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Effect of soy food intake on mineral status in women of childbearing age

Ying Zhou

Iowa State University

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Effect of soy food intake on mineral status in women of childbearing age

by

Ying Zhou

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Nutritional Sciences

Program of Study Committee:
Manju B. Reddy, Major Professor
D. Lee Alekel
Philip M. Dixon

Iowa State University
Ames, Iowa
2009

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Lastly, I would like to thank everyone in the Department of Food Science and Human Nutrition and Interdepartmental Program in Nutritional Sciences who have given me kind help during my study at Iowa State University.
GENERAL INTRODUCTION

Thesis Organization

This thesis starts with a general introduction including hypothesis, objectives, specific aims, limitations, and significance of the proposed study followed by a review of literature and a manuscript to be submitted to the Journal of Nutrition.

Hypothesis

Daily consumption of two servings of soy foods for 10 wk does not significantly affect iron and zinc status or thyroid status but may have beneficial effects on bone turnover in women of childbearing age.

Objectives

1. The primary objective is to determine the effect of consuming two servings of soy foods per day for 10 wk, compared to animal food, on iron and zinc status in women of childbearing age.

2. The secondary objective is to investigate whether the consumption of two servings of soy foods per day for 10 wk, compared to animal food consumption, affects biochemical markers of bone and thyroid hormones in women of childbearing age.

Specific Aims

1. To determine the effect of soy food consumption on iron status as measured by serum iron, transferrin saturation, total iron binding capacity, transferrin receptor, ferritin, transferrin receptor-ferritin index (calculated based on ferritin and transferrin receptor), hemoglobin, and hematocrit.
2. To determine the effect of soy food consumption on zinc status as measured by serum and urinary zinc, as well as serum alkaline phosphatase concentrations.

3. To test the effect of soy food consumption on biochemical markers of bone as measured by serum bone-specific alkaline phosphatase (bone formation marker) and CTx (bone resorption marker).

4. To determine the effect of soy food consumption on thyroid status as measured by thyroid stimulating hormone and free thyroxine.

Limitations

There are several limitations of this study. A 10-wk intervention may be not long enough to result in significant changes in the outcomes we measured. Nutrient intakes of subjects were not strictly controlled during the intervention, since they maintained freely chosen diets except for the meals we provided, the prohibition of additional soy food intake, and meat intake restriction (<3 oz/d). In addition, subjects were required to consume only 3 out of 14 provided meals on site each week; thus, compliance for most meals except for the consumption of test meals might have affected our results. We could not assess dietary zinc, iodine and phytate intake due to lack of information in the dietary analysis software. Thus, the change in dietary intake of zinc, iodine and phytate may have influenced our results.

Significance

Single meal studies have shown apparent inhibition of soy food consumption on iron and zinc absorption. However, data are limited and conflicting from long term studies. Thus, it is important to investigate the effect of incorporating commonly consumed soy foods to daily diets on mineral status in women of childbearing age who are at risk of mineral
deficiencies. The possible benefits of soy consumption on biochemical markers of bone as well as the potential risk of an anti-thyroid effect were also determined.
REVIEW OF LITERATURE

I. IRON METABOLISM

Importance of Iron

Iron is an essential nutrient for human health because it plays an important role in many biochemical processes to maintain normal cellular functions, which include electron transport (cytochrome, iron-sulfur proteins), handling of molecular oxygen (peroxidase, catalase), oxygen transport and storage (hemoglobin and myoglobin), porphyrin metabolism, collagen synthesis, lymphocyte and granulocyte function, and neurotransmitter anabolism and catabolism (Pollitt & Leibel, 1976; Cammack et al., 1990).

Both iron deficiency and excess have harmful effects on human health. Severe iron deficiency causes anemia and results in developmental delay and cognitive impairment in children and infants (McCann & Ames, 2007), fatigue and reduced work capacity in adults (Haas & Brownlie, 2001), as well as adverse pregnancy outcomes in women (Ronnenberg et al., 2004; Lee et al., 2006). Iron supplementation increased night sleep duration and decreased night waking in infants and improved cognition in older children (Gera & Sachdev, 2009; Kordas et al., 2009) in fact suggests the importance of iron. In case of genetic or secondary iron overload, excessive iron is deposited in parenchymal cells of the liver and other organs, damaging organ structure and function (Crichton, 2006). For example, in hereditary hemochromatosis, the absorption of dietary iron increases abnormally so that iron accumulates in organs such as the liver and pancreas, leading to cirrhosis and diabetes (Hash, 2001; Kohgo et al., 2008). Moreover, iron was found to increase with age and iron excess has
been associated with neoplasia (Weinberg, 1994), atherosclerosis (Sullivan, 2008), and neurodegenerative diseases (Sipe et al., 2002).

**Body Iron Distribution**

Total iron in the body is approximately 3 to 4 g depending on age, weight, and gender (Wood & Ronnenberg, 2005). It may be divided into three major compartments: functional iron, storage iron, or transport iron, as described in Table 1 (Crichton, 2006). Functional iron consists of iron bound to hemoglobin in the erythrocytes, myoglobin in muscles, and iron-containing enzymes in all cells of the body, respectively accounting for about 65%, 10% and 2% of body iron. Storage iron associated with ferritin and hemosiderin is primarily located in the liver, comprising approximately 20% of body iron. Transport iron is bound to transferrin in plasma. Although it accounts for only about 0.8% of body iron, as much as 30 mg of iron cycles through this compartment daily to meet the erythropoietic needs. Additionally, some intracellular iron (2.2%) termed as ‘labile iron’ is readily utilized within the cells, acting as both functional and storage iron in the body (Fairbanks, 1999; Wood & Ronnenberg, 2005).

<table>
<thead>
<tr>
<th>Table 1. Typical Iron Distribution in Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Men</strong></td>
</tr>
<tr>
<td>mg Iron/kg Body Weight</td>
</tr>
<tr>
<td><strong>Functional iron</strong></td>
</tr>
<tr>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Myoglobin</td>
</tr>
<tr>
<td>Iron-containing enzymes</td>
</tr>
<tr>
<td><strong>Storage iron</strong></td>
</tr>
<tr>
<td>Ferritin (and hemosiderin)</td>
</tr>
<tr>
<td><strong>Transport iron</strong></td>
</tr>
<tr>
<td>Transferrin</td>
</tr>
</tbody>
</table>

Source: (Crichton, 2006)
Iron Absorption, Regulation, and Distribution

There are two types of dietary iron: heme iron mainly existing in hemoglobin and myoglobin from animal sources, but also present in plants in a low concentration (Kundu et al., 2003), and non-heme iron derived from both plant and animal foods (about 35-40% of iron in meats, fish, and poultry exists as non-heme iron) (Monsen, 1988). Heme and non-heme iron are absorbed by different pathways. After digestion, heme iron is released from globin (Grasbeck et al., 1982) and enters enterocytes as an intact metaloporphyrin (Conrad et al., 1967) possibly through a receptor-mediated endocytosis or heme transporter (West & Oates, 2008a). Iron (ferrous) is then released from the porphyrin ring by heme-oxygenase, entering the labile iron pool (Carpenter & Mahoney, 1992; West & Oates, 2008b). In contrast, non-heme iron is liberated from foods after gastric digestion and present as ferric and ferrous iron in the small intestine (alkaline conditions). In the small intestine, ferric iron readily precipitates, but ferrous iron remains relatively soluble and can be absorbed into enterocytes by a divalent metal transporter-1 (DMT1). Ferric iron may also be absorbed after reduction to ferrous iron by duodenal cytochrome-b (Dcytb). Ferrous iron from both heme and non-heme iron enters the labile iron pool, which can be either stored as ferritin in the enterocytes or exported across the basolateral membrane of enterocytes into circulation by ferroportin-1 (FPN-1) (Crichton, 2006; Dunn et al., 2007). Iron is also released from catabolized senescent erythrocytes (consists of 80% of the circulating iron) and the liver (Knutson & Wessling-Resnick, 2003). In circulation, iron is transported by transferrin and before incorporation into apotransferrin, ferrous iron is oxidized into ferric iron by hephaestin (a membrane-bound protein) or serum ceruloplasmin (Crichton, 2006; Dunn et
al., 2007). Iron (iron-bound transferrin) is then taken up by cells through transferrin receptor-mediated endocytosis (Aisen, 2004) for use or storage.

In iron deficient conditions, iron regulatory protein binds to iron response element and regulates transferrin receptor (TfR) and ferritin expression by stabilizing TfR against degradation or inhibiting ferritin translation so as to increase iron uptake and decrease storage; while in iron sufficient conditions, iron regulatory protein reacts inversely (Dunn et al., 2007). Hepcidin produced by the liver is another important modulator for iron absorption and mobilization. In iron replete conditions, hepcidin expression increases; it directly binds to FPN-1 and causes FPN-1 to be internalized and degraded so as to inhibit intestinal iron transport and the release of iron from the reticuloendothelial system that recycles iron from senescent erythrocytes (Donovan et al., 2000; Fleming & Bacon, 2005).

Heme iron has higher bioavailability than non-heme iron because the heme pyrrole structure protects against the interference from iron chelators such as polyphenols and phytate (Andrews, 2005). Heme iron absorption is enhanced by peptides and amino acids produced from meat hydrolysis (Carpenter & Mahoney, 1992) and inhibited by dietary calcium intake (Gleerup et al., 1995). Non-heme iron absorption is affected by many dietary factors such as polyphenols, phytate and calcium acting as inhibitors and ascorbic acid and ‘meat factors’ as enhancers (Davidsson et al., 1998; Baech et al., 2003; Hurrell et al., 2006). Iron absorption is also influenced by some specific conditions such as iron deficiency and the late stage of pregnancy (Wood & Ronnenberg, 2005). For example, a single meal study with a group of women showed that heme iron was significantly better absorbed than non-heme iron (16.1% vs 4.6%); the non-heme iron absorption increased in iron deficient women
compared to normal women (9.5% vs 4.6%) (Ekman & Reizenstein, 1993). It is estimated that heme iron contributes to approximately two-thirds of the individual’s total iron stores despite constituting only one-third of dietary iron in western countries (Carpenter & Mahoney, 1992).

Generally, about 1-2 mg iron is absorbed from the gastrointestinal (GI) tract when dietary iron intake is 10 to 15 mg. Iron absorbed from GI tract and released from catabolized senescent erythrocytes and iron stores is delivered by transferrin to bone marrow for hemoglobin synthesis (70-90%) and other tissues for utilization (synthesis of myoglobin and iron-containing compounds) or to the liver for storage (Figure 1) (Conrad & Umbreit, 2000).

![Figure 1. Schematic for Iron Kinetics](source: Conrad & Umbreit, 2000)

**Iron Requirements**

In healthy individuals, daily iron losses are estimated to be 1 mg in men and 1.5 mg in women, one half of which are lost through the intestine, including occult blood losses.
(0.35 mg/d), sloughed enterocytes as ferritin iron (0.10 mg/d), and iron in biliary secretions (0.2 mg/d). Other iron losses are via urine (0.08 mg/d), the shedding of skin cells (0.2 mg/d), and sweat in smaller amounts (Cook et al., 1992; Wood & Ronnenberg, 2005). Additionally, a significant amount of iron is lost during menstruation in women of childbearing age, placing them at greater risk of iron deficiency (Wood & Ronnenberg, 2005).

Apart from obligatory iron losses, infants and children need additional iron for growth and development. Based on 10% absorption efficiency of iron, the current recommended iron intake is 8 mg/d for adult males and postmenopausal women, 18 mg/d for premenopausal women (Food and Nutrition Board & Institute of Medicine, 2000). Details on the dietary reference intake of iron are shown in Table 2 (Food and Nutrition Board & Institute of Medicine, 2000).

<table>
<thead>
<tr>
<th>Table 2. Dietary Reference Intakes (DRI) Across the Life Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRI (mg/d iron)</td>
</tr>
<tr>
<td><strong>Infants</strong></td>
</tr>
<tr>
<td>0-0.5 yr</td>
</tr>
<tr>
<td>0.27 (AI)</td>
</tr>
<tr>
<td>0.5-1 yr</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td><strong>Children</strong></td>
</tr>
<tr>
<td>1-3 yr</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>4-8 yr</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>9-13 yr</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td><strong>Males</strong></td>
</tr>
<tr>
<td>14-18 yr</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>&gt;19 yr</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td><strong>Females</strong></td>
</tr>
<tr>
<td>14-18 yr</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>Pregnant</td>
</tr>
<tr>
<td>27</td>
</tr>
<tr>
<td>Lactating</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>19-50 yr</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>Pregnant</td>
</tr>
<tr>
<td>27</td>
</tr>
<tr>
<td>Lactating</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>&gt;50 yr</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>

Source: (Food and Nutrition Board & Institute of Medicine, 2000)
Dietary iron intake

Diet generally contains 5-7 mg iron per 1000 kcal. When consuming a western-style diet to meet energy requirements, adults are estimated to ingest 10-20 mg iron per day. However, in some developing countries where the diet is mainly based on plants, the dietary iron intake may be adequate but the absorption might be low due to the high content of inhibitors, such as phytate and polyphenols present in foods, and low intake of animal tissues and ascorbic acid (Wood & Ronnenberg, 2005).

Meat, fish and poultry are rich sources of iron containing both heme and non-heme iron. Cereals, breads, and grain products that are enriched with iron are very good sources of non-heme iron. Legumes and green leafy vegetables are also good source of non-heme iron. The contribution of these foods to the US dietary iron intake is shown in Figure 2 (Haymes, 2006).

![Figure 2. Constitution of US Dietary Iron Intake](source: Haymes, 2006)

Assessment of Iron Status

Stages in developing iron deficiency are shown in Figure 3 (Herbert, 1987). Individuals are in normal iron status when their functional needs for iron are satisfied.
Besides that, they have sufficient iron stores to meet the increased iron requirements in specific conditions, such as pregnancy (Bothwell, 1996). Iron stores vary among individuals but the average iron stores for iron replete subjects with western diets are 1000 mg in males and 300 mg in females (Bothwell et al., 1979). Iron depletion is the state when storage iron is used up but the functional iron has not been impaired; thus, no adverse consequences are expected at this stage. Further reduction in body iron leads to iron deficient erythropoiesis, since iron is insufficient for the needs of the erythroid marrow, which is associated with an increase in erythrocyte zinc protoporphyrin and TfR. Hemoglobin may still be in the normal range but anemia gradually develops with time. In severe iron deficiency, individuals develop anemia. Since the production of hemoglobin is impaired, individuals manifest with microcytic and hypochromic anemia, showing clinical signs of deficiency (Bothwell, 1996).

Biochemical indicators used for the assessment of iron status are described in the following text.

![Figure 3. Sequential Stages of Iron Status](Herbert, 1987)
Serum Ferritin

Serum ferritin is widely used as a marker of storage iron, and it is particularly useful in the identification of mild iron deficiency. Although iron stores can also be assessed by bone-marrow and liver biopsies or quantitative phlebotomy, these methods are invasive and thus not performed routinely (Baynes, 1996). Ferritin is primarily stored in liver but a small amount of ferritin also circulates in blood, the concentration of which reflects body iron stores. In healthy individuals and those at the early stage of iron deficiency, serum ferritin concentration is proportional to iron stores. For example, 1 µg/L represents 8-10 mg of storage iron in healthy adults. When serum ferritin level drops below 12 µg/L, it indicates the depletion of iron stores (Bothwell et al., 1979; Bothwell, 1996). Infants, children, and adolescents generally have a low serum ferritin concentration between 20 and 30 µg/L since most absorbed iron is utilized for growth and development. Ferritin concentrations increase in adult males until reaching a plateau of about 100 µg/L by 45 y. However, ferritin remains low in adult females until menopause; thereafter serum ferritin concentration gradually rises to concentrations comparable with adult men (Cook et al., 1976; Bothwell, 1996). Besides age and gender, routine oral contraceptive use may also influence serum ferritin concentration (Mooij et al., 1992).

Serum ferritin concentration may increase independently of iron stores in some conditions such as chronic inflammation (Kukulj et al., 2009), infection (Chaiyaratana et al., 2008), and neoplasia (Hearnshaw et al., 2006). In inflammation/infection, IL-6 mainly produced by hepatic Kupffer cells induces hepcidin expression (Ganz, 2003), which then blocks iron absorption and release from iron stores, resulting in a low plasma iron and a high
serum ferritin concentration. As a result, patients under some conditions may have higher serum ferritin but may be actually iron deficient.

For more complete assessment of iron status, serum ferritin is usually used in combination with other indicators reflecting functional iron compartments, such as hemoglobin and TfR, especially in populations with low iron stores like young children or with other disease conditions (Baynes, 1996).

**Serum Iron, Transferrin Saturation, and Total Iron-binding Capacity**

Serum iron is measured as the iron bound to serum transferrin, an iron transport protein. It is usually used in conjunction with transferrin saturation and total iron-binding capacity (TIBC) (CDC, 2008). Serum transferrin has two iron-binding sites and normally only one third of transferrin is saturated with iron (Crichton, 2006). TIBC measures serum transferrin after all available binding sites are saturated with iron; transferrin saturation is calculated as the ratio of serum iron to TIBC. TIBC increases with the depletion of storage iron and declines in chronic inflammation, infection and malignancies. In iron deficiency, serum iron declines but TIBC generally increases (>400 µg/dL), resulting in a low transferrin saturation (<16%). In iron overload, transferrin saturation is usually greater than 60%. Although serum iron and transferrin saturation are widely used in iron deficiency screening, their use is limited by the daily fluctuation of serum iron (CDC, 2008; Zimmermann, 2008).

**Transferrin Receptor**

Transferrin receptor, which reflects tissue iron sufficiency, is a sensitive indicator of functional iron deficiency (Skikne *et al.*, 1990) and in differentiating iron deficiency anemia from anemia caused by liver injury, inflammation, infection, or malignancy (Ferguson *et al.*, 1989).
A quantitative phlebotomy study showed that serum TfR increased in proportion to the extent of iron deficiency after the depletion of iron stores (serum ferritin <12 µg/L) (Skikne et al., 1990). Therefore, TfR is particularly useful in the evaluation of iron status in young children, adolescents, pregnant women, and trained athletes who commonly have low iron stores. Although expanded erythroid bone marrow may cause elevated TfR without functional iron deficit, these conditions may be distinguished from iron deficiency by testing serum ferritin concentration (Baynes, 1996).

**Serum Transferrin Receptor-Ferritin Ratio**

Since serum ferritin and TfR, respectively, reflect storage iron and functional iron deficits, the ratio of serum TfR-to-ferritin has been used to quantitatively estimate total body iron in infants (Olivares et al., 2000), children (Malope et al., 2001) or adults (Skikne et al., 1990; Cook et al., 2003). This ratio is particularly helpful in the calculation of iron absorbed over time for intervention trials to improve iron status. The use of this method is limited because serum ferritin and TfR concentrations are affected by some disorders independent of body iron status. In addition, this ratio is assay specific, since the TfR assay has not been standardized (Cook et al., 2003).

Apart from the TfR-to-ferritin ratio, the TfR-ferritin index (the ratio of serum TfR-to-log transformed ferritin, TfR-F index) has also proved to be a good indicator of iron depletion (Punnonen et al., 1997; Punnonen et al., 1998; Suominen et al., 1998). Punnonen et al. (Punnonen et al., 1997) had evaluated the diagnostic efficiency of lab tests for iron depletion among 129 consecutive anemic patients [48 with iron deficiency anemia (IDA), 64 with anemia of chronic disease (ACD), and 17 with depleted iron stores and an infectious or
inflammatory condition (COMBI)]. They found that TfR-F index not only had the highest sensitivity (the proportion of actual positive values which are correctly identified as positive) and specificity (the proportion of negative values which are correctly identified) to identify iron depletion out of a variety of indicators, including mean corpuscular volume, serum iron, transferrin, ferritin, transferrin saturation, TfR, TfR-to-ferritin ratio, and TfR-F index, but also was effective in distinguishing among IDA, ACD, and COMBI.

**Red Blood Cell Indices**

Red blood cell indices can be measured to assess the iron depletion in functional compartments, because iron deficiency anemia manifests as microcytic and hypochromic anemia with reduced hemoglobin, hematocrit (the proportion of blood volume occupied by red blood cells), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). However, the specificity of red blood cell indices is low because microcytic anemia can be caused by other conditions, such as lead poisoning, as long as hemoglobin synthesis is impaired. In addition, hemoglobin concentration differs with age, sex, and race and is affected by smoking, pregnancy and many other factors. Thus, the sensitivity of hemoglobin is also low due to the large overlap in values between normal and anemic populations (Cook et al., 1971). Since zinc is incorporated into protoporphyrin for hemoglobin synthesis when iron is insufficient, erythrocyte zinc protoporphyrin (ZPP) concentration is also a sensitive indicator of iron deficiency. Similarly, it is influenced by many factors, such as malaria, inflammation, and lead poisoning (Wood & Ronnenberg, 2005; Zimmermann, 2008).

Another limitation of these indicators is that they become abnormal relatively late in the development of functional depletion due to the long survival (~120 days) of red blood cells.
(Crichton, 2006). Reticulocyte hemoglobin content measures hemoglobin in reticulocytes (red blood cells one to two day old) rather than hemoglobin in all red blood cells between 1-120 days; thus, it is sensitive enough to reflect iron deficient erythropoiesis within days of onset. The drawback of this method is that false normal values may be caused by increased MCV (Wish, 2006; Zimmermann & Hurrell, 2007).

Iron Deficiency

Definition

Iron deficiency is a functional deficit of iron resulting from depleted iron stores and changes in iron metabolism and iron-related biochemical indices. It can be classified into latent iron deficiency (LID, iron deficiency without anemia) and iron deficiency anemia (IDA) (Wood & Ronnenberg, 2005).

Prevalence

Iron deficiency (ID) is the most prevalent nutritional disorder in the world, affecting approximately 2 to 5 billion people globally (Wood & Ronnenberg, 2005). The prevalence of ID is estimated to be ~50% and ~10%, respectively, in developing and developed countries (Denic & Agarwal, 2007). According to the 1999-2000 National Health and Nutrition Examination Survey in the United States, ID incidence was high in toddlers aged 1 to 2 y (7%) and females 12-49 y (9 to 16%) compared to other age and sex groups. Additionally, the prevalence of ID is twice as high among non-Hispanic black and Mexican-American females (19-22%) compared to non-Hispanic white females (10%). Further investigation found that overall IDA incidence (Table 3) was around 3% among these vulnerable ethnic groups (Looker & Cogswell, 2002).
Table 3. Prevalence of Iron Deficiency Anemia in Selected Populations in the United States, NHANES 1999-2000*

<table>
<thead>
<tr>
<th>Sex/Age group (yrs)</th>
<th>No.</th>
<th>%</th>
<th>(95% CI\textsuperscript{\dagger})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>319</td>
<td>2\textsuperscript{§}</td>
<td>(0-4)</td>
</tr>
<tr>
<td>Females\textsuperscript{¶}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-49</td>
<td>1950</td>
<td>3</td>
<td>(2-4)</td>
</tr>
<tr>
<td>12-19</td>
<td>1001</td>
<td>2</td>
<td>(1-3)</td>
</tr>
<tr>
<td>20-49</td>
<td>949</td>
<td>4</td>
<td>(2-5)</td>
</tr>
<tr>
<td>50-69</td>
<td>611</td>
<td>3\textsuperscript{§}</td>
<td>(0.5-5)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>394</td>
<td>1\textsuperscript{§}</td>
<td>(0-2)</td>
</tr>
</tbody>
</table>

*All racial/ethnic groups.
\textsuperscript{\dagger}Confidence interval.
\textsuperscript{§}Unreliable; relative standard error (i.e., standard error/prevalence estimate) is >30%.
\textsuperscript{¶}Nonpregnant only.
Source: (Looker & Cogswell, 2002)

**Causes**

Primary causes of ID in vulnerable groups are described in Table 4 (Zimmermann & Hurrell, 2007; WHO, 2008). People with monotonous plant-based diets with little meat, such as vegetarians, are at risk of ID because non-heme dietary iron has low absorption efficiency (generally less than 10%). In addition, the absorption of non-heme iron is inhibited by phytate, polyphenols and calcium (Zimmermann & Hurrell, 2007), commonly found in foods, with phytate being particularly high in the plant-based diets. For example, phytate ubiquitously exists in plants including nuts, cereals, legumes, and oilseeds (Weaver & Kannan, 2001); polyphenols are present in green and black teas, coffee, fruits, vegetables, olive oil, red and white wines (Perron & Brumaghim, 2009). Impaired iron absorption may also result in ID. Because non-heme iron absorption is facilitated by gastric acid that helps to dissolve and reduce dietary iron (Bezwoda \textit{et al.}, 1978), impaired gastric secretion may contribute to ID, as noted in achlorhydria and mucosal atrophy. Atrophic gastritis is common in the elderly, thus they are vulnerable to ID. For infants, iron stores accumulated during
gestation are soon used very early in life due to rapid growth and ID will develop if iron-fortified formula/foods are not provided. Similar to infants, young children and early adolescents are also at risk of ID because of rapid growth and relatively low body iron stores. Premenopausal women are vulnerable to ID primarily due to menstrual iron losses, which are estimated to be 0.5 mg iron/ml blood (Harvey et al., 2005). Pregnant women have higher iron requirements because of expanded blood volume and fetal growth. In addition, excessive iron losses may occur due to increased bleeding with gastrointestinal parasitic infection and some gastrointestinal diseases. Thus, patients with these diseases are vulnerable to ID (Herbert, 1987; Zimmermann & Hurrell, 2007).

**Table 4. Causes of Iron Deficiency**

<table>
<thead>
<tr>
<th>Primary causes</th>
<th>Vulnerable groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insufficient iron</strong></td>
<td></td>
</tr>
<tr>
<td><strong>intake/absorption</strong></td>
<td>Low dietary iron intake</td>
</tr>
<tr>
<td></td>
<td>Impaired iron absorption due to substances interfering with iron absorption, such as phytate or phenolic compounds in diets</td>
</tr>
<tr>
<td></td>
<td>Disease conditions like achlorhydria, mucosal atrophy</td>
</tr>
<tr>
<td></td>
<td>People in developing country</td>
</tr>
<tr>
<td></td>
<td>Population with monotonous plant-based diets with little meat and vegetarian based diets</td>
</tr>
<tr>
<td></td>
<td>Elder people and patients with celiac disease</td>
</tr>
<tr>
<td><strong>High iron</strong></td>
<td></td>
</tr>
<tr>
<td><strong>requirement</strong></td>
<td>Rapid growth</td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Menstruation</td>
</tr>
<tr>
<td></td>
<td>Infants, children, and adolescents</td>
</tr>
<tr>
<td></td>
<td>Pregnant women</td>
</tr>
<tr>
<td></td>
<td>Premenopausal women</td>
</tr>
<tr>
<td><strong>Excessive iron</strong></td>
<td></td>
</tr>
<tr>
<td><strong>losses</strong></td>
<td>Gastrointestinal parasitic infection</td>
</tr>
<tr>
<td></td>
<td>Bleeding from gastrointestinal tract caused by diseases</td>
</tr>
<tr>
<td></td>
<td>People in endemic areas of parasitic infection</td>
</tr>
<tr>
<td></td>
<td>Patients with gastrointestinal diseases</td>
</tr>
</tbody>
</table>

Source: (Zimmermann & Hurrell, 2007; WHO, 2008)

**Consequences**

IDA has been associated with impaired cognitive and physical development in infants and children (WHO, 2001). Even iron deficiency without anemia has a detrimental effect on cognition and behavior in infants. Recent studies found that poor iron status (IDA and LID)
adversely affected infant motor development and social-emotional behavior in a dose-dependent manner (Lozoff et al., 2008; Shafir et al., 2008). IDA during pregnancy may result in premature delivery and low birth weight (Lee et al., 2006). In addition, maternal iron deficiency negatively affected mother-child interactions as reflected by significantly lower score on maternal sensitivity and child responsiveness in IDA women than iron sufficient women at 10 wk or 9 mo postpartum. Iron supplements (125 mg FeSO₄/d) for 9 mo corrected the problem (Murray-Kolb & Beard, 2009). Additionally, IDA is associated with fatigue and reduced work productivity in adults due to impaired capacity in oxygen transport and oxidative metabolism (Haas & Brownlie, 2001; WHO, 2001).

ID is one of the ten leading risk factors for global burden of disease (impact caused by mortality and morbidity; measured by disability-adjusted life year (DALY) combining years of life lost due to premature deaths and years of life living in states of less than full health). It is estimated that ID causes almost a million deaths per year, accounting for 1.5% of deaths worldwide. The DALY resulting from ID is even greater. ID accounts for 2.4% of global DALYs. Particularly, one-fifth and one-tenth of perinatal and maternal deaths, respectively, in developing countries are attributed to ID (WHO, 2002).

Diagnosis

The indicators of ID are shown in Table 5 (Zimmermann & Hurrell, 2007; Zimmermann, 2008). ID can be diagnosed if serum ferritin concentration is less than 12 µg/L. However, serum ferritin may still be high in several conditions independent of iron status, as previously mentioned. Therefore, serum ferritin is generally examined to identify iron stores in combination with TfR and/or ZPP, considering that TfR and ZPP are sensitive
indicators of iron deficiency erythropoiesis, to determine if the reduced erythropoiesis is due to iron deficiency (Sherwood et al., 1998). TfR is not influenced by the acute phase response (Ferguson et al., 1992; Asobayire et al., 2001), while ZPP increases in ACD and myelodysplastic syndromes. TfR is usually <80 µmol/mol heme, not as high as the value observed in IDA (>100 µmol/mol heme) (Sherwood et al., 1998). A previous study (Skikne et al., 1990) demonstrated that the combination of transferrin receptor and ferritin provided an accurate diagnosis (92%) of ID. The specificity and sensitivity of several conventional tests are shown in Table 6. In case of combined ID with ACD, a higher cutoff value of serum ferritin (such as 60 µg/L) can be used to diagnose ID. Additionally, transferrin saturation <15% together with TIBC >70 µmol/L is diagnostic of ID. Other indicators may also be used to assist in the diagnosis. Although lead poisoning may result in >1000 µmol/mol heme, examination of blood lead along with high ZPP in a patient can quickly confirm the diagnosis (Sherwood et al., 1998).

Iron deficiency is common in developing countries. Severe ID causes anemia and results in a number of adverse outcomes. Infants, children, and adolescents are at risk of ID due to increased iron requirements. Women at childbearing age are also vulnerable to ID because of menstrual iron losses and poor dietary habits. Even in the developed countries, ID

<table>
<thead>
<tr>
<th>Test</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>&lt;20 µg/L</td>
<td>71</td>
<td>87</td>
</tr>
<tr>
<td>ZPP</td>
<td>&gt;100 µmol/mol heme</td>
<td>95</td>
<td>80</td>
</tr>
<tr>
<td>Iron</td>
<td>&lt;10 µmol/L</td>
<td>78</td>
<td>40</td>
</tr>
<tr>
<td>TIBC</td>
<td>&lt;45 µmol/L</td>
<td>76</td>
<td>90</td>
</tr>
<tr>
<td>Saturation (%)</td>
<td>&lt;15</td>
<td>90</td>
<td>62</td>
</tr>
</tbody>
</table>

Source: (Sherwood et al., 1998)
is a health problem for women of childbearing age. To improve accuracy, the diagnosis of ID is usually achieved by using a combination of several indicators.
<table>
<thead>
<tr>
<th>Table 5. Indicators of Iron Deficiency</th>
<th>Selected cutoff values to define ID</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoglobin (g/L)</strong></td>
<td>6 mo-5 y &lt;110 &lt;br&gt; 6-11 y &lt;115 &lt;br&gt; Non-pregnant women &lt;120 &lt;br&gt; Pregnant women &lt;110</td>
<td>When used alone, it has low specificity and sensitivity.&lt;br&gt;Thresholds vary by age, gender, pregnancy, altitude, ethnicity.</td>
</tr>
<tr>
<td><strong>Mean corpuscular volume (MCV) (fL)</strong></td>
<td>Children older than 11 y and adults &lt;82</td>
<td>A reliable, but late indicator of iron deficiency.&lt;br&gt;Low values can also be due to thalassaemia and/or inflammation.</td>
</tr>
<tr>
<td><strong>Haematocrit (%)</strong></td>
<td>6 mo-59 mo &lt;0.33 &lt;br&gt; 5-11 y &lt;0.34 &lt;br&gt; 12-14 y &lt;0.36 &lt;br&gt; Non-pregnant women &lt;0.36 &lt;br&gt; Pregnant women &lt;0.33 &lt;br&gt; Men &gt;15 y &lt;0.39</td>
<td>Same as hemoglobin</td>
</tr>
<tr>
<td><strong>Reticulocyte haemoglobin content (CHr) (pg)</strong></td>
<td>In infants and young children &lt;27.5 &lt;br&gt; In adults ≤28.0</td>
<td>A sensitive indicator that falls within days of onset of iron-deficient erythropoiesis.&lt;br&gt;False normal values can occur when MCV is increased and in thalassaemia. Wider use is limited because it can only be measured on a few models of analyser.</td>
</tr>
<tr>
<td><strong>Erythrocyte zinc protoporphyrin (ZPP) (µmol/mol haem)</strong></td>
<td>5 y or younger &gt;70 &lt;br&gt; Children older than 5 y &gt;80 &lt;br&gt; Children older than 5 y on washed red cells &gt;40</td>
<td>It can be measured directly on a drop of blood with a portable haematofluorometer.&lt;br&gt;A useful screening test in field surveys, particularly in children, in whom uncomplicated iron deficiency is the primary cause of anaemia. Red cells should be washed before measurement because circulating factors, including serum bilirubin, can spuriously increase values. Lead poisoning can increase values, particularly in urban and industrial settings.</td>
</tr>
<tr>
<td><strong>Serum or plasma iron (µg/L)</strong></td>
<td>&lt;40-50</td>
<td>Varies diurnally and after meals.&lt;br&gt;Low values in chronic disease.</td>
</tr>
<tr>
<td><strong>Transferrin saturation</strong></td>
<td>&lt;16%</td>
<td>It is inexpensive, but its use is limited by diurnal variation in serum iron and by many clinical disorders that affect transferrin concentrations.</td>
</tr>
<tr>
<td><strong>Serum or plasma total iron binding capacity (µg/dL)</strong></td>
<td>&gt;400</td>
<td>Large overlap between normal values and values in iron deficiency.</td>
</tr>
<tr>
<td>Table 5. (continued)</td>
<td></td>
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<tr>
<td>---------------------</td>
<td></td>
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</tr>
<tr>
<td><strong>Serum transferrin receptor (sTfR)</strong></td>
<td>Cutoff varies with assay, and with patient age and ethnic origin</td>
<td>Main determinants are erythroid mass in bone marrow and iron status; thus, sTfR is increased by enhanced erythropoiesis and iron deficiency. sTfR is not substantially affected by the acute-phase response, but it might be affected by malaria, age, and ethnicity. Its application limited by high cost of commercial assays and lack of an international standard.</td>
</tr>
<tr>
<td><strong>Serum ferritin (SF) or plasma ferritin (µg/L)</strong></td>
<td>5 y or younger &lt;12 Children older than 5 y &lt;15 In all age groups in the presence of infection &lt;30</td>
<td>It is probably the most useful laboratory measure of iron status; a low value of SF is diagnostic of iron deficiency anaemia in a patient with anemia. In healthy individuals, SF is directly proportional to iron stores: 1 µg/L SF corresponds to 8-10 mg body iron or 120 µg storage iron per kg body weight. As an acute-phase protein, SF increases independent of iron status by acute or chronic inflammation; it is also unreliable in patients with malignancy, hyperthyroidism, liver disease, or heavy alcohol intake.</td>
</tr>
<tr>
<td><strong>sTfR-to-SF ratio</strong></td>
<td>This ratio is a quantitative estimate of total body iron; the logarithm of this ratio is directly proportional to the amount of stored iron in iron-replete patients and the tissue iron deficit in iron deficiency. In elderly people, this ratio might be more sensitive than other laboratory tests for iron deficiency. This ratio cannot be used in individuals with inflammation because SF might be high independent of iron stores. This ratio is assay specific. Although it is only validated for adults, this ratio has been used in children.</td>
<td></td>
</tr>
<tr>
<td><strong>Body iron stores (mg/kg weight): [log (TfR/ferritin ratio)-2.8229]/0.1207</strong></td>
<td>Negative values indicate tissue iron deficit</td>
<td>Cannot be used in individuals with inflammation because the SF may be elevated independent of iron stores. Is assay specific Although only validated for adults, is often used in children.</td>
</tr>
</tbody>
</table>

Source: (Zimmermann & Hurrell, 2007; Zimmermann, 2008)
II. ZINC METABOLISM

Importance of Zinc

As an essential nutrient for human health, zinc plays an important role in normal growth and development (Gardner et al., 2005; Islam et al., 2009), immune function (Ibs & Rink, 2003), as well as reproduction (Bedwal & Bahuguna, 1994) by either participating in enzyme function and gene regulation or maintaining protein structure and stability. For example, more than 300 enzymes require zinc for their catalytic activity and the expression of metallothionein (MT, protein important in maintaining oxidoreductive homeostasis) is induced by the binding of zinc to the metal response element transcription factor (MTF1) (McCall et al., 2000; King & Cousins, 2005). Zinc deficiency could result in impaired immune function (Prasad, 2008), delayed growth, and increased susceptibility of respiratory infection, malaria, and diarrheal disease (WHO, 2002). Additionally, zinc supplementation improved growth in children and infants (Lind et al., 2004; Mozaffari-Khosravi et al., 2009), reduced malarial episodes (Zeba et al., 2008), and the duration/incidence of diarrhea (Baqui et al., 2002; Roy et al., 2007), suggesting the importance of zinc.

Body Zinc Distribution

The human body contains approximately 1.5-2.5 g zinc, making it the second most abundant trace mineral in the body, only slightly lower than iron (King & Cousins, 2005). As described in Table 7 (Rink & Gabriel, 2000), muscle and bone account for the majority of body zinc. However, some organs such as the prostate also have high concentrations of zinc and erythrocytes contain 15~30 mg zinc. In addition, most (95%) of the body zinc is intracellular, primarily existing in the cytosol (King & Cousins, 2005).
Zinc is absorbed in the small intestine primarily through an active saturable process. It is transported into enterocytes by ZIP4 (Zrt/Irt-like protein 4) and ZNT5 (zinc transporter 5) and then is exported into circulation by another zinc transporter ZNT1 (Lichten & Cousins, 2009). As zinc concentration in the intestinal lumen exceeds the capacity of active transport, zinc diffuses directly into circulation through the tight junctions between enterocytes (Grider, 2006). Circulating zinc is then delivered to cells primarily by albumin for utilization (Figure 4) (King & Cousins, 2005). Surplus zinc can be stored in bone and spleen (Emsley, 2001). Previous studies have found rapid zinc turnover (about 12.5 d) in the liver, pancreas, kidney and spleen and slow zinc turnover (about 300 d) in muscle, red blood cells, bone and nervous system. Zinc is recycled from senescent erythrocytes similar to iron through the reticuloendothelial system (King & Cousins, 2005). Zinc excretion is mainly through feces (90%; from pancreatic and biliary secretions, gastroduodenal secretions, and transepithelial flux from enterocytes (Krebs, 2000)), urine (5%), and sweat (5%) (Emsley, 2001; Alpers et al., 2008). Other zinc losses include epithelial cell desquamation, semen,
menstruation, parturition, and lactation (Food and Nutrition Board & Institute of Medicine, 2000).

Zinc homeostasis can be generally maintained through adjustments in zinc absorption and endogenous intestinal excretion as well as other changes (King et al., 2000; King & Cousins, 2005). Zinc absorption adjusts in response to the individual’s zinc status (King & Cousins, 2005), dietary zinc content (Chung et al., 2008), and the bioavailability of soluble zinc (influenced by many dietary enhancers or inhibitors) (Lonnerdal, 2000; Hunt et al., 2008). For example, a group of Korean women was found to have lower zinc absorption (22% vs 43%) when consuming a diet high in phytate compared to a low phytate diet (Kim et al., 2007). In addition, dietary zinc is positively related to fecal zinc losses (King & Cousins, 2005). It was reported that 25 d of dietary zinc deprivation (from 85 µmol/d to 12 µmol/d) with healthy male subjects (n=4) resulted in significantly reduced fecal and urinary zinc losses with no significant change in plasma zinc concentration (Taylor et al., 1991).

Additionally, zinc homeostasis is regulated by MT. When intracellular zinc concentration is high, zinc binds to MTF1 and activates MT, which in turn sequesters zinc to reduce the intracellular zinc concentration (Lichtlen & Schaffner, 2001). It was also reported in rats that intestinal MT synthesis was induced by a high zinc diet but inhibited by a low zinc diet (Hempe & Cousins, 1992).

Zinc absorption is affected by many dietary factors, with animal protein acting as promoter and phytate and calcium as inhibitors (Krebs, 2000). Phytate reduces zinc absorption by binding to zinc and forming poorly soluble complexes. The molar ratio of phytate-to-zinc is usually used to assess zinc bioavailability and absorption. Diets with a
molar ratio <5 have high availability and the absorption is around 50% when dietary zinc intake meets the body requirement. At molar ratios between 6 and 10, zinc absorption begins to decline; when the ratio exceeds 15 as is the case with soy products, zinc availability is low and the absorption is typically lower than 15% (Sandström, 1989; FAO/WHO, 2004).

Generally, 3-4 mg zinc is absorbed from the intestine per day with an average efficiency of 30-40% based on 15 mg of dietary zinc intake (King & Cousins, 2005; Alpers et al., 2008). Based on 7-15 mg intake, about 3.0-4.6 mg of zinc is lost per day (King & Cousins, 2005).

**Figure 4.** Schematic of Zinc Kinetics  
Source: (King & Cousins, 2005)
Zinc Requirements

The dietary zinc requirement has been estimated based on a factorial approach, in which total zinc requirement for tissue growth, maintenance, metabolism, and endogenous losses was divided by an average fractional absorption value in the population (King & Cousins, 2005). Table 8 (Food and Nutrition Board & Institute of Medicine, 2000) indicates the dietary reference intakes for zinc.

Table 8. Dietary Reference Intake for Zinc

<table>
<thead>
<tr>
<th></th>
<th>DRI (mg/d)</th>
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</thead>
<tbody>
<tr>
<td><strong>Infants</strong></td>
<td></td>
</tr>
<tr>
<td>0-0.5 yr</td>
<td>2 (Al)</td>
</tr>
<tr>
<td>0.5-1 yr</td>
<td>3</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
</tr>
<tr>
<td>1-3 yr</td>
<td>3</td>
</tr>
<tr>
<td>4-8 yr</td>
<td>5</td>
</tr>
<tr>
<td>9-13 yr</td>
<td>8</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
</tr>
<tr>
<td>≥14 yr</td>
<td>11</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
</tr>
<tr>
<td>14-18 yr</td>
<td>9</td>
</tr>
<tr>
<td>Pregnant</td>
<td>12</td>
</tr>
<tr>
<td>Lactating</td>
<td>13</td>
</tr>
<tr>
<td>≥19 yr</td>
<td>8</td>
</tr>
<tr>
<td>Pregnant</td>
<td>11</td>
</tr>
<tr>
<td>Lactating</td>
<td>12</td>
</tr>
</tbody>
</table>

Source: (Food and Nutrition Board & Institute of Medicine, 2000)

Dietary Zinc Intake

According to NHANES III in the United States, the median dietary zinc intake ranged from 5.4 mg in non-breastfeeding infants to 9-11 mg in adolescents and adults. Most infants and more than half of the males aged 4-70 y met the recommended dietary allowance (RDA) of zinc, but only 18.9% of young children aged 1-3 y, 39% of female adolescents, and 44% of male elderly aged >71 y satisfied their RDA (Briefel et al., 2000).
Lean red meat, whole grain cereals, pulses, and legumes are the best dietary sources of zinc providing 25-50 mg/kg. Processed cereals with low extraction rates, polished rice, chicken, pork, or meat with a high fat content are also good sources of zinc with 10-25 mg/kg (FAO/WHO, 2004). However, due to the presence of phytate (a dietary inhibitor of zinc absorption), grains and plant foods have lower zinc bioavailability than animal foods (FNB & IOM, 2000). The zinc content of fish, green leafy vegetables, and fruits is low with <10 mg/kg (FAO/WHO, 2004). Globally, pulses and cereals are the major zinc sources. However, in the US, they account for 30% of dietary zinc. Meat and dairy products, respectively, provided 50% and 20% of dietary zinc (Maret & Sandstead, 2006).

**Assessment of Zinc Status**

No reliable and accurate indicators are available to reflect individual zinc status. Daily zinc losses can be estimated from fecal zinc excretion, but this method is cumbersome and not routinely used. When zinc deficiency is suspected based on the evidence from low dietary intake, poor bioavailability, or suggestive clinical signs, definitive diagnosis can be reached by a symptomatic response after zinc supplementation. However, this method is limited by cost and time requirement (King & Cousins, 2005; Alpers et al., 2008).

**Plasma Zinc**

Plasma zinc is widely used as the screening test, although it is a relatively insensitive indicator of zinc status. Plasma zinc concentration is generally stable but decreases after several weeks of severe dietary restriction when tissue zinc concentrations are reduced. Thus, it poorly reflects tissue zinc status and cannot be used to identify marginal zinc deficiency. In addition, it is affected by other conditions independent of zinc status. For example, plasma
zinc concentration declines after food intake and in stress situations, such as fever and infection. In contrast, hemolysis and long-term fasting increase plasma zinc concentration (Alpers et al., 2008). However, reduced plasma zinc concentration may indeed be indicative of zinc-responsive growth reduction in a population (FAO/WHO, 2004). Serum zinc concentration can also be measured to assess zinc status, but false elevation may occur due to contamination (FNB & IOM, 2000).

**Lymphocyte Zinc Content**

Zinc status can be assessed by measuring lymphocyte zinc content (Terwolbeck et al., 1992). This method has been reported to be sensitive to marginal zinc intake in small, well-controlled studies. However, the effectiveness has not been verified in larger groups and the difficulty in separating white blood cells limits its utilization (Grider, 2006). In addition, this method needs a relatively large amount of blood sample, also limiting its utilization in small children (Eaton et al., 2004).

**Hair Zinc Concentration**

Hair zinc concentration is sensitive in detecting marginal zinc deficiency in children in the absence of protein-energy malnutrition. However, factors such as age, sex, and hair growth rate must be considered in the interpretation of results (Gibson et al., 2008). Besides, normal hair zinc concentration does not rule out zinc deficiency due to the possible contamination from shampoo (Eaton et al., 2004).

**Functional Indicators**

Functional changes in growth, body composition, cell-mediated immunity, neurological performance/recognition, neuromotor function, dark adaptation, taste, and smell
acuity may be observed well before the reduction in plasma zinc in zinc deficiency. Hence, symptomatic response after zinc supplementation is useful in the diagnosis of zinc deficiency. Well controlled observation is necessary when using these functional indicators because they are not specific for zinc deficiency (Maret & Sandstead, 2006).

Other Indicators

Urinary zinc excretion declines in severe zinc deficiency (FAO/WHO, 2004) and responded quickly after dietary zinc changes (Ruz et al., 1991). Thus, urinary zinc is also used as a marker for zinc status, but it is affected by diseases independent of zinc status, such as renal disease. Muscle biopsy is another method to assess zinc status but it is invasive and samples may be contaminated by fat, collagen, or blood (Eaton et al., 2004). Additionally, zinc status may be assessed by measuring erythrocyte zinc, MT (Grider et al., 1990; Caulfield et al., 2008) or zinc-dependent enzymes, such as serum alkaline phosphatase (Rothbaum et al., 1982; Samman et al., 1996).

Zinc Deficiency

Zinc deficiency is a condition where inadequate zinc is available for metabolic needs. It is usually nutritional in origin but may also be secondary to conditions such as malabsorption syndrome and chronic liver disease (Prasad, 2003).

Prevalence

Due to the lack of suitable biomarkers for zinc status, the prevalence of zinc deficiency has not been adequately investigated (Hambidge, 2000). Based upon food availability data, zinc deficiency is estimated to affect one-third of the world population with an average prevalence of 31% globally, ranging from 4% to 73% across the WHO subregions.
In North America where the mean daily per capita zinc intake is estimated to be ~11-12 mg and more than half of the zinc is from animal sources (Brown et al., 2001), apparent zinc deficiency is uncommon (Food and Nutrition Board & Institute of Medicine, 2000).

#### Causes

Primary causes of zinc deficiency are inadequate dietary intake or low absorption, but also include excessive zinc losses. Individuals mainly with plant-based diets, such as vegetarian and people in impoverished areas, are at risk of zinc deficiency. Although their

<table>
<thead>
<tr>
<th>Table 9. Causes of Zinc Deficiency</th>
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<tbody>
<tr>
<td><strong>Primary causes</strong></td>
</tr>
<tr>
<td><strong>Insufficient intake/ Low bioavailability</strong></td>
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<tr>
<td></td>
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<tr>
<td><strong>Impaired absorption</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>High requirement</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Excessive losses</strong></td>
</tr>
<tr>
<td><strong>Unexplained reasons</strong></td>
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</tbody>
</table>

Source: (Yanagisawa, 2008)
dietary zinc intake may be adequate, the amount of zinc absorbed may be not sufficient due to the presence of inhibitors, such as phytate and fiber from plant products (Caulfield & Black, 2004). A long-term crossover study reported that consuming a lactoovovegetarian diets (molar ratio of phytate-to-zinc=14) for 8 wk reduced zinc absorption by 35% and plasma zinc concentration by 5% in women (n=22) (Hunt et al., 1998). Infants, children, adolescents and pregnant women are also vulnerable to zinc deficiency due to increased zinc demand for rapid growth (FAO/WHO, 2004). Additionally, zinc deficiency may result from impaired zinc absorption, excessive zinc losses, and/or unknown causes as described in Table 9 (Yanagisawa, 2008).

Consequences

Zinc deficiency causes growth retardation in infants (Islam et al., 2009), children, and adolescents (Nishi et al., 1989; Siklar et al., 2003), and negatively affects sexual development in adolescents (Fons et al., 1992). Maternal intake of zinc during pregnancy and lactation was reported to be negatively associated with neonate focused attention and infant motor performance (Kirksey et al., 1994). Maternal zinc deficiency may result in a number of adverse outcomes including spontaneous abortion, congenital malformations, low birth weight/intrauterine growth retardation and preterm delivery (Wells et al., 1987; Jameson, 1993). Additionally, zinc deficiency impairs cognitive function in pregnant women (Stoecker et al., 2009) and psychological dimensions in the elderly (Marcellini et al., 2006). It also compromises immune function by affecting all types of immune cells (Ibs & Rink, 2003).

Zinc deficiency is significant cause of respiratory infections, malaria, and diarrheal disease. Out of twenty leading risk factors for diseases in the world, zinc deficiency ranks
11th. It contributes to 16% of lower respiratory tract infections, 18% of malaria, 10% of diarrheal disease, and 1.4% (0.8 million) of deaths (1.4% for male and 1.5% for female). Approximately 2.9% (28 million) of DALYs in the world are attributed to zinc deficiency (WHO, 2002). Among children between 6 mo and 5 y, zinc deficiency was responsible for 14.4% diarrheal deaths, 10.4% malarial deaths and 6.7% pneumonia-related deaths, contributing to 4.4% of childhood deaths (453207 deaths) and 3.8% of disease burden in Latin America, African, and Asia in 2004 (Fischer Walker et al., 2008).

**Diagnosis**

Zinc deficiency is suspected in cases of low dietary zinc intake, poor zinc bioavailability and absorption or suggestive clinical signs as indicated in Table 10 (King & Cousins, 2005).

<table>
<thead>
<tr>
<th>Table 10. Clinical Manifestation of Marginal to Severe Human Zinc Deficiency</th>
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<tbody>
<tr>
<td>Growth retardation</td>
</tr>
<tr>
<td>Delayed sexual maturation and impotence</td>
</tr>
<tr>
<td>Hypogonadism and hypospermia</td>
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<tr>
<td>Diarrhea and intestinal inflammation</td>
</tr>
<tr>
<td>Alopecia</td>
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<tr>
<td>Acroorificial skin lesions</td>
</tr>
<tr>
<td>Other epithelial lesions: glossitis, alopecia, nail dystrophy</td>
</tr>
<tr>
<td>Immune deficiencies: lymphopenia, thymic defects, reduced phagocytosis, depressed T-cell function, impaired cytokine production</td>
</tr>
<tr>
<td>Behavioral disturbances, including impaired hedonic tone</td>
</tr>
<tr>
<td>Impaired taste (hypogeusia)</td>
</tr>
<tr>
<td>Delayed healing of wounds, burns, and decubitus ulcers</td>
</tr>
<tr>
<td>Impaired appetites and food intake</td>
</tr>
<tr>
<td>Eye lesions, including phophobia and lack of dark adaptation and photic injury</td>
</tr>
</tbody>
</table>

Source: (King & Cousins, 2005)

Cousins, 2005). To diagnose zinc deficiency, a number of lab indicators (described in assessment of zinc status section) and a symptomatic response with zinc supplementation are usually monitored (Roth & Kirchgessner, 1999; King & Cousins, 2005; Alpers et al., 2008). Zinc-binding capacity and serum/plasma alkaline phosphatase activity prior to and after zinc
supplementation (zinc tolerance test) are considered to be most useful lab parameters (Roth & Kirchgessner, 1999). Sweat mineral analysis was not only non-invasive, convenient, and repeatable, but also more sensitive than blood or hair analysis. Thus, sweat mineral analysis may be suitable in clinical practice for auxiliary diagnosis of marginal zinc deficiency. Similarly, Bryce-Smith taste test has also been suggested as useful in the diagnosis of zinc deficiency, although not as accurate as sweat mineral analysis (Eaton et al., 2004).

Zinc deficiency is prevalent in developing countries. It has been associated with a number of adverse consequences, since zinc is an essential nutrient for human health. The primary causes of zinc deficiency are insufficient zinc intake and low zinc absorption. Apparent zinc deficiency is not common in the western world, whereas marginal zinc deficiency may be a problem. Infants, children, adolescents and pregnant women are vulnerable to zinc deficiency due to increased zinc demand. No completely reliable and accurate indicators are available to reflect individual zinc status, but diagnosis of zinc deficiency can be achieved by examining the symptomatic response to zinc supplementation.
III. SOY CONSUMPTION

Soy Foods and Soy Components

Soy (soybean), belonging to the leguminous family, constitutes a major component of the Asian diet. It has been used in various forms, such as soy milk, tofu, miso, yuba, and tempeh (Fournier et al., 1998). In the American diet, except for tofu and soy milk, soy products such as soy flour, soy protein concentrate, and soy protein isolate are generally used as the primary ingredient of many meat and dairy substitutes. Soy food consumption is not so common in the US except for vegetarians and infants consuming soy formula (Erdman & Fordyce, 1989). Average daily intake of protein from soy foods is approximately 20-80 g for Asians and 1-3 g for Americans (Fournier et al., 1998).

Soy protein offers almost a complete protein profile with an adequate amount of essential amino acids based upon ideal patterns published by the Food and Nutrition Board or the Food and Agriculture Organization. Only the sulfur-containing amino acids methionine plus cystine in most soy proteins are lower than the recommended amount (Erdman & Fordyce, 1989). Although the protein quality of soy is comparable to that of animal sources such as egg, milk and beef, the supplementation of methionine in soy protein products is necessary for infants and children (de Oliveira et al., 1981; Fomon et al., 1986) but not for adults (Wayler et al., 1983; Young et al., 1984; Endres, 2001).

Soy products not only provide an excellent source of protein, but are also rich in fibers and phytochemicals including isoflavones, phytate, and saponins (Mota et al., 2007). For example, soybean or soy products generally contain 1-3% phytate by weight (Anderson & Wolf, 1995) and soybean contains about 0.6-6.5% saponins by weight (Berhow et al.,
2006). However, it is important to note that soy products differ in their chemical composition (nutrients and bioactive components) due to various processing techniques. These differences make the interpretation and comparison of study data difficult, because traditional soy foods derived from whole or dehulled soy beans, such as tofu and soy milk, are generally examined in epidemiological studies, while soy concentrates, soy protein isolates, and isolated soy isoflavones are used in most animal and human intervention studies (Erdman et al., 2004). Dehulling, flaking, defatting, fermenting, and alcohol-washing result in lower isoflavone content than the native form of soy products. For example, soybeans contain 2-5 mg isoflavones per gram protein, higher than isolated soy protein; soy milk and tofu contain 2 mg isoflavones per gram protein; the isoflavone content of non-fermented soy foods, such as roasted soybeans, is 2-3 times as high as fermented soy foods, such as tempeh and miso (Wang & Murphy, 1994).

**Health Effects of Soy Consumption**

**Potential Benefits with Soy Consumption**

**Cardiovascular Diseases**

The beneficial effect of soy food consumption on cardiovascular disease (CVD) has been reported in epidemiological studies. The incidence of CVD in Asian populations who commonly consume soy foods has proven to be lower than in Western populations who rarely consume soy foods (Beaglehole, 1990). The substitution of animal products with soy foods resulting in low-fat, cholesterol-free, and high fiber diets generally leads to a hypocholesterolemic response (Carroll et al., 1979). A Japanese study showed an inverse association between soy food intake (soy, bean, miso soup) and the risk of cerebral and
myocardial infarction as well as CVD mortality in postmenopausal women (Kokubo et al., 2007).

Isoflavones in soy may also favorably affect lipoprotein profile, blood pressure, and oxidative stress (Djuric et al., 2001) to protect against CVD. The WHO-CARDIAC study demonstrated that coronary heart disease mortality rate was positively related to serum cholesterol concentrations but inversely related to 24-hr urinary isoflavone excretion (Yamori, 2000). A five-wk double-blind dietary intervention with middle-aged men at risk for CVD resulted in significant reductions in systolic blood pressure (BP), diastolic BP, total cholesterol (TC) and non-high density lipoprotein cholesterol in the soy group (soy powder containing at least 20 g soy protein and 80 mg isoflavones per day) compared to no changes in placebo group (Sagara et al., 2004). The TC and LDL-cholesterol lowering effect of soy isoflavones in mildly hypercholesterolemic individuals has been reported in a dose-dependent manner (Crouse et al., 1999), and this dose-dependent effect has been also supported by a meta-analysis study (Reynolds et al., 2006).

However, not all studies found beneficial effects of soy on lipoprotein profile. For example, another meta-analysis study did not show an improvement in TC and LDL-cholesterol in normocholesterolemic menopausal women with 1-3 mo ingestion of soy isoflavones (about 70 mg/d) (Taku et al., 2008), and two randomized controlled trial with 6 mo or 6 wk treatment did not observe effects of isoflavones on lipid profile in postmenopausal women (Engelman et al., 2005; Rios et al., 2008).

Other soy components such as phytate may also play a role in decreasing the risk of CVD. Phytate was found to reduce platelet aggregation (Vucenik et al., 1999), oxidative
stress (Porres et al., 1999) and hepatic/blood lipids (Onomi et al., 2004), though data are limited in humans. A 6-wk feeding study found significantly reduced total homocysteine concentration (a CVD risk factor) in postmenopausal women after daily consumption soy protein with native phytate and low isoflavones (40 g soy protein with 0.64 g phytate and 1.2 mg isoflavones) but not soy protein with native isoflavones and low phytate (40 g soy protein with 0.22 g phytate and 85.8 mg isoflavones), suggesting that phytate is another factor in soy that may reduce atherosclerotic CVD risk factors in postmenopausal women (Hanson et al., 2006).

Bone Health

Numerous epidemiological studies have shown beneficial effects of soy food consumption on bone. An observational study in Japan reported a positive association of soy food consumption with bone mass in postmenopausal women (Somekawa et al., 2001). A prospective cohort study in China observed an inverse association between soy food intake and bone fracture incidence in postmenopausal women (Zhang et al., 2005). A 2-y longitudinal study in young Korean women showed a positive change in bone mineral density with soybean consumption (Song et al., 2008). The improvement in bone after soy consumption is attributed to soy isoflavones and protein fraction of soy.

Soy isoflavones have shown to attenuate bone loss. A 6-mo randomized controlled trial (RCT) with postmenopausal women (n=30 subjects/group with 0, 84 or 126 mg isoflavones per day) found a significant dose-dependent effect of soy isoflavones on attenuating bone loss at the lumbar spine and femoral neck (Ye et al., 2006). This study indicated that the effect may be via the inhibition of bone resorption as reflected by lower
urinary total deoxypyridinoline (Dpyr, a bone resorption marker) excretion in the high dose group. Moreover, a meta-analysis based on nine long-term RCT (n=432) showed that the consumption of soy isoflavones significantly decreased urinary Dpyr concentration and increased serum bone alkaline phosphatase (BAP, a bone formation marker) concentration in peri- or postmenopausal women, compared with placebo treatment (Ma et al., 2008).

However, not all studies observed an effect of soy isoflavones on bone resorption or formation. For instance, significantly positive effect on lumbar spine bone mineral density and content was found in perimenopausal women with 24 wk intake of isoflavone-rich soy but not in those consuming isoflavone-poor soy or whey protein in another study (Alekel et al., 2000). However, no effect of soy isoflavones on bone resportion or formation was observed in the same study.

Additionally, some researchers had indicated that soy protein may protect against osteoporosis by inducing less urinary calcium excretion than animal protein due to the lower content of sulfur-containing amino acids in soy protein (Remer & Manz, 1994). Despite similar dietary calcium intakes, a meat-based diet caused significantly higher urinary and fecal calcium excretion than a soy-based diet (urine: 127 vs. 88 mg/d; fecal: 467 vs. 284 mg/d) (Pie & Paik, 1986).

Despite of positive results from some studies, effects of soy on bone are still inconclusive and controversial. For example, 2-y consumption of soy plus isoflavones (25 g soy protein with 90 mg isoflavones) was not found to favorably affect bone mineral density of lumbar spine and femoral neck, compared to milk protein (25 g casein and whey), in postmenopausal women (n=203) (Vupadhyayula et al., 2009). A recent meta-analysis (Liu et
al., 2009) of 10 RCTs did not find a significant increase in bone mineral density at lumbar spine, total hip or femoral neck in women (n=896) with at least 1 y isoflavone supplementation, and the author proposed that the benefit of soy isoflavone supplementation on bone health may only be transient, but not long-term.

Other Possible Health Benefits

Soy consumption may lower the risk of breast cancer as shown in numerous epidemiological studies. For example, a recent prospective cohort study (7.4 y) in China with 73,223 women reported a substantially reduced risk of premenopausal breast cancer with soy food intake (Lee et al., 2009). Similar results were found in another population-based case-control study, demonstrating the strongest protective effect for early-life soy intake (Korde et al., 2009). Additionally, soy food intake may protect against prostate cancer (Kurahashi et al., 2007; Yan & Spitznagel, 2009). These protective effects may be attributable to soy isoflavones, which have been shown to exert both anti-estrogenic and anti-carcinogenic properties. The regulation of cell proliferation and apoptosis in cancer cell lines by soy isoflavones has been demonstrated in a number of in vitro studies (Vinall et al., 2007; Imhof & Molzer, 2008; Singh-Gupta et al., 2009).

Soy formula has been shown to be hypoallergenic compared to cow’s milk. The incidence of food allergy was lower in infants fed soy formula (3-4% vs 25%) than those fed cow’s milk (Cantani & Lucenti, 1997). In addition, the beneficial effect of soy on glycemic control in type 2 diabetic patients has been reported (Jayagopal et al., 2002; Shahbazian et al., 2007). The glycemic response to soy foods may be attributable to their soluble fiber content (Mahalko et al., 1984; Thomas et al., 1988) and soy isoflavones, reported to inhibit
intestinal glucose update (Vedavanam et al., 1999) and stimulate insulin secretion (Jonas et al., 1995) in animals.

Potential Risks with Soy Consumption

Soy Allergy

Although soy formula has been successfully used in managing cow’s milk intolerance in infants, soy protein is one of the eight most significant food allergens listed by the Food and Agriculture Organization (FAO, 1995). A double-blind study found that 14% of children with IgE-associated cow’s milk allergy (CMA) developed a soy allergy after consuming soy formula for a year (Zeiger et al., 1999).

Mineral Balance

The concern related to mineral balance mainly focuses on negative effect of phytate on mineral bioavailability, because soy or soy products contain significant amount of phytate. Phytate binds to multivalent cations, especially zinc, iron, and calcium, and forms insoluble complexes in the small intestine, thereby decreasing their bioavailability (Zhou & Erdman, 1995). It is difficult to evaluate the effect of soy consumption on calcium balance since phytate inhibits calcium absorption, but soy protein consumption tends to facilitate urinary calcium retention compared to meat protein (Pie & Paik, 1986). Therefore, the effect of soy consumption on iron and zinc status is the focus of the following section.

Iron bioavailability has been demonstrated to not only be extremely poor in pure soy foods (Cook et al., 1981), but also poor in mixed diets incorporated with soy foods (Cook et al., 1981; Hallberg & Rossander, 1982; Morck et al., 1982). For instance, when full fat soy flour, textured soy flour, or isolated soy protein were substituted for egg albumin in a liquid
diet, the absorption reduced from 5.5% to 1%, 1.9%, and 0.4%, respectively (Cook et al., 1981). In a mixed diet with beef and soy flour (1:1), iron absorption was 2-fold lower than in a beef only diet (Lynch et al., 1985). Dephytinization improved iron absorption by four- to five-fold, but was still only half of the egg control in a single meal study. These results not only confirm the inhibitory effect of phyate, but also suggest the existence of dietary inhibitors in soy other than phytate (Hurrell et al., 1992).

Although iron absorption with soy is observed to be low in single meal studies, not all long-term feeding interventions reported a negative effect on iron status. Daily consumption of 40 g soy protein isolate for 6 mo showed no adverse effects on iron status in perimenopausal women with normal or compromised iron status (Swain et al., 2002). On the contrary, the same amount of protein for 6-wk negatively affected iron status in postmenopausal women who had moderately elevated iron stores (Hanson et al., 2006). Another 1 y feeding study also did not find significant differences on iron status in infants fed with a soy formula or an extensively hydrolyzed whey formula (Seppo et al., 2005). Since iron absorption is inversely related to iron status of individuals, the discrepancy of these results indicates that the effect of soy foods on iron balance may depend upon the iron status of individuals, the protein sources and duration of intervention.

Soy protein-based diets are generally considered to have low zinc bioavailability due to the high phytate-to-zinc molar ratio. For example, when cow’s milk had the same phytate-to-zinc molar ratio as soy formula after the addition of phytate, zinc absorption was reduced two-fold and became similar to the absorption from soy formula (Lonnerdal et al., 1984). The inhibitory effect of soy products on zinc absorption is apparent when soy is the sole
protein source, but is marginal in mixed diets in the presence of other protein sources, such as milk and meat (Solomons et al., 1982; Sandstrom et al., 1987). For example, zinc absorption was not significantly affected when milk proteins were substituted with 50% soy (Solomons et al., 1982). Similar to iron, zinc absorption was also improved after dephytinization, as reflected by increased zinc absorption from 11.3% to 20.1%, but was closer to 31.2% from cow’s milk in a single meal study (Zhao et al., 2003). The fact that human adaptation in zinc absorption with a low-zinc diet was absent if dietary phytate was high (Hunt et al., 2008), also emphasized the importance of dephytinization.

Due to the lack of a sensitive clinical indicator to assess zinc status, long-term feeding studies to assess the effect of soy on zinc balance are limited. Daily consumption of soy based diet for 3 mo (70 g soy protein/d) was reported to cause a negative zinc balance and a reduction in plasma (25%) and neutrophil (31%) zinc concentrations in a small group (n=5) of human subjects (Cossack & Prasad, 1982). Similar results were found in another study with daily consumption of a soy protein based diet (57 g soy protein/d) for 28 weeks in male subjects (n=5) (Rabbani et al., 1987). However, no effect of soy intake (0.8 g soy protein/kg weight) on zinc absorption or balance was found in another long-term (82 days) feeding study (Istfan et al., 1983).

Anti-Thyroid Effect

Soy consumption may have anti-thyroid effects, especially among those with inadequate intake of iodine. Before soy formula was supplemented with iodine in the mid 1960s, soy-induced goiter had been reported in infants fed with soy formula (Van Wyk et al., 1959; Hydovitz, 1960; Ripp, 1961). Feeding soy formula to infants with congenital
hypothyroidism resulted in prolonged increase in thyroid-stimulating hormone (TSH) (Conrad et al., 2004) and the discontinuation of feeding soy formula normalized the TSH concentration in three weeks (Jabbar et al., 1997). Even in healthy adults, daily consumption of soybeans (30 g/d) for 1 mo significantly increased TSH, although it was still within the normal range and no change in thyroid hormones were observed. Similarly, the cessation of soy consumption corrected the problem (Ishizuki et al., 1991).

The underlying mechanism for the inhibition of soy on thyroid function is closely related to iodine status. In the absence of iodide, the soy isoflavones, genistein and daidzein can irreversibly bind to thyroid peroxidase (TPO) and inhibit TPO-catalyzed iodination and coupling, thereby impairing thyroid hormone \( (T_3\) and \( T_4\)) synthesis. In presence of adequate iodide, genistein and daidzein act as alternate substrates for TPO to produce mono-, di-, and triiodoisoflavones (Divi et al., 1997). In addition, soy consumption may contribute to iron deficiency, which may in turn contribute to hypothyroidism since two important enzymes (TPO and hepatic 5’-deiodinase) in thyroid hormone synthesis are iron dependent. Hepatic 5’-deiodinase catalyzes the conversion of \( T_4\) to \( T_3\), the active thyroid hormone (Zimmermann, 2006).

Soy consumption is becoming popular in the Western world, especially among vegetarians and young persons, due to increased health consciousness. Most studies have focused on health benefits related to soy protein, isoflavones and phytate, but research on negative effects of soy food consumption is limited. It is important to investigate the effect of soy food consumption on women of childbearing age, who are at risk of mineral deficiencies due to menstrual losses and poor dietary habits.
REFERENCES


Effect of Soy Food Intake on Mineral Status in Women of Childbearing Age

A paper to be submitted to The Journal of Nutrition

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ABSTRACT

Many health benefits are attributed to soy food intake, but data on mineral balance are limited. Our main objective was to determine the effect of soy foods intake for 10-wk on iron or zinc status in women of childbearing age. We also tested the effect on bone and thyroid hormones. Women (18-28 y) non-smokers without chronic disease, anemia, pregnancy, and/or irregular menstrual cycles were randomly assigned to either a soy (n=31) or an animal food (n=32) group. Blood and urine samples and 3-d dietary records were collected prior to and after intervention. At baseline, iron and zinc status, biochemical markers of bone, and thyroid hormones were not different between two groups. After intervention, no significant changes were observed in hemoglobin, transferrin saturation, serum iron, ferritin, or transferrin receptor concentrations, as well as the transferrin receptor-ferritin index.

\textsuperscript{1}Funded by Iowa Soybean Association and Soyfoods Council
Intervention significantly decreased plasma zinc (5 µg/dl in each group) but not serum alkaline phosphatase (soy vs. animal food group: 1.1 vs. 1.2 IU/L) concentrations. Soy intake slightly increased (1.5 U/L) bone-specific alkaline phosphatase concentration, with the change being significantly different from the animal food group (-0.7 U/L). No significant changes were observed in bone resorption marker, thyroid stimulating hormone or free thyroxine after soy food intake. In conclusion, incorporating 2 servings/day of soy foods with ~20 g protein for 10-wk had no significant effect on iron and zinc status, bone resorption, or thyroid hormones and a beneficial effect on bone formation, compared to animal foods intake, in women of childbearing age.

KEY WORDS Women of childbearing age, soy foods, iron, zinc, TSH

INTRODUCTION

Soy consumption has been grown popularity in the western world for its health benefits. Soy has been reported to beneficially modify blood lipids and lipoproteins (1-3), decrease cancer risk (4-6), attenuate bone loss (7-9), and help controlling diabetes (10, 11). Despite benefits, soy consumption may impair mineral balance in humans. Soy and its components contain about 1-3% phytic acid by weight (12), which binds to minerals such as zinc, iron, and calcium, thereby decreasing their bioavailability.

Iron bioavailability has been shown to be extremely poor not only from soy foods (13) but also when soy was mixed other foods (13-15) in single meal feeding studies. For example, when full fat soy flour, textured soy flour, or isolated soy protein were substituted for egg albumin in a liquid diet, the absorption reduced from 5.5% to 1%, 1.9%, and 0.4%, respectively (13). In a mixed diet with beef and soy flour (1:1), iron absorption was 2-fold

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2 None of the authors had a personal or financial conflict of interest.
lower than in a beef only diet (16). However, not all long-term feeding studies reported a negative effect on iron status. Daily consumption of 40 g soy protein isolate for 6 mo showed no adverse effect on iron status in perimenopausal women with normal or compromised iron status (17). On the contrary, the same amount of protein intake for 6-wk negatively affected iron status in postmenopausal women who had moderately elevated iron stores (18).

Less data are available on the effect of soy foods on zinc absorption or balance. Soy protein-based diets are generally considered as having low zinc bioavailability due to the high phytate-to-zinc molar ratio (19). When the ratio exceeds 15 as is the case with soy products, zinc absorption is typically lower than 15% (19, 20). The inhibitory effect of phytate could be attenuated by dephytinization (21-23), or incorporating other protein sources, such as milk and meat to soy products (24, 25). Long-term feeding studies to assess the effect of soy on zinc balance are limited and conflicting. Daily consumption of a soy protein based diet for 3 mo was reported to cause a negative zinc balance and a reduction in plasma (25%) and neutrophil (31%) zinc concentrations in a small group (n=5) of human subjects (26), whereas no effect of soy on zinc absorption or balance was found in another long-term (82 days) feeding study (27).

Influences of soy consumption on bone and thyroid status have also been reported. Soy isoflavones were shown to attenuate bone loss from lumbar spine in perimenopausal women (28), possibly by inhibiting bone resorption (29, 30). Soy-induced goiter was reported in infants fed with soy formula without iodine supplement (31-33), probably because soy isoflavones impair thyroid hormone synthesis in the absence of iodide (34). Additionally, soy consumption may cause iron deficiency, which may in turn contribute to
hypothyroidism since two important enzymes (thyroid peroxidase and hepatic 5’-deiodinase) in thyroid hormone synthesis are iron dependent (35).

Overall, single meal studies clearly report an inhibitory effect of soy (mostly soy protein isolate) on iron and zinc absorption, but it is not clear whether consuming moderate amounts of soy foods daily will exert a significant impact on the iron and zinc status in healthy young adults. According to NHANES III in the United States, only 39% of female adolescents satisfied their recommended dietary allowance (Briefel et al., 2000). We therefore investigated the effect of incorporating commonly consumed soy foods daily for 10 weeks on iron and zinc status in young female subjects. The potential effects of soy food intake on biochemical markers of bone and thyroid hormones were also assessed.

SUBJECTS AND METHODS

Subject Selection

We recruited women aged 18-40 y by sending out emails and flyers throughout the Iowa State University. As shown in Figure 1, among the 195 subjects who responded, 87 were initially screened by telephone and 74 were eligible to participate. Women with chronic disease, gastrointestinal problems, anemia, pregnancy, smoking, irregular menstrual cycles (<24 or >32 d menstrual cycle), extreme BMI (≤18.5 or ≥30 kg/m²) or consumption of soy products more than two times per week were excluded. Women who met these criteria and were willing to be randomly assigned to either the soy (SF) or animal food (AF) group for 10-wk were included. After pregnancy test and chemistry screen, 70 subjects were selected based on inclusion/exclusion criteria. With 2 dropouts due to time conflict or consumption of mineral supplements and 5 lost to follow-up, we had a final sample size of 63 subjects.
Written informed consent was obtained from all subjects before baseline data collection. The study protocol, consent forms, and subject-related materials were approved by Iowa State University Human Subjects Review Committee (Institutional Review Board ID# 07-505).

**Study Design**

Once the subjects were selected based upon inclusion/exclusion criteria, they were randomly assigned to either the SF or AF group with foods (~two servings/d) provided by the study for 10 wk. Subjects came to the Human Metabolic Unit (HMU) on the Iowa State University campus three days per week (Monday, Wednesday and Friday) to eat their meals and to pick up the frozen meals to consume at home for the remaining days. Milk or yogurt was always consumed at home as a part of second meal every day. The weekly menu for subjects in each group is presented in Table 1; the soy protein content in SF group varied from 18 to 22 g per day, with an average of 19 g. In the meals we provided, total protein content and energy averaged 27 g and 2240 kJ per day for the AF group and 25 g and 2018 kJ for the SF group, respectively. Since the majority of subjects were students, we provided soy protein bars to the SF group and whey protein bars to the AF group during the 7-day spring break. Subjects in the SF group were instructed not to consume any additional soy foods and limited to 1 serving/d of animal products, whereas the AF group was instructed not to consume more than 1 serving/d of animal foods (excluding dairy) in addition to the meals we provided during the study period. In addition, subjects were instructed not to take vitamin/mineral supplements. Instructions were provided for 24-hr urine collection.
Data Collection

Questionnaires were administered to subjects at baseline to collect information on demographic characteristics, medical history, soy food intake history and reproductive history. Prior to and at the end of the intervention including the test meals, three-day dietary intake records were obtained; standing height, body weight, and waist and hip circumferences were measured by trained staff according to a standard protocol. Overnight fasting blood samples were drawn by phlebotomists and 24-hour urine samples were collected from each subject at baseline and the end of study. Aliquots of serum/plasma and urine samples were stored at -80°C until analysis.

Laboratory Measurements

Blood and urine samples were analyzed by a certified clinical laboratory (LabCorp, Kansas City, KS) for a general chemistry profile, complete blood count with differential, thyroid status (thyroid stimulating hormone and free thyroxine), iron status (serum iron, total iron binding capacity, transferrin saturation), and zinc status (plasma and urinary zinc, serum alkaline phosphatase). We measured serum ferritin using an immunoradiometric procedure and transferrin receptor concentrations using an enzyme immunoassay kit (TfR) following the manufacturer’s (Ramco Laboratories; Houston, TX) guidelines. Transferrin receptor-ferritin index (TfR-F index) was calculated as the ratio of serum transferrin receptor to log-transformed serum ferritin to determine non-anemic iron deficiency (36). Commercial ELISA kits were used to measure serum bone-specific alkaline phosphatase (BAP, bone formation marker) activity (Metra® BAP; Quidel Corporation; San Diego, CA) and serum cross-linked C-terminal telopeptides of type-I-collagen (C-Tx, bone resorption marker) concentrations (serum CrossLaps® ELISA; Nordic Bioscience Diagnostics; Denmark) with an automated micro-titer
plate reader (ELx808U with KC Junior software V 1.14, BIO-TEK INSTRUMENTS®, Inc.; Winooski, VT) according to the manufacturer’s guidelines. Urinary isoflavones were determined using a modified HPLC procedure (37). The intra-assay and inter-assay CVs (%) were 5.3% and 6.0% for ferritin, 4.1% and 11.6% for transferrin receptor, 2.1% and 9.5% for BAP, and 1.8% and 7.3% for C-Tx, respectively.

**Statistical Analyses**

Statistical analyses were performed using SAS (version 9.1, Cary, NC) with results considered statistically significant at $p \leq 0.05$. The normality of data was checked before analysis with the Kolmogorov-Smirnov test and by examining the distribution of data using histograms. Descriptive statistics included mean (range) for age, height, weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio; mean±SD for hemoglobin, serum iron, and serum alkaline phosphatase (ALP); and median (range) for serum ferritin, transferrin receptor, transferrin saturation, plasma and urinary zinc, and all dietary intakes. For normally distributed data, an unpaired student t-test was performed for between group comparisons and a paired student t-test was used for within group comparisons. For data that were not normally distributed, comparisons were conducted using a Mann-Whitney test between groups and a Wilcoxon signed-rank test within groups. Samples with serum ferritin values <1 µg/L (n=5 out of 126 measurements) were assigned to a value of 2 µg/L for statistical analysis.

**RESULTS**

**Subject Characteristics**

A total of 63 subjects completed the 10-week dietary intervention. Table 2 shows the baseline characteristics of subjects by group. At baseline, subjects did not differ significantly
in age, height or hip circumference between the two groups. The significant difference in weight, BMI and waist circumference between two groups was because one subject in the AF group was within the obese range with weight >90 kg, BMI >30 kg/m², and waist circumference>90 cm. The majority of the 63 subjects were Caucasian with 1 African American, 1 Hispanic/Latino, 11 Asian, and 2 with mixed race.

**Dietary Intakes**

Nutrient intakes were calculated from three-day dietary records using Nutritionist Pro (version 2.3.1, Stafford, TX). No significant differences between the groups were found in total dietary intake of energy, protein, carbohydrate, fat, iron, calcium, or vitamin C at baseline and the end of study ([Table 3](#)). However, the soy foods we provided to SF group contributed to the slightly higher intake of iron (5.2 mg vs 4.0 mg), calcium (395 mg compared to 319 mg), and vitamin C (6.2 mg vs 5.2 mg) than the AF group. However, total iron and calcium intake significantly increased in the AF group despite higher content of iron and calcium of soy foods. Dietary vitamin C intake decreased significantly in both groups post intervention (p<0.001).

Most subjects met the current Dietary Reference Intake (DRI) (38) of protein (46 g/d) and carbohydrate (130 g/d) prior to and after the intervention. However, more than half of the subjects had a dietary intake of iron and calcium lower than the DRI values (18 mg/d iron and 1000 mg/d calcium) before and after the intervention (39, 40). In addition, the number of subjects who did not meet the current DRI for vitamin C (75 mg/d) increased from 12 to 22 in the AF group and from 9 to 20 in the SF group.
Compliance

Since subjects only consumed 3 out of 14 provided meals per week at our HMU, compliance for soy food intake was checked on six subjects randomly selected in each group by assessing urinary isoflavones at baseline and post intervention. There was no significant difference between the two groups at baseline (AF vs. SF: 2524 vs. 742 µg/24 hr urine). As expected, the SF group had a higher urinary isoflavone excretion than the AF group after intervention. Total urinary isoflavones significantly increased by 11-fold (from 742 to 8052 µg/24 hr urine) in the SF group, whereas a 1.7-fold non-significant decrease (from 2524 to 1486 µg/24 hr urine) was observed in the AF group, indicating excellent compliance.

Effect on Iron Status

Iron status before and after soy feeding is presented in Table 4. Average hemoglobin concentration was 13 g/dl for both groups and no change was observed after intervention. The median values for serum iron, ferritin, transferrin receptor, and transferrin saturation were within the normal range at baseline and after intervention. Although serum iron declined in the SF group after intervention, the reduction was not significantly different from the AF group (-4.8 vs 3.2 µg/dl). Additionally, no changes were observed in transferrin saturation or transferrin receptor concentrations. Serum ferritin and transferrin receptor-ferritin index (TfR-F index) slightly decreased in both groups, but the changes were not significantly different between the SF and AF groups (serum ferritin: -1.2 vs -1.4 ng/ml; TfR-F index: -0.13 vs -0.18). After intervention, the number of subjects who had iron deficiency (ID) based on serum ferritin <15 ng/ml (41) increased from 10 to 11 in the SF group and remained as 11 in the AF group. If TfR >8.3 ng/ml was used to identify tissue iron deficit, the number declined from 7 to 3 in the SF group, and 9 to 3 in the AF group. If the TfR-F index ≥4.5 was
used to identify non-anemic ID (36), the number declined from 18 to 14 in the SF group and from 20 to 18 in the AF group. No treatment effect was found on serum ferritin after the adjustment of baseline ferritin in our study.

**Effect on Zinc Status**

Zinc status was assessed by measuring zinc concentration in the plasma and urine. The median plasma and urinary zinc concentrations were within the normal range for both groups prior to and at the end of intervention. As shown in Table 5, plasma zinc decreased significantly by 5 µg/dL in both groups. Although an increase (5 µg/g creatinine) in urinary zinc excretion occurred in the SF group, it was not significantly different either from baseline or compared with the change observed in the AF group (-18 µg/g creatinine). Moreover, serum ALP declined in the AF group and was significantly lower than in the SF group after intervention (p=0.017), but the ALP change did not differ between the SF (1.13 IU/L) and AF groups (-1.23 IU/L).

**Effect on Biochemical Markers of Bone and Thyroid Hormones**

Based upon the purported effect of soy isoflavones on bone, we assessed biochemical markers for bone formation (BAP) and resorption (C-Tx) (Figure 2). At 10-wk, BAP increased in SF group (1.5 U/L) but decreased in AF group (-0.7 U/L), and the change was significantly different (p=0.024) between two groups. We found no significant effect of treatment on C-Tx. To determine the effect of soy consumption on thyroid status, we tested thyroid stimulating hormone (TSH) concentration (Figure 3). After intervention, TSH declined by 13% in the AF group (p=0.018), whereas no significant change was observed in the SF group. The change in TSH (-0.39 vs 0.11 uIU/dL) was significantly different between
two groups. Further analysis of thyroxine ($T_4$) found no significant difference between the two groups and no change from baseline to post intervention within each group.

**DISCUSSION**

Although a body of evidence exists on the inhibitory effect of soy foods on iron and zinc bioavailability from single meal studies, data are limited from long-term studies. This 10-wk feeding study demonstrated that soy food intake had no detrimental effects on iron or zinc status in young women of childbearing age. At the end of the study, no significant changes were observed in macronutrient intake, reflecting that subjects adjusted their diets to maintain their habitual dietary intake and meals we provided did not contribute to additional nutrient intake. Iron intake was found to significantly increase only in the AF group, despite the higher iron content of the soy versus animal foods that we provided (5.2 versus 4.0 mg). It was likely that subjects in the AF group consumed fewer animal products before intervention and probably animal foods increased their iron intake. Similarly, the significant increase in calcium intake in the AF group may have been due to the increased dairy products intake during the study. Surprisingly, vitamin C intake declined in both groups, probably because of less consumption of vitamin C rich fruits and vegetables during the intervention. Since ascorbic acid may not markedly affect non-heme iron absorption from a complete diet (42), we do not expect that the reduction of dietary vitamin C had a significant effect on iron status.

It is important to investigate the effect of soy consumption on iron and zinc status among women of childbearing age, who are at risk of iron deficiency due to substantial menstrual iron losses and poor dietary habits (43). Based on a cutoff value of 12 g/dl hemoglobin (41), none of the subjects were anemic either at baseline or at the end of
intervention. After 10 wk of intervention, serum iron decreased by 7% in the SF group, but no significant changes in iron indicators (hemoglobin, serum iron, transferrin receptor, transferrin saturation, and serum ferritin) were observed in the AF or SF groups, indicating that soy feeding did not impair iron status in our study. Although serum iron and transferring saturation are widely used in iron deficiency screening, their use is limited by the daily fluctuation of serum iron (CDC, 2008; Zimmermann, 2008). Recently, TfR-F index was suggested to be a useful indicator for subclinical iron deficiency in healthy subjects (44, 45). If TfR-F index was used to classify subjects with non-anemic ID (36), the proportion of iron deficient subjects decreased by 6.5% and 12.5%, respectively, in the AF and SF group, showing an improvement in iron status with intervention. This is consistent with the findings from several but not all studies. A large scale cross-sectional study (1308 men and 1541 women aged over 20 y) in China (46) found the difference in mean serum ferritin concentration across quartiles of tofu intake was not significantly different. In addition, our previous study also demonstrated that the consumption of 40 g soy protein isolate daily for 24 wk did not have significant effect on iron status in perimenopausal women, though treatment reduced serum iron by 11% and transferrin saturation by 3-7% (n=69) (17). Overall, we did not find negative effect of soy food intake on iron indices.

However, these results contradicted our another study with postmenopausal women (n=15) with 6-wk soy protein isolate intake (40 g/d) showing a significant decrease in transferrin saturation (28%) and serum ferritin (30%) as well as a moderate reduction in serum iron (7%) (18). The disparity may be attributed to the different iron status of subjects at baseline (average serum ferritin concentrations of perimenopausal women compared to postmenopausal women: 15.5 vs. 65.4 ng/ml), in addition to the amount of soy protein intake
and the duration of intervention, because iron absorption in subjects with low iron stores may be higher than those with high iron stores (47). In our study, iron status of subjects with low iron stores were improved compared to those with high iron stores at the end of study. The fact that postmenopausal women generally have high iron stores compared to premenopausal women, as reflected by serum ferritin concentrations (48) may be one of the reasons why a significant reduction in iron status was observed in postmenopausal women but not in premenopausal or perimenopausal women with soy consumption.

Zinc deficiency is not common in North America due to good dietary habits (40). In the present study, no subjects were zinc deficient (based on criteria of plasma zinc <70 µg/dl and urinary zinc <100 µg/g creatinine (49)) at baseline and only two subjects developed zinc deficiency in the AF group after intervention. A long-term crossover study reported that lactoovovegetarian diets for 8 wk in women (n=22) reduced zinc absorption by 35% and plasma zinc concentration by 5% (50). Our study confirmed these results by showing an 8% reduction in plasma zinc concentration (p=0.04), with the values being normal after soy feeding. Plasma zinc is a relatively insensitive indicator for zinc status, although is widely used as screening test. Plasma zinc concentration is generally stable but decreases after several weeks of severe dietary restriction when tissue zinc is reduced. Thus, it poorly reflects tissue zinc status and cannot be used to identify marginal zinc deficiency (51). In our study, the reduction in plasma zinc may not have physiological significance, although it was statistically significant. We did not observe a compensatory reduction in urinary zinc excretion or decreased activity in serum ALP, despite a decline in plasma zinc concentration. Another feeding study observed a more marked reduction in plasma zinc concentration after daily consumption of a soy protein based diet for 3 mo in human subjects (n=5), causing a
negative zinc balance and a 25% reduction in plasma zinc concentration (26). The lower intake of soy protein per day (19 vs. 69 g) and the shorter study duration (10 vs. 12 wk) may have contributed to the moderate influence of soy on zinc status in our study compared to the previous study. Dietary zinc intake may also affect zinc status; however, we could not assess the dietary zinc intake in our subjects because the dietary analysis software does not provide this information. Additionally, our subjects were premenopausal women with lower plasma zinc than male subjects used in the previous study; whether sex and baseline zinc status influence the effect of soy consumption on zinc status has not been investigated thoroughly.

On the other hand, we were surprised to find a 9% reduction (p=0.048) in plasma zinc in the AF group with animal foods intake, considering that meat is a good source of zinc. Although dietary zinc data were not available, a slight decrease in urinary zinc concentration paralleled with a slight reduction in the activity of serum ALP (a zinc dependent enzyme) in the AF group after intervention, suggesting that dietary zinc intake might be slightly reduced after feeding, because previous study reported that urinary zinc declined proportionately to the reduction of dietary zinc intake (52). If endogenous fecal zinc excretion and dietary zinc intake were assessed, together with other zinc indicators, we would have been able to better evaluate zinc status in these subjects.

Some (53, 54), but not all (55, 56), human studies reported that the consumption of soy isoflavones increased bone formation and decreased bone resorption. We did not find significant changes in bone formation or resorption after intervention, indicating that our intervention with soy foods might not have been long enough to exert a significant effect on bone. However, we were surprised to find that serum BAP increased with soy foods intake but declined with animal foods intake. The significant difference in BAP changes between
two groups may be due to the different types of protein they consumed during intervention. In addition, no evidence of soy affecting TSH and T₄ was found in our study. This was consistent with the results of a recent study, showing no significant effects on serum thyroid hormones after 57 d of soy protein isolate intake in young men (n=35) (57). Surprisingly, TSH significantly declined in the AF group after intervention, indicating an improvement in thyroid hormone status. We speculated that this was due to the increased consumption of dairy products, which are a good source of dietary iodine in the United States (58).

In conclusion, we found no detrimental effect on iron status and a marginal effect on zinc status after consuming moderate amount of soy foods that are currently available in the market to women of childbearing age. We observed an improvement in bone formation after soy food intake compared to animal food consumption. However, no significant effects on thyroid hormones or bone resorption were observed with soy consumption in this study.

Acknowledgements

YZ conducted the study, collected and analyzed samples/data, and drafted the manuscript. MBR designed the study, secured funding, supervised the study, data analysis, and the manuscript writing. DLA provided technical support, analyzed biochemical markers of bone, and refined the manuscript. UG analyzed data. MM contributed to the study design and provided technical support. We sincerely acknowledge the Nutrition & Wellness Research Center at Iowa State University for facility support, and Archer Daniels Midland Company, Burke Corporation, HyVee, Revival Soy, Swiss Valley, SoyNut Butter Company, Sunrich, Wildwood, and WhiteWave Foods for food donations. We also appreciate Jeanne Stewart and Dr. Kathy Hanson for coordination during the study and Dr. Philip Dixon for support in statistical analysis.
Table 1

Weekly menu for subjects in animal and soy food groups

<table>
<thead>
<tr>
<th></th>
<th>AF</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>chili without beans</td>
<td>soy chili</td>
</tr>
<tr>
<td></td>
<td>crackers/peanut butter</td>
<td>crackers/soynut butter</td>
</tr>
<tr>
<td></td>
<td>yogurt</td>
<td>soy yogurt</td>
</tr>
<tr>
<td>Tuesday</td>
<td>meat balls</td>
<td>soy meat balls</td>
</tr>
<tr>
<td></td>
<td>pasta with sauce</td>
<td>pasta with sauce</td>
</tr>
<tr>
<td></td>
<td>milk</td>
<td>soy milk</td>
</tr>
<tr>
<td>Wednesday</td>
<td>taco with beef</td>
<td>taco with soy</td>
</tr>
<tr>
<td></td>
<td>yogurt</td>
<td>soy yogurt</td>
</tr>
<tr>
<td>Thursday</td>
<td>beef</td>
<td>tofu</td>
</tr>
<tr>
<td></td>
<td>rice</td>
<td>rice</td>
</tr>
<tr>
<td></td>
<td>stir fried peas and carrots</td>
<td>stir fried peas and carrots</td>
</tr>
<tr>
<td></td>
<td>milk</td>
<td>soy milk</td>
</tr>
<tr>
<td>Friday</td>
<td>burger/bun</td>
<td>soy burger/bun</td>
</tr>
<tr>
<td></td>
<td>milk</td>
<td>soy milk</td>
</tr>
<tr>
<td>Saturday</td>
<td>green peas</td>
<td>edamame</td>
</tr>
<tr>
<td></td>
<td>rice</td>
<td>rice</td>
</tr>
<tr>
<td></td>
<td>carrots</td>
<td>carrots</td>
</tr>
<tr>
<td></td>
<td>peanuts</td>
<td>soy nuts</td>
</tr>
<tr>
<td></td>
<td>yogurt</td>
<td>soy yogurt</td>
</tr>
<tr>
<td>Sunday</td>
<td>chicken</td>
<td>soy chicken</td>
</tr>
<tr>
<td></td>
<td>pasta/sauce</td>
<td>soy pasta/sauce</td>
</tr>
<tr>
<td></td>
<td>milk</td>
<td>soy milk</td>
</tr>
</tbody>
</table>

\(^1\)AF= animal foods; SF= soy foods; milk and yogurt were consumed as second serving and always consumed at home; condiments were provided with each meal to subjects when they consumed at site.
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>AF (n=31)</th>
<th>SF (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21 [18-27]</td>
<td>22 [18-28]</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 [1.54-1.76]</td>
<td>1.65 [1.54-1.77]</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>64.0 [51.1-96.2]</td>
<td>60.0 [49.0-76.8]</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>23.3 [18.5-33.9]</td>
<td>22.0 [18.5-27.2]</td>
</tr>
<tr>
<td>Waist Circumference (cm)*</td>
<td>72.6 [61.2-98.6]</td>
<td>69.6 [58.7-78.2]</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>100.3 [89.4-123.2]</td>
<td>97.5 [86.4-107.7]</td>
</tr>
<tr>
<td>Waist-to-Hip Ratio</td>
<td>0.72 [0.63-0.83]</td>
<td>0.71 [0.65-0.77]</td>
</tr>
</tbody>
</table>

Table 2
Baseline characteristics of the subjects by group¹

¹Mean [min-max]; AF = animal foods; SF = soy foods; student’s t-test was used to compare the differences between groups for height, BMI and waist to hip ratio; Mann-Whitney test was performed for comparison of age, weight, waist circumference and hip circumference between groups; *p<0.05.
<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AF (n=31)</td>
<td>SF (n=32)</td>
<td></td>
</tr>
<tr>
<td><strong>Energy (kJ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>6665 [3483-10320]</td>
<td>6958 [4023-10802]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>6866 [4421-13083]</td>
<td>7034 [4375-9965]</td>
<td></td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>69 [42-189]</td>
<td>68 [42-97]</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>233 [82-321]</td>
<td>226 [158-414]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>231 [134-330]</td>
<td>222 [116-391]</td>
<td></td>
</tr>
<tr>
<td><strong>Total Fat (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>51 [24-112]</td>
<td>53 [17-100]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>57 [31-122]</td>
<td>57 [24-99]</td>
<td></td>
</tr>
<tr>
<td><strong>Iron (mg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>13.7 [6.8-36.5]</td>
<td>14.0 [7.7-32.8]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>14.4 [7.4-31.3]&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.2 [8.5-31.9]</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>725.6 [285.8-1803]</td>
<td>827.5 [312.2-1580]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>854.5 [446.5-1942]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>931.2 [466.6-1330]</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>98.1 [21.4-434.2]</td>
<td>97.0 [10.9-322.1]</td>
<td></td>
</tr>
<tr>
<td>post</td>
<td>51.2 [18.0-129.9]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.6 [14.5-229.0]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Median [min-max]; AF= animal foods; SF= soy foods; non-parametric tests were used to compare the difference within the group or between groups; letters represent differences between baseline and the end of study within each group; <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001; no significant difference was found in any dietary factors between groups at baseline and then end of study; the change of any dietary factor did not differ between two groups.
### Table 4
Iron status of subjects at baseline and the end of study

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AF (n=31)</td>
<td>SF (n=32)</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dl)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>13.19 ± 0.76</td>
<td>13.28 ± 0.58</td>
</tr>
<tr>
<td>post</td>
<td>13.39 ± 0.62</td>
<td>13.45 ± 0.58</td>
</tr>
<tr>
<td>change</td>
<td>0.02 ± 2.77</td>
<td>-0.53 ± 2.60</td>
</tr>
<tr>
<td><strong>Serum Iron (μg/dl)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>99.3 ± 39.7</td>
<td>94.8 ± 51.4</td>
</tr>
<tr>
<td>post</td>
<td>102.5 ± 34.3</td>
<td>90 ± 43.7</td>
</tr>
<tr>
<td>change</td>
<td>3.2 ± 44.2</td>
<td>-4.8 ± 65.4</td>
</tr>
<tr>
<td><strong>Transferrin Saturation (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>27 [10-53]</td>
<td>23 [10-82]</td>
</tr>
<tr>
<td>change</td>
<td>3 [-24-23]</td>
<td>2 [-52-41]</td>
</tr>
<tr>
<td><strong>Transferrin Receptor (μg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>6.39 [2.82-10.84]</td>
<td>6.16 [4.27-10.86]</td>
</tr>
<tr>
<td>change</td>
<td>-0.11 [-4.37-4.85]</td>
<td>-0.16 [-2.77-3.62]</td>
</tr>
<tr>
<td><strong>TfR-Fer Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>5.15 [1.84-22.82]</td>
<td>4.86 [2.68-26.65]</td>
</tr>
<tr>
<td>change</td>
<td>-0.18 [-17.10-12.55]</td>
<td>-0.13 [-4.17-19.16]</td>
</tr>
<tr>
<td><strong>Serum Ferritin (ng/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>21.2 [2.0-110.8]</td>
<td>23.6 [2.4-77.8]</td>
</tr>
<tr>
<td>post</td>
<td>18.8 [2.0-91.1]</td>
<td>22.5 [2.0-65.7]</td>
</tr>
<tr>
<td>change</td>
<td>-1.4 [-35.2-26.8]</td>
<td>-1.2 [-46.7-27.4]</td>
</tr>
</tbody>
</table>

1Median [min-max]; 2Mean±SD; AF= animal foods; SF= soy foods; student’s t-test was used for comparison of hemoglobin and non-parametric tests were utilized for comparison of iron indices; no significant difference was found between two groups for any parameter at baseline and the end of study; no significant changes were observed in any parameter at the end of study.
Table 5
Zinc status of subjects at baseline and the end of study

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Normal Range</th>
<th>AF (n=31)</th>
<th>SF (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma Zinc (μg/dl)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>baseline</td>
<td>80.0 [60-142]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>post</td>
<td>73.0 [52-115]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>change</td>
<td>-5 [-49-46]^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine Zinc (μg/g Creatinine)</td>
<td>100 - 900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>baseline</td>
<td>265 [50-838]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>post</td>
<td>259 [37-860]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>change</td>
<td>-18 [-579-487]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum Alkaline Phosphatase (IU/L)^2</td>
<td>25 - 150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>baseline</td>
<td>55.19 ± 10.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>post*</td>
<td>53.97 ± 11.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>change</td>
<td>-1.23 ± 7.24</td>
</tr>
</tbody>
</table>

1Median [min-max]; 2Mean±SD; AF= animal foods; SF= soy foods; student’s t-test was used for comparison of serum alkaline phosphatase, and non-parametric tests were utilized for comparison of serum and urinary zinc; letters represent differences between baseline and the end of study within each group; ^ap<0.05; symbols represent differences between groups; *p<0.02.
Figure 1
Subject recruitment\(^1\)

Subjects Responded  
\(n=195\)

Assessed for Eligibility via Telephone Screening  
\(n=87\)

Completed Pre-Baseline Screening:  
Initial Eligibility Confirmed  
\(n=73\)

Excluded  
\(n=14\)  
- Extreme BMI (6)  
- Work schedule conflict (6)  
- Irregular menstruation (1)  
- Consumption of soy foods >2 times/wk (1)

Completed Blood Screen and Pregnancy Test:  
Randomized to Treatment  
\(n=70\)

Excluded  
\(n=3\)  
- Chronic disease (3)

SF Group  
\(n=37\)

Lost to Follow-up  \(n=3\)
Dropped Out  \(n=2\)  
- Wanted to take iron supplement (1)  
- Time conflict (1)

AF Group  
\(n=33\)

Lost to Follow-up  \(n=2\)

Completed Treatment  
SF Group  
\(n=32\)
AF Group  
\(n=31\)

\(^1\)AF= animal foods; SF= soy foods.
Figure 2
Effect of soy food consumption on biochemical markers of bone

A. 

Figure 2
Effect of soy food consumption on biochemical markers of bone

A. 

B. 

1 Animal foods; Soy foods; Median+Interquartile range; bone formation and resorption prior to and at the end of study were reflected by BAP and C-Tx respectively; non-parametric tests were performed for comparison; n=31 for animal food group and n=32 for soy food group; *p<0.05.
Figure 3
Effect of soy food consumption on thyroid-stimulating hormone

<table>
<thead>
<tr>
<th>TSH (uIU/dL)</th>
<th>Baseline</th>
<th>Post</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td><img src="Baseline" alt="Baseline" /></td>
<td><img src="Post" alt="Post" /></td>
<td><img src="Change" alt="Change" /></td>
</tr>
</tbody>
</table>

Animal foods; Soy foods; Mean±SEM; Student’s t-test was used to examine the difference within group and between groups; n=31 for animal food group and n=32 for soy food group; *p<0.05, #p<0.02.
LITERATURE CITED


