Effects of Protein Source and Calcium Level on the Utilization of Minerals in Adult Men

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Chapter I

Introduction

Protein is one of several dietary components known to affect mineral utilization in man. Vegetable protein is the main protein source of many underdeveloped countries; furthermore, due to advances in food technology, the consumption of soy protein products and extenders have increased steadily in the United States and other developed countries in the last decade (Bodwell, 1983). The lower bioavailability of minerals, especially trace minerals, from vegetable proteins, as compared to animal proteins is one area of nutritional concern and debate.

Iron deficiency anemia is recognized as a major nutrition related health problem in the United States and the world. Cook et al. (1981) observed that iron absorption was significantly reduced in humans fed isolated soy protein, as compared to animal protein. These researchers also found that a soy replacement of 30% of the meat protein in a conventional diet resulted in a significant reduction in iron absorption.

Marginal zinc status has also been reported among children eating diets of mostly plant proteins, specifically bread and beans (Prasad, 1963). These boys, characterized by dwarfism and hypogonadism, showed a growth and maturation response to zinc supplements. Other researchers have observed a low rate of weight gain and plasma zinc response in infants recovering from malnutrition that were fed soy formula, as compared to those fed

cow's formula (Golden and Golden, 1981).

Although the availability of copper from soy or animal protein has been researched considerably less than that of the other trace minerals, investigators studying rats have reported significantly lower liver copper uptake from a soy milk formula as compared to a cow's milk formula (Lonnerdal et al., 1985).

Other researchers have also suggested that the absorption of magnesium has decreased in subjects who consumed a diet with isolated soy protein as opposed to one with casein-lactalbumin protein (Stephenson et al., 1979).

The low availablity of these minerals to animals and humans fed diets high in foodstuffs derived from plant seeds has been attributed to the presence of phytic acid in these seeds. The high amounts of fiber in these diets has also been implicated in reducing mineral utilization. Furthermore, recent studies have suggested that high levels of dietary calcium may enhance the action of phytate by forming insoluble calcium-phytate complexes that bind with other minerals and further decrease mineral absorption (Oberleas, 1981). The current promotion in the media to increase calcium and fiber intakes may have negative implication on trace mineral utilization.

The effect of protein source on calcium bioavailability has not been researched to the same extent as that on trace mineral bioavailability; however, epidemiologial studies have suggested that the incidence of osteoporosis is lower in countries where vegetable, rather than animal

protein is consumed. Although many studies have suggested that high intakes of protein, mostly in the form of purified proteins and sulfur amino acids, cause hypercalciuria (Linkswiler, 1974; Allen, 1982), Watkin et al. (1985) have suggested that average intakes of meat protein, as opposed to soy protein, have also caused increased amounts of acid and calcium in the urine. The researchers claim that excessive nitrogen and/or sulfur from a protein diet increases tha amount of acid formed in the body, which may cause mobilization of calcium from the bone.

In light of this research, protein source may have important implications for mineral utilization. The purpose of this study was to determine the effects of three protein sources, soy, dairy, and meat protein, as well as two levels of calcium on the utilization of zinc, iron, copper, magnesium, and calcium in adult men.

Chapter II

Review of Literature

Zinc

Metabolism

Zinc is truly a ubiquitous trace mineral within human and animal tissues. As a component of numerous metallo-enzymes, it plays a key role in protein and nucleic acid metabolism, growth and wound healing, development of the male reproductive organs, and the immune system (Linder, 1985). Among the most abundant zinc enzymes are erythrocyte carbonic anhydrase, essential for acid-base balance; alcohol dehydrogenase which catalyzes the oxidation of ethanol and other primary and secondary alcohols and reduces retinene; carboxypeptidase A and B which are implicated in proteolysis and digestive processes; and superoxide dismutase which found in all cells is thought to play a defensive role in the disposal of damaging superoxide anions (Prasad, 1976). From these observations, it is evident that zinc has a broad involvement in metabolism.

Only a small percentage of zinc, ranging from 20 -30%, is absorbed from dietary zinc. The rate of absorption is greatest from the duodenunm and varies directly with dietary intake, as well as the physiological need for zinc (Suitor and Crowley, 1984). Utilization of zinc depends on a myriad of relationships among nutrients, including the effect of one nutrient upon another and the effect of other components of the food upon one or more nutrients. The

presence of fiber and phytate tend to reduce zinc absorption; in addition, calcium can join with the zinc-phytate complex and decrease its absorption further. Since zinc competes with other transition metal ions for binding sites at the surface or within the epithelial cells, large supplements of iron or copper may also reduce zinc absorption (Solomons, 1982). Dietary factors that significantly increase zinc absorption are unknown; however, human milk contains a zinc-binding ligand that is thought to enhance the absorption of zinc, and wine apparently contains a non-alcoholic substance that promotes zinc absorption (Suitor and Cowley, 1985). Large quantities of lactose and EDTA, a food preservative, have also been found to enhance zinc absorption (Solomons, 1982).

Once absorbed, almost all zinc is carried in the blood bound to proteins, where it can be distributed through the body or stored in the liver (Solomons, 1982). About 80% is probably bound loosely to albumin which appears to be concerned primarily with transport, while the remaining zinc is bound to globulin proteins. Unlike iron, the body stores of zinc are not easily mobilized and, hence, there is an unusual dependence upon a regular, exogenous supply of the element (Goodhart and Shils, 1980).

Pancreatic secretions, the main route of zinc excretion, release a considerable amount of zinc into the intestine. Fecal zinc (which ranges between 5-10 mg) consists predominantly of unabsorbed dietary zinc although a small amount is derived from endogenous secretions, as

well as sloughed cells (Underwood, 1977). Urinary excretions contain about 0.3 -0.5 mg/day, an amount apparently independent of intake and urinary volume. The loss of zinc through the sweat ranges from 0.5-1.3 mg/day (Goodhart and Shils, 1980).

The average American consumes about 10 mg of zinc daily, which is lower than the recommended dietary allowance (RDA) of 15 mg/day, established by the Food and Nutrition Council (1980). In a review article, Solomons (1982) points out that the customary intake of zinc does not approach the RDA level; in fact, the mean intake of adults of different ages and physiological states (taken from various survey data) ranged from 46 - 63% of the RDA. Flesh foods, as well as oysters and shellfish are the most reliable sources of zinc since they contain high amounts of zinc without the substances that are thought to inhibit its absorption. The main vegetable sources high in zinc are whole grains, cereals, and legumes (Suitor and Crowley, 1985).

The vital role that zinc plays in DNA synthesis explains the rapid onset of biochemical changes following the induction of zinc deficiency. Prominent signs of zinc deficiency are loss of apetite, failure to grow, skin changes, impaired healing of wounds and decreased taste acuity (Prasad, 1976).

The Effect of Protein Intake on Zinc Bioavailability

Animal Studies

Studies examining the effects of different levels of

dietary protein on the absorption and utilization of zinc have produced conflicting results. Van Campen and House (1974) observed that rats fed a 15% casein diet retained more zinc than animals fed a protein-deficient diet of 5%. Zinc concentrations in the plasma and the liver were also reduced in rats fed the low-protein diet. Wallwork et al. (1983) also found that rats fed deficient levels of protein (6% egg white) had lower zinc absorption than those fed adaquate levels.

To study the effects of high levels of protein, Chan et al. (1979) reported that zinc retention, plasma, and bone concentrations were significantly higher in rats fed a high protein diet (60% egg white) as compared to a normal protein diet (20% egg white). The zinc retention actually increased from 10 to 60%. Snedeker and Greger (1983) also observed that adult male rats fed a high protein diet (45% lactalbumin) had higher liver zinc than those fed an adaquate protein diet (15% lactalbumin). Methfessel and Spencer (1968), on the other hand, reported that high levels of dietary protein actually reduced the absorption and tissue uptake of ^{65}Zn in rats.

Human Studies

In pre-adolescent girls, zinc retention was improved when nitrogen, in the form of intact protein, ammonium citrate or synthetic amino acids, was added to a basal diet of approximately 25 g of protein (Price and Bunce, 1972). The subjects of a study conducted by Greger and Snedeker

(1980) absorbed more dietary zinc when fed a 150 g protein diet, as opposed to a 50 g diet. The researchers added casein, lactalbumin, dried egg whites, and wheat gluten to increase the protein intakes. Those subjects on the high protein diet not only had higher levels of serum zinc and reduced levels of fecal zinc, but, they had increased levels of urinary zinc. Allen et al. (1979) also observed that subjects lost more zinc in their urine in the 2 - 4 hours after they consumed a test breakfast which contained 54 g of protein than in the hours after they consumed a test breakfast which contained 18 g protein. When Mahalko et al. (1983) increased the protein intake from 65 to 95 g/day in adult men, an increase in urinary zinc was also reported; however, no significant differences in zinc absorption was seen. In adult women, Colin et al. (1983) observed similiar results.

On the other hand, Spencer and Samachson (1970) reported that adult males fed low protein diets (.5 g/kg daily) had higher 65Zn concentrations in their plasma following administration of the isotope, than did those on a diet providing 1.0 g/kg daily. Even higher levels (2.0 g/kg daily) resulted in lower plasma 65Zn levels and increased fecal excretion.

The Effect of Source of Protein on Zinc Bioavailability

Animal Studies

It has only been in the last fifty years that researchers discovered the link between the dietary source of

protein and zinc bioavailability. Tucker and Salmon (1955) cured para-keratosis in pigs with supplements of zinc. What fascinated the researchers was that the animals were fed a diet with adaquate amounts of zinc, but the protein source was plant in origin, specifically corn and soybean meal. Later, in 1957, O'Dell et al. founhat the zinc in soybean protein was less available to chickens than the zinc in casein. The authors cite other research with pigs and rats that showed similar results. Forbes et al. (1960) observed that rats fed a protein diet consisting of casein or egg whites required 12 ppm of zinc for normal growth, while rats fed a soybean diet required 18 ppm of zinc for normal growth. The rats absorbed nearly twice the amount of zinc from the animal diet than from the soy-based diet.

Stuart et al. (1986) also found that rats retain more zinc from animal protein than from plant protein. These researchers not only investigated the bioavailability of zinc from defatted soy flour and dried chicken meat, but also a mixed ratio (50:50) of both in an egg-white-based diet, using radioisotope 65Zn. Retention of the 65Zn from the chicken test meal was significantly higher than that of the soy test meal. The mixed diet had retention values midway (85%) between the chicken-based diet (93%) and the soy-test meal (76%). The authors point out that it has not been determined whether the differences in zinc bioavailability are due to the presence of phytate in the soy flour or to an unidentified absorption enhancement factor in the animal protein. In another study, Greger and Mulvaney (1985)

reported a higher apparent absorption in rats fed a 30% lactalabumin diet, as compared to a 30% soy diet.

Human Studies

Because of the complications with metabolic balance and radioisotope studies, the influence of protein source on zinc bioavailability in humans has been researched considerably less than in animals and remains controversial. Originally, Prasad et al. (1963) researched a zinc deficiency in Egyptian boys whose diet consisted mostly of plant proteins, specifically bread and beans. These boys, characterized by dwarfism and hypogonadism, showed a growth response to zinc supplementation. In agreement, Lonnerdal et al. (1984) observed a low bioavailabilty of zinc from a soy formula as compared to a milk formula in adult men and women. Zinc absorption from milk was found to be 41%, while the absorption from soy formula was only 14%. When a cereal-cow's milk formula was given, with a respective ratio of 25:75, zinc absorption was found to be 22%, midway between the other two treatments. In another study, Golden and Golden (1981) observed a low rate of weight gain and plasma zinc response in infants recovering from malnutrition that were fed soy formula as compared to those fed cow's milk formula.

Bodwell et al. (1983), on the other hand, found no significant difference in zinc absorption in 17 adult males who were fed high levels of protein (about 1.6g per kg of body weight) with 70% of the protein either texturized soy,

soy isolate, or animal protein. The average zinc intake was 15.5 mg. Young et al. (1981) in a similiar metabolic study with 5 adult men studied the bioavailability of zinc from a soy isolate diet, a mixed soy isolate/milk diet, and a milk diet. With an average zinc intake of 15.4 mg/day from each of the 3 diets, the zinc bioavailability was 37% from the soy, 47% from the mixed, and 51% from the milk diet. There were no significance differences among the three diets, but the authors note a pattern and suggest a larger population size might produce different results. In another metabolic study, Turnland and King (1983) found zinc absorption equivalent in five women fed diets containing 70% animal protein or 70% vegetable protein for 21 days. The types of proteins were not specified.

The Effect of Phytate on Zinc Bioavailability

O'Dell (1969) and other researchers attributed the decrease in zinc bioavailability in the soy-based meals to the presence of phytate, a phosphorous storage compound found in plant seeds which forms insoluble zinc-phytate complexes. Soybean products usually contain considerable quantities of phytic acid, with values ranging from 1.4 - 2.2 % (Forbes, 1979). Animal products do not contain phytate. To test whether phytate itself actually decreased zinc bioavailability, O'Dell (1979) fed chicks a casein-derived protein diet with added phytates. The chicks had the same growth response as chicks fed a soy-based diet. When fed a soy-based diet that was first autoclaved to

destroy the phytate and a traditional casein-based diet with no added phytate, there was no difference in the growth response in the chicks. More recently, House et al. (1982) measured zinc metabolism in male rats by both the balance method and an analysis with the radioisotope 65Zn. The rats were fed an egg-albumin basal diet with or without 2% phytate. The apparent and true absorption (13% and 32%) of zinc intake for rats fed phytate was significantly lower than the apparent and true absorption (19% and 46%) of zinc intake in rats fed the basal diet. The researchers question whether or not this level of phytate would be injested by humans.

In human studies, the effect of phytate on zinc absorption has been less clear. Ellis and Morris (1981) found no difference in apparent zinc absorption in patients fed a regular, an ovo-lacto-vegetarian, or a soy-meat substitute hospital diet, providing 377, 440, and 1144 mg of phytic acid, respectively. Lonnerdal (1984), on the other hand, found that the addition of phytic acid to a milk-based formula significantly reduced zinc absorption in adults.

Oberleas and Harland (1981) summarized the zinc/phytate contents of selected foods and calculated this ratio in many vegetarian diets and nonvegetarian diets. The ratio acceptable for adaquate Zn absorption is 15.

The Effect of Phytate and/or Fiber on Zinc Bioavailability

Although it was originally thought that phytate binds dietary zinc, many researchers question the role of dietary

fiber in decreasing zinc absorption. Reinhold (1972) found 48 out of 59 poor Iranian school children with low plasma zinc concentrations, as well as iron and calcium deficiencies. As in Prasad's study, they had adaquate intakes of calcium, iron, magnesium, and zinc in their diet which consisted mostly of unrefined stone-ground wheat breads. The researchers first attributed low zinc absorption to the presence of phytate; however, later, they found that feeding an equivalent amount of purified phytates as was in the wheat bread, had little effect on zinc balance, implicating fiber as the culprit for reducing zinc retention (Reinhold et al., 1973). Evidence from in vitro research also suggests that zinc bound to wheat fiber, unlike phytate, is not degraded by digestive secretions. While starch, wheat proteins, fiber, and phytate, the major components of the whole wheat, removed considerable amounts of 65Zn and stable zinc from a buffered saline solution, only the fiber remained zinc-bound when treated with pancreatic amylase (Reinhold, 1975).

Researchers have also questioned whether different types of fiber may act differently on zinc absorption. Sandstead et al. (1978) found that 26 g of added wheat bran to an American "middle-class" diet decreased the retention of zinc in adult males; however, the addition of corn bran had no significant effect on zinc balance. In a 21-day metabolic study, Drews et al. (1979) found that 14.2 g of hemicellulose significantly decreased zinc fecal loss, as compared to the basal diet without added fiber; however,

cellulose and pectin had no significant effect. Klevay et al. (1979) reported that zinc balance was significantly lower in male subjects who consumed a mixed diet with added fiber from fruits and vegetables. This fiber contained more lignin and cellulose and less hemicellulose than would an equivalent amount of fiber from bran.

Other authors disagree that fiber should be implicated in reducing zinc absorption. King et al. (1982) studied fecal mineral excretion of 10 young men fed diets high in phytate or fiber components. For 63 days, two groups of men, confined to a metabolic unit, were fed an egg white formula with added fibers such as cellulose, xylan, pectin, or corn bran, or added phytate. Results showed that positive balance was maintained and fecal zinc was unchanged with group I who consumed the added fibers. The second group fed the diet with added phytate had a significant increase in fecal zinc, causing a negative balance of zinc. The results showed that phytate, not dietary fiber, can alter the absorption of zinc in humans, although the small sample size of 5 subjects per group should be noted.

In another similar study from the same lab, Turnland et al. (1984) studied four healthy young men confined to a metabolic unit for again 63 days and fed a semipurified liquid formula diet with egg albumen as the protein source, providing 15 mg of zinc. To this basal diet, phytate or a-cellulose was added, as well as 67Zn, a stable isotope of zinc, to reflect zinc absorption. Average zinc absorption was 34.0% from the basal diet without added phytate or a-

cellulose, 33.8% with the added a-cellulose, and a significantly reduced 17.5% from the diet with added phytate. It should be noted that the phytate used in this study was in a purified form and the results may not have been the same if the phytate were in a natural food diet.

To study the effect of phytate and fiber in a natural diet, Anderson et al. (1981) studied the zinc and iron status of fifty-six Seventh-Day Adventist women, the average of whom followed a vegetarian diet for nineteen years. Iron, zinc, fiber, and protein intakes were calculated from a three day dietary record. Even though the group consumed a high fiber and phytate diet, with plant products fulfilling 72 - 92% of their protein needs, as well as zinc intakes lower than the recommended dietary allowance, all women had adaquate zinc and iron status, as determined by by blood plasma levels. Balance data was not obtained.

The Combined Effect of Phytate and Calcium on Zinc Bioavailability

The mechanism by which calcium may decrease zinc absorption is still questioned; however, the calcium ion, may be involved in the formation of the insoluble phytate-zinc complex. (Oberleas and Prasad, 1976). In 1960, Forbes et al. noted that high levels of calcium by itself do not inhibit zinc absorption, but the combination of calcium, phytate, and possibly phosphorous decrease zinc bioavailability. In experiments with rats, chickens, and pigs, O'Dell et al. (1969) fed a casein-based protein diet

with and without phytic acid. They found that excess calcium depressed growth rates only with the addition of phytates in the diet. Heth and Hoekstra (1965), on the other hand, found that high doses of calcium inhibit zinc absorption in rats even with low amounts of phytates.

In humans, little information is available on the effect of calcium on zinc metabolism and what information is available has been controversial. Recently, Sandstead et al. (1984) studied the effects of fiber and phytate sources and diet calcium on absorption, retention, and requirements for zinc of 45 men fed a conventional diet with either 40g or 75g of protein. Twenty-six grams of fiber and phytate sources (corn bran, soy hulls, apple powder, carrot powder, several wheat brans, and wheat germ) were fed in bread for 26-30 day intervals. Using regression analysis, the authors found that dietary calcium alone did not affect zinc absorption, but, the interaction of phytate and calcium impaired zinc retention at both protein levels. The dietary fiber also significantly decreased zinc absorption.

Using a radionuclide technique with 65Zn, Sandstrom et al. (1980) measured zinc absorption from composite meals of conventional foods with various main protein sources. The test meals consisted of meat patties of either 100% beef or chicken or a mixture of beef or chicken with soy flour. In one test meal, subjects drank 125 ml of milk to test the effect of dietary calcium and phytate on zinc absorption. The researchers found that the absorption from

the soybean meal did not differ from an animal protein meal with comparable zinc values; however, lower zinc retention was found when the calcium content of the soybean meal was increased by the addition of milk. The authors question whether the calcium itself lowered zinc absorption or whether the effect was the result of a possible phytate-calcium complex or even of the type of food.

The Effect of Calcium on Zinc Bioavailability

Spencer et al. (1965) agree that high levels of calcium alone have no significant effect on zinc absorption in humans. In their study, they orally administered a tracer dose of 65Zn to 5 patients who received 12-15 mg per day of dietary zinc. The intake of the low calcium diet provided an average of 258 mg of calcium, while the high calcium diet was supplemented with calcium gluconate tablets to allow the calcium intake to average 1983 mg per day. Results showed that the total zinc absorption averaged 35.7% during the low calcium intake and 31.3% during the high calcium intake. The authors point out that even though the calcium intake increased 8-fold, there was no significant difference in zinc absorption. Snedeker et al. (1982) even increased calcium intake to 2382mg from 780mg during a 39-day metabolic balance study and found no changes in zinc absorption. In both studies, the phytate level was very low. Spencer argues that the difference in results obtained with a high calcium intake in animals and in the present study in humans can be explained by the fact that

the amount of calcium used in animal studies was considerably higher relative to the total body weight than the calcium used in humans.

In a later study, Spencer et al. (1984) studied the effects of three levels of calcium: 200 mg, 800 mg, and 2000mg and two levels of phosphorous: 800 mg and 2000 mg per day on zinc balance. In a tightly controlled study with 36 men, there were no significant changes in urine or fecal zinc. Also, zinc balance and absorption did not vary significantly for any of the levels of calcium or phosphorous; however, average total zinc balance changed from +2.6 for the low calcium intake to +1.1 for the medium intake (which equaled the RDA), to -1.1 for the high calcium intake. Although there is no significant difference in absorbances, the negative zinc balance observed with the high calcium intake might suggest a lowering effect on zinc balance.

Contrary to these studies, Pecoud et al. (1975) reported that dietary calcium had a negative effect on zinc absorption in volunteers who injested single doses of 50 mg of zinc sulfate with a test meal of 200 ml of milk and 50 g of cheese, as compared to one of 60 g of dry meat only.

Iron

Metabolism

Iron, the most abundant trace element in the human and animal body, functions primarily in oxygen transport, as a component of hemoglobin, and oxygen utilization, as a constituent of many enzymes such as the heme-containing cytochromes and the iron-sulfur proteins of the electron transport and oxidative phosphorylation, as well as the liver enzymes, catalases, peroxidases, and others. Larger amounts are found in the form of myoglobin which stores oxygen in muscles for use in contraction. Extra iron is stored, primarily in the liver, spleen, and bone marrow, in the form of ferritin and hemosiderin (Linder, 1985).

Iron absorption is affected by numerous factors, but the primary control is the physiological need of the individual. Consequently, iron homeostasis is largely controlled by regulation of absorption rather than excretion. A larger proportion of dietary iron, ranging from 30 - 60%, is absorbed in a deficiency state, but only 10 - 15% of dietary iron is absorbed when the body has ample stores of the mineral. Linder (1985) notes that higher concentrations of available iron in the diet will result in greater absorption, even when the iron stores of the body are high. However, there are limits to the extent that iron absorption can be controlled; in fact, iron overload is possible during long-term high intakes (Prasad, 1977).

Although the detailed mechanism of iron absorption is

still somewhat controversial, it is generally agreed that iron in the ferrous state (Fe ++) enters the mucosal cells of the duodenum and jejunum and either passes directly to the blood, by a presumably energy-dependent process, or forms complexes with apoferritin in mucosal cells. The amount of dietary iron, the iron status of the individual, the red blood cell production rate, the number of iron receptors on the mucosal cells, and the composition of intestinal excretions are just a few factors that regulate the amount of iron transported across the intestinal mucosa. Once in the blood, iron is bound to transferrin, a protein which transports iron to the bone marrow, liver, or other tissues, where it can be used for synthesis of hemoglobin and other iron-containing compounds (Suitor and Crowley, 1984).

Most of the iron released from hemoglobin and porphyrin of degraded red blood cells is reabsorbed, with very little excreted. True excretory iron, derived from bile and desquamated intestinal cells, is estimated at 0.2 - 0.5 mg/day. Between 6 - 16 mg of dietary iron, depending on the amounts ingested, are excreted daily through the feces. Urinary losses of iron range from 0.2 - 0.3 mg, while sweat losses are estimated at 0.5 mg per day.

When discussing iron absorption, it is essential to distinguish between heme iron and non-heme (inorganic) iron. Heme iron contains a form of iron chelate more available for absorption than inorganic iron or iron in plant-derived foods. The absorption of heme iron, which

accounts for an average of 40% of the iron present in flesh foods, is more complete than non-heme iron. For example, if heme iron absorption is 23%, then non-heme iron would be absorbed in the range of 3 - 8%. Furthermore, heme iron is absorbed as the iron porphyrin complex and its availability is little affected by meal composition, unlike non-heme iron which is affected by dietary factors (Bodwell, 1983).

Phytate, oxylate, tannins, and even phosphates in the diet, all of which are present in various plant foods, tend to form insoluble iron precipitates that render non-heme iron unavailable for absorption. Calcium, due to its high affinity for phytate, may further decrease iron absorption through the formation of calcium-phytate-iron complexes (Monsen, 1978). High intakes of zinc, cadmium, copper, and manganese also interfere with iron absorption through competition for the protein-binding sites in the intestinal mucosa (Bothwell, 1979). Counteracting these factors, chelating agents, like ascorbic acid and fructose, tend to enhance iron absorption by reducing iron from a higher oxidation state (ferric) to a lower one (ferrous), thereby increasing its solubility and absorption. The addition of heme protein in general makes non-heme iron more available for absorption, as well as the amino acids lysine, histidine, cysteine, and methionine, which act as buffering and chelating agents and help transport non-heme across the gut mucosa (Prasad, 1978).

The presence of precipitating or chelating agents

within a given food could be altered substantially if eaten in a composite meal containing one or more additional foods. For example, the inhibiting effect of phosphoprotein in egg yolk is not limited to the iron in egg, but depresses iron absorption from all foods taken with it. Similiarily, the enhancing effect of ascorbic acid would increase iron absorption in a given meal. Using radioiron measurements, it has been shown that non-heme iron destined for absorption behaves as though derived from a single pool consisting of vegetable iron, non-heme iron in meat, and any soluble inorganic salts that may be added or present in the meal. (Bjorn-Rasmussen, 1974).

The average diet contains about 10 -15 mg of iron, the amount necessary to meet the daily requirements of normal men and non-menstruating women (RDA = 5 - 10 mg). Although there is a great deal of variation between individuals, these requirements are based on the assumption of 10% absorption. Iron requirements are increased with increased rates of growth, especially in infancy and the growth spurt of adolescence, as well as for menstruating and pregnant women when iron intakes are most critical and deficiencies are prevalent (Suitor and Crowley, 1984).

Flesh foods with residual blood or muscle cells are generally rich in iron, as well as egg yolk, dried legumes, shellfish, and dried fruit. Poor sources include milk and milk products, polished rice, and potatoes.

Iron deficiency (hyposiderosis), a prevalent and widespread deficiency, is the most common cause of anemia. It

results from a relative lack of iron in the diet and/or a substantial loss of iron from the body through bleeding, pregnancy, and other routes.

The Effect of Dietary Protein Source on Iron Bioavailability

With the consumption of soybeans and soy-containing products increasing in the United States and developing countries, the effect of protein source on iron availability has been the subject of much research and remains controversial. It is generally concluded that iron in the form of heme iron in animal foods has a higher biavailability than non-heme iron in plant foods. This reduced availability may be offset, in part, by the high iron content of the soybeans.

Animal Studies

Erdman and Thompson (1984), using extrinsically radiolabeled iron, reported that rats absorb significantly less iron from a diet of soy protein isolate than one of casein. Furthermore, rats fed a soy-based meal before or after the casein-based meal had less iron retention compared to those only fed the casein-based diet. Chauson et al. (1988) found similiar results in young chicks who consumed feed ingredients of plant or animal origin. The chicks absorbed 45 and 68% of iron from the soybean meal and the poultry-by-product meal, respectively. Fitch et al. (1964) also observed a lower absorption of iron in rhesus monkeys fed

soybean protein than those fed casein. In a study with healthy and anemic rats fed a standard diet with meat protein, meat substituted with whole wheat, or only whole wheat, Thannoun et al. (1988) reported that the absorption of non-heme by both groups decreased as the proportion of heme iron decreased, signifying the importance of heme for iron retention. Since iron absorption depends on iron status, it seems reasonable that anemic rats absorbed significantly more non-heme iron than the healthy rats, although both groups absorbed similiar amounts of heme iron.

Contrary to these findings, Greger and Mulvaney (1985) reported no significant difference in iron absorption among male weanling rats fed a 30% lactalbumen or 30% soy assay protein diet. In addition, there were no changes in hemoglobin or liver iron. Schricker et al. (1982) found that iron-deficient rats absorbed significantly more iron in soy and casein-based meals than healthy rats; however, iron from meals made with soy flour, soy protein concentrate, isolated soy protein, or casein were absorbed equally well within each group. Furthermore, Steinke and Hopkins (1978), who measured iron assessment by hemoglobin repletions in rats, observed a relatively high availability (61%) of the iron in isolated soy protein. Weaver et al. (1984) found similiar results in both healthy and anemic rats using whole-body retention curves of radioisotope iron.

Human Studies

A Comparison of the Effect of Soy and Animal Proteins on Iron Bioavailability

Studies employing single food items tagged biosynthetically with radioiron have shown that absorption from vegetable or cereal foods is usually less than 5% as compared with 15 - 20% absorption from animal sources such as beef, liver, and fish (Martinez-Torres, 1973). Layrisse et al. (1973) have shown that the absorption of a small quantity of inorganic iron is more than 10-fold greater when ingested with a meal with veal than a meal of maize. The iron in black beans has also been shown to increase almost three-fold when eaten with fish or veal (Martinez-Torres, 1973). Not only is there a higher percentage of heme iron in the meat, but the heme has been found to enhance the non-heme protein. In addition, soy products contain factors that have been found to inhibit non-heme iron.

In 1981, Cook et al. published results from a series of studies that showed several soy products to inhibit markedly the absorption of non-heme iron in human subjects. Performing radioiron absorption studies to determine non-heme iron absorption, the researchers recruited thirty-six iron-replete men. In the first study, the effects of different semi-purified proteins: albumen, casein, and isolated soy protein on iron absorption were compared. When egg albumen and casein were substituted in protein equi-

valent quantities in a semi-synthetic meal, similiar mean iron absorptins of 2.5 and 2.7% were observed, respectively; however, the mean absorption of isolated soy protein was only 0.5%. In a second study, the influence of various soy products on non-heme iron absorption was studied. The iron absorptions were again markedly lower in the test meals containing full-fat soy flour, textured soy flour, and isolated soy protein than in the test meal based on egg albumen. In fact, the soy product with the highest protein content gave the lowest iron absorption, indicating an inhibitory effect by a wide range of soy products. The authors suggest that the processing of the whole beans to obtain the three products may also alter their influence on food iron absorption. Bodwell et al. (1979) also observed negative iron retention in men fed textured soy (-1.4 mg) and isolated soy protein (-7.9 mg); however, the subjects fed the mixed-animal protein were also in negative balance. Even though these men were fed 1.6 g of protein per kg of body weight daily, they were all in negative balance which seems unlikely according to the widely held beliefs about iron metabolism. Derman et al. (1987) compared the iron absorption in multiparous Indian women fed a soybean-based or milk-based infant formula, by measuring erythrocyte utilization of radioactive iron. Results showed that the mean absorptions were significantly higher in the group fed the milk-based formula than the soybean formula. Even with the addition of 5.8 mg of ascorbic acid to the test diet, no changes in absorption were reported for the soy-based

formula, although the absorption of iron increased significantly in the dairy-based formula. To test whether the increased iron absorption in subjects was due to some effect of the milk protein, the authors compared the absorption of the soybean formula in water to that in milk. No difference were seen. Ashworth and March (1973) also reported a reduced iron availability in Jamaican infants fed iron-fortified dried skim milk or a maize-soybean-milk mixture.

On the other hand, Young and Janghorbani (1981) reported no significant differences in iron absorption in men who received diets containing protein either from soy isolate, milk protein, or a mixture of them both. Using a stable isotope of iron, the authors found that iron from soy protein isolate was readily available. The authors point out, however, that the conditions of the experiment were designed to incorporate conditions that favor iron availability. Inhibitory factors, such as dietary fiber, phytate, and tea, were not provided in the diet; however, ascorbic acid, which has been found to enhance iron absorption was included in the meals. In another study, when soy flour was added to a mixed Latin American diet composed of maize, rice, and black beans, a slight improvement was noted in iron absorption (Hallberg and Rossander, 1984). The authors suggested that this increase was due to the contribution of soluble iron from the soy protein flour and the presence of enhancing factors like ascorbic acid.

The Effect of Soy Extenders on Iron Absorption

Due to the increased use of soy extenders, Cook et al. (1981), using radioiron absorption studies, also examined iron absorption in men fed meals containing animal protein (hamburger, milk shake, and french fries) and those partially extended with textured soy isolate. With a ratio of 3:1 and 2:1 of meat to textured soy protein, absorption decreased by 61 and 53% respectively. In a similiar study, Hallberg et al. (1982) reported that the absorption of iron in adult men who were fed a hamburger meal with half of the meat protein substitiuted with defatted soy flour was reduced from 8.4 to 5.4%. The authors point out that the amount of non-heme iron absorbed was unchanged due to the high iron content of the soy flour; however, the total amount of iron absorbed, including the heme iron, was significantly reduced when the meat was substituted with soy. The meat protein not only stimulates absorption of non-heme iron, but provides heme iron which is absorbed more efficiently.

However, when 25% of the meat in a conventional diet was replaced with soy isolate, Sandstrom et al. (1986) observed no differences in iron absorption in eight ileostomy patients. Van Stratum and Rudrum (1979) also found no differences in iron absorption in 138 healthy volunteers fed a conventional diet and one replaced with 25% soy.

A Comparison of the Effect of Different Animal Proteins on Iron Absorption

In a study with seventy females, Cook and Monsen (1976) studied the absorption of iron from various animal proteins as evaluated by radioiron absorption measurements. Protein equivalent substitutions of nine animal foods: beef, pork, lamb, liver, chicken, milk, cheese, egg, and ovalbumin were made in meals of both high and low iron availability to compare their effect on the assimilation of non-heme iron. The effect of substituted various animal proteins for the beef meal on iron absorption was nearly the same for pork, lamb, liver, chicken, and fish, but markedly reduced for milk, cheese, egg, and ovalbumin. Similiar results were found when these foods were substituted for the ovalbumin, the meal of low iron availability. The authors point out that all sources of animal protein are not equivalent in their enhancing effect. A more valid distinction can be made between animal tissues and other sources of dietary protein.

It should be noted that iron from human milk, in contrast to cow's milk, is absorbed well in infants, probably due to the ligand lactoferrin to which iron is attached (Underwood, 1977).

The Effect of Phytate on Iron Absorption

Because ferric iron, like other trace minerals, form highly insoluble complexes with phytate, one might expect a low availability of iron in foods like soybeans which are

rich in phytate and iron; however, the effects of phytate on the absorption of iron remains unclear.

The postulate that phytate inhibits iron absorption appears to be based on the inhibitory effect of purified sodium phytate on iron absorption in rats (Davies and Nightingale, 1975). These researchers found that the addition of 1% phytate to an egg albumin diet significantly reduced the whole body retention of iron. In agreement, Mahoney and Hendricks (1978) reported that the addition of different phytate salts to diets fed to weanling rats caused decreased iron absorption, hemoglobin concentration, and liver iron values.

Other researchers have found conflicting results. In a recent study, Beard et al. (1988) studied the effects of hydroponically-grown soybeans, adjusted to produce seeds with high, intermediate, and low phosphate and phytate content, on iron absorption in rats and humans. By using a radioiron technique, the researchers reported that increasing the concentrations of phytate in whole bean flour had no significant effect on iron absorption in the rats or humans. In a similiar study, Welch and Van Campen (1975) observed that a group of rats absorbed 32% of a single dose of radiolabeled iron in immature soybean seeds, which contained 0.61% phytic acid, as compared to another group which absorbed 52% of the iron in mature seeds containing 1.71% phytic acid, a higher percentage than the immature seeds.

Similiar results were observed in a study with chicks,

in which researchers compared the iron absorption of different plant-derived feeds. Both sesame and rice bran meal had high phytate percentages, 3.97 and 5.78, respectively; however, in spite of their high phytate content, the relative iron availability of these plant sources was significantly higher than soybean or ground corn meal which contained 1.6 and 0.75% phytate respectively. The sesame and rice bran meal also contained more total fiber, specifically hemicellulose, than the other two meals, suggesting that other factors besides fiber and phytate play a role in reducing iron bioavailability (Chausow and Czarnecki-Maulden, 1988).

Morris and Ellis (1982) observed that the effect of phytate on iron absorption depends upon the nature of the iron-phytate complex. Monoferric phytate, the major fraction of iron in wheat bran, is soluble and a highly bioavailable form of dietary iron to animals and humans, in contrast to the insoluble di- or tetraferric phytate. To support these observations, they found that the hemoglobin response of rats and dogs to monoferric phytate and ferrous ammonium sulfate were equal; however, the hemoglobin response of the animals to di- or tetraferric phytate was significantly lower. Similiar results were seen in dogs. In a human study, Simpson et al. (1981), using the extrinsic tag method, measured iron absorption in humans fed meals with either monoferric phytate or iron chloride, each labeled with different isotopes. They found no significant differences.

The Effect of Phytate and Calcium on Iron Absorption

As mentioned earlier, phytate (which is considered to be a substituted cyclic polyphosphate) has a high affinity for calcium. What effect this complex may have on iron absorption has not been investigated to the extent of the zinc-phytate-calcium complex. Zemel and Bidari (1983) reported that orthophosphates, hexametaphosphates, and polyphoshates all reduced iron absorption in male weanling rats: furthermore, iron absorption was significantly lowered when calcium intake was increased from 0.53 to 1.06%. Similiarily, data from human studies indicate that orthophosphates depress iron absorption only when calcium intake was concommitantly elevated (Monsen and Cook, 1976).

The Effect of High Calcium Intakes on Iron Absorption

Few studies have investigated the effects of high intakes of calcium on iron nutriture. Greger and Snedeker (1982) conducted one thirty-nine day balance study with adult males fed a conventional diet. Calcium levels were increased from 780 mg to 2382 mg daily with supplements, while iron intakes remained at about 17 mg daily and kilocalories were calculated at 3050 mg daily. Dietary treatments had no effect on plasma iron levels. Subjects excreted significantly more iron in the urine, but there were no significant difference in the feces. Apparent retention of iron decreaesd from 0.56 to - 0.44 for the medium and high calcium intake, respectively; however, this was not a significant difference.

In a recent study, Dawson-Hughes et al. (1986) found that iron absorption was significantly reduced in postmenopausal women who injested standard meals radiolabeled with iron and capsules containing 500 mg of calcium. Monsen and Cook (1976) observed that retention of nonheme iron was impaired by 53 to 73% when moderate amounts of both calcium (178 mg) and phosphorous were added to a test meal, whereas iron retention was not affected by addition of an equivalent amount of either calcium or phosphate alone.

The Effect of Fiber on Iron Retention

The results of many human balance studies indicate that fiber has different effects on iron balance. In one of the first studies, Widdowson and McCance (1942) reported that iron was absorbed less from a diet containing brown bread than from one containing white bread, even though brown bread supplied 50% more iron than the white bread. Other researchers also found a direct negative relation between the absorption of nonheme iron and the amount of wheat bran added to the bread (Bjorn-Rasmussen, 1974, Reinhold, 1974). In another study, the absorption of iron, as measured by serum iron levels, was less when wholemeal flour was eaten in the place of white flour (Dobbs and Baird, 1977).

On the other hand, Morris and Ellis (1976) observed that adult men who consumed 36 g of wheat bran per day had positive iron balances which were not increased when dephytinized bread was consumed. Sandstead et al. (1978)

reported that the addition of 26 g of soft white wheat bran or corn bran to a basal diet containing 11 to 20mg of iron had no effect on iron balance. Kelsay et al. (1979) also reported that iron balance was not affected by the inclusion of fruits and vegetables to a diet.

Copper

Metabolism

Copper, as an essential component of key metallo-enzymes, plays a vital role in numerous biochemical and physiological functions in higher animals. The copper-containing enzyme ceruloplasmin is involved with iron metabolism; cytochrome C is important in the oxidation of vitamin C and ATP synthesis; tyrosinase functions in the formation of melamin; and other copper-enzymes are necessary for the development of connective tissue, collagen, and elastin (Mason, 1979).

The absorption of copper, regulated at the level of the intestinal mucosa, occurs primarily in the stomach and duodenum. It is absorbed quite efficiently, ranging from 30 - 50 % of dietary copper. While vitamin C, phytate, and high zinc intakes tend to decrease copper absorption, L-amino acids and fresh vegetables tend to increase it. At least two absorption mechanisms, one involving amino acids and a second in which copper is bound to metallothioneins, have been described (Burch et al. 1975). Zinc and other trace minerals also compete for the binding sites on this metal-binding ligand, possibly explaining the antagonistic relationship between zinc and copper. Once absorbed, copper is bound to amino acids or albumin and transported to the liver where more than one-third of the bodies supply of copper is stored.

Excretion is predominately through the intestinal

tract, either via the bile (a major factor) or as non-absorbed copper. Of an average intake of 4 mg/day, nearly 3.5 mg is excreted through the feces. Urinary excretion, neglible in humans (.05 mg/day), is about 1 to 2% of dietary intake and sweat losses range from 0.04-0.4 mg/day (Mason, 1979).

Copper is ubiquitous in plants and animals. The richest sources are shellfish, especially oysters, and organ meats, followed by nuts and dried legumes. The poorest sources are dairy products. In the past, researchers believed that common foods in our everyday diet (Western diet) provided enough copper to cover and even excede the RDA of 2 mg/day; however, contemporary analyses of the copper content of diets suggest that many provide less than 2 mg of copper daily (Sandstead, 1980). Furthermore, in a study that analyzed fifteen diets typically consumed in the U.S., Klevay et al. (1980) revealed that eight of them contained less than 1.0 mg of copper. Only two of the diets actually had more than 2.0 mg copper.

Sandstead (1980) reports that copper deficiency can lead to anemia, since copper plays an integral role in iron metabolism; skeletal defects due to defective collagen formation; poorly pigmented hair due to lack of melamin; degeneration of the the nervous system; cardiovascular lesions; and impaired immunity. Klevay et al. (1975) observed one man fed approximately 0.8 mg/day of copper, in a diet of conventional foods for 16 weeks. Mild copper deficiency signs of reduced serum copper and erythrocyte

superoxide dismutase, as well as increases in serum cholesterol were seen. Even abnormalities of heart beats on the ECG were observed, possibly implicating copper deficiency in heart disorders. All indices returned to normal after copper supplementation. With many diets providing less than 2.0 mg of copper a day, either mild deficiencies of copper are a frequent occurence or the copper requirement is actually less than 2.0 mg/ day. More data are needed on the copper requirement of both sexes at all ages consuming different diets, as well as factors affecting availability.

The Effect of Protein Intake on Copper Bioavailability

Animal Studies

One factor that affects copper status is the level of protein in the diet. In animal studies, the addition of more protein or sulfur amino acids to the diets of rats and chicks has been found to depress the concentration of copper in the liver of animals (Jensen,1979). McCall and Davis (1961) have even observed a protective effect of high protein intakes against the development of copper toxicity in rats, probably through the formation of macromolecular complexes of copper and protein.

In other animal studies, Snedeker and Greger (1983) also observed that male rats fed a high protein diet (45% lactalbumin) had slightly lower liver copper than those fed an adaquate protein (15 % lactalbumin) diet. However, Sherman et al. (1985) revealed that high protein feedings (16% - 32% casein) in young, mature, and aged rats, produc-

ed no changes in liver copper. Van Campen and House (1974) reported an increase in kidney copper in rats fed a 15% casein diet, as opposed to a 5% one. As far as inade- quate levels of protein, Wallwork et al. (1983) reported that weanling rats on a 6% egg white diet had lower liver copper than those fed moderate levels of copper.

Human Studies

Results from human studies differ somewhat from those of animals. Species and experimental design differences most likely account for these discrepancies. In a study with men fed a Western diet for 26-30 days, Sandstead et al. (1982) revealed that higher protein intakes (ranging from 40 -100 gms) increased copper absorption and reduced copper requirement by 15%. The authors believe that the enhanced absorption is probably due to the increased intake of L-amino acids. Greger and Snedeker (1980) found similar results in a 51-day metabolic study with adult males. Apparent absorption and retention of copper were significantly greater in subjects who were fed a high mixed-protein diet (150 gms) rather than the low protein diet (50 gms). Subjects lost significantly more copper in their feces when fed the low protein diet, while urinary losses were small and not affected by dietary treatments, as was serum copper.

On the other hand, Mahalko et al. (1983) found no differences in urinary and fecal copper levels, as well as copper absorption or balance when they measured mineral

balance in adult men fed 65 or 95 g of protein from a conventional diet. Colin et al. (1983) found similar results in adult women fed 50 g or 95 g of food protein per day. In agreement, Price and Bunce (1970) reported that the addition of nitrogen, in the form of ammonium citrate or synthetic limiting amino acids, to a basal diet of 24 g of protein did not affect copper retention in preadolescent children.

The Effect of Dietary Protein Source on Copper Balance Animal Studies

Davis et al. (1961) demonstated that soy protein reduced the availability of copper in chicks, as compared to an egg-white protein. Nwoholo et al. (1975) also reported that only 51% of copper from soybean meal was biologically available to broiler chicks. Using a suckling rat pup model, Lonnerdal et al. (1985) compared copper bioavailability from various infant diets. Results showed that liver copper uptake from the milk formula was 25%, whereas the soy formula resulted in a significant lower liver uptake of 10%. The cereal/milk formula diet resulted in a 17% uptake, mid-way between the other diets.

In another study with rats, Greger et al. (1985) found that animals consuming a lactalbumin diet actually absorbed significantly less copper than those fed soy-based diets. The rats on the animal protein diet, however, absorbed zinc more efficiently than the other animals which the authors suggest may have depressed copper absorption.

Human Studies

Engel et al. (1967) reported that copper absorption was equivalent in pre-adolescents fed a diet of mixed vegetable proteins, such as nuts and legumes, and those fed a mixed-animal protein diet. Butler et al. (1973) also reported that the absorption and retention of copper in ten young women was not significantly different from a diet consisting of animal protein (casein-lactalbumin) or vegetable protein (peanut flour). However, subjects consuming the animal protein absorbed a higher percentage of copper and excreted less fecal copper than those eating the vegetable protein, even though the average copper intake was higher in the peanut flour protein (4.3 mg) than the casein-lactalbumin protein (3.9 mg).

When soy products have been partially replaced for animal protein, the effect on copper absorption has been confusing. With the use of the stable isotope 65Cu, Turnland et al. (1983) determined copper absorption in women fed two different diets: one provided approximately 70% of the protein from animal products (beef, turkey, and milk) and 30% from plant sources (lentils, kidney beans, and wheat products) and the other had the same food items, but the percentages were reversed. The subjects absorbed 41.2% of the from the animal protein diet, but only 33.8% from the plant diet; however, the actual amount absorbed was higher from the plant protein diet since it contained considerably more copper than the animal protein diet. If the intakes of copper had been the same from each diet,

the results might have been significant.

Greger et al. (1978) studied the utilization of dietary copper among adolescent girls fed standard lunch menus with texturized soy protein that was substituted for 0, 15, or 30% of the meat. The replaced soy, even at the highest levels, had no effect on urinary or fecal excretion of copper. However, this partial substitution of soy for meat resulted in the addition of only 81 mg of phytate to the diet which is less than 0.02% phytate daily. Perhaps the amount of phytate was not large enough to produce an effect on copper retention, since levels of phytates between 0.4-2.0% of the diet have been found to impair zinc absorption in animals (O'Dell, 1979).

The Effect of Phytate on Copper Availability

In a study with rats, Davies and Nightingale (1975) reported that the addition of 1% dietary phytate to an egg albumin diet significantly reduced the average daily accumulation and whole-body retention of copper. The mechanism by which phytate reduces the availablity of copper probably results from the ability of these trace metals to form metal-phytate complexes which are stable within the intestinal tract. The authors note that copper is only second to zinc in the formation of these stable complexes.

Contrary to these results, Lo et al. (1984) reported that copper from isolated protein was as available as copper from copper carbonate in 150 hypoculemic rats who

were fed copper-repleted diets containing 0, 0.5, 1.0, or 2.0 ppm of copper from copper carbonate or isolated soy protein. The presence of phytate did not affect the absorption of copper.

The Effect of Fiber and/or Phytate on Copper Availability

Although there have been very few human studies investigating the role of phytate alone on copper balance, Sandstead (1982) added 26 gms of fiber (wheat germ, corn bran, soy protein, and pectin, and others) to a mixed Western diet for intervals of 26-30 days. The extra fiber reduced the absorption of copper, although the author points out that phytate, present in most of these sources of dietary fiber, may have impaired the retention of copper, as well as the combination of the two. Turnland and King (1983) found that neither phytate nor a-cellulose added to an egg-albumin diet affected the copper absorption in three out of four subjects; however, the sampling size was too small to draw conclusions.

In another experiment, Sandstead et al. (1978) found that 26 g of soft white wheat bran added to a conventional diet actually improved copper absorption, while 26 g of added corn bran had little effect on copper balance. Conducting a study with adolescent boys, Drews et al. (1979) investigated the effects of three fibers (hemicellulose, cellulose, and pectin) at a level of 14.2 gm/day on copper utilization. In spite of the use of short-term periods, fecal copper excretion showed a signi-

ficantly higher value when the hemicellulose supplements were fed, in comparison to the basal diet without any added fibers. The other fibers also increased fecal excretion, but not significantly. From these studies, it can be concluded that the effect of fiber, with and without phytate, needs to be qualified when discussing copper bio-availability.

The Effect of Phytate and Calcium on Copper Availability

The effect phytate has on copper availability seems to be enhanced with the addition of calcium. Moody and Oberleas (1981) studied the in-vitro complexation of copper with phytate with and without calcium. Only minimal amounts of precipitate formed when only copper and phytate were added together; however, with the addition of calcium, a maximum precipitate formed at ph 6 which contained 76% of copper. In weanling rats, Zemel and Bidari (1983) observed that supplementing a low calcium diet with hexameta-phosphate did not affect fecal or urine copper. But the addition of higher levels of copper with the phosphate caused a 17% increase in fecal copper, thereby signifi-cantly reducing copper absorption. The authors suggest that the decreased absorption may be due to the formation of stable calcium-polyphosphate complexes resistant to attack by intestinal phosphatases. When Wise et al. (1963) supple-mented rats with calcium, they observed a reduction in phytic acid hydrolysis in the intestinal microflora.

The Effect of Calcium on Copper Availability

Without added phytates, increased levels of calcium may possibly enhance copper bioavailability in animals. Diets high in calcium increased copper toxicity in pigs, presumably by reducing the the zinc-copper antagonism and lowering zinc availability (Suttle, 1966). Similiarly, calcium can alleviate meat anemia in mice by increasing the the availability of copper (Sandstead, 1982).

In humans, there seems to be little effect of increased intakes of calcium on copper balance. Price and Bunce (1970) observed no significant differences in copper balance in preadolescent girls fed two levels of calcium (260 mg or 620 mg). In adult males, Spencer et al. (1979) varied the calcium level of a mixed diet from 200 mg to 800 mg, and even 1500 mg in one subject. The researchers observed no difference in copper absorption for any of the subjects. Although, it should be noted that all individuals in the study were in negative balance, presumably due to the low intake (< 1 mg) of copper. Snedeker et al. (1982) also found no significant difference in nine adult males fed mixed diets with 2382 mg or 780 mg of calcium.

The Effect of Zinc Intakes on Copper Balance

High intakes of zinc have been found to reduce the bioavailabiliuty of dietary copper; in fact, evidence suggests that a Zn:Cu ratio of greater than 10:1 can produce a nutritional impact. Prasad et al. (1978)

described the occurence of copper deficiency in a patient treated with high levels of zinc in the treatment of sickle cell anemia. Using the activity of superoxide dismutase as an index for metabolically available copper, Fischer et al. (1984) reported that ingestion of high amounts of zinc (50 g) by humans decreased copper status. Klevay (1980) states that relative or absolute deficiency of copper characterized by a high Zn:Cu ratio can result in ischemic heart disease, and more specifically hypercholesterolemia and myocardial and arterial damage. In a recent study, Bialkowska et al. (1987) found that the zinc hair concentrations and Zn/Cu ratios in survivors of myocardial infarctions was significantly higher in comparison to controls.

On the other hand, Taper et al. (1980) observed that dietary zinc did not influence copper retention in adult women who were fed dietary regimes that provided 2.0 mg copper and levels of zinc that are representative of the range of intakes found in the diets of the general population, specifically 8, 16, or 24 mg of zinc. Conducting a twenty-four day metabolic study, Colin et al. (1983) also observed no significant effect of varied levels of zinc (9.5 -19.9 mg) in twenty-four adult women fed mixed diets with 2.0 mg copper.

Greger et al. (1978) disagree. They reported that adolescent girls fed two levels of zinc (11.5 and 14.7 mg), both of which are consumed in a normal diet, excreted significantly more copper in their feces when fed the

higher levels of zinc. Sandstead (1982) also observed in adult men a higher requirement for copper, as zinc levels increased from 5 to 20 gms.

Calcium

Metabolism

Calcium is the most abundant mineral in the body, 99% of which is in the skeleton and teeth. Although only a small percentage of it is in the blood, this plasma calcium, regulated precisely between 9-11 mg/d, plays a major role in nerve transmission, muscle contraction, membrane permeability, blood clotting, metabolic pathways, and bone formation.

The absorption of calcium varies with each individual and the physical state of the body. During periods of growth, as well as pregnancy and lactation, absorption is about 50-60% of intake. This decreases to 30% for adults, and even 20% for post-menopausal women. Although calcium is absorbed at a rate 1/50th of that of sodium, it is absorbed at a greater rate than that of other divalent cations, such as iron and zinc.

Vitamin D, probably the most important factor in calcium absorption, is metabolized to its active form, 1,25 cholecalciferol, which regulated by the parathyroid hormone, acts on the intestine to increase calcium absorption. Calcitonin decreases this absorption. Other factors such as vitamin C and lactose tend to increase calcium absorption, while a high fat diet may decrease absorption due to the formation of insoluble calcium soaps excreted in the feces. Both calcium and phosphorous are better absorbed if ingested together, but high intakes of phoshorous can decrease calcium absorption.

Once absorbed in the small intestine, calcium is transported across the gut mucosa by both active and passive transport, as well as passive diffusion. Calloway (1987) reports that as calcium intake increases, the active process is supressed which results in a smaller percentage of absorbed calcium; however, the total amount absorbed does increase.

Allen (1982) reports that fecal loss is composed of unabsorbed dietary calcium, plus an endogenous fecal excretion of 100-130 mg/ day. Urinary calcium which averages about 150 mg/day is not influenced by the diet or blood level, but by the efficiency of calcium absorption in the kidneys. Although discussed in detail later, the effect of a high protein diet has been implicated in reducing the renal reabsorption of calcium, therefore increasing urinary calcium.

With sweat loss of about 15 mg/ day, the total obligatory endogenous loss equals 250-280 mg / day. To cover this loss, the Recommended Daily Allowance for calcium has been set at 800 mg/day, assuming an average absorbance of 30% for adults (Food and Nutrition Council, 1980).

Although the RDA is 800 mg/day for the adult, there is still controversy as to the adaquate level for calcium balance. The FAO/WHO committee on calcium requirements suggests that 400-500 mg/day is a more practical estimate, whereas others feel that 800 mg is not enough to satisfy the needs of the elderly and other vulnerable groups.

To determine the amount of calcium needed by adults to attain calcium equilibrium or positive balance, Spencer et al. (1984) investigated the effects of six intakes of calcium ranging from 234 - 2320 mg/day in a total of 181 studies with adult men between the ages of 34-71 years. The authors reports that calcium balance is negative during low calcium intakes and becomes slightly positive (average = 22 mg/day) with intakes of 800mg, although many of the subjects were in negative balance. When the calcium from the diet is insufficient to meet the needs of the individual, calcium may be withdrawn from the bone, which over a period of time can lead to osteoporosis. Increasing intake to 1200 mg/day resulted in a significant increase in calcium balance, with the average balance being equal to 100 mg/day; however, intake over 1200 mg/day did not further improve calcium balance. Spencer suggests that a calcium requirement of 1200 mg/day would be preferable to the present recommendation of 800 mg/day.

The main food source of calcium is milk. Other dairy products and canned fish with edible bones are also good sources. Dark green vegetables also contain calcium, but in a form that is less available than that in milk products. Ways in which dairy proteins may proteins differ from other sources of protein, such as red meat or soy protein in regard to calcium availability will be discussed in this chapter.

The Effect of Protein Intake (Level and Source) on Calcium Utilization

The effect of protein level on calcium utilization remains controversial. Many researchers including Johnson et al. (1970), Margen et al. (1974), Linkswiler et al. (1974), and Allen et al. (1979) agree that high intakes of protein, mostly in the form of amino acid supplements or purified proteins such as lactalbumen and gluten, have significantly increased urinary calcium and decreased calcium absorption with little or no effect on fecal calcium; however, these researchers disagree over the mechanism of hypercalciuria. Schuette and Linkswiler (1980) suggest that the nitrogen and/or sulfur content of protein increases the amount of acid in the body which not only decreases renal absorption of calcium, but may leach the mineral from the bone. Spencer and Kramer (1978 and 1983), on the other hand, have found that protein, in the form of red meat, consumed in large amounts does not affect calcium absorption or excretion. They insist that protein in its "natural state" does not cause hypercalciuria, probably because of the high phosphorous content of red meat.

The effect of soy, dairy, or meat protein on calcium absorption when consumed at recommended daily intakes has not been investigated to the same extent as high protein levels; however, the role of phytate and fiber in soybeans seems to be a major factor in decreasing calcium bioavailability.

Increased Protein Intake and Hypercalciuria

As early as 1920, Sherman had observed that the addition of meat to a diet containing 390 mg of calcium in humans caused an increase in urinary calcium without an increase in calcium absorption. More recently, Margen et al. (1974) found that varying protein intake from 0 to 90 g per day in adult males results in approximately an 800% increase in calcium excretion, regardless of calcium intake. Studying the effects of two protein levels (48g and 141g daily) on urinary and fecal calcium and calcium retention in six males, Johnson et al.(1970) found similiar results. Using ordinary foods such as lean ground beef, turkey, grains, fruits, vegetables, and powdered milk for basal protein diets, the researchers added lactalbumen, gluten, and egg whites to increase protein intake. They reported that the increased protein level caused a two-fold increase in urinary calcium (from 175 to 338 mg), even though calcium intake was held constant at 1400 mg. The mean daily calcium balance for the low protein diet was 10mg, while the subjects on the high protein diet were in negative balance with a mean of -84 mg. There was no significant change in fecal calcium.

In a similar study, Linkswiler et al. (1974) demonstrated in young men that calcium balance could be achieved with a calcium intake of 500 mg, provided the protein level was 47 g. However, when protein intake was increased to 142 g, 30 of the 33 young men could not achieve calcium balance even at 1400 mg of calcium daily,

which suggests that the magnitude of the calciuric effect depends directly on the level of protein, rather than the amount of calcium in the diet. Linkswiler et al. have estimated that consumption of 95 grams of protein daily, along with 500 mg of calcium would produce a bone loss of 21 grams of calcium yearly. A mineral loss of this magnitude over a period of time has severe implications in the development of osteoporosis.

Mechanisms of Hypercalciuruia

Increased Protein Intake and Renal Acid Excretion

Like Linkswiler, Schuette et al. (1980) also found that increasing protein intake in human subjects from 47 to 112 g/day while maintaining calcium, magnesium, and phosphorous intakes constant, caused an increase in urinary calcium and a decrease in calcium retention; however, what they found most interesting was a significant increase in the total renal acid and sulfur excretion in the urine. The researchers suggest that the excessive nitrogen and/or sulfur from a high protein diet increases the amount of acid formed in the body, which may leach out calcium from the bone. Furthermore, this increased acid affects kidney function; Schuette et al. also observed a decrease in the fractional renal tubular reabsorption of calcium, as well as an increase in the glomerular filtration rate.

Wachman and Bernstein (1968) also hypothesized that bone dissolution is a possible mechanism to buffer the fixed acid load imposed by ingestion of an acid-ash diet.

When Lutz et al. (1984) fed a high protein diet (44g vs. 102g) to post-menopausal women, they also demonstrated a significant increase in net renal acid excretion. However, when six women ingested small amounts of bicarbonate (1mEq/kg bw), the researchers found that urinary calcium actually decreased and calcium balance became positive on the high protein intake. Also, with the addition of the sodium bicarbonate to the high protein diet, essentially all of the urinary acid was neutralized. Lutz et al. believe that these results support the premise that increased protein intakes represent a form of acid loading which may help explain the high incidence of osteoporosis in individuals living in developed countries where intaked of protein are high.

High Intakes of Sulfur Amino Acids and Hypercalciuria

Whiting and Draper (1981) suggest that the mechanism of increased protein and hypercalcuria is related to the sulfur amino acid content of animal proteins which they also found decreases renal tubular reabsorption of calcium in rats. In a human study reported by Lemann et al. (1979), adult males were fed a large amount of the sulfur-containing amino acid DL-methionine. The researchers reported an increase in urinary calcium, inorganic sulfate, and net acid within twenty-four hours. They note that when sulfur amino acids are metabolized, two equivalents of the hydrogen ion are produced per mole of oxidized sulfur, possibly causing metabolic acidosis in high amounts.

Other researchers disagree that sulfur amino acids increase calcium excretion. Margen et al (1974) found no relationship between urinary calcium and the sulfur amino acid content of various amino acid mixtures fed to humans. Block and Allen (1979, 1982) also showed that sulfur amino acid supplements had no effect on calcium excretion or absorption, although they agree that high protein intakes increase urinary calcium. These researchers fed meals containing 15 g of milk protein, 45 g of milk protein, or 15 g milk protein plus sulfur amino acids equivalent to those in the high protein diet, to twelve subjects. By measuring urinary excretion postprandially, the researchers showed that the increased consumption of dietary milk protein reduced renal fractional absorption of calcium; however, sulfur amino acids did not affect calcium excretion or its reabsorption by the kidney. The authors point out that the consumption of sulfur amino acids might cause a metabolic acidosis in high amounts as Lemann (1979) had showed; however, they evaluated the effect of protein that might be realistically consumed in a single meal. Furthermore, there was no difference due to the diets in the excretion of net renal acid or urine pH. These researchers argue that the mechanism of hypercalciurua is not acidosis or sulfur amino acids, but an increased insulin response which decreases the renal absorption of calcium and increases calcium excretion.

The Effect of High Intakes of Red Meat Protein on Calcium Balance

Not all researchers agree that higher intakes of protein have deleterious effects on calcium metabolism. Spencer et al. (1984) argue that many of the studies demonstrating high protein intakes and reduced calcium absorption have used protein supplements, specifically purified proteins, such as lactalbumins, gluten, and egg whites as the main protein source. However, the use of a intact protein, specifically red meat, which is part of a usual diet, does not increase urinary calcium excretion, nor does it change the intestinal absorption of calcium. In 1978, Spencer et al. observed that a high protein diet (2g/kg/ body weight) of red meat as the sole source of protein did not lead to a significant loss of calcium or urinary or fecal calcium. There was no change in calcium balance in the 14 fully ambulatory adult male patients who consumed 800mg of calcium per day; however, urinary calcium increased when the subjects consumed 1100 mg or 2000 mg of calcium per day.

Furthermore, in a more recent study, Spencer et al. (1983) studied the long-term effects of two levels of meat protein (76 gm/day and 142 gm/day) in four adults for 78 to 132 days. All subjects consumed an average of 835 mg calcium. Results showed no difference in calcium aborption or fecal and urine calcium levels. Spencer suggests the lack of any increase in the urinary calcium is probably due to the phosphorous content of the high protein meat diet.

Hegsted et al. (1981) have also found that adding increased amounts of phosphorous to high protein diets results in increased calcium absorption; but Schuette and Linkswiler (1982) caution that diets low in calcium, but high in phosphorous and protein, have consistently caused negative calcium balances.

Addressing Spencer's charge that purified proteins cause hypercalciuria, as opposed to intact meat protein, Schuette and Linkswiler (1982) compared a high protein diet (146 gms/day) of red meat to a high protein diet of purified proteins in human subjects. Both diets caused increased urinary calcium and decreased absorption as compared to controls; however, subjects on the simulated high protein diet had a significantly lower balance (-59 mg/day), as compared to those consuming the high red meat diet (-17 mg/day).

Using human subjects in a 51-day metabolic study, Hegsted et al. (1981) also compared the differences between a protein diet consisting of only red meat and one with added sulfur amino acids. The diets containing either 8 gm or 24 gm of nitrogen from red meat or 8 gm of nitrogen plus sulfur amino acids equivalent to those in the 24 gm nitrogen diet. The added sulfur amino acids caused an increase in urinary calcium and a decrease in calcium balance, but not to the extent of the meat protein intake.

The Effect of Source of Protein at Recommended Levels of Intake on Calcium Balance

Controlled Studies

All the research mentioned so far has dealt with increased levels of animal protein or protein supplements on calcium absorption, with little consideration of other protein sources or recommended levels of dietary protein. Watkins et al. (1985), however, studied the effects of recommended daily intakes of soy protein versus meat protein upon urinary acid and calcium in nine human adults, aged 22-69. The subjects were fed diets of baked chicken or soybeans, both of which provided 80 gms protein, 450 mg of calcium, 1900 mg phosphorous, and 2300 gms of sulfur. The meat diet resulted in significantly greater titratable acid, sulfur, and calcium excretion in the urine compared with the soy diet. Since the soy diet and the chicken diet had comparable amounts of sulfur, the authors like Schuette and Linkswiler (1982), suggest that the higher ratio of sulfur containing amino acids in the meat protein may have caused the increased calcium loss in the urine. Meat protein contains five times more methionine than bean protein.

In a study with nine postmenopausal women, Howe et al. (1985) fed subjects three sources of protein: cottage cheese (15g,45g), beef (15g,45g), and soy protein (45g) in a single meal, then measured their total and ionized urinary calcium every half hour after ingestion for three hours. All the meals contained 288 mg calcium. The inges-

tion of 45 g of protein from the meat or cottage cheese significantly increased the excretion of ionized calcium above that for the basal meal which contained no protein. Neither the 15 g of protein as cottage cheese or beef, or the 45 g of soy protein, differed significantly from the basal diet as far as a calciuretic response. The researchers note that there was no difference in serum calcium in individuals consuming the different meals.

As far as calcium absorption, Sandstrom et al. (1986) measured the apparent absorption of calcium in eight ileostomy subjects who were fed diets containing 60 g of meat protein, soy isolate protein, or a combination of meat and soy isolate protein (in which soy was 25% of the diet). The apparent absorption of calcium decreased from 2.5 mmol for the meat diet to 1.6 mmol for the combination diet, to 0.6 mmol for the soy isolate diet. The authors conclude that a 25% soy replacement in the diet represents an upper limit for protein intake if mineral absorption is to remain positive. Possibly the increased fiber and phytate in the soybeans interfered with the absorption of calcium. Van Stratum and Rudrum (1979) also reported a positive calcium balance in 44 men fed a meat diet and one replaced with 25 % soy protein. They, in fact, reported no changes in urine or fecal calcium excretion or absorption between the two groups.

Epidemiological Studies

In their study of the effects of vegetable protein and

animal protein on calcium excretion, Blockis et al. (1984) compared two population groups: lacto-ovo Seventh-Day Adventists and the meat-eating Mormons. Diets of these two groups are similiar except for the animal protein. Diets of thirty females and males from each group were analyzed and the level of total protein (animal and vegetable), as well as calcium was calculated. Twenty-four hour urine specimens were collected and assessed for calcium and uric acid. The authors reported that uric acid was significantly higher in the meat-eaters than in the vegetarians and that the level of urinary calcium increased with higher amounts of animal protein in the diet.

In a study with 168 Roman Catholic nuns, Heaney et al. (1982) also found a significantly negative association between calcium balance and nitrogen intake. The magnitude of the effect observed was such that a 50% increase in intake of nitrogen above the group mean intake value (10.89 g/day) would be predicted to result in a calcium balance shift of - .032 gm/day.

Comparing protein intake and incidence of osteoporosis, researchers have found that women of less developed countries eat considerably less meat and have a lower incidence of osteoporosis. Walker et al. (1970) studied rural Bantu women in South Africa who consumed half the calcium (250-400 grams/day) and considerably less protein in their vegetarian diet than Caucasian women. Interestingly, the researcher reported fracture rates to be ten times greater among the South African Caucasians eating

a meat-rich European-type diet.

In the United States, Marsh et al. (1980) have published a study comparing the bone density of Seventh-Day Adventists who were lacto-ovo vegetarians with age, weight, and sex-matched omnivore controls. There was no significant difference in bone mineral mass between the two groups in the third, fourth, and fifth decades; however, they found significantly increased average bone density of S.D.A. women in the sixth decade and later: during the post-menopausal years.

These findings correspond with observations by Mazess et al. (1974) who found that the bone mineral mass of the elderly Eskimo women was significantly lower than that of Caucasian omnivores of the same age. The Eskimo diet, very high in protein, consists of abundant supplies of fish, reindeer, moose, caribou, and other meats. The authors note that as early as the fourth decade of life, Eskimo women had bones with less than 85% of the density of age and sex-matched white women living in the United States.

The Effect of Phytate and Fiber on Calcium Balance

Although very little has been written on calcium absorption and soy protein, the effect of phytate and fiber, both contained in soybeans, has been investigated. In rats, Forbes et al. (1979) reported that the effect of phytic acid on calcium bioavailability seems to be the same from a casein diet as from a high phytate soy concentrate. After adding calcium supplements to these two diets, the

researchers found that the rats utilized calcium equally well from each protein source. These results suggest that fortification of soy products will result in good utilization of the mineral, despite claims that phytate binds calcium. Graf and Eaton (1985) also reported no impairment of calcium absorption when 45Ca and phytate was administered to mice by a gavage feeding.

In humans, the role of phytate or fiber on calcium balance is less clear. Berlyne et al. (1973) found that the consumption of unleavened whole-wheat bread high in fiber and phytate caused osteomalacia in adults. Reinhold et al. (1973) also showed that eating a diet with this unleavened bread caused a negative calcium balance of 200 to 300 mg/day; however, they found that feeding an equivalent amount of purified phytate had a negligible effect on calcium balance which suggests that the fiber in the whole-meal reduced calcium absorbance. Contrary to these results, King et al. (1982) reported that young men fed an egg white formula high in fiber or phytates had a significant increase in fecal calcium and a negative calcium balance when fed the high fiber diet.

Kies et al. (1985) conducted a series of studies at the University of Nebraska in which a total of 285 human subjects were fed different fiber sources, including wheat bran, pectin, and cellulose. Although all of the fibers caused some increase in fecal calcium, the wheat bran caused a significant fecal calcium loss (not urinary) and a negative balance. When Cummings et al. (1979) added 52 g of

fiber to a meat diet providing 136 g of protein, the subjects went from a positive to a negative balance, even though calcium intake was 1302 mg.

On the other hand, King et al. (1982) who studied the fecal mineral excretion of 10 young men fed diets high in fiber (0.5 g/kg) which consisted of cellulose, pectin, and corn bran or phytate (13 g/day) , reported that phytate, not fiber, caused a significant increase in fecal calcium and a subsequent negative balance.

Conducting two metabolic balance studies with 22 men, Morris and Ellis (1985) wanted to explore the effects of phytate further by varying the levels of phytate and calcium in an ordinary diet; the role of fiber was not discussed. The authors found that the higher the molar ratio of phytate to calcium, the lower the absorption of calcium. With high intakes of calcium (1100 mg), the subjects absorbed calcium equally well when fed the high, medium, and low levels of phytate; however, when the calcium intake was low (740 mg), calcium absorption decreased proportionately with the increasing phytate levels.

Although the calcium-phytate complex is unabsorbable, Allen (1982) reports that phytate is digested in the lower intestine to varying degrees in humans, possibly releasing calcium. Furthermore, Kies (1985) believes that fiber itself inhibits the digestion of phytate, thus preventing the release of calcium. She found increased phytate excretion with high-fiber diets, as opposed to lower ones.

Thus, it may be that the combination of a high fiber and phytate diet is more detrimental to calcium absorption than either of these components alone.

Magnesium

Metabolism

In spite of the fact that magnesium is the fourth most abundant cation in the mammalian body after calcium, sodium, and potassium, comparatively little is known about its regulation. Sixty percent of the magnesium in the body is located in the bone, as part of the crystalline mineral and the hydrated crystal surface. Most of this magnesium is not freely exchangeable, as are calcium and phosphorous (Wacker, 1980). The remainder of magnesium is distributed in the soft tissues and fluids where it is essential for over three hundred different enzyme systems. It is indispensible to the metabolism of ATP and, consequently, many metabolic processes such as glucose utilization and synthesis of fat, protein, and nucleic acids. Magnesium also plays a role in neuromuscular transmission and activity, acting in concert with, or against the effects of calcium (Linder, 1985).

Magnesium absorption ranges from 20 - 40% and occurs throughout the small intestine, although the jejunum and ileum appear to be the major sites of absorption. The percentage of magnesium an individual absorbs rises and falls inversely with magnesium intake; with a very low dietary intake of magnesium (< 1 mmol/d), 75% of the ingested magnesium was absorbed in adult men, while a high intake (25 mmol/d) resulted in a 24% decrease in absorption (Aikawa, 1981). Various studies in animals have suggested that magnesium and calcium are transported across the

intestinal wall and into the renal tubule cells by the same mechanism (Hendrix, 1963; MacIntyre, 1960); although the data is not conclusive (Wacker, 1980). There is no unequivocal evidence that magnesium is actively transported across the intestinal wall (Aikawa, 1981). Thus, the net amount of dietary magnesium absorbed is directly related to intake, the time available for absorption from the small intestine, and the magnesium status of the individual. High intakes of calcium, vitamin D, protein, fats, and alcohol tend to decrease absorption. Phytates may also interfere with magnesium absorption by forming a complex with magnesium in the intestinal lumen (Prasad, 1978).

Secretions of magnesium into the intestinal tract from the bile and from pancreatic and intestinal juices are followed by almost complete reabsorption. About one-tenth to one-third of the ingested magnesium is excreted through the kidney, which is the major regulator of body magnesium content. As with phosphorous, homeostasis occurs via adjustment of urinary excretion: when dietary intake of magnesium is increased or decreased, urinary excretion is increased or decreased respectively without any significant changes in the magnesium plasma level (Aikawa, 1981). Actively reabsorbed from the proximal and distal tubule, magnesium competes with calcium for transport site. PTH tends to enhance tubular reabsorption of magnesium, although the relationship between magnesium, PTH, and calcitonin is not well understood (Wacker, 1980).

The feces do not contain appreciable quantities of

endogenous magnesium, but average about 200 mg from unabsorbed dietary magnesium. Excretion of magnesium also occurs in sweat. When individuals are exposed to high temperatures for several days, 10 - 15% of the total output of magnesium is recovered in sweat (Aikawa, 1981).

From results of balance studies carried out on healthy young adults, the National Research Council (1980) has stated that the recommended dietary allowance for magnesium is 350 mg/day for adult men and 300 mg/day for adult women, with an extra 150 mg/day in pregnancy and lactation. Contrary to these findings, Seelig (1986) in an extensive review of balance studies presented in the literature, observed that 83% of the men and 73% of the women who consumed the RDA intake or below, were in negative balance. She suggests that the daily recommended magnesium intake should not be less than 6 mg/kg/day, and can be as high as 7 to 10 mg/kg/day.

Magnesium is widely distributed in foods: whole grains, nuts, seeds, green vegetables, and cocoa are especially rich sources. However, refining and cooking may substantially diminish the magnesium content. When the wheat germ and bran are stripped away to make refined white flour, nearly 85% of the magnesium is also stripped. In addition, individuals with declining calorie needs have problems obtaining optimal quantities of magnesium (Passwater and Cranton, 1983).

Significant magnesium deficiency in normal individuals due to simply dietary restrictions is not common because of

the exceedingly efficient renal mechanisms for conservation. Acute magnesium deficiency probably develops only in pathological conditions and during prolonged dietary insufficiency. Magnesium deficiency has been discussed as a possible contributory factor in the development of atherosclerosis, myocardial damage, hypertension, and kidney stones (Aikawa, 1981).

The Effect of Protein Level on Magnesium Bioavailability

Whether the amount of protein in the diet causes positive or negative magnesium balance seems to depend on the relative amounts of each in the diet. Male subjects on a low magnesium intake and a very low protein intake had improved magnesium retention when protein intake was increased to a marginally adaquate level (Seelig, 1986). Mahalko et al. (1983) observed no significant difference in magnesium balance in men fed a conventional diet with protein intakes of either 65 or 94 g protein. The mean magnesium intake of 243 mg was adaquate for positive balance. In a one-year study, Lakshmanan et al. (1984) observed conflicting balance results in thirty-two men and women who consumsumed self-selected diets. A significant positive effect of protein level which ranged from 27 - 96 g and magnesium absorption was seen in young women under the age of thirty-five, while a negative effect was seen women over thirty-five. There was no significant correlation between the protein level and magnesium absorption in the group of males, even though their protein intakes

ranged from 58 - 149 g.

The Effect of Protein Source on Magnesium Bioavailability

Animal Studies

Researchers have observed that magnesium utilization in rats fed soy protein was equivalent to those fed the highly available organic magnesium carbonate (Forbes, 1979; Erdman, 1980). In agreement, Lo et al. (1980) reported no significant changes in magnesium bioavailability in rats fed incremental levels of magnesium carbonate added to isolated soy protein, casein, or beef protein. Using invitro studies, Geunter and Sell (1974) reported that magnesium availability in soybean meal was as high as in skim milk.

In contrast, Forbes et al. (1964) found that magnesium absorbance, but not balance, was reduced in young rats fed isolated soy protein diets in comparison to those fed casein and egg white diets.

Human Studies

Like calcium, a limited number of human studies have been conducted which tests the effect of protein source on magnesium absorption. Stephenson et al. (1970) conducted a twenty-seven day study with ten women who were fed diets containing 5.7 mg magnesium per kg body weight and 3.79 g nitrogen daily from beef, casein-lactalbumin or peanut flour. Mean positive nitrogen balances were maintained throughout the study. A significantly higher positive mean magnesium balance was found in the subjects consuming the

casein-lactalbumin diet than those fed the beef or peanut flour diet. Balance did not vary between the beef and peanut flour period.

In adolescent girls fed diets in which defatted soy protein was substituted for 0, 15, or 30% of the meat in lunch menus, Greger et al. (1978) found no significant effects of soy protein on magnesium retention; however, twelve of the fourteen girls were in negative balance. The authors point out that the magnesium requirement of adolescents, who had daily magnesium intakes of 3.3 to 5.6 mg/kg/body weight, appear to be higher than that of adults. In agreement, Van Stratum and Rudrum (1979) observed no changes in magnesium retention in adult men fed a conventional diet with 25% of the meat substituted with soy isolate. Sandstom et al. (1986) also reported no impairment of magnesium absorption in ileostomy patients fed a standard diet replaced with 25% soy protein.

The Effect of Fiber on Magnesium Bioavailability

Like the other minerals studied, high levels of fiber have conflicting effects on magnesium retention. In a one study, magnesium intake and fecal and urinary excretion increased significantly when the amount of dietary fiber (wheat bran) was increased in the diet of young men; however, there was no effect of fiber on magnesium balance (Van Dokkum et al, 1982). Drews et al. (1979) reported that magnesium retention was significantly lower in adolescent boys whose diet was supplememtned with 21 g of

hemicellulose, as opposed to 21 g of pectin or cellulose. In another study, men fed high levels of fiber (55.2 g/day) from fruits and vegetables had significantly lower balances than men given a low fiber (9.2 g/day) diet (Kelsay et al., 1979). In agreement, Reinhold et al. (1976) found that magnesium absorption decreased significantly in men fed wheat bread than those fed the same amount of white bread, even though the magnesium intakes were nearly double from the wheat bread.

The Effect of Phytate on Magnesium Availability

Very few animal or human studies have investigated the effects of phytate on magnesium absorption. In rats, Lo et al. (1980) observed that magnesium bioavailability was equivalent in two groups of rats fed a soy protein diet with phytate intact and one in which the phytate was autoclaved and destroyed.

Conducting a sixty-three day metabolic study with adult men, King (1982) reported that phytate, not fiber, significantly increased fecal magnesium, causing negative balances in the subjects. The men were fed an egg-white diet formula with either cellulose (0.5 g/kg) or phytate (3 g/day) added.

The Effect of Calcium on Magnesium Bioavailability

Animal Studies

Nugara and Edwards (1963) observed that the magnesium requirement of the chick increased from 461 to 594 ppm with

increased intakes of calcium. Chico et al. (1973) conducted a series of experiments with growing sheep in which high dietary levels of calcium depressed magnesium levels in bone and plasma. Excess dietary magnesium reduced plasma calcium and appeared to increase fecal calcium excretion.

Human Studies

In humans, increasing the calcium intake does not seem to have any striking effect on magnesium balance in subjects on normal or high magnesium intake. Spencer et al. (1986), by using both the balance method and doses of radioisotope magnesium, observed that high calcium intakes of up to 2000 mg/day does not interefere with the intestinal absorption of magnesium in healthy adult men. Observations by Greger et al showed that a high intake of calcium (2382 mg) as compared to a low calcium intake (780 mg) had no significant effect on magnesium retention. Lakshmanan et al. (1984) also found that increased daily intakes of calcium (265 - 1080 mg/day) had little effect on magnesium balance in women consuming self-selected diets.

On the other hand, Seelig (1964) presented data indicating that magnesium balance of men decreased at calcium intakes above 10 mg/day, but excretions were unaffected. In agreement, Linkswiler and Kim (1980) found that increasing the calcium intake from 800 to 2400 mg /day significantly decreased magnesium retention; however, there was no effect on urine excretion. In college women, Leichsenring et al.

(1951) observed that there was a significant negative effect of dietary calcium on urinary magnesium excretion when a diet containing 260 mg magnesium, 300 mg calcium and 800 mg phosphorous was supplemented to provide 1500 mg of calcium and 1400 mg of phosphorus. Magnesium balance did not significantly change, however.

Chapter III

Experimental Procedure

Experimental Design

This experiment was designed to test the effect of three sources of protein: soy, dairy, and meat protein, and two levels of calcium on zinc, iron, copper, calcium, and magnesium retention in young adult males. Changes in retention of the minerals under the different dietary conditions were observed to gain insight into the nutrient intakes necessary to achieve optimal balance in this specific age group. The study also assessed the nutritional status of the subjects with respect to the minerals by measuring the concentrations of minerals in the plasma.

Subjects

Twenty-four healthy young men between the ages of 20 and 34 years were recruited by posters and flyers placed on the campus of Virginia Tech in Blacksburg (Appendix I and II). The subjects were screened by obtaining information about age, weight for height and age, dietary habits and health practices, as well as a personal and family history of health problems (Appendix III). Individuals were confirmed to be in good health by a clinical evaluation performed by a physician at the University Health Center. Once accepted in the study, the interested young men were given a written and oral explanation of the experimental procedure and the outline of objectives and design

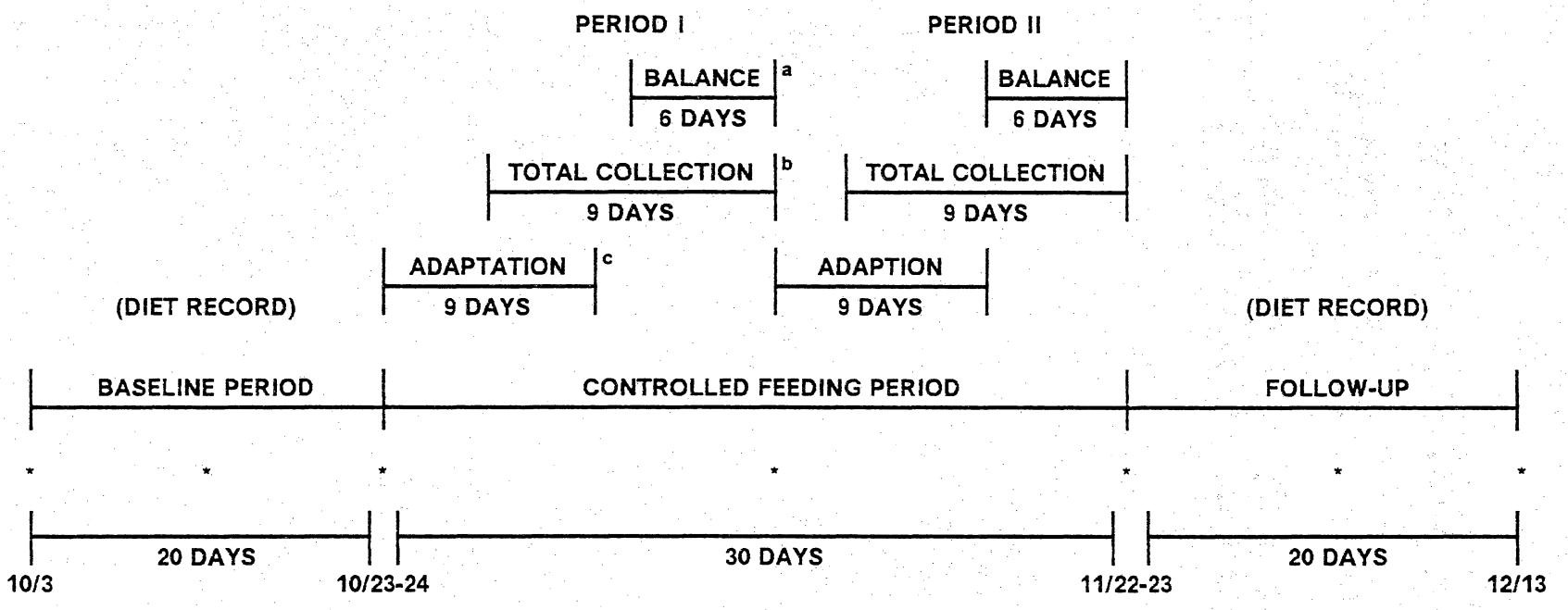
(Appendix IV), and signed the necessary consent form, as required by the Institutional Review Board for Research with Human Subjects of Virginia Tech (Appendix V). All subjects understood the importance of their commitment to the study and their strict adherence to the diet provided and the experimental procedures.

Design

The metabolic study was seventy-two days in duration with a twenty day baseline period, a thirty day controlled feeding period and a twenty day follow-up period (Table 1, Appendix VI). During the pre- and post-treatment period, the subjects consumed their normal dietary intake. They recorded two separate five-day food records which were used to gain information about a subject's usual diet and caloric intake (Appendix VII). During the controlled feeding period, the twenty-four subjects were randomly assigned to one of three dietary treatment groups which differed in respect to protein source. Subjects remained on the same dietary treatment throughout the controlled feeding period and were instructed to consume only the foods and drink provided by the metabolic unit.

To test the effect of calcium level on mineral retention, the controlled feeding period was divided into two periods: Period I, in which the subjects consumed moderate levels of calcium (mean= 1206.77 $\mp$ 193.29 mg/day) and Period II, in which the subjects consumed high levels of calcium (mean = 2134.51 $\mp$ 164.63 mg/day). Balance data were

TABLE 1



[a]November 2-8 (PERIOD I); November 17-23 (PERIOD II)

[b]October 30-November 8 (PERIOD I); November 14-23 (PERIOD II)

[c]October 23-November 2 (PERIOD I); November 8-17 (PERIOD II)

*Blood samples, blood pressure, and weight taken every two weeks

determined from the fecal, urine and food composites of the last six days of the nine-day collection; the first three days of collection allowed subjects to practice collecting excreta, minimizing any problems or mistakes. Two nine-day adaption periods preceded the two nine-day collection periods, respectively. Blood samples, as well as blood pressure and body weights were taken every two weeks.

All food for the subjects was prepared, weighed, and served in the metabolic unit. No water other than the deionized water provided by the study could be consumed during the controlled feeding period. The kitchen staff were very careful in measuring and preparing exact portions of food to the nearest g to insure that appropriate foods were eaten by the subjects at each meal. This was noted by the subjects who filled out a meal card at each meal which was checked and initialled by the meal supervisor (Appendix VIII). Attendance sheets were also signed for each subject at each meal and proper procedures were carried out for food spills and food refusals (Appendix VIII). Daily status sheets were filled out at breakfast by all subjects in order to have a record of any illness, medications taken, or errors in sample collection. Subject weights were recorded daily and monitored for weight loss or gain so caloric intake could be adjusted accordingly.

Throughout the study, the subjects were required to collect all urine and fecal excretion in containers provided by the investigators. Acid-washed and labeled urine bottles were picked up each morning after breakfast

during the collection period and used during the entire day, then returned the next morning at breakfast. Plastic-lined fecal containers were left in designated recepticals throughout the day.

Diets

All diets were isocaloric and isonitrogenous between treatments and provided all nutrients at, or above, recommended levels of intake (Table 2, Appendix IX). The three dietary treatments consisted of (1) a dairy product diet, providing 70% of the 70 g of dietary protein from dairy products, (2) a soy product diet providing 67% of the 70 g of dietary protein from soy products, and (3) a meat product diet providing 70% of the 70 g of dietary protein from red meat products. Other protein sources were held to a minimum to achieve the desired goal of providing different protein sources with all other nutrient variables remaining constant.

The diets consisted of conventional foods that were prepared with deionized water. Three menus were served for each dietary treatment and cycled every three days throughout the study. (Appendix X). For subjects who required calories in excess of those provided by the base metabolic diet, jelly beans, as well as food supplements, consisting of bread and butter/margarine, were given. The number of supplement units needed by individual subjects differed, although the supplements remained consistent across periods (Appendix XI).

TABLE 2

NUTRIENT CONTENT OF DAILY DIETS[1]

	DAIRY	SOY	MEAT
CALORIES (kcal)	3406	3529	3172
TOTAL PROTEIN (g)	74 (9%)[2]	77 (9%)	69 (9%)
DIETARY TREATMENT PROTEIN (g)	52	52	49
% DIETARY TREATMENT PROTEIN	70	67	70
FAT (g)	149 (39%)	149 (38%)	142 (40%)
P/S RATIO*	0.42	0.44	0.51
CARBOHYDRATES (g)*	443 (52%)	469 (53%)	405 (51%)
CHOLESTEROL (mg)*	507	506	484

[1]Food supplements included

[2]( ) percent of total calories

*Composition of Foods, 1980.

The diets, as well as the food and mineral supplements supplied the total intake of minerals (Table 3). During the first period, calcium supplements were given to subjects in the soy and meat treatment groups only, for the purpose of balancing the higher calcium intakes derived from the dairy diet. During the second period, subjects in all treatment groups received calcium supplements, although the amounts varied again, in order that total intakes were balanced across dietary treatments. The calcium supplements in period II also provided 10 mg of zinc and 266.6 mg of magnesium since high intakes of calcium have been found to have a negative effect on both zinc and magnesium absorption (Sandstrom et al., 1980; Seelig, 1964). Zinc supplements (10 mg) were also given to all subjects in the first period in order that the supplemental zinc intake was equal for both periods. To ensure that recommended dietary allowances for all vitamins were met, a One-A-Day multivitamin tablet was given daily at breakfast.

Sample Collection, Preparation, and Analysis

Diet

Prior to meal preparation, six clean trays, two for each of the three diet groups, were set up and appropriately labeled with the diet group (Dairy, Soy, Meat), diet day (1,2,3), and duplicate letter (A,B). After all the meals were served to the subjects, the composite trays were set up with all the items indicated on the meal card. Care was taken to scrape all the food contents from the dupli-

TABLE 3

TOTAL MINERAL INTAKE FROM DIETARY TREATMENT, FOOD SUPPLEMENTS, AND MINERAL SUPPLEMENTS

	DIETARY TREATMENT			FOOD SUPPLEMENTS			MINERAL SUPPLEMENTS			TOTAL INTAKE		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
CALCIUM (mg)												
PERIOD 1	1061	923	531	192	226	126	—	250	300	1279	1399	942
PERIOD 2	1078	918	549	190	223	113	917	1167	1267	2182	2308	1913
MAGNESIUM (mg)												
PERIOD 1	165	291	172	50.7	60.7	32.9	—	—	—	215	352	199
PERIOD 2	167	289	187	50.7	60.7	24.4	266	266	266	484	617	473
ZINC (mg)												
PERIOD 1	5.4	7.8	10.1	1.2	1.3	0.73	10	10	10	16.6	19.1	20.7
PERIOD 2	8.2	9.9	11.2	1.0	1.3	0.64	10	10	10	19.1	21.1	21.7
IRON (mg)												
PERIOD 1	6.9	14.7	7.5	4.4	6.1	3.0	—	—	—	11.5	20.8	10.2
PERIOD 2	8.8	12.3	10.1	4.3	6.0	2.6				13.2	18.3	12.4
COPPER (mg)												
PERIOD 1	0.72	1.2	0.80	0.21	0.33	0.12	—	—	—	0.93	1.5	1.0
PERIOD 2	0.43	1.0	0.52	0.23	0.33	0.12				0.67	1.3	0.62

cate dairy, meat, or soy meal into two separate plastic-lined labelled containers each meal. At the end of the day, after all three meals for each treatment had been added to the collection container, the contents were emptied separately into a tared five-quart Waring blender and homogenized with the addition of deionized water. Weight of the blender contents were recorded after adding water. After the food was blended at high speeds for 5 minutes, five percent samples (about 150-200 gms) were taken immediately from each duplicate meal, stored separately in two one-liter acid washed plastic bottles, and frozen overnight. This process was repeated each day until food samples for the entire collection period (6 days) were added to each one-liter bottle. All jars were labelled with the appropriate period, dietary treatment, and date, and frozen until further analysis (Appendix XIII). A total of six food composite bottles were filled during each period with duplicate samples of the dairy, soy, and meat diet.

At the conclusion of the study, food samples were thawed, mixed and analyzed for mineral content using atomic absorption spectrophotometry. Food composites were prepared for mineral analysis as follows: 1.5-2.0 g duplicate samples of the homogenized food were weighed into 100-ml acid-washed glass beakers, covered with acid-washed watchglasses, and dried overnight at 60°C. 1.5 milliliters of redistilled nitric acid and 1.0 milliliter of 70 % reagent grade perchloric acid were added carefully to the beakers and the samples were allowed to digest on a hot

plate in the perchloric hood. The temperature of the hot plate was initially 150°F, but then increased slowly to 325° F to allow samples to reflux in the perchloric acid. Beakers were watched closely at all times and temperatures were modulated to maintain a controlled reaction. When samples were completely digested, i.e. when all organic matter had been oxidized and dense white fumes generated, the watch glasses were removed to allow the remaining liquid to evaporate. Once the dried samples were removed from the hotplate and allowed to cool, the ash was rehydrated with 10 milliliters of 10% 6 M hydrochloric acid. To prevent contamination, all beakers were covered with parafilm, then allowed to sit for approximately thirty minutes, until the minerals dissolved in the acid. The resulting solution was then transferred to contaminant-free, labeled plastic tubes, sealed, and then refrigerated until mineral determination on the atomic absorption spectrophotometer.

Samples were appropriately diluted with redistilled hydrochloric acid for determination of zinc, iron, and copper (1:10), and for determination of calcium and magnesium (1:100). In addition, sample dilutions of calcium and magnesium contained 0.3% lanthanum to prevent phosphate interference with calcium absorption. Absorbances were measured by aspiration into a Perkin-Elmer Model 406 Atomic Absorption Spectrophotometer. The fuel for the spectro-photometer was acetylene and the oxidant was air (Appendix XIII). Mineral concentrations were calculated by comparison

of sample absorbances to a standard absorbance curve using linear regression.

Food samples were also analyzed in duplicate for nitrogen using a modified Kjeldahl-Gunning-Arnold method (A.O.A.C., 1970) , (Appendix XIII).

Urine

During each collection period, the subjects collected urine into labelled, wide-mouthed, acid-washed, one-liter polyethylene bottles. All clean bottles contained 10 ml of 10% hydrochloric acid to prevent bacterial growth. Collections for both periods were made on a twenty-four hour basis beginning after the first voiding of one day and including the first voiding of the following day. When a problem arose, detailed notes were taped to the urine specimen and logged if necessary.

Every morning of each collection period, subjects brought in their twenty-four-hour urine sample which was composited daily. The urine from each collection bottle was emptied into a 2000 ml acid-washed plastic graduated cylinder and the volume was recorded. Deionized water was used to bring urine volume up to 2 or 3 liters depending on the volume of the sample. The diluted urine was mixed in a plastic acid-washed gallon jug by securing the lid and inverting five times. Duplicate 5% aliquots were then measured in an acid-washed 100 ml graduated cylinder and poured into two separate one-liter acid-washed polyethylene bottles labelled with the subject's name and number. Daily

aliquots of urine were pooled for each period and frozen (-20°C) for future mineral analysis.

Before they were returned to the subjects, the urine bottles were washed three times in hot tap water and then two times in deionized water. Uric acid deposits were removed from the bottle using the alkaline cleanser Alconox. To prevent cross-contamination, the graduated cylinders were also washed thoroughly for use between each subject.

For mineral analysis, frozen pooled urine composites were thawed and mixed. Undiluted samples were directly aspirated into the atomic absorption flame for determination of zinc. For both calcium and magnesium determinations, samples were diluted with a solution of 0.3% lanthanum and 10% hydrochloric acid at 1:80 and 1:200 respectively. Due to the negligible amounts of iron and copper in the urine, these minerals were not measured (Appendix XIII).

Feces

All feces were collected in 1-pint or 1-quart cardboard plastic-lined cups (mineral-free) labeled with the date, time of collection and subjects' name and number. Each day of the two collection periods, used fecal containers were collected from designated recepticles at the site of study and frozen (- 20°C) until the end of the study at which time they were composited for each subject. Upon completion of the study, the subject's fecal samples from the first six-day collection period were weighed in a tared

five quart blender, then partially thawed and thoroughly blended for about five minutes with the addition of deionized water. As with the procedure for food composites, two duplicate five percent aliquots of the homogenized fecal composite (125 g) were added to two 250 ml acid-washed polyethylene bottles and frozen (- 20°C) for subsequent mineral analysis. To avoid contamination, rubber gloves were worn throughout the procedure and the blenders were washed thoroughly with deionized water between samples. The same procedure was performed for the second collection period.

Mineral content was determined by the same procedures described for food. Absorbances for iron and copper were read without making dilutions, while zinc was diluted to 1:100 using 5 % redistilled hydrochloric acid as diluent. Samples for calcium and magnesium analysis were both diluted at 1:1000 with a 0.3% lanthanum and 5% hydrochloric acid solution. Mineral analysis of samples were repeated for food, urine, and fecal samples if the values obtained for duplicates differed by amounts greater than 5%. Recovery samples consistently run at intervals in this laboratory indicated recoveries between 95 and 99% (Appendix XIII).

Blood

Two blood samples were taken after a 14-hour fast from each subject by a registered medical technician. Approximately 20 milliliters of blood were withdrawn from the

antecubital vein using a vacutainer, which was treated for trace mineral analysis, and a 1 1/2 inch, 21 gauge needle. Blood was taken over a period of 3 to 4 minutes with minimum vacuum to reduce hemolysis. The blood was then placed into two heparinized plastic tubes which contained plastic beads to facilitate mixing and also minimize hemolysis. The tubes were inverted several times to mix the blood and the anticoagulant, and centrifuged for 25 minutes at 2000 x g. The plasma was carefully pipetted off the red cells into plastic tubes and frozen (-20°C) for mineral analysis.

Plasma was diluted to 1:1 and 1:5 for copper and zinc, respectively, using deionized water. Standards for copper and zinc analysis were made to final concentrations of 10 and 5% glycerol, respectively, in order to match the viscosity of the diluted plasma. In atomic absorption, less sample will enter the flame with increasing viscosity resulting in lowered absorbance readings. Dilutions of 1:50 in 0.3% lanthanum were made for the determination of calcium and magnesium. Due to the high content of iron in the blood, plasma samples were wet-ashed before mineral determination. No dilutions were made.

Statistical Analysis

An analysis of variance procedure (SAS) was used to determine significant differences among the three dietary treatments, across the two periods, and to identify the effect of interaction between dietary treatment and period

on mineral urine and fecal excretion and retention. Student's t-tests ($p < 0.01$) were used to locate the significant differences based on the results of the ANOVA ($p < 0.05$). All means are expressed as Least Square Means due to unequal observations per group. Absorption and retention values were derived from measured data in the following manner:

$$\text{Apparent Absorption (\%)} = \frac{\text{Intake - Fecal excretion}}{\text{Intake}} \times 100$$

$$\text{Retention} = \text{Intake} - (\text{Fecal} + \text{Urinary Excretion})$$

Chapter IV

Results

Descriptive Data of Subjects

The subjects, all adult males, ranged in age from 20-34 years. Pre-, mid-, and post-study weights remained constant, with means of 73.8 ± 11.7, 73.6 ± 11.5, and 74.0 ± 11.1 kg, respectively (Appendix II). All twenty-four males completed the study; however, data for subject #5 were deleted from the results due to several incomplete urine and fecal collections. His aberrant retention values were also affected by his weight lifting activity to which he devoted two to four hours per day (Appendix XV - XIX). As a result of his exclusion, data from seven subjects were analyzed for the meat treatment group, as compared to eight subjects for the other treatments.

Medications taken by the subjects are listed in Appendix XIV. Subject #4 took two prescribed medications: lithium and tetracycline. The latter has an inhibiting effect on mineral absorption. In fact, the subject was in negative balance for all the minerals during the first period, and in negative balance for iron and copper during the second period. Subject # 9 also took tetracycline for two days, but it probably had little effect on mineral balance since it was not taken during a balance period. Other medications taken included aspirin and other over-the-counter drugs used for cold, cough, and headache relief. According to Colin (1983), none of these contained

significant levels of minerals that might increase total mineral intake.

Nutrient Intakes

The nutrient content of the diet was originally calculated to provide fats, carbohydrates, and protein in a ratio of 40:50:10, respectively, and dietary treatment protein as 70% of the total protein. Table 3 shows that the actual determined percentages were very close to the desired goals and intakes were consistent across treatment groups. The calories, which were based on the energy needs of the individuals, varied slightly among treatment groups due to the addition of food supplements, which were given to subjects for weight maintenance throughout the study. The polyunsaturated:saturated fat ratio, cholesterol, and dietary fiber were also calculated to be consistent across groups, although slight variations occurred.

Zinc Intake

Mean values for zinc intake, excretion, retention, and absorption are shown in Table 4 and individual values are shown in Appendix XV. Mean daily zinc intakes which ranged from 16.5 to 21.7 mg $\pm$ 0.2 mg were just above the recommended dietary allowance of 15 mg/day. Zinc intakes remained constant among the three treatment groups for a period II; however, zinc intake from the dairy diet in period I was significantly lower than that of the soy or meat diet in period I or the dairy diet in period II ($p < 0.01$).

TABLE 4

MEAN ZINC INTAKE, URINARY AND FECAL EXCRETION, RETENTION, AND APPARENT ABSORPTION IN SUBJECTS CONSUMING TWO LEVELS OF CALCIUM AND THREE SOURCES OF PROTEIN

	PERIOD I			PERIOD II			POOLED MEAN		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
INTAKE	16.6[a1]*	19.1[b]	20.7[b]	19.1[2]	21.1	21.7	17.9[a]	20.1[b]	21.2[b]
mg/day	±0.15	±0.15	±0.16	±0.15	±0.15	±0.16	±0.11	±0.11	±0.11
URINE	0.87	0.81	0.59	0.91	0.74	0.61	0.89	0.78	0.60
mg/day	±0.15	±0.15	±0.16	±0.15	±0.15	±0.16	±0.10	±0.10	±0.11
FECES	14.7	17.2	16.0[1]	12.3[a]	16.5[b]	21.6[c2]	13.5[a]	16.9[b]	18.8[b]
mg/day	±0.84	±0.84	±0.90	±0.84	±0.84	±0.90	±0.59	±0.59	±0.63
RETENTION	1.1[1]	1.1	4.1[1]	6.0[a2]	3.8[a]	-0.5[b2]	3.5	2.5	1.8
mg/day	±0.84	±0.84	±0.90	±0.84	±0.84	±0.90	±0.59	±0.59	±0.64
APPARENT ABSORPTION (%)	11.4	9.9	22.7	35.6	21.8	0.46	24.6	15.9	11.3

*Values reported indicate mean ± SD.

[a]Values with different superscripts within periods are significantly different ($p < 0.01$).

[1]Values with different numbers within the same dietary treatments between periods are significantly different ($p < 0.01$).

Zinc Excretion

Mean daily urinary zinc excretions ranged from 0.6 to 0.9 mg ± 0.2 mg, with individual values ranging from 0.3 to 2.0 mg per day. No significant differences were seen between periods, within treatments, or among treatment groups.

Mean daily fecal zinc excretions ranged from 12.3 ± 0.8 to 21.6 ± 0.9 mg for both periods. Individual values ranged from 8.5 to 26.0 mg/day. Results showed that fecal zinc excretion was higher in subjects fed the meat diet in period II, as compared to those in period I, even though intakes were consistent ($p < 0.01$). No differences in fecal zinc were seen between the dairy or soy groups across periods. When comparing treatment groups within each period, fecal zinc excretion remained constant in period I. There were, however, significant differences among all the treatment groups in period II, even when intakes remained constant. The subjects in the meat group excreted significantly more fecal zinc than those in the soy or dairy group ($p < 0.01$); and subjects fed the soy diet excreted significantly more fecal zinc than those in the dairy group ($p < 0.01$).

Zinc Retention

Mean daily zinc retention values ranged from -0.5 ± 0.9 mg to 5.95 ± 0.8 mg for both periods, with individual values ranging from -4.5 to 9.8 mg/day. Five out of twenty-three subjects were in negative balance during period I and

five different subjects were in negative balance during period II. Individuals fed the dairy diet in period II retained significantly more zinc than those in period I ($p < 0.01$). Significantly less zinc was retained in subjects who consumed the meat diet in period II than those during period I ($p < 0.01$). No significant differences among dietary treatments were found within period I. In period II, however, zinc retention was significantly higher in the dairy group than the meat group ($p < 0.01$), even though zinc intake from the meat diet was higher than that of the dairy diet.

Iron Intake

Mean values for iron intake, excretion, retention, and absorption, are shown in Table 5 and individual values are shown in Appendix XVI. Mean daily iron intakes which ranged from 11.5 to 20.8 $\pm$ 0.6 mg were higher than the recommended dietary allowance of 5 - 10 mg/day for normal men and non-menstruating women. Iron intakes remained constant from the meat and dairy diet for each of the periods; however, iron intakes from the soy diet were significantly higher than the other treatments for both periods.

Iron Excretion

Mean daily iron excretion values ranged from 11.5 to 20.7 $\pm$ 0.9 mg, while individual excretion values ranged from 8.6 to 25.9 mg/day. Because no significant differences were seen for any of the dietary treatments across

TABLE 5

MEAN IRON INTAKE, FECAL EXCRETION, RETENTION, AND APPARENT ABSORPTION IN SUBJECTS CONSUMING TWO LEVELS OF CALCIUM AND THREE SOURCES OF PROTEIN

	PERIOD I			PERIOD II			POOLED MEAN		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
INTAKE	11.4[a]*	20.8[b]	10.2[a]	13.2[a]	18.3[b]	12.4[a]	&12.3[a]	19.5[b]	11.3[a]
mg/day	±0.64	±0.64	±0.68	±0.64	±0.64	±0.68	±0.45	±0.45	±0.48
FECES	11.5[a]	20.7[b]	12.2[a]	12.9[a]	20.4[b]	15.6[a]	12.2[a]	20.5[b]	13.9[a]
mg/day	±0.93	±0.93	±0.99	±0.93	±0.93	±0.99	±0.66	±0.66	±0.70
RETENTION	-0.05	0.08	-2.0	0.27[a]	-2.1	-3.2[b]	0.11[a]	-0.98	-2.6[b]
mg/day	±0.71	±0.71	±0.76	±0.71	±0.71	±0.76	±0.50	±0.50	±0.54
APPARENT ABSORPTION (%)	-0.88	0.48	-19.6	2.3	-11.5	-25.8	0.81	-5.1	-23.0

*Values reported indicate mean ± SD.

[a]Values with different superscripts within periods are significantly different ($p < 0.01$).

periods, pooled values were used in statistical analysis. Subjects in the soy treatment group excreted significantly more fecal iron during both periods than those in the other treatment groups ($p < 0.01$).

Iron Retention

Pooled mean iron retention values ranged from -2.6 to 0.1 $\pm$ 0.5 mg/day, while individual values ranged from -6.7 to 3.9 mg/day, with 17 out of 23 subjects in negative balance during period I and II. There was no significant difference in retention among subjects in the soy group and those in the other two treatments; however, as mentioned earlier, iron intake from the soy group was nearly double that of the dairy or meat group. Yet, subjects in the soy group had a mean negative retention. Iron retention in subjects in the meat group was also significantly lower than in the dairy group ($p < 0.01$).

Copper Intake

Mean values for copper intake , excretion, retention, and absorption for copper are shown in Table 6 and individual values are shown in Appendix XVII. Mean daily copper intakes which ranged from 0.6 to 1.6 $\pm$ 0.04 mg were below the recommended dietary allowance of 2 mg/day. Intakes of copper varied significantly between periods and among dietary treatment groups ($p < 0.01$). Within periods, copper intakes remained constant in the the meat and dairy groups, but copper intakes from the soy group, like that of iron

TABLE 6

MEAN COPPER INTAKE, FECAL EXCRETION, RETENTION, AND APPARENT ABSORPTION IN SUBJECTS CONSUMING TWO LEVELS OF CALCIUM AND THREE SOURCES OF PROTEIN

	PERIOD I			PERIOD II			POOLED MEAN		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
INTAKE	0.93[a1]*	1.6[b]	0.95[a1]	0.67[a2]	1.3[b]	0.62[a2]	0.80[a]	1.4[b]	0.79[a]
mg/day	±0.04	±0.04	±0.04	±0.04	±0.04	±0.04	±0.03	±0.03	±0.03
FECES	0.82[a1]	1.7[b1]	0.91[a]	0.58[a2]	1.4[b2]	0.78[a]	0.70[a]	1.5[b]	0.84[a]
mg/day	±0.07	±0.07	±0.07	±0.07	±0.07	±0.07	±0.05	±0.05	±0.05
RETENTION	0.11	-0.08	0.04	0.09[a]	-0.05	-0.15[b]	0.10[a]	-0.07[b]	-0.06
mg/day	±0.07	±0.07	±0.07	±0.07	±0.07	±0.07	±0.05	±0.05	±0.05
APPARENT ABSORPTION (%)	11.8	-6.3	4.2	13.4	-7.7	-25.8	12.50	-7.1	-6.3

*Values reported indicate mean ± SD.

[a]Values with different superscripts within periods are significantly different ($p < 0.01$).

[1]Values with different numbers within the same dietary treatments between periods are significantly different ($p < 0.01$).

intake, was significantly higher than the other treatments within both periods ($p < 0.01$). Across periods, however, copper intakes in the meat and dairy groups both decreased significantly from period I to II ($p < 0.01$).

Copper Excretion

Mean fecal copper excretions ranged from 0.6 to 1.7 $\pm$ 0.1 mg/day. Individual fecal copper excretions ranged from 0.5 to 2.0 mg / day. Within period I and II, there was no significant difference in excretion between the meat and dairy treatment group; however, like iron, there was a significant increase in fecal copper excretion in the soy group. Significantly less copper was excreted in subjects fed the dairy and the soy diet in period I than in period II.

Copper Retention

Pooled mean copper retention values ranged from -0.1 to 0.1 $\pm$ 0.1 mg/day, with individual values ranging from -0.4 to 0.4 mg/day. Eight out of the 23 individuals in period I and 13 out of 23 subjects in period II were in negative balance, with 5 subjects in negative balance for both periods. Copper retention in subjects consuming the soy diet was significantly lower than that of subjects in the dairy group, although the soy diet contained nearly double the copper levels of the other two treatments.

Calcium Intake

Mean values for calcium intake, excretion, retention, and absorption are shown in Table 7; individual values are shown in Appendix XVIII. Mineral intakes of calcium remained constant within each period, except for the meat treatment group in Period I. Intakes nearly doubled between the two periods , as was determined in the experimental design. All intakes exceeded the recommended dietary allowance of 800 mg/ day.

Calcium Excretion

Mean urinary calcium excretions ranged from 108 $\pm$ 15 to 147 $\pm$ 14 mg/day. Individual values ranged from 72 to 251 mg/day. Excretions of urinary calcium remained constant among dietary treatments and across treatments. No significant differences were seen even when calcium intakes were doubled.

Mean fecal calcium excretions for period I ranged from 653 $\pm$ 70 to 1060 $\pm$ 66 mg/day. In period II, mean fecal calcium excretions ranged from 1340 to 1665 $\pm$ 66 mg/day, a significant increase for all dietary treatments which reflected the high supplemented intakes. Individual means of fecal calcium excretion ranged from 426 to 1205 mg/day for period I and 1080 to 2010 mg/day for period II. Within both periods, fecal calcium excretion was significantly higher in the soy treatment group ($p < 0.01$), even though intakes were not significantly different.

TABLE 7

MEAN CALCIUM INTAKE, URINARY AND FECAL EXCRETION, RETENTION, AND APPARENT ABSORPTION IN SUBJECTS CONSUMING TWO LEVELS OF CALCIUM AND THREE SOURCES OF PROTEIN

	PERIOD I			PERIOD II			POOLED MEAN		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
INTAKE	1278^{a1}*	1399^{a1}	942^{b1}	2182^{2}	2308^{2}	1913^{2}	1731^{a}	1853^{a}	1428^{b}
mg/day	±24.4	±24.4	±26.0	±24.4	±24.4	±26.0	±17.2	±17.2	±18.4
URINE	141	122	108	147	123	119	144	123	113
mg/day	±13.8	±13.8	±14.8	±13.8	±13.8	±14.8	±9.8	±9.8	±10.5
FECES	815^{a1}	1060^{b1}	653^{a1}	1340^{a2}	1665^{b2}	1539^{a2}	1077^{a}	1363^{b}	1096^{a}
mg/day	±65.7	±65.7	±70.3	±65.7	±65.7	±70.3	±46.5	±46.5	±49.7
RETENTION	323^{1}	216^{1}	181	696^{a2}	520^{a2}	256^{b}	509	368	218
mg/day	±64.4	±64.4	±68.9	±64.4	±64.4	±68.9	±45.6	±45.6	±48.7
APPARENT ABSORPTION (%)	36.2	24.2	30.6	38.6	27.9	19.6	37.8	26.4	23.2

*Values reported indicate mean ± SD.

[a]Values with different superscripts within periods are significantly different ($p < 0.01$).

[1]Values with different numbers within the same dietary treatments between periods are significantly different ($p < 0.01$).

Retention

Retention means varied from 180 ± 68 to 322 ± 64 mg/day for period I and increased to 256 to 695 ± 69 mg/day for period II. Individual values during period I ranged from -25 to 528 mg/day, with only two subjects in negative balance, one of whom was subject # 4 who took the tetracycline medication. All subjects were in positve balance in period II with values ranging from 23 to 974 mg/day. Retention values increased significantly in period II from period I for subjects in the dairy and soy treatments ($p < .01$). Although retention values for the meat group were also higher in this period, there was no significant difference. Within periods, the dietary treatments had no effect on calcium retention in period I. In period II, retention was significantly lower in subjects fed the meat diet than in those fed the other treatments.

Magnesium Intake

Mean values for magnesium intake, excretion, retention, and absorbance are shown in Table 8 and individual values are shown in Appendix XIX. Magnesium intake increased significantly from period I to period II because of the magnesium supplement given in the second period. Mean magnesium intakes for period I ranged from 199 to 352 ± 8 mg/day, with most subjects consuming intakes below the recommended dietary allowance of 350 mg/day. Mean intakes for period II ranged from 484 to 617 ± 8 mg/day which were well above the RDA for adult males.

TABLE 8

MEAN MAGNESIUM INTAKE, URINARY AND FECAL EXCRETION, RETENTION, AND APPARENT ABSORPTION IN SUBJECTS CONSUMING TWO LEVELS OF CALCIUM AND THREE SOURCES OF PROTEIN

	PERIOD I			PERIOD II			POOLED MEAN		
	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT	DAIRY	SOY	MEAT
INTAKE	216^{a1}*	352^{b1}	199^{a1}	484^{a2}	617^{b2}	471^{a2}	350^{a}	484^{b}	336^{a}
mg/day	±7.8	±7.8	±8.3	±7.8	±7.8	±8.3	±5.5	±5.5	±5.9
URINE	113	120	95.9	121	127	115	117	124	106
mg/day	±8.0	±8.0	±8.5	±8.0	±8.0	±8.5	±5.7	±5.7	±6.0
FECES	154^{a1}	306^{b1}	179^{a1}	257^{a2}	409^{b2}	350^{c2}	206^{a}	358^{b}	265^{c}
mg/day	±12.9	±12.9	±13.7	±12.9	±12.9	±13.7	±9.1	±9.1	±9.7
RETENTION mg/day	-51.9^{a1} ±16.4	-74.6^{a1} ±16.4	-76.1^{a1} ±17.6	106^{a2} ±16.4	80.4^{a2} ±16.4	6.9^{b2} ±17.6	26.8 ±11.6	2.9 ±11.6	-34.6 ±12.4
APPARENT ABSORPTION (%)	28.7	13.1	10.1	46.9	33.7	25.7	41.1	26.0	21.13

*Values reported indicate mean ± SD.

[a]Values with different superscripts within periods are significantly different ($p < 0.01$).

[1]Values with different numbers within the same dietary treatments between periods are significantly different ($p < 0.01$).

Excretion

Mean urinary magnesium values ranged from 96 ± 9 to 127 ± 8 mg/day for both periods. Individual values ranged from 73 to 163 mg/day for both periods. Urinary excretion of magnesium was constant across periods and within dietary treatments.

For period I, mean excretions of fecal magnesium ranged from 154 to 306 ± 13 mg/day. For period II, mean values ranged from 257 ± 13 to 409 ± 14 mg/ day which are significantly higher than those of period I ($p < 0.01$). Individual values ranged from 114 to 359 mg/day for period I and 172 to 445 mg/day for period II. Mean fecal magnesium excretion was significantly higher between periods for all treatment groups. Within periods, fecal excretion of magnesium was significantly higher in the soy groups than either of the other treatments. This reflects the increased intake of magnesium in the soy diet. In period II, excretion of magnesium was significantly higher in those subjects consuming the meat diet than those on the dairy diet, even though intakes were essentially the same.

Retention

In period I, mean retention values of magnesium ranged from -52 ± 16 to -76 ± 18 mg/day, with individual values ranging from -132 to 21 mg/day. Twenty-two of the 23 subjects were in negative balance. Retention means for period II ranged from 7 ± 18 to 106 ± 16 mg/day, with individual values ranging from -7 to 194 mg/day. Only 4 out

of the 23 subject were in negative balance in Period II. There was a significant increase in magnesium retention across periods for each dietary treatment ($p < 0.01$). Within groups, mean magnesium retention remained constant among the three diets in period I; retention in period II was significantly lower in subjects fed the meat diet than those fed the soy or dairy diets.

Plasma Mineral Concentrations

Plasma zinc, copper, iron, calcium, and magnesium were measured at the beginning, the middle, and the end of the 30-day collection period (Table 9). No significant differences were seen among the mean plasma values at the different times within or between the specific dietary treatment groups. Four of the five minerals were within normal plasma range: calcium (8.5 - 10.5 mg/dl), magnesium (1.4 - 3.7 mg/dl), zinc (75 - 125 ug/dl), and iron (60 - 200 ug/dl), while plasma copper was below the normal levels of 110 - 160 ug/dl.

TABLE 9

MEAN PLASMA MINERAL VALUES

DIETARY TREATMENT	TIME*	CALCIUM mg/dl	MAGNESIUM mg/dl	ZINC μg/dl	IRON μg/dl	COPPER μg/dl
DAIRY	1	9.2† ±0.15	1.9 ±0.05	96.4 ±3.5	167 ±13.7	82.8 ±5.1
	2	9.2 ±0.15	1.9 ±0.05	91.4 ±3.5	123 ±13.7	93.1 ±5.1
	3	9.4 ±0.15	2.0 ±0.05	88.3 ±3.5	153 ±13.7	88.3 ±5.1
SOY	1	8.9 ±0.15	1.8 ±0.05	94.9 ±3.5	165 ±13.7	85.6 ±5.1
	2	9.1 ±0.15	1.9 ±0.05	92.8 ±3.5	150 ±13.7	84.4 ±5.1
	3	9.3 ±0.15	2.0 ±0.05	89.3 ±3.5	148 ±13.7	84.2 ±5.1
MEAT	1	9.2 ±0.15	1.9 ±0.05	90.3 ±3.5	153 ±13.7	75.0 ±5.1
	2	9.2 ±0.15	2.0 ±0.05	86.4 ±3.5	152 ±13.7	82.6 ±5.1
	3	9.5 ±0.15	2.1 ±0.05	86.0 ±3.5	122 ±13.7	78.1 ±5.1

*Numbers represent the intial, middle, and end of the controlled feeding period, respectively.

†Values reported indicate mean ± SD.

Chapter V

Discussion

Zinc excretion and balance

Mean urinary zinc excretion, consistent among the three dietary treatments, was not affected by protein source or calcium level. In period I, there was also no significant difference in fecal zinc excretion and retention among subjects in the three treatment groups, although the retention values were higher in the subjects fed the meat diet. Bodwell (1981) also reported no significant difference in zinc retention in men who consumed a soy isolate diet, a textured soy, or one with mixed animal proteins. Janghorbani et al. (1982) also found zinc absorption to be equivalent in men fed a diet with chicken, soy, or a chicken/soy mixture as the protein source. Other researchers who substituted 25 - 30% of meat protein in a diet with soy isolate reported no differences in zinc absorption (Van Stratum, 1979; Greger et al, 1978; Sandstrom, 1980). It is important to note that our subjects had zinc intakes above the recommended dietary allowance; lower zinc intakes, at or below the RDA may have produced different results.

The effect of high calcium intakes ($\bar{x}$ = 2134 mg/day) on zinc excretion and retention in the subjects was conflicting. The calcium supplements had a significant enhancing effect on zinc retention in individuals fed the dairy diet, a significant negative effect on those fed the meat diet,

and no significant effect on those fed the soy diet. Results from the literature have been just as confusing. Both Spencer et al. (1984) and Snedeker et al. (1982) observed no effect of calcium supplements (over 2000 mg) on urinary or fecal zinc excretion or zinc retention in men fed a mixed protein diet. Sandstrom (1980) found that calcium in the form of milk enhanced zinc absorption in men fed wholemeal bread high in both phytate and fiber; however, in another study, Sandstrom (1980) reported that zinc absorption was significantly decreased in men fed a soybean protein diet with the addition of 125 ml of milk. The author points out that these differences in absorption may be due to the type of phytate found in the two meals. In agreement, Morris and Ellis (1976) reported that 50% of the phytate in wholemeal bread is soluble and more easily absorbable than the phytate in soybeans, a possible explanation for the increased absorption in the wholemeal bread.

In our study, high levels of calcium given to subjects fed the soy diet had no significant effect on zinc absorption. The phytate amount in our soybean diet was not determined; however, a level of 200 to 250 mg was estimated, assuming that 1.0 to 1.4 % of textured soy protein contains phytate (Bodwell, 1983). Sandstrom reported a higher phytate level (480 mg) in his soybean diet which may explain the negative influence of calcium they found on zinc retention. The enhancing effect that calcium had on zinc absorption in the dairy group may be related to the form in which calcium is presented in the diet. Although

milk contains high levels of calcium, it also contains lactose and casein, two factors that may enhance zinc absorption.

It is puzzling why the subjects consuming the meat protein with the high calcium intake had a mean negative retention, as well as a significantly higher fecal zinc excretion than those in the other treatments. Most researchers agree, as we saw in the first period, that zinc is well absorbed from animal protein diets, possibly due to the ability of amino acids to chelate zinc and increase its absorption (Solomons, 1982). In addition, meat does not contain the fiber and phytate that have been implicated in binding calcium and reducing zinc absorption.

The increased amount of heme iron and/or total iron from the meat may have affected zinc absorption, since iron and zinc compete for binding sites at the surface of the epithelial cells; however, the negative effect that iron may have on zinc is usually at a Fe:Zn ratio greater than 2:1 (Solomons, 1982). The ratio in this diet was 1/1.8. In addition, the soy diet contained significantly more iron than the meat diet and no negative effect on zinc retention was seen.

The negative zinc absorption in the meat group may be explained by exposing some of the inherent problems manifested in a metabolic balance study. Although we tried to minimize any errors in our study by analyzing everything in duplicate and repeating samples with duplicate differences greater than 5%, errors in compositing, wet

ashing, and diluting, may have occured. Composites of food, feces, and urine may have represented incomplete collections, thereby affecting results. In addition, the smaller sampling size in the meat group, individual variations, and the short adaptation periods could have all influenced the results. Collectively, these factors could have been significant.

Iron Excretion and Retention

Since there was no significant differences in excretion of iron in subjects across periods, the means for both periods were pooled for analysis. Fecal excretion was significantly higher in the soy group than the other two treatments. The higher intake of iron from the soy diet was a factor in the high excretions of fecal iron; however, inhibiting factors in the soy protein may have played a role, since the iron retention was negative and the intake was more than two times the recommended intake for iron (Food and Nutrition Board, 1980). Animal studies with rats, chickens, and monkeys have also shown that iron retention is significantly lower from soy protein products than from casein, the main protein in milk (Erdman and Thompson, 1984; Chauson et al, 1988; Fitch et al, 1964) Human studies have shown similiar results (Cook et al., 1981). In agreement, Derman et al. (1987) found that women absorbed significantly more iron from a milk-based infant formula than a soy-based one.

If the iron intakes were consistent in our study, the

iron retentions in the soy group may have been significantly lower. Why the soy diet had such high levels of iron is still unanswered. Our values were based on the literature provided by the food companies that manufactured the products. Since we did not analyze mineral content of the soy products by atomic absorption spectrophotometry prior to the study, we could not insure equal intakes among the treatment groups.

As far as the the effect of high levels of calcium, the soy group in period II given the high calcium supplements had a lower iron retention than the same group in period I who did not consume the extra calcium, although the results were not significant. No significant differences were seen between periods for the dairy or meat group either; therefore, the pooled means were used for analysis. Greger et al. (1982) also found that calcium supplements as high as 2000 mg had no effect on iron retention in subjects fed a mixed protein diet. In agreement, Monson and Cook (1976) reported that iron retention in men and women was not affected when moderate levels of radiolabeled calcium were added to a test meal.

The negative retention found in the group fed the meat diet is difficult to explain, since most research suggests the contrary. In fact, heme iron from meat is the best absorbed iron (Prasad, 1977). The meat used in our diets was ground beef, roast beef, pork, and other heme iron foods, that should have a positive effect on iron retention. As with zinc retention, the aberrant iron values may

be explained by problems within the balance study that may have affected this data.

Copper Excretion and Balance

Unlike iron, the intakes of copper from the three treatment groups were much lower than the recommended dietary allowance of 2 mg; however, the excretion and retention results of copper were very similiar to those of iron. The pooled mean retention values for copper were used since the differences between period I and II for each of the treatment groups were not significant.

The subjects in the soy protein group, most of whom were in negative balance, ingested significantly more copper from this diet and excreted significantly more fecal copper than the other subjects. According to the pooled means, the men in the soy group retained significantly less copper than those in the dairy group, although the data is difficult to interpret due to the high intakes of copper from the soy diet. These results, however, are consistent with many animal studies that also suggest that copper is not absorbed as well from soy products as from dairy products (Lonnerdale, 1985; Davis, 1961; Davies and Nightingale, 1975). Results from human studies are less clear, since very few studies with humans have compared the effects of only soy protein and dairy protein on copper balance; instead, diets containing mixed animal proteins (cheese, milk, eggs, and meat) and mixed vegetable proteins (legumes and cereal) have been used. Although these

studies suggest a trend that copper is absorbed better from a mixed animal protein diet than one with plant proteins, most of the results are not significant.

Price and Bunce (1970) observed that copper retention in adolescents fed diets of mixed animal and plant sources was slightly higher than those fed just plant proteins; however, the mixed diet had higher levels of protein than the plant diet. Butler et al (1973) found that copper absorption and retention from an animal-protein and a plant-protein diet did not significantly differ in young girls; however, copper intake from the protein diet was higher in the plant-protein diet. In addition, Turnland et al. found that copper absorption from a mixed animal protein diet was higher than that from a plant protein diet, but the actual amounts absorbed were similiar due to the higher levels of copper in the plant-based diet.

The pooled mean copper retention in subjects fed the meat-protein diet was not significantly different than those in the dairy or soy group; however, subjects in the meat group had a mean negative balance. This trend has been seen for both iron and zinc in this study. For copper, however, animal studies have shown that the addition of protein and/or sulfur amino acids have depressed the concentration of copper in the liver of rats (Jensen, 1979). In addition, McCall and David (1961) have observed a protective effect of high protein intakes against the development of copper toxicitiy in rats, possibly through the formation of macromolecular complexes of copper and

protein. Such results have not been seen in humans, however. In fact, Sandstead et al (1982) revealed that moderate to high levels of animal protein enhance copper absorption and even reduced copper requirement by 15%.

The high levels of zinc in the meat diet may have been a factor in reducing copper retention. Researchers have found that the high levels of zinc may reduce copper absorption in animals and man by competition for binding sites on metallothionein in the intestinal mucosal cells (Greger et al., 1978; Klevay, 1975; Burke et al., 1981). The zinc:copper ratio of the meat group (27:1) was higher than that of the dairy diet (22:1); however, it should be noted that both ratios are over 20:1, the level that has been found necessary to inhibit absorption (Prasad, 1977).

Whether the calcium supplements given in the second period had a significant effect on fecal copper excretion is difficult to determine due to the varying intakes of copper between the two periods; however, calcium supplements had no effect on copper retention. Price and Bunce (1972) also found that varying calcium intake from 300 - 1300 mg had no significant effect on balance in young girls fed a mixed protein source diet. Spencer et al. (1979, 1984) and Snedeker (1982) also found no significant effects of high levels of calcium on copper absorption.

Our results indicate that copper intakes of 0.93 and 0.94 from a dairy and meat-protein diet, respectively, are adequate amounts to maintain copper balance, even with zinc intakes nearly 30% over the recommended dietary allowance;

however, copper intakes of 1.57 mg from a soybean diet were not adequate to maintain balance. These results over a longer adaptation and collection period may have been different.

Calcium Excretion and Retention

Our results showed that urinary calcium excretion was not affected by protein source or calcium level. Most of the literature suggests that hypercalciuria occurs with high protein intakes in the form of meat protein, amino acids, and purified proteins (Linkswiler et al., 1974; Margen et al., 1974; Johnson et al., 1970; Allen et al., 1982); however, Watkins et al. (1985) found that men who ingested 80 g of chicken protein, as compared to 80 g of soybean protein excreted significantly more calcium in their urine. These researchers attribute the hypercalciuria to the higher levels of sulfur amino acids in the chicken diet. Howe et al. (1985) also found that 45 g of both cottage cheese and beef in a single meal caused increases in urinary calcium in nine postmenopausal women, as compared to 45 g of soy protein.

Our results are consistent with other researchers who have found no significant relationship between urinary calcium excretion and the sulfur amino acid content of various amino acid mixtures fed to humans (Margen et al., 1974). In addition, Block and Allen (1979, 1982) reported no differences in urinary calcium excretion when men were fed a meal of 45 g of milk protein or one of 15 g of milk

protein with added sulfur amino acids. Spencer (1978) also found no increases in urinary calcium in men fed a red meat diet of 1 g/kg/day, as compared to one with 2g/kg/day.

Fecal calcium was the main route of calcium excretion. When consuming moderate and high levels of calcium, the subjects in the soy group excreted significantly more fecal calcium than the other two groups, although there were no significant differences in calcium retention. Very few animal or human studies have investigated the effects of soy protein on calcium retention; however, Sandstrom et al. (1986) found that 60 g of soy protein significantly decreased calcium absorption in eight ileostomy patients, as compared to 60 g of meat protein. When 25% of the meat protein was replaced with soy protein, there was no significant effect on calcium absorption. Van Stratum and Rudrum (1979) found similiar results.

Increasing the supplementation of calcium in the second period significantly enhanced fecal excretion in all the treatment groups and increased calcium retention in all subjects, although the effect on the meat group was not significant. Spencer et al. (1984) also found that fecal excretions in men increased significantly when calcium intakes ranged from 200 to 2000 mg/day; however, the retention values did not increase after intakes of 1200 mg, a level which represents the threshold of calcium absorption. The reason our retention values were so high may be related to the calcium status of the individuals, although the short adaptatation period was the probable reason.

The increase in the calcium:phosphorous ratio from 1:1 in the first period to 2:1 in the second period may have also played a role in the increased retention of the calcium; however, Spencer found no significant effect on calcium excretion when the Ca:P ratio ranged from .5/1 - 2/1 (Spencer et al. , 1984).

It should be noted that deviations of 25% have been found in fecal collections when fecal markers were not used when determining calcium balance (Allen, 1982).

Magnesium Excretion and Retention

As with the other minerals, no difference in urine excretion was seen among the treatments within or between the periods.

In period I, magnesium intakes from the meat and dairy diets were well below the recommended dietary allowance of 350 mg/day. As a result, the magnesium retention of subjects from both of these treatment groups was negative. The soy diet, on the other hand, provided levels of magnesium equal to the RDA; however, the magnesium retention of these subjects was also negative, suggesting that those on a diet based on vegetable protein may require higher magnesium intakes than those on an animal-protein diet.

When magnesium intakes were increased significantly in period II, all subjects were in positive balance. Those in the soy group excreted significantly more fecal magnesium than those in the other two groups, although there was no

significant difference in retention between the men in the soy or dairy group. Forbes et al. (1964) also found no significant difference in balance in young rats fed a soy isolate diet in comparison to those fed a casein diet. In humans, however, Stephenson et al. (1970) found that magnesium was absorbed better from a casein-lactalbumin diet than from a peanut flour or beef diet. The peanut flour may have had higher levels of phytate than a soybean diet, therefore affecting magnesium absorbance. We also found that the men had significantly lower retentions in the meat diet, as opposed to the dairy or soy diet; however, these low retentions have been seen throughout the study, as mentioned earlier.

The effect of high levels of calcium on magnesium balance is difficult to determine due to the varied levels of magnesium between the periods.

Plasma Minerals

All plasma values remained consistent throughout the thirty day collection period. Values for plasma calcium, magnesium, zinc, and iron all were within their normal ranges. Copper was slightly lower than the accepted range, which may be a reflection of the inadequate intake of copper in both period I and period II.

Chapter VI

Summary and Conclusions

A thirty-day metabolic balance study was conducted with young men between the ages of 20 - 34 to determine the effect of three types of protein and two levels of calcium on zinc, iron, copper, calcium, and magnesium retention. All subjects were apparently in good health. Twenty-four subjects were randomly assigned to three treatment groups. The dairy treatment group was fed a diet in which 70% of the dietary protein was derived from dairy products; the soy treatment group was fed a diet in which 67% of the dietary protein was derived from soy products; and the meat treatment group was given a diet in which 70% of the dietary protein was provided by animal meat products.

The controlled feeding period was divided into two periods: period I, in which the subjects consumed moderate levels of calcium (mean = 1206 + 193 g/day) and period II, in which the subjects consumed high levels of calcium (mean = 2134 + 165 mg/day). Balance data was calculated from the last six days of the nine-day collection period in which the subjects collected all urinary and fecal excretions. Both period I and II had a nine-day adaption period. Fasting blood samples were collected at the beginning, the middle, and the end of the controlled feeding period.

Urine and food were composited each day and pooled for each 6-day period. Feces were composited at the end of the study for each 6-day collection period. Atomic Absorption Spectrophotometry was used to analyze urine for calcium,

magnesium, and zinc concentrations, and food and feces for zinc, copper, iron, calcium, and magnesium concentrations. Nitrogen content of the food was determined by the Kjeldahl method. Plasma was analyzed for zinc, iron, copper, magnesium, and calcium concentrations.

There were no differences seen in urinary zinc, magnesium, or calcium excretion among the treatments or between the two periods.

Soy Treatment Group

At moderate intakes of calcium, in period I, fecal calcium, magnesium, iron, and copper were significantly higher in the soy group than either the dairy or meat groups. These high excretions reflected the high intake of these minerals in the soy group. Although positive retentions were seen in these subjects for calcium, zinc, and iron, the intakes of these minerals were well above the RDA. A negative mean retention for copper was seen; the intake was below the RDA.

The high intake of calcium did not have a significant effect on iron utilization in the men consuming the soy diet; however, the positive retention in period I was significantly reduced in period II, even though the iron intakes remained constant between the two periods.

Dairy Treatment Group

In general, the subjects in the dairy group absorbed all the minerals well, except magnesium; however, the

magnesium intakes were well below the RDA. When the magnesium intakes were increased, the men had positive balances. The high levels of calcium did not appear to have any significant effect on these subjects, except for the enhanced retention for both zinc and calcium.

Meat Treatment Group

In the first period, the men in the meat group had adequate utilization of zinc, copper, and calcium. Since the magnesium intakes were below the RDA, they had negative retentions, like the other treatments. Questions about the validity of data from the meat group are asked when looking at the data for the second period. The mean retention values for iron, copper, and zinc were negative. The mean retention value for calcium and magnesium were also lower than the other two groups. Inherent problems of the metabolic balance study were probably the main cause of the disparate values. Incomplete food, fecal, or urine composites, errors in analysis, variation between the subjects, and the short adaptation period could have all affected the data.

If I were to do the study over, I would have given the subjects a fecal marker to insure the beginning of collection period. I would have also analyzed all the diets prior to the study for mineral content using atomic absorption spectrophotometry, instead of calculating them from food book values. These procedures would insure more accurate intake values, as well as fecal composites.

Implications of the Study

Mineral bioavailability of soy protein seems to be adequate when minerals intakes are in excess of the RDA; however, a diet based on vegetable protein with low levels of mineral intake, may significantly reduce retention. We saw this for the minerals copper and magnesium. In addition, high supplememts of calcium for individuals consuming a diet high in vegetable protein may also negatively affect iron utilization.

We did not find in our study that meat protein had a positive effect on urine calcium. This could be an area of future research.

Chapter VII

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Appendix I

Advertisement Flyer Used For Recruitment of Subjects

EAT FREE FOR 4 WEEKS

1985 HNF DAIRY STUDY

Wanted: Dedicated non-smoking male volunteers in good health and of normal weight, between the ages of 20 and 35, to participate in a Department of Human Nutrition and Foods (HNF) Metabolic Feeding Study.

The study will run from September to December, 1985. For 4 weeks during the October and November subjects will eat 3 meals a day free at the department of HNF Metabolic Kitchen (Solitude).

During the entire study (September to December) measurements of blood cholesterol and minerals, and blood pressure will be made at regular intervals.

All subjects will receive monetary compensation upon successful completion of the study. For further information contact the Department of Human Nutrition and Foods at 961-5549.

Appendix II

Description of Subjects

DESCRIPTION OF SUBJECTS

NUMBER	NAME	AGE	HEIGHT (cm)	PRE-STUDY WEIGHT (kg)	MID-STUDY WEIGHT (kg)	POST-STUDY WEIGHT (kg)
1		22	168.0	60.8	61.2	61.2
2		27	171.0	112.6	110.8	108.6
3		34	175.0	67.1	67.6	67.1
4		21	169.0	76.6	76.2	76.6
5		21	173.5	91.4	92.1	94.1
6		32	183.5	66.2	65.7	65.6
7		20	184.0	70.7	69.3	72.1
8		20	172.0	77.0	76.5	77.2
9		33	169.5	68.7	67.9	67.7
10		28	176.5	68.0	68.0	69.7
11		33	173.5	67.5	66.6	66.6
12		21	181.5	80.4	79.4	78.3
13		23	175.0	68.0	67.7	69.0
14		22	176.5	74.7	74.7	76.4
15		30	168.0	85.8	85.8	85.9
16		26	181.0	78.4	78.6	80.2
17		26	175.0	69.9	72.3	70.9
18		34	181.0	64.9	65.3	64.5
19		33	182.0	79.6	78.5	79.2
20		26	176.5	64.3	64.0	64.7
21		23	182.5	53.2	52.9	55.5
22		23	186.0	83.5	81.9	81.6
23		27	174.0	65.2	63.8	66.0
24		29	175.0	78.8	79.3	77.5
Mean				73.8	73.6	74.0
Standard Deviation				±11.7	±11.5	±11.1

Appendix III

Screening Forms

SCREENING QUESTIONNAIRE
1985 DAIRY STUDY

Please answer the following questions. All responses will remain strictly confidential.

Name _____ Age _____

Social Security Number _____ Date _____

1. History of cardiovascular disease. (If yes, specify what type of condition.)

   Personal _____
   Father _____
   Mother _____
   Grandfather, maternal _____
   paternal _____
   Grandmother, maternal _____
   paternal _____
   Brother(s) _____
   Sister(s) _____
   Uncle(s) _____
   Aunt(s) _____
   Other _____
   No known history _____

2. Have you ever been diagnosed as having high cholesterol?
   yes _____ no _____
   If yes, specify when. _____

3. Have you evern been diagnosed as having hyperlipoproteinemia?
   yes _____ no _____
   If yes, specify when. _____

4. Have you ever been diagnosed as having hyperglycemia (high blood sugar) or diabetes mellitus?
   yes _____ no _____

5. Are you taking any medication (prescription or non-prescription on a regular basis?
   yes _____ no _____
   If yes, specify the name and dosage of the medication(s).

   _____

6. Are you on a special diet?
   yes _____ no _____
   If yes, specify the type. _____

7. Do you supplement your diet with vitamins, minerals, protein and/or fiber?
   yes _____ no _____
   If yes, specify the name and dosage of each supplement.

   _____

8. Do you smoke?
   yes _____ no _____
   If yes, which of the following do you use, and how often do you use it (them)?
   cigarette _____ pipe _____
   cigar _____ chewing tobacco _____

9. Do you use any recreational drugs (i.e., marijuanna, LSD, cocaine, etc.)?
   yes _____ no _____

10. Are you allergic to any foods?
    yes _____ no _____
    If yes, list the foods to which you are allergic. _____
    _____
    _____
    _____
    _____
    _____

11. Would you like the results of your blood analyses?
    yes _____ no _____

FOOD PREFERENCE QUESTIONNAIRE
1985 DAIRY STUDY

Name _____ Date _____

The foods and beverages listed below are part of the metabolic feeding study menu. Therefore, you may be required to eat many of these foods very often. Please draw a line through any items that you are unable to eat (i.e., any to which you are allergic, or any that you definitely dislike).

Coffee
Tea
Orange Juice
Apple Juice
KoolAid
Lemonade
Tang
Oranges
Grapefruit
Canned Pears
Canned Peaches
Strawberries
Canned Pineapple
Lettuce
Tomato
Cucumber
Green Pepper
Carrots
Celery
Broccoli
Mushrooms
Green Beans
Potatoes
Potato Chips
Pizza
Tomato Sauce
Spaghetti Sauce
Chocolate
Cookies
Gelatin
Tossed Salad

Roast Beef
Ground Beef
Roast Pork
Ham
Canadian Bacon
Sausage
Tuna
Scallops
Shrimp
Chili
Eggs
Cheddar Cheese
Mozzarella Cheese
Cream Cheese
Cottage Cheese
American Cheese
Parmesan Cheese
Ice Milk
Ice Cream
Sour Cream
Vegetable Soup
Quiche
Mayonnaise
Spaghetti Noodles
Egg Noodles
Bread
Butter
Margarine
Jelly
Pudding
Dry Cereal

CLINICAL EVALUATION

I, _____ authorize _____, M.D.
Print Name Print Physician's Name

to release requested information about my health to Forrest W. Thye, Ph.D., Department of Human Nutrition and Foods, Virginia Tech.

Signed_____
Signature of Subject

I have recently given a physical examination and/or reviewed the medical record of _____ and ☐ Do not find
Please print
☐ Do find

a medical or physical condition that precludes his participation in the Human Nutrition and Foods human feeding and metabolic study of 1985.

_____, M.D.
Signature of Physician

Office Phone _____

Address _____

Appendix IV

Dairy Study Information and Procedures

1985 HNF DAIRY STUDY INFORMATION

I. Research Objectives
To investigate the effects of different sources of dietary protein and two levels of dietary calcium adult males on:
a. plasma lipid and lipoprotein levels
b. serum mineral levels
c. mineral balance
d. blood pressure levels

II. Study Date
The study will be conducted from October 3 to December 13, 1985.

III. Subject Qualifications
- male
- 20 to 35 years of age
- in good health
- non-smoker
- sedentary to moderate exercise pattern
- no personal history of heart disease
- taking no high blood pressure medication
- blood cholesterol level greater than 200 mg/determined at study screening)
- taking no blood lipid medication

IV. Study Format
The study consists of 3 periods:
a. preliminary period
   - subjects eat usual diets
   - subjects keep 5-day dietary records
   - subjects have blood drawn and blood pressures and body weights measured approximately bi-weekly
b. controlled feeding period
   - subjects randomly assigned to 1 of 3 diet groups (red meet, dairy, or soy group)
   - subjects eat only food and beverages supplied by study
   - subjects eat meals at HNF metabolic unit (Solitude)
   - subjects have blood drawn and blood pressures and body weights measured approximately bi-weekly
   - subjects provide total fecal and urine collections for two 9-day periods
c. follow-up period
   - subjects eat usual diets
   - subjects keep 5-day dietary records
   - subjects have blood drawn and blood pressures and body weights measured approximately bi-weekly

V. Subject Constraints
- must be in town during entire controlled feeding period (Oct. 24 to Nov. 22, 1985)
- must eat all meals (3 per day) at Solitude during controlled feeding period
- must consume ALL food and beverages provided by study during controlled feeding period
- must consume NO food or beverages (except water) other than those provided by study during controlled feeding period
- must not change exercise habits during entire study period (Oct. 3 to Dec. 13, 1985)
- must not lose or gain weight during entire study period (Oct. 3 to Dec. 13, 1985)

- must collect ALL urine and feces during the 2 total collection periods during controlled feeding period
- must be present at all appointments to have blood drawn and blood pressures and body weights measured during entire study period
  (Oct. 3, Oct. 4, Oct. 14, Oct. 15, Oct. 23, Oct. 24, Nov. 7, Nov. 8, Nov. 22, Nov. 23, Dec. 2, Dec. 3, Dec. 12, Dec. 13, 1985)

VI. Subject Stipend

Each subject to successfully complete the entire study will receive $225.00.

DAIRY STUDY PROCEDURES

FALL 1985

Overall Procedures

1. All subjects must be present at all laboratory sessions during the entire study (i.e. to draw blood, to take blood pressures and to obtain body weights).
2. All subjects must eat all meals at Solitude during the 30-day controlled feeding period, from October 24 through November 22.
3. All subjects must not gain or lose more than ± 2 kg of weight during the entire study from October 3 to December 13.
4. All subjects must maintain their normal exercise and activity level during the entire study from October 3 to December 13.
5. All subjects must complete two 5-day dietary records during the study, one during the preliminary and one during the follow-up period.
6. All subjects must have a physical examination or medical record check prior to the beginning to the study (October 3), and have clearance from a physician for participation in the study.
7. All subjects must sign a consent of participation form prior to the beginning of the study (October 3).

Lab Day Procedures

1. Arrive at Wallace Hall room 306 at your designated time. It is very important that you be on time. If you will be late for any reason, call 961-5375.
2. Have your weight taken, without shoes and heavy sweater or coat.
3. Sit quietly for 10 minutes in preparation for your blood pressure measurement.
4. Have your blood pressure taken.
5. Have your blood drawn.
6. Eat breakfast (at Wallace during the preliminary and follow-up periods; at Solitude during the controlled feeding period).

   Approximately 50 ml of blood will be taken at each lab session. This blood will be analyzed for total cholesterol, lipoprotein cholesterol, and minerals.

Dietary Record Procedures

A dietary record is a detailed written record of everything that is consumed (food, beverages, supplements, medications) for a specific length of time.

Two 5-day dietary records are part of the study, one before and one after the controlled feeding period; October 8 through October 12 and December 1 through December 5.

The main purpose of the records is to gain information about your normal diet so that its estimated nutrient content can be compared to that of the controlled feeding diet.

A second purpose of the record kept prior to the controlled feeding period is to gain information about your usual caloric intake, so that your required caloric needs for the controlled feeding period can be estimated.

For the above reasons, the dietary records must be a complete and accurate as possible, so it is important that you follow the steps listed below:

1. Record your intake on the dietary record sheets provided. Fill our the forms completely.
2. Eat your usual diet during the dietary record periods, i.e. do not consciously alter your intake of food items or quantities because of the need to record them.
3. Fill our the food record form as soon as possible after each time that you eat or drink something. Do not wait to complete the food record until evening. It is too easy to forget something that you consumed earlier during the day.
4. Measure food and beverage items as often as possible with standard measuring cups and spoons. Estimate amounts at other times, trying to be as precise as possible.
5. Record all measurements and estimates in specific quantities. i.e. cups, milliliters, ounces, grams, numbered quantities.
6. When indicating numbered quantities, also indicate sizes, i.e. 1 small apple - 3″ in diameter.
7. Indicate whether recorded quantities are actual measurements or estimates.
8. Indicate methods of food preparation and recipe ingredients whenever possible.
9. Record all toppings, condiments, sauces, etc. and indicate their amounts.
10. Record if salt is added to food during cooking or at the table.
11. Record the time food and beverages are consumed and where. If you eat at a restaurant give its name.
12. Obtain and record exact quantities of food items and specific recipe ingredients whenever possible when you eat at a restaurant.
13. Record all dietary supplements, vitamin and mineral preparations and medicines consumed, and indicate the dose, Brand name and formulation whenever possible.

Food and Beverage Procedures

1. All subjects consume their normal diets during the preliminary and follow-up study periods.
2. Only food and beverages provided by the study can be consumed during the controlled feeding period.
3. No water other than the deionized water provided by the study can be drunk during the controlled feeding period. Deionized water from other sources on campus is not permitted. A container will be provided to each subject for this water, and can be refilled as often as needed. Carrying cases will be available for water bottles if desired.
4. You can pour water to drink into a plastic glass at home. The glass, however, must first be rinsed thoroughly with some of the deionized water. Do not use any containers other than plastic. Do not use glass containers. Do not use ice cubes made at home, unless you make them with deionized water and freeze the in pre-rinsed plastic trays. Do not use metal trays.
5. Use deionized water for rinsing when brushing your teeth. It is important that you do not swallow any toothpaste.
6. No chewing gum or chewing tobacco are permitted during the 30-day controlled feeding period.
7. No vitamin or mineral supplements are permitted during the entire study, October 3 to December 13.
8. Non-prescription medicine should be avoided as much as possible during the 30-day controlled feeding period. Any medicine which is consumed, both prescription and non-prescription, must be reported to the study.

Mealtime Procedures

For the study to run smoothly, and for subject diets to be consistent and accurate, it is vital that all subjects follow the procedures listed below.

1. All meals will be served at Solitude for the 30-day controlled feeding period.
2. The meal times are scheduled as follows:

	Monday - Friday	Saturday - Sunday
Breakfast:	7:15 - 8:30 (8:45 on lab days)	7:45 - 9:00
Lunch:	11:30 - 1:00	12:00 - 1:30
Dinner:	5:00 - 6:00	5:00 - 6:00

3. If you are going to be late for a meal for any reason call Solitude at 961-5987.
4. When entering Solitude, do so by the door facing the duck pond.
5. Drop off your fecal collection cup(s) in the designated receptical on the porch. (Only during the total collection periods).
6. Place your urine collection bottle(s) in the designated place. (Only during total collection periods).
7. Tell the kitchen supervisor on duty your name, and the study group to which you belong (Dairy, Meat, or Soy Group).
8. Have your weight taken by the kitchen supervisor or designee. (Only at breakfast). Do not weight yourself. Remove your shoes and any heavy sweater or coat before being weighed.
9. Report any medications that you consumed in the previous 24 hours.
10. Wash your hands thoroughly in the second flood bathroom. Do not apply hand cream.
11. Go to the dining area and sit at any of the places designated for your study group.
12. Before beginning to eat, check the items on your tray card against those on your tray. Report any discrepancies to the kitchen supervisor immediately. Do not clean up the spill before the supervisor sees it.
13. While eating use your rubber spatula to clean each dish completely. Use break to absorb grease and sauces. Use deionized water to rinse all dishes and cups clean and then drink the water.
14. When you have finished eating, have the kitchen supervisor check your tray and remove your tray card.
15. Pick up any snack you are scheduled to receive.
16. Carry your tray from the dining area. Place spatulas and non-disposable dishes in the designated area. Place disposable items (paper products and plastic utensils) in the designated receptical on the porch. Place your tray in the designated area.
17. Have your water bottle refilled as needed.
18. Pick up your clean urine bottle(s) and fecal collection containers. (Only during total collection periods).

Total Urine and Fecal Collection Procedures

Two 9-day total collection periods are part of the study. They are from October 30 to November 8 and November 14 to November 23.

1. Labeled collection bottles are provided for urine collection.
2. A clean labeled urine bottle (two if necessary) is to be picked up each morning after breakfast during the collection period, used during the entire day, and returned to Solitude the next morning at breakfast.

3. Each 24-hour urine collection must be kept separate. A 24-hour urine collection is from the second voiding of one morning through the first voiding of the next morning. The urine collection days are as follows:

Period	Day	From Second Voiding On:	Through First Voiding On:
I	1	Oct. 30	Oct. 31
	2	Oct. 31	Nov. 1
	3	Nov. 1	Nov. 2
	4	Nov. 2	Nov. 3
	5	Nov. 3	Nov. 4
	6	Nov. 4	Nov. 5
	7	Nov. 5	Nov. 6
	8	Nov. 6	Nov. 7
	9	Nov. 7	Nov. 8
II	1	Nov. 14	Nov. 15
	2	Nov. 15	Nov. 16
	3	Nov. 16	Nov. 17
	4	Nov. 17	Nov. 18
	5	Nov. 18	Nov. 19
	6	Nov. 19	Nov. 20
	7	Nov. 20	Nov. 21
	8	Nov. 21	Nov. 22
	9	Nov. 22	Nov. 23

4. Clean urine bottles will contain 10 ml of 10% hydrochloric acid. Therefore caution must be exercised when urinating in the bottles. The HCL must remain in the bottles as it prevents bacterial growth.
5. The fecal collection periods are as follows:

Period	From	To
I	Noon, Oct. 30	Noon, Nov. 8
II	Noon, Nov. 14	Noon, Nov. 23

6. Partially labeled contains are provided for fecal collection.
7. Each fecal excretion must be collected in a separate container, and each container must be labeled with the subject number and name and the date and time of the fecal excretion.
8. The label will look as follows:

Subject # _____ Subject name _____

Date _____ Time: _____ a.m. _____ p.m.

Subjects must fill out the label completely for each fecal excretion. A black waterproof marking pen will be provided.

9. Bring used fecal containers to Solitude when you come for meals, and place them in the designated receptical on the porch.
10. Carrying cases will be provided for carrying urine bottles and fecal containers.
11. At the end of each collection period return all empty urine bottles, fecal collection containers and marking pens.

Appendix V

Consent of Participants

DAIRY STUDY

CONSENT OF PARTICIPATION

FALL 1985

I have received an explanation of the Department of Human Nutrition and Foods human feeding and metabolic study and understand the following:

As a subject, I will be on a controlled diet during the period from October 24 to November 22, 1985. I understand that I am to consume only food and beverages provided by the study. Diets will provide nutrients at or above levels recommended by the National Research Council, Food and Nutrition Board (1980). The diets will contain approximately 500 mg of cholesterol per day.

A physical examination or medical record check must be conducted on each subject prior to the study by university health services or a personal physician. If at any time the study investigators, a physician or the subject himself believes that his health may be impaired by remaining in the study, the subject may drop from the study.

No compensation or medical treatment, other than that normally available through student health services and emergency service by the rescue squad, is available if unjury is suffered as a result of the research.

Venous samples of blood, approximately 50 ml, will be drawn a total of 14 times throughout the study. Seven two-consecutive day samples, separated by 9 or more days, will be the blood sampling schedule. Blood samples will be taken by qualified medical personnel. Individual serum cholesterol data will be available to each subject.

Mineral balance will be determined for two 9-day periods during the controlled feeding period. The data will be based on dietary intake and total urine and fecal output.

Subjects will be paid $225.00 for successful completion of the entire study. A subject may drop from the study at any time for health or personal reasons.

All subjects are invited to ask any questions about procedures at any time.

I understand the above and agree to participate in the Department of Human Nutrition and Foods human feeding and metabolic experiment from October 3 through December 13, 1985.

_____ _____

Signature of Subject Date

F. W. Thye, Principle Investigator
961-6620
961-5549

C.D. Waring, Chairman
IRB
961-5284

Appendix VI

Dairy Study Timetable

DAIRY STUDY TIMETABLE

FALL 1985

September 25	Subject Orientation
October 3	Beginning of Preliminary Period Lab Day
October 4	Lab Day
October 8 - 12	Dietary Record
October 14	Lab Day
October 15	Lab Day
October 23	End of Preliminary Record Lab Day
October 24	Beginning of Controlled Feeding Period Lab Day
Oct. 30 - Nov. 8	Total Urine and Fecal Collection
November 7	Lab Day
November 8	Lab Day
Nov. 14 - Nov. 23	Total Urine and Fecal Collection
November 22	End of Controlled Feeding Period Lab Day
November 23	Beginning of Follow-Up Period Lab Day
Dec. 1 - Dec. 5	Dietary Record
December 2	Lab Day
December 3	Lab Day
December 12	Lab Day
December 13	End of Follow-Up Period Lab Day

Appendix VII

Dietary Record

DAIRY STUDY - FALL 1985

DIETARY RECORD

DATE:_____ NAME:_____

1. Record all the food and beverages you have consumed for the entire day. Fill out the form completely.
2. After you have recorded your intake answer the following questions:
   A. Was this how you usually eat? yes no
   B. If no, why was it unusual? _____
3. Use as many dietary record sheets as you need to complete each day's intake.

Time	Place	Food and Beverages	Quantities	Method of Preparation and/or Recipe Ingredients

Appendix VIII

Procedures for Meals

DAY 3 - DINNER

MEAT DIET

NAME _____ DATE _____

CANTONESE PORK AND VEGETABLES		_____
MIXED GREEN SALAD		_____
THOUSAND ISLAND SALAD	15 g	_____
FLAT BREAD	50 g	_____
BUTTER	5 g	_____
CHOCOLATE CHIP SUNDAE		_____
ICED TEA	22 g	_____

OTHER _____

_____

MEAL COMPLETELY EATEN _____ SUPERVISOR _____

PROCEDURES FOR FOOD REFUSALS

There may be instances when because of illness a subject is unable to eat part or all of a meal or snack. If this happens, notify the study supervisor. If the study supervisor is unavailable follow the procedures listed below:

1. Save the uneaten food just as it is.
2. Cover it and refrigerate or freeze it, as appropriate.
3. Indicate on the subject's meal card what food was refused.
4. Indicate on the subject's next meal card what food was refused.
5. Offer the refused food again at the next meal.
6. If at the end of the day, the subject has been unable to eat his entire diet do the following:
   a. estimate and record the amount of refused food on a blank sheet of paper, along with the date, the subject's name and diet group and the diet day.
   b. staple this paper to the dinner meal card of the subject.
   c. place the refused food in a clean polyethylene bag closing it securely.
   d. label the bag "Food Refusal", and indicate the date, the subject's name and diet group and the diet day.
   e. Freeze the refused food in the food composite freezer.

PROCEDURES FOR SPILLED FOOD OR BEVERAGES

All subjects have been instructed to report any spilled food or beverage to the kitchen supervisor immediately. If spillage occurs notify the study supervisor. If the study supervisor is not available follow the procedures listed below:

1. If the precise quantity and composition of the food that was spilled can be determined, replace it.
2. If a fairly good estimate of the spillage can be made, replace it.
3. If it is possible to recover the spillage, bag it in the same manner as a diet refusal.
4. Any spillage which in even the smallest way affects the accuracy of the subject's intake is considered an error an is recorded as such.
5. All spills and the subsequent action taken must be reported to the study supervisor.

Appendix IX

Nutrient Content of the Diets

NUTRIENT CONTENT OF DAILY DIETS

	DAIRY*	SOY	MEAT
Calories (kcal)	2630	2621	2680
Total Protein (g)	68	69	65
Dietary Treatment Protein (g)	52 (8%)†	52 (8%)	48 (7%)
Fat (g)	117 (40%)	112 (39%)	121 (41%)
P/S Ratio	0.41	0.44	0.51
Carbohydrates (g)	342 (52%)	351 (53%)	341 (51%)
Cholesterol (mg)	499	497	479

*Without food supplements.

† ( ) percent of total calories.

NUTRIENT CONTENT OF FOOD SUPPLEMENTS

	FOOD Supplement (1 unit)	TOTAL FOOD SUPPLEMENTS		
		DAIRY	SOY	MEAT
Calories (kcal)	217	776	908	492
Total Protein (g)	1.8	6.4	7.5	5.1
Fat (g)	8.9	31.8	37.3	20.2
P/S Ratio	0.50	0.50	0.50	0.50
Carbohydrates (g)	28.2	101	118	64.0
Cholesterol (g)	2.2	7.9	9.2	5.0

Appendix X

Menus

DAIRY DIET: DAY 1

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		34
Cnd Pears, light syrup, drained		75
Creamy Strawberry Delight:	1% Cottage Cheese	30
	Powdered Sugar	2
	Strawberry Jam	30
Welsh Rarebit:	1% Cottage Cheese	55
	Cheddar Cheese	5
	Sour Cream Sub	40
	Egg Yolk, frozen, raw	15
	Corn Oil Margarine	10
	White Flour	2.5
	Dry Mustard	.21
	Worchestershire Sauce	.25
	Cayenne Pepper	.02
Low Protein Bread		50
Butter		5
LUNCH		
Toasted Cheese Sandwich:	American Cheese	25
	White Bread, 2 slices	56
	Butter	5
Potato Chips		15
Vegetables with Dip:	Carrot, pared, raw	30
	Celery, raw	30
	1% Cottage Cheese	55
	Sour Cream Sub	20
	Dry Onion Soup Powder	4.4
Cream Cheese Tart:	1% Cottage Cheese	68
	Neufchatal Cheese	56
	Clover Honey	21
	Imitation Vanilla	.44
	Low Protein Gel Powder	7
	Deionized Water	7.5
	Graham Cracker Crumbs	5
	Corn Oil Margarine	2.5
Lemanade Powder (swtnd)		30
DINNER		
Spaghetti Sauce:	Tomato Sauce	120
	Italian Seasoning	.05
	Onion Powder	.07
	Garlic Powder	.09
	Oregano	.05
	Basil	.05
	Granulated Sugar	2.5
	Corn Oil Margarine	11.5
	Olive Oil	4
1% Cottage Cheese		75
Spaghetti, ckd al denta		97.5
Tossed Salad:	Iceburg Lettuce	40
	Carrot, pared, raw	20
	Tomato, raw	30
	Frozen Egg Yolk, raw	12.5
Creamy Italian Dressing:	Mayonnaise	15
	Red Wine Vinegar	1.8
	Onion Powder	.03
	Garlic Powder	.03
	Italian Seasoning	.04
	Granulated Sugar	7
Low Protein Flat Bread		50
Garlic Butter:	Butter	5
	Garlic Powder	.1
Vanilla Ice Milk		100
Low Protein B'scotch Cookies		28
Ice Tea Powder (lemon & sugar)		22

DAIRY DIET: DAY 2

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		34
Cnd Mandarin Oranges, light syrup, drained		60
Cnd Grapefruit, light syrup, drained		40
Orange Cream Sauce:	1% Cottage Cheese	30
	Clover Honey	5
	Powder Sugar	8
	Orange Juice, cnd (unswtnd)	5
	Imitation Vanilla	.2
Broccoli Cheese Quiche:	Frozen Egg Yolk, raw	25
	1% Cottage Cheese	75
	Sour Cream Substitute	60
	Broccoli, froz, ckd	30
	MBF B'fast Strips, ckd	3
	White flour	5
	Corn Oil Margarine	5
	Worchestershire Sauce	.2
	Onion Powder	.1
	Basil	.05
Low Protein Flat Bread		50
Butter		5
LUNCH		
Cottage Cheese Salad:	1% Cottage Cheese	120
	Iceburg Lettuce	20
Vegetable Soup A:	Beef Broth, condensed	75
	Deionized Water	65
	Cnd Tomatoes, drained	25
	Low Protein Spaghetti Rings, dry	10
	Carrots, cnd. drained	20
	Green Beans, cnd. drained	15
	Corn Oil Margarine	5
	Coconut Oil	4
	Tomato Paste	15
Low Protein Flat Bread		60
Corn Oil Margarine		5
Vanilla Ice Milk		100
SS Choc Chips		15
Lemonade Powder (swtnd)		30
DINNER		
Vegetable Stroganoff:	1% Cottage Cheese	55
	Sour Cream Substitute	55
	Red Wine	2.5
	Onion Powder	.02
	Dill Weed	.04
	Soy Sauce	1.3
	Safflower Oil	2.5
	Corn Oil	2.5
	Egg Noodles, ckd al dent	120
Tossed Salad:	Iceburg Lettuce	40
	Carrot, pared, raw	20
	Tomato, raw	30
	Frozen Egg Yolk, raw	5
CO French Dressing:	French Dressing	30
	Corn Oil	3
Low Protein Flat Bread		50
Corn Oil Margarine		5
Chocolate Cheese Mousse:	1% Cottage Cheese	60
	Neufchaetel Cheese	56
	Powdered Sugar	10
	Imitation Vanilla	2.2
	Low Protein Orange Gel Powder	3.5
	Cocoa, high-med fat	.4
Vanilla Wafers		8
Ice Tea Powder (lemon & sugar)		22

DAIRY DIET: DAY 3

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		25
Frozen Strawberries, slightly thawed (unswtnd)		75
Creamy P'apple Sauce:	1% Cottage Cheese	35
	Clover Honey	4
	Powdered Sugar	4
	Cnd P'apple Tidbits, in juice, drained	15
Neufchatel Cheese		28
Dry Cereal, Rice Krispies		22
Polyrich		150
White Bread, 1 slice		28
Butter		5
LUNCH		
Pizza Sauce:	Tomato Sauce, @1/8c	26
	Tomato Paste	4.1
	Coconut Oil	3
	Cnd Mushrooms, drained	15
	Garlic Powder	.1
	Oregano	.05
	Basil	.05
Mozzarella Sauce		10
Low Protein Flat Bread		105
Tossed Salad:	Iceburg Lettuce	40
	Tomato, raw	30
	Carrot, pared, raw	20
Vinegar & Oil Dressing:	Wine Vinegar	5
	Olive Oil	5
Cottage Cheese Salad:	1% Cottage Cheese	100
	1% Cot. Cheese, dry curd	30
Peach Cheese Delight:	Cnd Peaches, light syrup drained	25
	Low Protein Gel Powder	22
	Deionized Water	115
	1% Cottage Cheese	35
	Neufchatel Cheese	25
	Imitation Vanilla	.25
	Clover Honey	
Lemonade Powder (swtnd)		30
DINNER		
Vericheesey Casserole:	Potatoes, cnd. drained	100
	Frozen Egg Yolk, raw	20
	1% Cottage Cheese	105
	Low Protein Flat Bread Crumbs	10
	Coconut Oil	5
	Soybean Oil	6.5
	Olive Oil	5
	Onion Powder	.1
	Basil	.05
	Low Protein Flat Bread Crumbs	10
	Parmesan Cheese	5
	Butter	5
Mixed Green Salad:	Iceburg Lettuce	40
	Cucumber, pared, raw	26
	Frozen Egg Yolk, raw	8.3
Thousand Island Dressing		20
Olive Oil		5
White Bread, 2 slices		56
Butter		12.5
Choc Chip Sundae:	Vanilla Ice Milk	100
	SS Choc Chips	15
	Non-Dairy Whip Topping	10
Ice Tea Powder (lemon & sugar)		22

MEAT DIET: DAY 1

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		34
Cnd Pears, light syrup, drained		75
Creamy Strawberry Delight:	1% Cottage Cheese	25
	Powdered Sugar	2
	Strawberry Jam	30
Brown N Serve Sausage, ckd (@ 4 links)		84
Low Protein Flat Bread		50
	Corn Oil Margarine	5
LUNCH		
Ham Sandwich: Boiled Ham, 5% fat		90
	White Bread, 2 slices	56
	Iceburg Lettuce	10
	Prepared Mustard	5
Potato Chips		15
Vegetables with Dip:	Carrot, pared, raw	30
	Celery, raw	30
	Sour Cream Substitute	40
	Dry Onion Soup Powder	4.4
Strawberry Sorbet	@ 1/4 carton	100
Vanilla Wafers		8
Lemonade Powder (swtnd)		30
DINNER		
Spaghetti Sauce w/Meat: Grnd Beef, 10% fat		
	braised, drained	65
	Tomato Sauce	120
	Italian Seasoning	.05
	Onion Powder	.07
	Garlic Powder	.09
	Oregano	.05
	Basil	.05
	Granulated Sugar	2.5
	Safflower Oil Margarine	2.5
	Corn Oil Maragarine	3
Spaghetti, ckd al denta		96
Tossed Salad:	Iceburg Lettuce	40
	Carrot, pared, raw	20
	Tomato, raw	30
	Ham, boiled, 5% fat	25
	Frozen Egg Yolk, raw	25
Creamy Italian Dressing:	Mayonnaise	15
	Red Wine Vinegar	1.8
	Onion Powder	.03
	Garlic Powder	.03
	Italian Seasoning	.04
	Granulated Sugar	7
Low Protein Flat Bread		50
Garlic Butter:	Butter	5
	Garlic Powder	.1
Fruit Flavored Gel:	Low Protein Gel Powder	28.4
	Deionized Water	150
	Non Dairy Whip Topping	5
Low Protein B'Scotch Cookies		28
Ice Tea Powder (lemon & sugar) (in 10 oz. water)		22

MEAT DIET: DAY 2

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		25
Cnd Mandarin Oranges, light syrup, drained		40
Cnd Grapefruit, light syrup, drained		40
Orange Cream Sauce:	Sour Cream Substitute	25
	Clover Honey	2.5
	Powder Sugar	8
	Orange Juice, cnd (unswtnd)	5
	Imitation Vanilla	.2
Canadian Bacon, unheated		65
Dry Cereal, Puffed Rice		11.3
Polyrich		150
Low Protein Flat Bread		40
Corn Oil Maragarine		5
LUNCH		
Roast Beef Sandwich:	Roast Beef, round, ckd	60
	White Bread, 2 sl	56
	Iceburg Lettuce	15
	Mayonnaise	15
Vegetable Soup b:	Beef Broth, condensed	75
	Deionized Water	65
	Cnd Tomatoes, drained	25
	Coconut Oil	5
	Low Protein Spaghetti Rings, dry	10
	Carrots, cnd. drained	20
	Green Beans, cnd. drained	15
	Tomato Paste	15
Vanilla Pudding Supreme:	Vanilla Pudding Powder	22
	Polyrich	120
	Cornstarch	8
	SS Choc Chips	15
	Shredded Coconut	5
	Non-Dairy Whip Topping	10
Lemonade Powder (swtnd)		30
DINNER		
Pork Stroganoff:	Pork Center Loin, rsted	50
	Sour Cream Substitute	50
	Corn Oil	1
	Red Wine	2.5
	Onion Powder	.02
	Dill Weed	.04
	Soy Sauce	1.3
	Low Protein Tagliatelle, dry	42.5
	Coconut Oil	5
Tossed Salad:	Iceburg Lettuce	40
	Carrot, pared, raw	20
	Tomato, raw	30
	Frozen Egg Yolk, raw	25
French Dressing		15
Low Protein Flat Bread		40
Butter		5
Peach Sorbet @ 1/4 carton		100
Vanilla Wafers		8
Ice Tea Powder (lemon & sugar)		22

MEAT DIET: DAY 3

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		34
Frozen Strawberries, slightly thawed (unswtnd)		75
Creamy P'apple Sauce:	Sour Cream Substitute	25
	Clover Honey	8
	Powdered Sugar	4
	Cnd P'apple Tidbits, in Juice, drained	15
Boiled Ham, 5% fat, unheated		28.4
Dry Cereal, Rice Krispies		28.4
Polyrich		165
Low Protein Flat Bread		50
Corn Oil Margarine		5
LUNCH		
Chili with Meat:	Ground Beef, 10% fat braised, drained	70
	Tomato Paste	20
	Cnd Tomatoes, drained	100
	Juice from Cnd Tomatoes	45
	Coconut Oil	3
	Olive Oil	5
	Chili Powder	2
	Ground Cumin	.3
	Onion Powder	.25
	Garlic Powder	.35
Tossed Salad:	Iceburg Lettuce	40
	Cucumber, pared, raw	26
	Carrot, pared, raw	20
Vinegar & Oil Dressing:	Wine Vinegar	5
	Olive Oil	5
Low Protein Flat Bread		50
Garlic Butter:	Butter	2.5
	Garlic Powder	.03
Peach Gel Delight:	Cnd Peaches, light syrup drained	25
	Low Protein Gel Powder	21.3
	Deionized Water	115
	Non-Dairy Whip Topping	10
Leomade Powder (swtnd)		30
DINNER		
Cantonese Pork & Veg:	Pork Loin, cubed, rstd	60
	Brocolli, froz, ckd	15
	Olive Oil	2
	Butter	5
	Onion Powder	.1
	Ground Ginger	.05
	Chix Broth, condensed	25
	Deionized Water	25
	Cornstarch	2
	Soy Sauce	2
	Long Grain Rice, boiled	155
Mixed Green Salad:	Iceburg Lettuce	40
	Cucumber, pared, raw	26
	Frozen Egg Yolk, raw	23.5
Thousand Island Dressing		15
Low Protein Flat Bread		50
Butter		5
Choc Pudding Sundae:	Choc Pudding Powder	28.3
	Polyrich	120
	SS Choc Chips	15
	Non-Dairy Whip Topping	10
Ice Tea Powder (lemon & sugar)		22

SOY DIET: DAY 1

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		34
Cnd Pears, light syrup, drained		75
Creamy Strawberry Delight:	Sour Cream Substitute	20
	Powdered Sugar	2
	Strawberry Jam	30
Shrimp Style Rarebit:	Kokeyal Shrimp Style	70
	Sour Cream Substitute	60
	Frozen Egg Yolk, raw	15
	Dry Mustard	.21
	Worchestershire Sauce	.25
	Cayenne Pepper	.02
Low Protein Flat Bread		50
LUNCH		
Chicken Style Salad:	Naturalean	100
	Mayonnaise	21
	Sour Cream Substitute	28
	Onion Powder	.15
	Iceburg Letture	10
	Low Protein Flat Bread	50
Potato Chips		15
Vegetables with Dip:	Carrot, pared, raw	30
	Celery, raw	30
	Sour Cream Substitute	28
	Coconut Oil	2
	Dry Onion Soup Powder	4.4
Strawberry Sorbet	@ 1/4 carton	100
Vanilla Wafers		8
Lemonade Powder (swtnd)		30
DINNER		
G'burger Spaghetti Sauce Sc:	Granburger, dry	16
	Beef Broth, condensed	20
	Delonized Water	20
	Tomato Sauce	120
	Italian Seasoning	.05
	Onion Powder	.07
	Garlic Powder	.09
	Oregano	.05
	Basil	.05
	Granulated Sugar	2.5
	Butter	5
	Coconut Oil	6
	Corn Oil	1.5
Spaghetti, ckd al denta		97.5
Tossed Salad:	Iceburg Lettuce	30
	Carrot, pared, raw	20
	Tomato, raw	30
	Frozen Egg Yolk, raw	20.5
Creamy Italian Dressing:	Sour Cream Substitute	28
	Red Wine Vinegar	1.8
	Onion Powder	.03
	Garlic Powder	.03
	Italian Seasoning	.04
	Granulated Sugar	7
Low Protein Flat Bread		50
Garlic Butter:	Butter	5
	Garlic Powder	.1
Vanilla Almond Tofutti:	@ 1/4 carton	85
Low Protein B'Scotch Cookies		28
Ice Tea Powder (lemon & sugar) (in 10 oz. water)		22

SOY DIET: DAY 2

BREAKFAST		**WEIGHT (GRAMS)**
Tang Powder		25
Cnd Mandarin Oranges, light syrup, drained		40
Cnd Grapefruit, light syrup, drained		40
Orange Cream Sauce:	Sour Cream Substitute	25
	Coconut Oil	3
	Clover Honey	2.5
	Powder Sugar	8
	Orange Juice, cnd (unswtnd)	5
	Imitation Vanilla	.18
Grnd Beef Style Hash:	Granburger, dry	36.5
	Beef Broth, condensed	40
	Deionized Water	40
	Potatoes, cnd. drained	100
	Coconut Oil	5
	Olive Oil	5
	Worchestershire Sauce	.2
	Onion Powder	.1
	Basil	.05
Low Protein Flat Bread		40
Butter		5
LUNCH		
Tuna Salad:	Tuna	57
	Mayonnaise	15
	Sour Cream Substitute	10
	Onion Powder	.15
	Pickle Relish	10
	Iceburg Lettuce	15
	White Bread, 2 sl	56
Vegetable Soup b:	Beef Broth, condensed	75
	Deionized Water	65
	Coconut Oil	5
	Cnd Tomatoes, drained	25
	Low Protein Spaghetti Rings, dry	10
	Carrots, cnd. drained	20
	Green Beans, cnd. drained	15
	Tomato Paste	15
Vanilla Pudding Supreme:	Vanilla Pudding Powder	22
	Polyrich	120
	Cornstarch	8
	SS Choc Chips	10.5
	Shredded Coconut	5
Lemonade Powder (swtnd)		30
DINNER		
Seafood Style Stroganoff:	Kokeyal Scallop Style	85
	Butter	7.5
	Sour Cream Substitute	55
	Red Wine	2.5
	Onion Powder	.02
	Dill Weed	.06
	Soy Sauce	1.3
	Low Protein Tagliatelle, dry	42.5
Tossed Salad:	Iceburg Lettuce	40
	Carrot, pared, raw	20
	Tomato, raw	30
	Kokeyal Shrimp Style	21
	Frozen Egg Yolk, raw	25
Creamy French Dressing:	French Dressing	15
	Sour Cream Substitute	10
Low Protein Flat Bread		40
Butter		5
Peach Sorbet @ 1/4 carton		100
Vanilla Wafers		8
Ice Tea Powder (lemon & sugar)		22

SOY DIET: DAY 3

BREAKFAST		WEIGHT (GRAMS)
Tang Powder		25
Frozen Strawberries, slightly thawed (unswtnd)		75
Creamy P'apple Sauce:	Sour Cream Substitute	25
	Clover Honey	8
	Powdered Sugar	4
	Cnd P'apple Tidbits, in juice, drained	15
Dry Cereal, Rice Krispies		28.4
Polyrich		175
Sausage Style Patty:	Naturalean	50
	Low Protein Flat Bread Crumbs	10
	White Flour	7.5
	Frozen Egg Yolk, raw	5
	Coconut Oil	2.5
	Cayenne Pepper	.03
	Sage	.1
	Ground Thume	.1
	Summer Savory	.1
Low Protein Flat Bread		40
Butter		5
LUNCH		
Granburger Chili:	Granburger, dry	40
	Beef Broth, condensed	50
	Deionized Water	50
	Tomato Paste	20
	Cnd Tomatoes, drained	100
	Juice from Cnd Tomatoes	45
	Butter	5
	Coconut Oil	6
	Olive Oil	2.5
	Chili Powder	2
	Ground Cumin	.3
	Onion Powder	.25
	Garlic Powder	.35
Tossed Salad:	Iceburg Lettuce	40
	Cucumber, pared, raw	26
	Carrot, pared, raw	20
Vinegar & Oil Dressing:	Wine Vinegar	5
	Olive Oil	5
Low Protein Flat Bread		40
Garlic Butter:	Butter	5
	Garlic Powder	.1
Peach Gel Delight:	Cnd Peaches, light syrup drained	25
	Low Protein Gel Powder	21.3
	Deionized Water	115
	Non-Dairy Whip Topping	10
Leomade Powder (swtnd)		30
DINNER		
Shrimp Style Cantonese:	Kokeyal Shrimp Style	65
	Brocolli, froz, ckd	15
	Butter	11.5
	Coconut Oil	3
	Onion Powder	.1
	Ground Ginger	.05
	Deionized Water	50
	Cornstarch	2
	Soy Sauce	2
	Long Grain Rice, boiled	155
Mixed Green Salad:	Iceburg Lettuce	40
	Cucumber, pared, raw	26
	Frozen Egg Yolk, raw	27.5
Thousand Island Dressing		20
Low Protein Flat Bread		40
Butter		5
Chocolate Sundae:	Choc Supreme Tofutti	86
	SS Choc Chips	15
	Non-Dairy Whip Topping	10
Ice Tea Powder (lemon & sugar)		22

Appendix XI

Food Supplements

DAILY TOTAL INTAKE OF FOOD SUPPLEMENT UNITS*

SUBJECT NUMBER	DIETARY TREATMENT	PERIOD I		PERIOD II
1		3		2.8
8		5		5
11		1.5		1.7
12		5		5
18	DAIRY	2		2
19		5.5		5.5
20		2.8		2.7
22		3.8		4
Mean		3.6		3.6
Pooled mean		±1.4	3.6	±1.4
2		6.3		6.6
4		3		2.8
6		3.7		4
7	SOY	4.7		4.8
15		3.8		3.8
17		4.8		4.5
23		4.1		4.1
24		3.2		2.8
Mean		4.2		4.2
Pooled mean		±1.0	4.2	±1.1
3		0.66		0
5		5.3		4.9
9		0		0.66
10	MEAT	0		0
13		3.5		3.2
14		4		4.6
16		4		4.6
21		0.66		0
Mean		2.3		2.3
Pooled mean		±2.0	2.3	±2.1

*1 Unit Represents: 2 slices of white bread (59 g)
Parkay margarine (8 g)
Butter (1 g)

Appendix XII

Mineral Supplements

DAILY MINERAL SUPPLEMENTS

	DAIRY	NO.	SOY	NO.	MEAT	NO.
PERIOD I	One-a-day*	1	One-a-day	1	One-a-day	1
	Zinc Sup	1	OsCal	1	Cal Sup	1
	Zinc Sup	1	Zinc Sup	1		
Calcium (mg)	—		250		300	
Magnesium (mg)	—		—		—	
Zinc (mg)	10		10		10	
PERIOD II	One-a-day	1	One-a-day	1	One-a-day	1
	Combo	2	Combo	2	Combo	2
	Oste Shell	1	Os-Cal	2	Cal Sup	2
Calcium (mg)	917		1167		1267	
Magnesium (mg)	267		267		267	
Zinc (mg)	10		10		10	

*Contains 100% of RDA for all essential vitamins.

Mineral and Vitamin Supplements

1. Oscal (made from Oyster Shell):

   125 mg Calcium
   62.5 IU of Vit D

2. COMBO

   33.3 mg Calcium
   133.3 mg Magnesium
   5 mg Zinc

3. Cal Sup

   300 mg Calcium w/Glycine

4. Oste Shell

   250 mg Calcium
   125 IU Vitamin D

5. Zinc Supplement

   10 mg

6. One-A-Day Vitamins

   (contains 100% of RDA for vitamins listed below)

Vitamin A	5000 IU	Niacin	20 mg	Folic Acid	0.4 mg
Vitamin C	60 mg	Vitamin D	400 IU	Vitamin B-12	6 mg
Vitamin B-1	1.5 mg	Vitamin E	30 IU	Panthothenic Acid	10 mg
Riboflavin (B-2)	1.7 mg	Vitamin B-6	2 mg		

Appendix XIII

Methodology

PROCEDURES FOR PREPARING METABOLIC DIET COMPOSITES

I. Compositing Diets
1. Prior to beginning meal preparation, set up 6 clean trays, 2 trays for each of the 3 diet groups.
2. Label the trays with diet group (Dairy, Meat, Soy), diet day (1, 2, 3), and duplicate letter (A, B).
   Example: Dairy A - Day 1
3. Place an appropriate meal card on each tray, labeled as in #2.
4. As menu items are prepared for a meal place required items on the diet composite trays.
   a. Place items which do not need to be refrigerated directly on the trays as they are prepared.
   b. Label items that need to be refrigerated or frozen as "Composite Item", and place into refrigerator or freezer.
5. After all the subjects have finished eating the meal, set up the composite trays with all items indicated on the meal cards.
   Note: Do not add water to Tang, lemonade and ice tea powders.
6. Assemble six 1-gallon cardboard containers, which have been lined with 2 polyethylene bags, and labeled like the composite trays and meal cards.
   Note: An entire day's menu (breakfast, lunch, and dinner) for 1 diet goes into 1 container.
7. From 1 composite tray at a time, empty each menu item completely into the appropriately container.
8. **IMMEDIATELY** after each item is placed into the container, check that item off on the appropriate meal card.
9. After all menu items from the tray have been placed into the appropriate container, place the lid securely on the container and place the container into the food composite refrigerator.
10. Initial the meal card.
11. Repeat steps 7 through 10 for each composite tray.
12. After all the diets have been composited for the meal, file the composite meal cards.

II. Weighing and Blending Composited Diets
1. Remove the diet composites from the refrigerator and allow them to come to room temperature.
2. Weight a large pyrex bowl on the digital top loader balance and record the weight in the Diet Composite Notebook.
3. Tare the large pyrex bowl.
4. Remove the lid from the composite container and tie the 2 polyethylene bags tightly closed with the twist tie.
5. Carefully light the diet composite in the bags into the tared pyrex bowl and record the weight.
6. Subtract 8.24 gm (weight of 2 bags + tie) from the weight obtained in step 5.
7. Record this weight as the diet composite weight.
8. Compare the diet composite weight from step 7 to the expected weight of the diet.
9. If the 2 weights differ by more than 10 grams, the diet composite is discarded, and the study supervisor is informed.
   Note: DO NOT DISCARD the diet composite until you have weighed the duplicate diet composite. If both diet composites (A and B) differ by more than 10 grams from their calculated weights, neither of them is discarded.
10. If the 2 weights differ by less than 10 grams, remove the composited diet from the pyrex bowl.

11. Place the composite (still in the bags) back into the composite container.
12. Untie the bags and smooth the excess over the lip of the container.
13. Re-tare the pyrex bowl and carefully empty the diet composite into the bowl. BE SURE TO HOLD THE PLASTIC BAGS SECURELY IN PLACE.
14. Rinse the inner polyethylene bag several times with small amounts of deionized water until the bag is clean, adding this water to to diet composite. Use a rubber spatula to scrape the sides clean.
15. Rinse the spatula with deionized water into the bowl.
16. Make sure enough deionized water is added to ensure thorough blending of the diet.
17. Record the weight of the diet + water.
18. Empty all of the diet composite into the large stainless steel blender labeled "Food Composites".
19. Place lid securely on blender.
20. Run blender on low speed for 1 minute or until contents are thoroughly mixed.
21. Then run the blender at speed 2 for 1 minute.
22. Then run the blender at high for 4 minutes, or longer for complete homogenization.
23. While the blender is running, calculate a 30% aliquot of the weight obtained in step 17.
24. Record this weight.
25. As soon as the composite is blended and the blender is turned off, IMMEDIATELY place the aliquot into an acid-washed, appropriately labeled 1-liter polyethylene bottle.
26. Place lid securely on bottle and place into food composite freezer.
27. Repeat steps 2 through 26 for all of the diet composites for the day.

PROCEDURES FOR DETERGENT AND ACID WASHING

I. Detergent Washing
   1. Place about 1 Tbs. of Alconox into each container that is to be washed.
   2. Fill with hot tap water.
   3. Scrub with bottle brush.
   4. Rinse well in tap water.

II. Acid Washing
   1. Wear heavy rubber gloves for entire procedure.
   2. Make enough 20% nitric acid solution for entire session.
      (200 ml nitric acid diluted with deionized water to 1000 ml)
   3. Pour 20% nitric acid solution into containers to be acid washed until they are COMPLETELY full.
   4. If washing bottles - put tops securely on bottles, and invert bottles 4 times.
   5. Let stand for 30 minutes.
   6. Pour nitric acid solution into next set of containers.
      Note: Same nitric acid solution can be used 3 times.
   7. Rinse empty containers 6 times with deionized water.
   8. Drain thoroughly by turning upside down on clean brown paper towels.
   9. Dispose of used nitric acid solution by pouring it down the drain and running additional tap water after it.

PROCEDURES FOR COMPOSITING URINE

1. Empty all the urine from one 24-hour collection period for 1 subject into that subject's acid-washed 2000 ml polyethylene graduated cylinder.
2. Add deionized water to bring the volume up to 2 liters, or a convenient volume greater than 2 liters if the initial urine volume is greater than 2 liters.
3. Record this volume in the Urine Composite Notebook.
4. Mix the diluted urine by pouring it into an acid-washed 1-gallon polyethylene jug, placing the lid on the jug securely, and inverting the jug 4 times.
5. Calculate 5% of the total volume from step 3, and record this volume.
6. Measure duplicate 5% aliquots into an acid-washed 100 ml graduated cylinder.
7. Pour the aliquots into 2 separate acid-washed 1-liter polyethylene bottles, which have been labeled with the subject's name, number, urine composite period and day.
8. Rinse the empty urine bottle(s) 3 times with hot tap water.
9. Remove uric acid deposits (red film) from used bottles as necessary, by cleaning with a solution of Alconox (1 Tbs. Alconox per urine bottle, filled with tap water, and scrubbed with a bottle brush).
10. Rinse the cleaned urine bottle(s) again 3 times with hot tap water.
11. Then rinse 2 times with deionized water.
12. Turn rinsed urine bottle(s) upside on trays covered with clean brown paper towels to dry.
13. Dispose of the unused urine of the subject by pouring it down the drain.
14. When dry add 10 mls 10% HCl to the bottle(s) with an automatic pipette.
15. Cap the bottle(s) securely and place on the shelve for reuse.
16. Rinse the acid-washed graduated cylinders used to measure urine 3 times with deionized water and turn upside down on clean brown paper towels to dry.
17. Repeat all steps for each subject's urine.

PROCEDURES FOR FECAL COMPOSITING

1. All feces will be collected into 1-pint cardboard cups (mineral free) labeled with the date, time of collection, and subject's name and number.

2. Fecal collections will be stored in a freezer until they are composited.

3. Feces will be composited as follows: All of the feces for a subject will be partially thawed. Feces will be separated by period on the basis of a predetermined time schedule.

4. The feces for a given period will be weighed after the addition of deionized water (to a convenient weight and to allow for a free flowing mixture after blending).

5. Feces and deionized water will be homogenized in a specifically designated 5-quart blender for a total of 5 minutes or until thoroughly blended.

6. Two labeled 250 ml acid washed polyethylene bottles will be filled with a 5% aliquot (or reasonable percentage) of the homogenized fecal composite. This will be frozen subsequent mineral analysis.

7. Care will be taken to minimize extraneous mineral contamination of composites by using plastic gloves when handling feces (if necessary) and rinsing the blender cup with deionized water in between composites.

8. Thawed fecal composites will be analyzed in duplicate for the desired nutrients using the same procedures described for food. The sample sizes for nitrogen and mineral determinations will be 10 and 2.5 grams respectively. The thawed composite will be shaken vigorously before each duplicate sample in order to prevent errors due to settling of the composite. Samples will be reanalyzed if values obtained for specific samples do not duplicate within 5%.

NITRIC-PERCHLORIC WET ACID OXIDATION

1. Homogenize samples by grinding tissues with a teflon pestle in polycarbonate specimen cups.
2. Weigh 0.1 - 0.5 g samples into 50 ml acid-washed (10% HCl) beakers and cover with watch glasses. Run blanks along with samples.
3. Add 1.5 ml of redistilled nitric acid and 1.0 ml of 70% perchloric acid and heat at 150°F.
4. Increase temperature slowly to 325°F and allow samples to reflux in perchloric acid until clear.
5. Remove watch glasses and dry down to a white ash. If charring occurs add nitric acid dropwise and allow to reflux.
6. When samples have dried to a white ash dissolve in 10 ml of 10% HCl (ultrapure).

ATOMIC ABSORPTION

To turn on:
Turn hood on.

1. Turn power on by depressing button.
2. Install the proper lamp (for each element examined) by sliding it into the holder and securing the catch release. Plug the lamp into the power source, being careful to align the pins.
3. Depress "Absorbance" button on right front panel.
4. Set the "Slit" selector knob.
5. Select and depress the correct range on the left front panel: UV or Visible range. If the wavelength is above 650 mm, also depress the "Filter" button.
6. Set wavelength just above or below the valve given in the manual.
   UV: wavelength number = wavelength, nm
   Visible: wavelength number = 1/2 wavelength, nm
   (e.g., if reads 211.0, L=422nm)

   Note: Standard conditions (steps 3-5) for each element are given in Analytical Methods for Atomic Absorption Spectropholometry
7. Slowly increase source control until the milliammeter (in the lamp box) reaches the operating current indicated on the lamp label. DO NOT EXCEED maximum current rating at any time.
8. Slowly increase "gain" control until the energy meter moves half of the way to the green region of the scale.
9. Move the "fine wavelength" control slowly to obtain the maximum needle deflection to the right on the "Energy Meter." DO NOT EXCEED the bright green area of the scale. If necessary, reduce "Gain" control.
10. Check lamp position by moving the vertical and horizontal alignment screws to obtain maximum energy output (see step 9). Clove cover.
11. Adjust "Gain" control so that the "Energy Meter" needle falls just to the left of the green region.
12. Turn on the fuel source (i.e., open the tank) by turning valve on top of tank 1/4 turn counterclockwise. IF PRESSURE OF THE ACETYLENE AT THE TANK IS LESS THAN 75 PSIG, DO NOT USE. Replace the tank.
13. Adjust the acetylene cylinder output pressure at 10 psig.
14. Press the "Fuel Flow Check" and "Oxidant Flow Check" buttons simultaneously. The instrument settings should read as follows:

Oxidant pressure gauge	30 psi
Auxillary oxidant plowmeter	55 psi
Fuel pressure gauge	8 psi
Fuel flowmeter	32 psi

15. Flip the "Gases" control to "ON" and ignite the flame by depressing the "Ignite" button.

16. Insert sampling capillary tube into a beaker of deionized water. Allow burner apparatus to warm-up approximately 5 minutes before beginning measurement.

17. Flame mixture can be varied to maximize meter readout by adjusting fuel or oxidant flow (see manual).

18. Routine measurements are usually made with the Int 1 Sec button depressed. Push "Read" and then "Auto Zero" while aspirating ion-free water. The instrument should be zeroed in this manner after each measurement.

19. Read samples, standards, and blanks. (AA gives you absorbance readings). It is best to read standards both before and after samples.

INSTRUMENT SHUTDOWN:

1. Aspirate ion-free water for a minimum of 1 minute before burner shutdown.

2. Remove capillary tube from deionized water.

3. Switch "Gases" toggle valve to shutdown position.

4. Turn "source" and "gain" controls full counterclockwise to stop.

5. Turn off fuel source.

6. "Bleed" fuel from fuel lines by holding "Fuel Flow Check" until Fuel Flowmeter and acetylene regulator gauges register zero.

7. Close acetylene cylinder regulator valve by turning counterclockwise.

8. Depress power button.

9. Turn hood off.

CALCULATIONS:

Do linear regression program to obtain concentrations using absorbance of the blank as "0" concentration.
Metal (mineral) content in μg/gm is calculated by multiplying the diluted concentration by the dilution factor and dividing by the sample weight.

PROXIMATE ANALYSIS OF FOODS

General Information

A routine analysis consists of nitrogen (protein), moisture, ether extract (lipid), crude fiber and ash. "Nitrogen Free Extract" is the total of these (expressed in %) subtracted from 100%. All analyses are run in duplicate and the two duplicates should check. We will determine nitrogen, moisture, ether extract and ash on the food sample selected.

Percent error allowed:
If analytical result is between:

0 - 2%	10% error is allowed
2 - 4%	6% error is allowed
4 - 6%	5% error is allowed
6 or more	3% error is allowed

To calculate error:

$$\frac{\text{difference between two}}{\text{smallest value}} \times 100 = \%\ \text{error}$$

The Kjeldahl room should be kept neat and clean at all times. Everyone who uses it is responsible. All glassware is to be kept chemically clean (i.e. washed and rinsed with hot soapy water and then rinsed with distilled water). Any equipment borrowed should be returned promptly.

Glassware, laboratory equipment and solutions for the procedure will be provided at the site of analysis.

Each student will choose the food to be sampled and analyzed. Various foods from the four food groups will be used.

Protein or Nitrogen

Principle:

The nitrogen is oxidized to $(NH_4)_2SO_4$ by digestion with concentrated H_2SO_4. The digest is made alkaline with concentrated NaOH and the NH_3 is distilled into a 2 percent solution of boric acid. The ammonium borate produced is titrated with standard HCl. The amount of nitrogen obtained is multiplied by the factor 6.25 to arrive at the crude protein content of the sample.

Procedure:

Sample Size: 0.5 - 2.2 g (approximately 1.0 g) (equivalent to 15 to 40 mg of nitrogen). Either use a weighing pan or weigh the sample onto a filter paper. Prepare one blank (include filter paper is used).

Digestion: Place sample is the Kjeldahl flask (if filter paper was used, fold sample and paper and place in flask). Add 2 glass beads about 10 g of Na_2SO_4, 0.2 g $CuSO_4$ (already mixed in) and 25 ml of concentrated H_2SO_4. Run the acid down the sides of the flask to remove any

part of the sample which may stick to neck of flask. If sample is larger than 2.2 g add 10 ml of H_2SO_4 for each additional g of sample. Heat gently until frothing stops, then briskly until solution turns a clear green. Continue heating for about 30 to 60 minutes after solution clears. Samples usually take 2 hours. During the early stages the flask should be turned occasionally to rinse down carbonaceous matter from the sides of the flask. After digestion is completed allow flask to cool at least 20 to 30 minutes. After the digested sample has cooled completely, cautiously add about 250 ml of distilled water. Stopper, check identification and leave for second day.

Distillation:

A. Prepare receiving flask.

   Into a 500 ml Erlenmeyer flask add 25 ml of 4% boric acid.
   Measure with graduated cylinder. Add about same amount of water.
   Add 4 drops of mixed indicator (methyl red-methylene blue).
   Place receiving flask on the distillation rack and insert delivery tube below surface of liquid. TURN ON WATER TO CONDENSER.

B. Turn distillation burners on low heat (1 or 2) to let apparatus warm up. To the diluted samples add about 60 ml of 50% (w/w) sodium hydroxide slowly and gently down the side of the Kjeldahl flask allowing the NaOH to form a layer below the sulfuric acid digest. There must be more than enough NaOH to neutralize the sulfuric acid to allow the ammonia to distill (there should be a blue line between layers).

C. Be sure that water is circulating through the condenser. Add a few granules of Zn just before stoppering to prevent bumping. After the flask is connected tightly to the distillation unit, shake slowly and then vigorously. Turn up heat immediately. If the mixture does not turn blue, there is insufficient NaOH.

D. Distill until approximately a total of 200 ml of liquid is collected in the receiving flask. Move the collection flask so that the tube is no longer under the liquid. Distill another 10 to 20 mls to rinse tube. Turn off heat and water.

Titration:

Titrate with standard HCl (about 0.1 N) until the green color changes to a grey lavender color. Titrate blank first (about 0.2 ml). Tirate all samples until the color matches end point of blank.

Note: End point of titration is partway between green and purple. Titrate until green color is no longer present-resulting color is grey, or true blue.

Calculation:

One equivalent of HCl reacts quantitatively with one equivalent of N as ammonium borate.

Normality of acid x 14.008 = mg N equivalent to 1 ml of acid or mg N/ml acid.

As protein contains about 16% N; mg N/ml acid x 6.25 = mg protein/ml acid.

Therefore:

$$\% \text{ protein} = \frac{(\text{ml HCl - ml blank}) \times (\text{mg N/ml acid} \times 6.25) \times 100}{\text{sample weight}}$$

Moisture (Air Drying)

Determine percent of moisture (H_2O) in each of four samples (2 in aluminum dishes and 2 in ashing crucibles) by the following method. Do all drying in the air drying over at 80-85°C. Make all drying periods at least 16-18 hours (overnight). Do weighing on the Mettler analytical balance (302) and open pan balance (207).

Procedure:

1. Dry two aluminum containers and two ashing crucibles.
2. Cool containers in dessicator for approximately 1 hour (or overnight).
3. Weigh dried and cooled containers.
4. Place approximately 3-4 g of dry or 5-10 g of wet ground (or chopped, but drying time increased) sample in the weighed dish. Weigh dish plus sample. 3-4 g of dry sample in duplicate is required for the ether extract procedure.
5. Dry sample plus container at 80-85°C.
6. Cool container plus sample in dessicator for approximately 1 hour (or overnight).
7. Weight dried sample plus container.
8. Calculate percent moisture in sample

$$\% \text{ moisture} = \frac{\text{wt. sample before drying} - \text{wt. sample after drying} \times 100}{\text{wt. sample before drying}}$$

Use average of duplicate values.

Don't touch containers with hands. Use tongs, as oil from hands increases weight of container, resulting in greater error. There is loss of moisture when samples are ground.

Ether Extract (Total Lipids)

Determine percent ether extract in duplicate by the following method. Do all weighings on open pan balance (207) for each sample.

Procedure:

1. Clean Goldfisch Fat Extraction beakers are put in air drying over at 100°C for overnight.
2. Cool beakers in dessicator for 1 hour (or overnight).
3. Weigh beaker and record weight and number.
4. Weigh dry finely ground sample (3-4 g) onto filter paper (Whatman # 1), fold and transfer to the fat extraction thimble. (A larger sample is required for substances low in fat).
5. Place thimble with sample into extraction cup and place in the fat extractor.
6. Start flow of water through the condenser slowly and adjust the flow of water (1/4 turn).

CAUTION: ETHYL ETHER IS HIGHLY VOLATILE AND FLAMMABLE. NO FLAMES OR SPARKS WHILE ETHER EXTRACTION IS IN USE. ALWAYS TURN OFF MAIN POWER SWITCH BEFORE CHANGING HEATER SWITCH SETTINGS.

7. Add 25-30 ml of anhydrous ethyl ether into tared beaker with flanged and ground top.
8. Lock beaker to condenser by means of the lock ring with cork gasket insert.
9. Raise hot plate to bottom of beaker. Turn heater to high. Then turn on main power switch.

10. Extract for 2 hours (check for tight fit and water flow during first 30 minutes and periodically thereafter).
11. Turn main power off. Remove thimble and cup and insert ether reclaiming cup. Reconnect.
12. Turn heater to low and turn main power switch on. Reclaim ether or extract sample down to about 10 ml of ether in the beaker. Do not let sample burn.
13. Turn main power off. Remove reclaimed ether and save ether for reuse.

Shut off water.

14. Turn main power on. Dry extraction beaker on extraction rack until all ether has evaporated using low heat.
15. Remove sample beakers and turn off main power switch.
16. Cool beaker containing sample in dessicator for 1 hour or overnight.
17. Weigh sample and beaker and record weight.

Calculations:

Calculate percent fat or lipid extract in original sample.

$$\%\ \text{lipid extract wet basis} = \frac{\text{wt. of lipid extract or fat}}{\text{wt. sample, wet}}$$

$$\%\ \text{lipid extract dry basis} = \frac{\text{wt. of lipid extract or fat}}{\text{wt. sample, dry}}$$

1. Dry Extraction - the material to be analyzed must be dried thoroughly in any oven and the solvent used anhydrous. Drying of the original wet tissues is necessary as it cannot be extracted sufficiently. Also it is necessary to avoid the presence of any water so that the water soluble materials are not extracted and determined with the fat. This is the type of extraction used in the Goldfisch apparatus.

2. Wet Extraction - Ex. Babcock method for determination of fat in milk.

Other solvents sometimes used: Acetone, chloroform, benzene, petroleum ether.

Ash

Determine percent ash on duplicate samples using the following method. Moisture has previously been expelled at 80 - 85°C and calculated using appropriate crucibles for ashing. Dry samples in crucibles have been stored in dessicators.

Procedure:

1. Samples should be in ground or powder form. Check sample weight after any manipulation.
2. Add a few drops of olive oil to dry material and heat slowly over and open flame until swelling and smoking stops. Do not allow to flame.
3. Place crucible in muffle furnace at about 550-600°C for at least 6-8 hours (overnight).
4. Cool in dessicator for 1 hour or overnight and weigh.

Calculations:

$$\% \text{ Ash} = \frac{\text{Crucible} + \text{ash} - \text{crucible wt.}}{\text{sample wt.}} \times 100$$

Nitrogen Free Extract

This value will be estimated by using your determinations of crude protein, water, ether extract and ash, approximating crude fiber from food composition tables and subtracting from total weight.

Calculations:

$$\% \text{ N.F.E.} = \text{Sample wt.} - \frac{(\text{protein} + \text{E.E.} + H_2 + \text{Ash} + \text{C.F.}) \times 100}{\text{Sample wt.}}$$

Appendix XIV

Medications

MEDICATIONS

SUBJECT NO.	MEDICATIONS	DATE
1	Allergy Shot	11/8,23 12/13
2	2 Actifed	10/26,27
4	Lithium Tetracycline	EVERY OTHER DAY
9	1 Antacid Tetracycline 2 Tylenol	10/30 11/13,14 11/20
10	Talwin (pain killer)	10/27 11/14
11	2 Drixoral	12/3
15	2 Aspirin 2 Drixoral 2 Tylenol	10/30 11/9 11/14
16	250 mg Penicillin (4x/day) Tussionex Pseudoephrine 2 Aspirins (3x/day) 4 Phenol Losenge Benylin Cough Syrup 2 Tylenol (2x/day)	10/25,26 10/25 10/25 11/11-15 11/11-20 11/13-20 11/15-20
18	Tylenol	11/23
22	1 Contac	10/30
23	Benedryl	11/23
24	2 Aspirin	11/2

Appendix XV

Zinc Intake, Urine and Fecal Excretion and Retention

ZINC INTAKE, URINE AND FECAL EXCRETION AND RETENTION

SUBJECT NUMBER	GROUP TOTAL	DIETARY TREATMENT	PERIOD I				PERIOD II			
			INTAKE	URINE	FECES	RETENTION	INTAKE	URINE	FECES	RETENTION
1		DAIRY	16.66	0.49	14.51	1.46	18.91	0.62	8.50	9.79
8		DAIRY	16.90	1.95	14.22	0.73	19.40	2.44	13.87	3.09
11		DAIRY	16.05	0.42	14.45	1.68	18.76	0.37	12.33	6.06
12	8	DAIRY	16.90	0.85	13.42	2.63	19.40	0.94	10.79	7.67
18		DAIRY	15.84	0.69	14.38	0.77	18.46	0.67	13.88	3.91
19		DAIRY	17.15	0.98	16.56	-0.39	19.40	0.78	12.54	6.08
20		DAIRY	16.59	0.49	16.43	-0.33	19.20	0.64	11.94	6.62
22		DAIRY	16.66	0.86	13.35	2.45	19.35	0.80	14.18	4.37
MEAN			16.59	0.87	14.67	1.13	19.11	0.91	12.25	5.95
±SD			±0.15	±0.15	±0.84	±0.84	±0.15	±0.15	±0.84	±0.84
2		SOY	19.65	1.63	17.18	0.84	21.81	1.36	21.26	-0.81
4		SOY	19.02	0.53	19.86	-1.37	21.01	0.40	19.79	0.82
6		SOY	18.87	0.80	14.68	3.39	21.03	0.78	14.04	6.21
7	8	SOY	19.16	0.74	20.33	-1.91	21.08	0.74	17.78	2.56
15		SOY	19.02	0.89	18.73	-0.61	20.98	0.72	16.04	4.22
17		SOY	19.21	1.00	17.14	1.07	21.17	0.70	16.52	3.95
23		SOY	19.02	0.49	13.45	5.08	21.03	0.67	11.35	9.01
24		SOY	19.02	0.40	16.20	2.42	20.68	0.55	15.38	4.75
MEAN			19.12	0.81	17.20	1.11	21.10	0.74	16.52	3.84
±SD			±0.15	±0.15	±0.84	±0.84	±0.15	±0.15	±0.84	±0.84
3		MEAT	20.38	0.91	15.17	4.30	21.22	0.83	18.80	1.59
5		MEAT	(21.68)*	(0.32)	(7.26)	(14.10)	(22.54)	(0.47)	(7.84)	(14.23)
9		MEAT	20.11	0.25	15.45	4.41	21.62	0.18	20.42	1.02
10		MEAT	20.11	0.56	13.94	5.61	21.22	0.74	23.08	-2.60
13	8	MEAT	21.34	0.31	14.86	6.17	22.31	0.35	17.51	4.45
14		MEAT	21.34	0.31	14.86	6.17	22.31	0.76	26.00	-4.45
16		MEAT	21.34	0.34	18.81	2.19	22.31	0.54	22.67	-0.90
21		MEAT	20.38	1.00	14.28	5.10	21.22	0.84	22.86	-2.48
MEAN			20.71	0.59	16.04	4.07	21.74	0.61	21.62	-0.48
±			±0.16	±0.16	±0.90	±0.90	±0.16	±0.15	±0.90	±0.90
Total Mean (Dairy, Soy, & Meat)			18.81	0.75	15.97	2.10	20.65	0.75	16.80	3.10
±SD			±0.09	±0.09	±0.50	±0.50	±0.09	±0.09	±0.50	±0.50

*( ) Values not included in mean.

Appendix XVI

Iron Intake, Fecal Excretion and Retention

IRON INTAKE, FECAL EXCRETION AND RETENTION

SUBJECT NUMBER	GROUP TOTAL	DIETARY TREATMENT	PERIOD I			PERIOD II		
			INTAKE	FECES	RETENTION	INTAKE	FECES	RETENTION
1		DAIRY	12.17	12.27	-0.10	13.18	9.81	3.37
8		DAIRY	12.75	11.31	1.44	14.05	11.35	2.70
11		DAIRY	9.55	10.07	-0.52	11.62	14.30	-2.68
12	8	DAIRY	12.17	12.27	-0.10	14.05	10.93	3.12
18		DAIRY	8.68	10.13	-1.45	10.56	12.44	-1.88
19		DAIRY	12.17	11.86	0.31	14.05	15.94	-1.89
20		DAIRY	11.88	11.99	-0.11	13.47	12.37	1.10
22		DAIRY	12.31	12.14	0.17	14.43	16.13	-1.70
MEAN			11.46	11.51	-0.05	13.18	12.91	0.27
±SD			±0.64	±0.93	±0.71	±0.64	±0.93	±0.71
2		SOY	23.57	24.40	-0.83	21.63	25.92	-4.29
4		SOY	19.93	22.01	-2.08	17.22	23.20	-5.98
6		SOY	19.83	17.96	1.87	17.51	16.69	0.82
7	8	SOY	21.23	21.57	-0.34	19.06	19.64	-0.58
15		SOY	20.00	21.22	-1.22	17.65	20.00	-2.35
17		SOY	21.47	22.12	-0.65	18.59	22.78	-4.19
23		SOY	20.30	16.45	3.85	17.51	16.31	1.20
24		SOY	19.93	19.86	0.07	17.22	18.24	-1.02
MEAN			20.78	20.70	0.08	18.30	20.35	-2.05
±SD			±0.64	±0.93	±0.71	±0.64	±0.93	±0.71
3		MEAT	8.66	11.98	-3.32	10.42	11.14	-1.02
5		MEAT	(12.75)*	(5.50)	(7.25)	(15.35)	(7.91)	(7.44)
9		MEAT	7.50	10.17	-2.67	11.86	13.53	-1.67
10		MEAT	7.50	8.57	-1.07	10.12	16.77	-6.65
13	8	MEAT	12.73	13.39	-0.66	14.77	15.96	-1.19
14		MEAT	13.10	17.14	-4.04	14.77	20.09	-5.32
16		MEAT	13.10	14.55	-1.45	14.77	17.56	-2.79
21		MEAT	8.66	9.64	-0.98	10.12	13.88	-3.76
MEAN			10.18	12.21	-2.03	12.36	15.56	-3.20
±SD			±0.68	±0.99	±0.76	±0.68	±0.99	±0.76
Total Mean (Dairy, Soy, & Meat)			14.14	14.80	-0.66	14.61	16.27	-1.66
±SD			±0.38	±0.55	±0.41	±0.38	±0.55	±0.41

*( ) Values not included in means.

Appendix XVII

Copper Intake, Fecal Excretion and Retention

COPPER INTAKE, FECAL EXCRETION AND RETENTION

SUBJECT NUMBER	GROUP TOTAL	DIETARY TREATMENT	PERIOD I			PERIOD II		
			INTAKE	FECES	RETENTION	INTAKE	FECES	RETENTION
1		DAIRY	0.96	0.98	-0.02	0.62	0.48	0.14
8		DAIRY	0.96	0.85	0.11	0.67	0.52	0.15
11		DAIRY	0.84	0.79	0.05	0.58	0.59	-0.01
12	8	DAIRY	0.96	0.78	0.18	0.67	0.49	0.18
18		DAIRY	0.80	0.76	0.04	0.51	0.61	-0.10
19		DAIRY	0.96	0.89	0.07	0.89	0.62	0.27
20		DAIRY	0.94	0.72	0.22	0.64	0.55	0.09
22		DAIRY	1.02	0.80	0.22	0.74	0.74	0.00
MEAN			0.93	0.82	0.11	0.67	0.58	0.09
±SD			±0.04	±0.07	±0.07	±0.04	±0.07	±0.07
2		SOY	1.74	1.87	-0.13	1.50	1.60	-0.10
4		SOY	1.48	1.74	-0.26	1.20	1.52	-0.32
6		SOY	1.53	1.71	-0.18	1.29	1.25	0.04
7	8	SOY	1.61	1.59	0.02	1.35	1.36	-0.01
15		SOY	1.54	1.68	-0.14	1.28	1.66	-0.38
17		SOY	1.62	1.97	-0.35	1.33	1.48	-0.15
23		SOY	1.56	1.13	0.43	1.29	0.95	0.34
24		SOY	1.49	1.50	-0.01	1.20	1.04	0.16
MEAN			1.57	1.65	-0.08	1.31	1.36	-0.05
±SD			±0.04	±0.07	±0.07	±0.04	±0.07	±0.07
3		MEAT	0.86	1.28	-0.42	0.52	0.70	-0.18
5		MEAT	(1.04)*	(0.37)	(0.67)	(0.76)	(0.37)	(0.39)
9		MEAT	0.80	0.78	0.02	0.60	0.61	-0.01
10		MEAT	0.80	0.55	0.25	0.52	0.81	-0.29
13	8	MEAT	1.08	0.84	0.24	0.73	0.66	0.07
14		MEAT	1.12	1.12	0.00	0.73	1.09	-0.36
16		MEAT	1.12	1.05	0.07	0.73	0.84	-0.11
21		MEAT	0.86	0.77	0.09	0.52	0.72	-0.20
MEAN			0.95	0.91	0.04	0.62	0.78	-0.15
±SD			±0.04	±0.07	±0.07	±0.04	±0.07	±0.07
Total Mean (Dairy, Soy, & Meat)			1.15	1.13	0.02	0.86	0.90	-0.03
±SD			±0.02	±0.04	±0.04	±0.02	±0.04	±0.04

*( ) Values not included in means.

Appendix XVIII

Calcium Intake, Urine and Fecal Excretion and Retention

CALCIUM INTAKE, URINE AND FECAL EXCRETION AND RETENTION

SUBJECT NUMBER	GROUP TOTAL	DIETARY TREATMENT	PERIOD I				PERIOD II			
			INTAKE	URINE	FECES	RETENTION	INTAKE	URINE	FECES	RETENTION
1		DAIRY	1329.28	149.66	769.40	410.22	2183.34	129.67	1080.00	973.67
8		DAIRY	1329.28	172.48	749.01	407.79	2221.06	174.06	1145.83	901.17
11		DAIRY	1216.12	251.39	772.35	192.38	2124.62	246.80	1251.66	627.16
12		DAIRY	1329.28	110.67	794.29	424.32	2221.06	129.26	1240.22	851.58
18	8	DAIRY	1136.40	97.01	757.64	281.75	2070.18	129.67	1396.50	544.01
19		DAIRY	1329.28	109.11	1062.99	157.18	2221.06	113.67	1801.14	306.25
20		DAIRY	1274.71	112.00	871.68	291.03	2195.91	108.50	1321.05	766.36
22		DAIRY	1287.28	128.80	741.25	417.23	2221.06	143.85	1481.89	595.32
MEAN			1278.95	141.39	814.83	322.74	2182.41	146.94	1339.79	695.69
±SD			±24.36	±13.84	±65.73	±64.42	±24.36	±13.84	±65.73	±64.42
2		SOY	1399.01	88.32	1118.49	192.20	2311.05	97.86	2010.00	203.19
4		SOY	1399.01	106.64	1317.75	-25.38	2298.43	100.67	1922.77	274.99
6		SOY	1399.01	147.95	892.73	358.33	2311.00	143.96	1412.39	754.65
7		SOY	1399.01	156.32	1204.69	28.00	2311.00	143.12	1714.58	453.30
15	8	SOY	1399.01	149.13	1051.46	198.42	2311.00	146.55	1585.98	578.47
17		SOY	1399.01	150.83	1118.37	129.81	2311.00	130.15	1786.54	394.31
23		SOY	1399.01	72.12	798.56	528.33	2311.00	96.04	1329.93	885.03
24		SOY	1399.01	98.13	981.01	319.87	2298.43	123.18	1560.59	614.66
MEAN			1399.01	122.43	1060.38	216.20	2307.86	122.69	1665.35	519.83
±SD			±24.36	±13.84	±65.73	±64.42	±24.36	±13.84	±65.73	±64.42
3		MEAT	881.28	109.44	784.11	-12.27	1816.26	96.75	1428.39	291.12
5		MEAT	(1057.31)*	(75.04)	(302.08)	(680.19)	(2042.50)	(92.94)	(735.88)	(1213.76)
9		MEAT	830.99	140.73	425.74	264.52	1891.70	154.34	1192.58	544.78
10		MEAT	830.99	171.20	519.83	139.96	1816.26	163.76	1629.38	23.12
13	8	MEAT	1057.31	33.06	699.79	324.46	2017.43	85.54	1478.92	452.97
14		MEAT	1057.31	86.64	738.40	232.27	2017.43	108.43	1779.90	129.10
16		MEAT	1057.31	125.10	822.26	109.95	2017.43	120.21	1634.98	262.24
21		MEAT	881.28	90.67	584.12	206.49	1816.26	101.71	1626.43	88.12
MEAN			942.35	108.12	653.46	180.77	1913.25	118.67	1538.65	255.92
±SD			±26.02	±14.79	±70.27	±68.87	±26.02	±14.79	±70.27	±68.87
Total Mean (Dairy, Soy, & Meat)			1206.77	123.98	842.89	239.90	2134.51	129.44	1514.59	490.48
±SD			±14.39	±8.18	±38.84	±38.07	±14.39	±8.18	±38.84	±38.07

*( ) Values not included in means.

Appendix XIX

Magnesium Intake, Urine and Fecal Excretion and Retention

MAGNESIUM INTAKE, URINE AND FECAL EXCRETION AND RETENTION

SUBJECT NUMBER	GROUP TOTAL	DIETARY TREATMENT	PERIOD I				PERIOD II			
			INTAKE	URINE	FECES	RETENTION	INTAKE	URINE	FECES	RETENTION
1		DAIRY	208.46	97.83	157.95	-47.32	469.58	103.33	182.92	183.33
8		DAIRY	237.44	130.38	114.04	-6.98	505.80	139.95	171.70	194.15
11		DAIRY	186.73	163.21	141.60	-118.08	462.33	161.04	260.16	41.13
12	8	DAIRY	237.44	80.04	144.71	12.69	505.80	116.69	250.72	138.39
18		DAIRY	179.48	86.56	168.78	-75.86	447.84	96.33	302.23	49.28
19		DAIRY	249.52	145.60	161.37	-57.45	517.88	137.93	304.30	75.65
20		DAIRY	206.05	83.80	216.03	-93.78	471.99	90.48	291.50	90.01
22		DAIRY	220.54	117.95	131.24	-28.65	491.31	125.13	293.80	72.38
MEAN			215.71	113.17	154.47	-51.93	484.07	121.36	257.17	105.64
±SD			±7.79	±7.98	±12.85	±16.43	±7.79	±7.98	±12.85	±16.43
2		SOY	382.60	133.30	295.06	-45.78	652.50	154.13	444.70	53.67
4		SOY	334.30	152.93	313.25	-131.88	596.96	154.38	429.77	12.81
6		SOY	343.96	110.68	264.62	-31.34	613.86	125.56	365.26	123.04
7		SOY	358.45	117.60	320.37	-88.52	625.94	149.32	411.67	64.95
15	8	SOY	346.38	111.50	359.30	-124.42	611.45	114.16	434.99	62.30
17		SOY	360.87	127.47	329.77	-96.37	621.11	113.37	433.64	74.10
23		SOY	348.79	73.35	254.12	21.32	613.66	90.13	370.80	152.93
24		SOY	336.72	134.50	302.13	-99.91	596.96	114.79	382.98	99.19
MEAN			351.51	120.17	305.95	-74.61	616.58	126.98	409.23	80.37
±SD			±7.79	±7.78	±12.95	±16.43	±7.79	±7.98	±12.85	±16.43
3		MEAT	182.15	97.20	186.75	-101.80	453.36	97.56	330.70	25.10
5		MEAT	(249.77)*	(64.09)	(128.77)	(56.91)	(518.57)	(109.95)	(149.28)	(259.34)
9		MEAT	172.49	99.91	202.45	-129.87	467.85	118.43	315.57	33.85
10		MEAT	172.49	92.75	145.71	-65.97	453.36	112.32	386.52	-45.16
13	8	MEAT	223.21	111.31	169.01	-57.11	492.00	127.05	289.27	75.68
14		MEAT	230.45	79.20	182.18	-30.93	492.00	123.33	376.70	-7.03
16		MEAT	230.45	97.16	201.31	-68.02	492.00	133.00	370.04	-11.04
21		MEAT	182.15	93.75	167.28	-78.88	453.36	95.55	380.92	-23.11
MEAN			199.06	95.90	179.24	-76.08	471.99	115.18	349.96	6.90
±SD			±8.32	±8.54	±13.74	±17.56	±8.32	±8.54	±13.74	±17.56
Total Mean (Dairy, Soy, & Meat)			255.42	109.74	213.22	-67.54	524.21	121.17	338.78	54.27
±SD			±4.60	±4.72	±7.59	±9.71	±4.60	±4.72	±7.59	±9.71

*( ) Values not included in means.

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Effects of Protein Source and Calcium Level
on the Utilization of Minerals in Adult Men

by

Sandra Porter Leon

(ABSTRACT)

The effect of three sources of protein: soy, dairy, and meat protein, and two levels of calcium on zinc, iron, copper, calcium, and magnesium retention in young adult men was determined in a 30-day metabolic balance study. The study was divided into a twenty-day baseline period, a thirty day controlled feeding period from which all the balance data was collected, and a twenty day follow-up period. During the controlled feeding period, twenty-four subjects were randomly assigned to one of three dietary treatment groups which differed in respect to protein source. The dairy treatment group was fed a diet in which 70% of the dietary protein was derived from dairy products; the soy treatment group was fed a diet in which 67% of the dietary protein was derived from soy products; and the meat treatment group was given a diet in which 70% of the dietary protein was provided by animal meat products.

To test the effect of calcium level on mineral retention, the controlled feeding period was divided into two periods: Period I, in which the subjects consumed moderate levels of calcium (mean = 1206.77 + 193.29 mg/day) and Period II, in which the subjects consumed high levels of calcium (mean = 2134.51 + 164.63 mg/day).