473 ± 0.008	2.928 ± 0.208	3.026 ± 0.043
	Male (9)	2.430 ± 0.077	2.364 ± 0.077	0.973 ± 0.002	0.490 ± 0.012	2.748 ± 0.144	3.197 ± 0.104
	Female (9)	2.272 ± 0.068	2.207 ± 0.068	0.971 ± 0.001	0.512 ± 0.019	2.767 ± 0.181	2.970 ± 0.093
Source of Variation		*P-values*	*P-values*	*P-value*	*P-values*	*P-values*	*P-values*
I		0.15	0.19	<0.04	<0.06	<0.04	0.53
Se		0.40	0.43	0.44	0.73	0.44	0.23
I*Se		0.11	0.12	0.46	0.24	0.46	0.55
Sex		<0.002	<0.002	0.43	0.31	0.43	0.74
I*Sex		0.99	0.87	<0.03	0.82	<0.03	0.27
Se*sex		<0.06	<0.07	0.87	<0.04	0.87	0.44
I*Se*Sex		0.50	0.56	0.13	0.08	0.13	0.21

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. TV = Total volume. BV = Bone volume. BV/TV = Bone relative volume. Cortical porosity = ((1- (BV/TV))*100. Cortical bone surface = TRI BV/(44*0.0165).

APPENDIX J

Effects of the diets on L_3 trabecular total volume, bone volume, bone volume fraction, connectivity density, and degree of anisotropy by microcomputed tomography

Diet	Gender (n)	L_3 TV (mm^3)	L_3 BV (mm^3)	L_3 BV/TV (1)	L_3 ConnD ($1/mm^3$)	L_3 DA (1)
+Se+I	Male (9)	12.638 ± 0.530	2.360 ± 0.186	0.185 ± 0.010	63.000 ± 3.334	1.840 ± 0.029
	Female (9)	11.281 ± 0.455	2.501 ± 0.123	0.221 ± 0.004	78.541 ± 2.567	1.909 ± 0.027
+Se-I	Male (8)	11.853 ± 0.887	2.509 ± 0.239	0.210 ± 0.010	72.394 ± 3.657	1.807 ± 0.030
	Female (8)	8.937 ± 0.535	2.185 ± 0.165	0.243 ± 0.007	77.437 ± 2.358	1.931 ± 0.024
-Se+I	Male (9)	11.479 ± 0.516	2.343 ± 0.143	0.204 ± 0.007	75.400 ± 2.211	1.783 ± 0.023
	Female (9)	9.664 ± 0.228	2.593 ± 0.111	0.268 ± 0.008	86.208 ± 2.292	1.945 ± 0.022
-Se-I	Male (9)	10.029 ±0.557	1.886 ± 0.156	0.186 ± 0.007	65.071 ± 3.749	1.807 ± 0.030
	Female (9)	9.664 ± 0.311	2.352 ± 0.150	0.243 ± 0.014	75.044 ± 2.207	1.868 ± 0.019
Source of variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		<0.07	0.30	0.77	0.18	0.49
Se		0.09	0.52	0.27	0.30	0.39
I*Se		0.46	0.43	<0.03	<0.005	0.67
Sex		<0.0001	0.37	<0.0002	<0.0005	<0.0002
I*Sex		0.82	0.54	0.77	0.22	0.71
Se*sex		0.10	<0.07	0.08	0.99	0.70
I*Se*Sex		<0.03	0.12	0.97	0.31	0.07

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. TV= Total volume. BV = Bone volume. BV/TV = Bone volume over total volume. ConnD = Connectivity density.

APPENDIX K

Effect of the diets on L_3 trabecular structural model index, number, thickness, separation, and bone surface over bone volume by microcomputed tomography

Diet	Gender (n)	L_3 SMI (1)	L_3 TbN (1/mm)	L_3 TbTh (mm)	L_3 TbSp (mm)	L_3 BS (mm^2)	L_3 BS/BV (1/mm)
+Se+I	Male (9)	1.236 ± 0.089	3.158 ± 0.071	0.070 ± 0.001	0.316 ± 0.008	78.590 ± 5.169	34.499 ± 0.816
	Female (9)	0.867 ± 0.048	3.567 ± 0.048	0.069 ± 0.001	0.274 ± 0.004	82.503 ± 3.830	33.518 ± 0.272
+Se-I	Male (7)	1.023 ± 0.085	3.435 ± 0.083	0.070 ± 0.002	0.286 ± 0.008	81.722 ± 6.659	33.622 ± 0.919
	Female (8)	0.368 ± 0.201	3.740 ± 0.062	0.070 ± 0.001	0.261 ± 0.004	68.656 ± 4.332	32.114 ± 0.756
-Se+I	Male (9)	1.028 ± 0.070	3.322 ± 0.048	0.068 ± 0.001	0.298 ± 0.005	78.437 ± 4.108	34.362 ± 0.643
	Female (9)	0.161 ± 0.140	3.842 ± 0.046	0.073 ± 0.001	0.253 ± 0.004	79.009 ± 2.470	30.787 ± 0.616
-Se-I	Male (9)	1.189 ± 0.039	3.271 ± 0.065	0.067 ± 0.001	0.303 ± 0.007	64.917 ± 4.945	35.494 ± 0.509
	Female (9)	0.556 ± 0.172	3.657 ± 0.073	0.073 ± 0.002	0.268 ± 0.006	72.587 ± 2.959	31.645 ± 1.018
Source of variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		0.68	0.27	0.79	0.16	0.13	0.78
Se		0.18	0.33	0.92	0.37	0.32	0.70
I*Se		<0.02	<0.006	0.54	<0.0006	0.57	0.18
Sex		<0.0001	<0.0001	<0.04	<0.0001	0.83	<0.0006
I*Sex		0.85	0.19	0.82	0.11	0.40	0.74
Se*sex		0.18	0.29	<0.02	0.47	0.17	<0.03
I*Se*Sex		0.14	0.87	0.69	0.74	0.07	0.93

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. SMI= Structural model index. TbN = Trabecular number. TbTh = Trabecular thickness. TbSp = Trabecular separation. BS = Bone surface. BS/BV = Bone surface over bone volume.

APPENDIX L

Effects of the diets on serum alkaline phosphatase, osteocalcin, tartrate resistant acid phophatase, ferric reducing ability of plasma, and liver thiobarbituric acid reactive substances

Diet	Gender (n)	Seruμm ALP (μmole/L)	Serum (osteocalcin ng/mL)	Serum TRAP (μmole/L)	Serum FRAP (μmole/L)	Liver TBARS (nmole/mg protein)
+Se+I	Male (9)	112.8 ± 8.4	33.1 ±1.7	7.09± 0.40	585.5 ± 39.5	0.269 ± 0.005
	Female (9)	86.9 ± 9.1	34.1 ± 2.0	6.16 ± 0.36	631.9 ± 87.8	0.261 ± 0.004
+Se-I	Male (8)	87.0 ± 8.3	47.7± 6.9	6.20 ± 0.90	644.2 ± 79.1	0.289 ± 0.005
	Female (8)	56.9 ± 6.2	56.0 ± 6.7	6.26 ± 0.42	1090.1 ± 117.4	0.251 ± 0.006
-Se+I	Male (9)	92.1 ± 5.3	25.0 ± 2.3	7.09 ± 0.37	502. 6 ± 46.5	0.297 ± 0.003
	Female (9)	64.4 ± 6.4	31.1 ± 1.7	5.33 ± 0.54	488.0 ± 63.8	0.269 ± 0.006
-Se-I	Male (9)	71. 5 ± 17.5	24.0 ± 2.0	5.97 ± 0.79	382.2 ± 24.1	0.311 ± 0.004
	Female (9)	78.1 ± 12.2	30.1 ± 3.5	5.81 ± 0.60	691.8 ± 107.3	0.286 ± 0.004
Source of variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		0.30	0.21	0.69	0.13	<0.03
Se		0.49	<0.04	0.67	<0.03	<0.0001
I*Se		0.31	0.17	0.82	0.18	0.17
Sex		<0.03	<0.02	<0.04	<0.02	<0.0001
I*Sex		0.27	0.21	<0.06	<0.03	0.20
Se*sex		0.32	0.96	0.46	0.54	0.62
I*Se*Sex		0.21	0.23	0.58	0.67	<0.06

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. -Se+I = Low selenium, adequate iodine. -Se-I = Low selenium, low iodine. ALP = Alkaline phosphatase. TRAP = Tartrate resistant acid phosphatase. FRAP = Ferric reducing ability of plasma. TBARS = Thiobarbituric acid reactive substances.

APPENDIX M

Effects of the diets on urinary, calcium, phosphorus, magnesium, creatinine, and deoxypyridinoline excretion

Diet	Gender (n)	Urinary Ca (mg/12 h)	Urinary P (mg/12 h)	Urinary Mg (mg/12 h)	Urinary creatinine (mmole/L)	Urinary DPD (µmole/mmole creatinine)
+Se+I	Male (9)	0.74 ± 0.12	9.57 ± 1.02	3.21 ± 0.48	3.36 ± 0.73	251.245 ± 34.20
	Female (9)	2.93 ± 2.14	8.71 ± 2.57	1.97 ± 0.21	2.62 ± 0.58	179 67 ± 23.71
+Se-I	Male (8)	0.81 ± 0.21	6.67 ± 0.88	2.76 ± 0.78	3.07 ± 0.57	154.67 ± 18.68
	Female (8)	0.71 ± 0.18	4.79 ± 0.88	1.87 ± 0.34	1.95 ± 0.25	205.48 ± 19.35
-Se+I	Male (9)	0.88 ± 0.09	8.97 ± 0.35	2.97 ± 0.29	2.30 ± 0.29	154.72 ± 10.31
	Female (9)	1.00 ± 0.21	6.37 ± 0.50	2.27 ± 0.34	2.06 ± 0.31	340.04 ± 91.40
-Se-I	Male (9)	0.98 ± 0.24	5.02 ± 0.54	1.57 ± 0.18	2.50 ± 0.54	111.50 ± 8.99
	Female (9)	0.86 ± 0.13	4.72 ± 0.58	1.53 ± 0.21	1.91 ± 0.31	261.35 ± 77.92
Source of variation	P-values	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		0.40	0.09	0.09	0.73	0.41
Se		0.46	0.11	0.28	0.25	0.74
I*Se		0.40	<0.07	0.27	0.69	0.82
Sex		0.51	<0.04	<0.007	<0.03	0.18
I*Sex		0.29	0.78	0.35	0.51	0.70
Se*sex		0.30	0.43	0.18	0.41	0.13
I*Se*Sex		0.37	0.92	0.69	0.90	0.49

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. Ca = Calcium. P = Phosphorus. Mg = Magnesium. DPD = Deoxypyridinoline.

APPENDIX N

Effects of the diets tibia length, body lean mass, body fat mass, liver weight, spleen weight, and heart weight

Diet	Gender (n)	Tibia length (mm)	Body lean mass (% BW)	Body fat (% BW)	Liver weight (% BW)	Spleen weight (% BW)	Heart weight (% BW)
+Se+I	Male (9)	39.28 ± 0.48	92.16 ± 0.33	7.85 ± 0.33	3.07 ± 0.12	0.252 ± 0.006	0.400 ± 0.022
	Female (9)	37.12 ± 0.30	92.35 ± 0.41	7.65 ± 0.41	3.44 ± 0.08	0.303 ± 0.010	0.455 ± 0.016
+Se-I	Male (8)	37.06 ± 0.54	91.60 ± 0.52	8.40 ± 0.52	3.07 ± 0.05	0.227 ± 0.006	0.392 ± 0.007
	Female (8)	35.14 ± 0.21	93.16 ± 0.50	6.84 ± 0.50	3.34 ± 0.05	0.274 ± 0.006	0.457 ± 0.014
-Se+I	Male (9)	37.71 ± 0.12	95.34 ± 0.36	8.66 ± 0.36	3.51 ± 0.04	0.240 ± 0.008	0.389 ± 0.009
	Female (9)	35.80 ± 0.29	92.24 ± 0.37	7.76 ± 0.37	3.72 ± 0.06	0.284 ± 0.004	0.462 ± 0.012
-Se-I	Male (9)	35.99 ± 0.36	92.59 ± 0.36	7.41 ± 0.36	3.13 ± 0.09	0.221 ± 0.007	0.396 ± 0.017
	Female (9)	34.50 ± 0.49	92.57 ± 0.67	7.43 ± 0.67	3.42 ± 0.20	0.270 ± 0.010	0.497 ± 0.039
Source of Variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-values*	*P-values*
I		<0.0006	0.27	0.27	<0.02	<0.004	0.64
Se		<0.007	0.80	0.80	<0.005	0.10	0.66
I*Se		0.3844	0.36	0.36	<0.06	0.41	0.52
Sex		<0.0001	<0.04	<0.04	<0.0004	<0.0001	<0.0006
I*Sex		0.55	0.71	0.71	0.97	0.99	0.58
Se*sex		0.49	0.47	0.47	0.63	0.84	0.57
I*Se*Sex		0.83	<0.07	<0.07	0.54	0.71	0.83

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. BW = Body weight.

APPENDIX O

Effects the diets on femur length (by caliper) and biomechanical properties by 3-point bending

Diet	Gender (n)	Femur length (mm)	Femur cortical thickness (mm)	Femur yield force (N)	Femur ultimate force (N)	Femur stiffness (N/mm)
+Se+I	Male (9)	34.27± 0.38	0.73 ± 0.06	77.63 ± 4.08	87.35 ± 4.21	84.37 ± 6.95
	Female (9)	32.70 ± 0.27	0.72 ± 0.05	76.96 ± 2.22	83.28 ± 2.03	88.35 ± 3.15
+Se-I	Male (8)	32.39 ± 0.41	0.68 ± 0.05	68.85 ± 3.96	76.37 ± 3.75	78.64 ± 5.57
	Female (8)	30.31 ± 0.30	0.71 ± 0.05	64.46 ± 2.89	70.65 ± 2.89	74.02 ± 5.17
-Se+I	Male (9)	32.83 ± 0.20	0.65 ± 0.04	65.08 ± 4.64	74.56 ± 2.83	77.23 ± 4.06
	Female (9)	31.25 ± 0.10	0.69 ± 0.02	66.57 ± 2.89	75.05 ± 2.25	92.16 ± 3.26
-Se-I	Male (9)	31.86 ± 0.30	0.58 ± 0.03	67.12 ± 3.86	74.91 ± 3.94	75.74 ± 3.93
	Female (9)	30.26 ±0.29	0.58 ± 0.04	64.18 ± 2.80	71.60 ± 3.03	70.36 ± 6.84
Source of Variation		*P-value*	*P-value*	*P-value*	*P-value*	*P-value*
I		<0.002	0.11	<0.07	<0.005	<0.02
Se		<0.03	<0.04	<0.03	<0.03	0.52
I*Se		0.12	0.41	<0.07	<0.03	0.83
Sex		<0.0001	0.69	0.53	0.17	0.54
I*Sex		0.43	0.97	0.45	0.55	<0.05
Se*sex		0.42	0.92	0.70	0.45	0.48
I*Se*Sex		0.43	0.50	0.99	0.81	0.42

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine.

APPENDIX P

Effects the diets on femur SMA, modulus of elasticity, yield stress, and ultimate stress by 3-points bending

Diet	Gender (n)	Femur SMA (mm^4)	Femur modulus of elasticity (N/mm^2)	Femur yield stress (N/mm^2)	Femur ultimate stress (N/mm^2)
+Se+I	Male (9)	8.12 ± 0.70	3053.67 ± 245.75	113.37 ± 4.76	128.04 ± 6.01
	Female (9)	6.63 ± 0.30	3880.00 ± 164.38	130.80 ± 4.93	141.61 ± 4.93
+Se-I	Male (8)	7.09 ±0.45	3209.33 ± 162.49	111.93 ± 4.17	124.38 ± 3.84
	Female (8)	5.77 ± 0.26	3704.44 ± 244.49	120.72 ± 5.01	132.36 ± 5.43
-Se+I	Male (9)	6.45 ± 0.36	3532.85 ± 267.89	112.60 ± 8.85	129.79 ± 6.98
	Female (9)	5.79 ± 0.16	4608.94 ± 201.87	124.39 ± 5.60	140.19 ± 4.46
-Se-I	Male (9)	6.60 ± 0.38	3369.93 ± 208.83	115.01 ± 7.27	128.32 ± 7.54
	Female (9)	5.43 ± 0.35	3899.33 ± 543.18	129.14 ± 9.33	144.51 ± 11.57
Source of Variation		*P-value*	*P-value*	*P-value*	*P-value*
I		0.22	0.27	0.82	0.61
Se		<0.05	<0.060.	0.82	0.40
I*Se		0.28	0.29	0.32	0.42
Sex		<0.0001	<0.0006	<0.008	<0.02
I*Sex		0.72	0.28	0.74	0.99
Se*sex		0.33	0.72	0.99	0.79
I*Se*Sex		0.60	0.79	0.56	0.56

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine. SMA = Second moment of area.

APPENDIX Q

Effects of the diets on L_3 average strain, total force, and Stiffness, Size Independent stiffness, and average von Mises stress using finite element analysis (FE) by MicroCT

Diet	Gender (n)	Average strain	Total force (N)	Stiffness (N/m x 10^3)	Size independent stiffness(N/m)	Average corrected von miss stress (MPa)
+Se+I	Male (9)	0.158 ± 0.020	1026.07 ± 158.28	283403.22 ± 42052.42	295.76 ± 50.55	60.228 ± 4.552
	Female (9)	0.237 ± 0.018	1742.48 ± 155.65	491572.44 ± 43028.72	547.60 ± 46.84	46.307 ± 2.096
+Se-I	Male (8)	0.200 ± 0.014	1459.69 ± 180.55	400147.57 ± 47642.24	437.36 ± 46.36	50.747 ± 5.435
	Female (8)	0.273 ± 0.017	2037.75 ± 151.15	672170.13 ± 37345.80	693.14 ± 48.88	43.822 ± 1.963
-Se+I	Male (9)	0.189 ± 0.010	1272.13 ± 98.01	361305.33 ± 25263.35	394.63 ± 32.54	53.806 ± 2.711
	Female (9)	0.272 ± 0.012	2180.64 ± 133.97	647332.56 ± 38578.16	759.28 ± 44.02	40.496 ± 1.788
-Se-I	Male (9)	0.185 ± 0.014	1222.91 ± 118.66	403434.56 ± 35430.45	369.92 ± 37.51	59.856 ± 5.065
	Female (9)	0.266 ± 0.023	2102.26 ± 275.33	662390.33 ± 92053.34	696.76 ± 88.71	44.541 ± 4.377
Source of Variation		*P-values*	*P-values*	*P-values*	*P-values*	*P-value*
I		0.17	0.21	0.24	0.23	0.84
Se		0.37	0.28	0.35	0.15	0.84
I*Se		<0.08	<0.08	0.34	<0.05	<0.07
Sex		<0.0001	<0.0001	0.09	<0.0003	<0.0005
I*Sex		0.86	0.72	0.84	0.84	0.66
Se*sex		0.80	0.30	0.72	0.27	0.48
I*Se*Sex		0.93	0.82	0.63	0.80	0.41

Values are least square means ± SEM. Effect is significant at P<0.05. +Se+I = Adequate selenium, adequate iodine. +Se-I = Adequate selenium, low iodine. –Se+I = Low selenium, adequate iodine. –Se-I = Low selenium, low iodine, L_3 = The 3rd lumbar vertebra.

VITA

Fanta Toure

Candidate for the Degree of

Doctor of Philosophy

Dissertation: EFFECTS OF IODINE AND SELENIUM DEPLETION ON GROWTH AND BONE QUALITY OF RATS

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ABSTRACT

Name: Fanta Toure Date of Degree: July, 2005

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECTS OF IODINE AND SELENIUM DEPLETION ON GROWTH AND BONE QUALITY OF RATS

Pages in Study: 156

Major Field: Nutritional Sciences

Scope and Method of Study: This project investigated the effects of experimental Iodine (I) and/or selenium (Se) depletion on bone density, structure, and strength in young rats. Dams were fed experimental diets beginning at week 1 of lactation. Pups were weaned at 3 wks of age and a sub-sample of males and females were fed the experimental diet of their mother for an additional 7 wks. I and Se status and growth in the animals were assessed. Bones quality of the rats was investigated for biochemical markers of bone metabolism, bone density, structure, and strength. Oxidative status was also assessed.

Findings: Weight gain was decreased by I depletion in all animals and by Se depletion in males. Thyroid weight was increased and serum thyroxine (T_4) was reduced by I deficiency. Se depletion was confirmed by lower hepatic glutathione peroxidase activity. Tri-iodothyronine (T_3) was higher in female rats when Se was deficient. I and Se depletion independently decreased bone length and mineral area (BMA) in tibia and femur and selenium depletion decreased femur cortical thickness. There was higher bone turnover and lipid oxidation, and lower bone ash weight in male rats. BMA and bone mineral density (BMD) in vertebra, and bone mineral content in tibia, femur and vertebra were decreased by Se depletion in male rats as measured by DEXA. Analysis of proximal tibia and L_3 trabecular bone by micro-computed tomography (μCT) showed a better bone microacthitecture in females than males and when Se was adequate, the deficiency of I increased the structural quality of both tibia and L_3 trabecular bone. Tibia cortical bone volume and thickness were higher in males when Se was adequate. The compressive strength of L_3 trabecular bone and the bending strength of femur were better in females. The breaking force of femur was higher in iodine adequate rats when Se was also adequate, and the adequacy of iodine increased femur stiffness in females. Iodine and/or selenium depletion impaired bone quality in growing rats.

Adviser's Approval:____Barbara J. Stoecker_____