INTRODUCTION
Man’s food whether of vegetable or animal origin, is derived from the soil and sea. It is composed of complex nutrients, which are carbohydrate, protein, fat, vitamins and minerals. All these five nutrients are necessary for proper growth and maintenance of the body.

Minerals may be defined as those elements which remain largely as ash when plant or animal tissues are burned. It is well known that the non combustible portion of the food, the mineral ash, is essential for growth during early life and thereafter for maintaining normal health. The human body contains more than 24 minerals, all of which must be provided by the diet. They include calcium, phosphorus, potassium, sodium, chloride, cadmium, selenium, silicon, vanadium and molybdenum etc. They do not supply any heat or energy to the human body. They are necessary for various functions like formulation of bone, constituent of soft tissues, control of acid-base balance, osmolarity of extra and intra cellular fluids, formulation of hormones, activation of enzymes etc. The body can tolerate a deficiency of vitamins for a relatively long period but slight changes in the blood concentration of the important minerals may rapidly make life endangered. Given below is the classification of essential minerals according to their function.

**Classification of essential minerals according to their function:**

<table>
<thead>
<tr>
<th>Function</th>
<th>Minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Structural function</td>
<td>→Calcium, magnesium, phosphate</td>
</tr>
<tr>
<td>-Involved in membrane function: principle cations of extracellular</td>
<td>→Sodium, potassium</td>
</tr>
<tr>
<td>intracellular fluids, respectively</td>
<td></td>
</tr>
<tr>
<td>-Function as prosthetic groups in enzymes</td>
<td>→Cobalt, copper, iron, molybdenum,</td>
</tr>
<tr>
<td></td>
<td>selenium, zinc</td>
</tr>
<tr>
<td>-Regulatory role or role in hormone action</td>
<td>→Calcium, chromium, iodine,</td>
</tr>
<tr>
<td></td>
<td>magnesium, manganese, sodium,</td>
</tr>
<tr>
<td></td>
<td>potassium</td>
</tr>
<tr>
<td>-May occur in foods and known to be toxic in excess</td>
<td>→Aluminum, arsenic, boron, lead, mercury,</td>
</tr>
<tr>
<td></td>
<td>silver</td>
</tr>
</tbody>
</table>

(www.cnn.com)
Many of the essential minerals are widely distributed in foods and most people eating a normal mixed diet are likely to receive adequate intakes. The amounts required vary from grams per day for sodium and calcium, through milligrams per day to micrograms per day for the trace elements. In general, mineral deficiencies are encountered when foods come from one region, where the soil may be deficient in some minerals (Harper, 2003).

Calcium is a major structural element in bones and teeth. The mineral component of bone consists mainly of hydroxyapatite crystals, which contain large amounts of calcium and phosphorus (about 40% calcium and 60% phosphorus). Bone cells called osteoclasts begin the process of remodeling by dissolving or reabsorbing bone. Bone-forming cells called osteoblasts then synthesize new bone to replace the bone that was reabsorbed. During normal growth, bone formation exceeds bone resorption. Osteoporosis may result when bone resorption exceeds formation.

Calcium plays a role in mediating the contraction and relaxation of blood vessels (vasoconstriction and vasodilation), nerve impulse transmission, muscle contraction, and the secretion of hormones, such as insulin. Excitable cells, such as skeletal muscle and nerve cells, contain voltage-dependent calcium channels in their cell membranes that allow for rapid changes in calcium concentrations. For example when a muscle fiber receives a nerve impulse that stimulates it to contract, calcium channels in the cell membrane open to allow a few calcium ions into the muscle cell. These calcium ions bind to activator proteins within the cell that release a flood of calcium ions from storage vesicles inside the cell. The binding of calcium to the protein, troponin-c, initiates a series of steps that lead to muscle contraction. The binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen to provide energy for muscle contraction.

Calcium is necessary to stabilize or allow for optimal activity of a number of proteins and enzymes. The binding of calcium ions is required for the activation of the seven "vitamin K-dependent" clotting factors in the coagulation cascade. The term, "coagulation cascade", refers to a series of events, each dependent on the other that stops bleeding through clot formation.

Calcium is needed for

- Regulating the heart beat
- Clotting the blood
- Proper thyroid function
- Nerve impulse transmission
- Building strong bones and teeth

**Food sources of calcium:**

<table>
<thead>
<tr>
<th>Household measure</th>
<th>Calcium (mg)</th>
<th>% of adult daily allowance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, fresh, whole</td>
<td>1 cup</td>
<td>291</td>
</tr>
<tr>
<td>Milk, nonfat dry, low-density</td>
<td>1/3 cup</td>
<td>279</td>
</tr>
<tr>
<td>Mustard greens, cooked</td>
<td>¼ cup</td>
<td>97</td>
</tr>
<tr>
<td>Ice cream</td>
<td>1/8 quart</td>
<td>87</td>
</tr>
<tr>
<td>Soybeans, mature, cooked</td>
<td>½ cup</td>
<td>73</td>
</tr>
<tr>
<td>Cottage cheese, creamed</td>
<td>½ cup</td>
<td>68</td>
</tr>
<tr>
<td>Orange, whole</td>
<td>1 medium</td>
<td>54</td>
</tr>
<tr>
<td>Sweet potato, boiled</td>
<td>1 medium</td>
<td>48</td>
</tr>
<tr>
<td>Egg, whole</td>
<td>1 medium</td>
<td>28</td>
</tr>
<tr>
<td>Cabbage, raw, shredded</td>
<td>½ cup</td>
<td>22</td>
</tr>
<tr>
<td>Carrots, cooked</td>
<td>½ cup</td>
<td>26</td>
</tr>
<tr>
<td>Bread, soft crumb type</td>
<td>1 slice</td>
<td>13</td>
</tr>
</tbody>
</table>

Milk is the outstanding source of calcium in the diet; without it, a satisfactory intake of calcium is extremely difficult. Whole or skimmed, homogenized or non-homogenized, plain or chocolate-flavored, sweet or sour milks are equally good. For the adult 2 to 3 cups milk daily and for the child 3 to 4 cups daily will ensure adequate calcium intake. Cheddar cheese is an excellent source of calcium.

All foods other than dairy products when considered together contribute not more than 200 to 300 mg calcium daily. Certain green leafy vegetables such as mustard greens, turnip greens are important sources of calcium when they are eaten frequently. Meats and cereal grains are poor sources. The use of nonfat dry milk, dough conditioners and mold inhibitors in bread enhances the calcium value of the diet. Calcium is also an optional enrichment ingredient in flours and breads.

Some of the main sources of calcium in American diets are dairy products. It has been estimated that as much as 75% of the calcium consumed in the U.S. comes from these sources. However, many people in the world are unable to digest milk and other dairy products due to a condition called lactose intolerance. In lactose intolerant individuals, not enough of the enzyme lactase is produced, the enzyme needed to break down lactose. Lactose is the sugar found in milk. Most of the races in the world, especially those where dairy products have never been a part of the native diets for
long periods, are lactose intolerant. Approximately 70-90% of adults of Asian, African, Native American and Mediterranean descent are lactose intolerant. People who are lactose intolerant or who for other reasons do not consume dairy products have a number of other options for getting calcium. These include:

Non dairy sources of calcium such as legumes, green leafy vegetables, tortillas made with limestone, tofu, nuts and foods where bone are consumed such as sardines and lactose reduced milk, lactose supplements, smaller portions of dairy products as the body allows. Yogurt with live cultures and aged cheeses are sometimes better tolerated than other dairy products such as milk calcium supplements.

Pumarino (1992) studied, a 61 year old man with a urinary stone having a daily calcium intake of about 400 mg. The serum calcium concentration was normal but urinary excretion of calcium was high (725 mg / day). This did not alter when dietary calcium was increased to 900 mg / day. Examination of spinal bone density showed it to be 51% of normal indicating severe osteoporosis. Administration of hydrocortisone (50 mg / day) reduced urinary calcium output by 25% and the addition of 1125 mg / day phosphorus brought it to normal levels.

**Daily allowances:**

A great deal of controversy exists concerning the requirement for calcium. The calcium balance technique for determining the calcium requirement has been criticized by many. People who ingest high levels of calcium are in negative balance if they suddenly shift to a lower intake, but in time most of them adjust to the lower level of intake. Adults throughout the world consume diets that often provide 400 mg or less of calcium, yet they do not show any adverse effects.

The recommended allowance for calcium is 800 mg for adults and for children 1 to 10 years; 1200 mg for boys and girls 11 to 18 years and for pregnant and lactating women; and 360 to 540 mg during the first year of life (1980). The FAO/WHO committee has recommended 400 to 500 mg calcium as a “practical allowance” for adults (FAO, 1974). Such levels can be realized in most countries for the entire population, whereas higher levels would be impractical in terms of available food supplies. The Canadian allowance is 800 mg for men and 700 mg for women, with an additional 500 mg allowance for pregnancy and lactation. The allowance for adults is based upon replacement of daily losses including 175 mg in the urine, 125 mg as endogenous loss from digestive juices in the feces, and 20 mg from the skin.
Assuming absorption to be 40 per cent, this daily loss of 320 mg would necessitate an allowance of 800 mg.

**Absorption:**

Calcium is absorbed by active transport, an energy requiring process, chiefly from the duodenum. Passive diffusion of calcium across the intestinal mucosa also occurs from the jejunum and ileum.

As people age they lose the ability to make vitamin D in their skin from sunlight. Because of this, getting enough vitamin D for the elderly may be as important as getting enough calcium to prevent weak bones. One research study found that women who took a vitamin D supplement had higher bone density than women who did not take extra amounts of the vitamin. Figure 1 shows the utilization of calcium.

**Factors favoring absorption:**

Body need is the major factor governing the amount of calcium that is absorbed. Healthy adults receiving a diet that meets the recommended allowances absorb approximately 20 to 30 per cent of their dietary calcium. At higher levels of intake the proportion that is absorbed is lower but the absolute amount that crosses the intestinal membrane depends upon need. In many areas of the world, the diet supplies low amounts of calcium. People in these low levels absorb a high proportion of the intake. During growth the absorption is increased to take care of increase in size and hardness of the skeleton. Thus children absorb proportionally more calcium than adults. Pregnancy and lactation are also two physiological states which trigger an increased absorption. It has also been found that men have a more efficient absorption rate than non pregnant women.

Dr. Oke (1990) reported the factors involved in calcium absorption and their influence on the bioavailability of the ion in normal healthy adults in terms of a positive or negative balance. Factors able to enhance calcium absorption include: calcitriol, luminal ionized calcium concentration, sodium, milk products (lactose, casein and derived phosphopeptides) and calcium citrate. Factors able to diminish the efficiency of absorption include: alcohol, hormones (calcitonin, glucocorticoid, thyroxine), genetic influences and drugs such as thiazides, diuretics and phenothiazines.

The levels of dietary calcium influences calcium absorption as high dietary levels depress the efficiency of absorption. This adaptation to intake is probably
Food Calcium

Small Intestine,

Body needs Gastric Acidity, Vitamin D, Lactose, Ascorbic acid, certain amino acids

Too little vitamin D
Phytic acid
Oxalic acid

Fecal Calcium

Intestinal juices

Calcium in Blood Plasma

Vitamin D
Ascorbic acid
Calcitonin

Parathyroid hormone
Excess vitamin D
Bone immobilization

Teeth

Bones

Urinary Calcium

Source: Robinson et al. (1986)

Figure 1: The Utilization of Calcium

The amount of phosphorus consumed along with the calcium influences the availability of the calcium. For adults 1:1 calcium to phosphorus ratio is recommended, although slight deviations from this ratio do not have a significant effect. Within the acceptable ratio, an increase in phosphorus intake tends to increase the efficiency of calcium absorption.

An acid reaction aids in the absorption of calcium since calcium salts are then more soluble. Once bile and pancreatic juice have mixed with the chyme, the reaction becomes strongly alkaline and the solubility of the calcium salt is reduced. The presence of ascorbic acid and some amino acids facilitates absorption by increasing the solubility of the calcium salts.

Several mechanisms control the amount of calcium that is absorbed. Two of these are the hormones secreted by the parathyroid gland, parathormone and vitamin D. Parathormone is secreted when the blood calcium level is lowered. One of it's functions is to stimulate the kidney to synthesize vitamin D hormone. This metabolically active vitamin D functions with parathormone to stimulate increased absorption from the intestine. Vitamin D is needed in order to adequately absorb calcium from the gastrointestinal tract. Vitamin D comes from two sources- diet and sunlight. Even with sufficient calcium, if vitamin D is not present, rickets and the adult version of rickets, osteomalacia, may result.

Legumes and cereal grains such as whole wheat, oats, rye and barley contain phytates, which may interfere with calcium absorption. Phytates are reduced by baking, sprouting and fermentation.

The ability of lactose to increase absorption has long been recognized. Being slowly absorbed, lactose favors the growth of intestinal microorganisms that increase the acidity of the intestinal contents (Condon et al, 1970). Lactose may promote absorption by interacting with the absorptive cells of the intestine to increase their permeability to calcium ions (Armbrecht and Wasserman, 1976). Buchowski and Miller (1991) concluded that lactose increased calcium bioavailability in all age groups. Bioavailability from milk was higher than those from lactose-hydrolyzed milk in all age groups. Lactose and glucose +galactose increased bioavailability over sugar-free CaCl2-casein mixture in all age groups. A high protein diet favors absorption of
calcium whereas a high cereal diet will diminish it. Certain amino acids and ascorbic acid are concerned with the optimum absorption of calcium (Adolph and Chen, 1932). **Factors interfering with absorption:**

A reduction in the amount of acid, sometimes found in elderly persons, reduces the solubility of the calcium salts. A marked increase in gastrointestinal motility reduces the length of time that calcium remains in contact with the intestinal mucosa. The lack of vitamin D seriously impairs the absorption of calcium. Such lack may arise from inadequate exposure to sunlight or failure to ingest vitamin D in some form.

Abdel et al (1982) reported that dietary deficiency of phosphorus reduced the efficiency of intestinal calcium absorption and was associated with a reduction in the plasma concentration of 1-hydroxy cholecalciferol.

Similarly, dietary protein levels influence calcium availability. When high protein diets (100gm or more) are consumed, calcium needs increase in order to maintain calcium balance. Approximately 100 gm protein per day is consumed by many persons in the United States. As the protein level is decreased to the RDA levels, calcium needs are similarly lowered. The effect of higher protein levels on calcium needs is greater with a diet high in dietary fiber.

Oxalate and phytate decrease the absorption of calcium. They combine with calcium to form calcium oxalate and calcium phytate, respectively, and these forms cannot be absorbed at all.

The presence of oxalic acid or phytic acid in foods and an abnormal calcium-to-phosphorus ratio are known to result in the formation of insoluble calcium complexes and thus interfere with calcium absorption.

Oxalic acid is a chemical substance. At high concentrations, it is a dangerous poison, but such immediately toxic levels are not found in foodstuff. It is also a naturally occurring component of plant, and is found in relatively high levels in dark-green leafy foods. In the human body, ingested oxalic acid is not (so far as is known today) a useful nutrient; so, like all such unneeded components of the diet, it is processed by the body to a convenient form and that byproduct is then excreted—in this case, in the urine. In the course of being processed by the body, oxalic acid combines with other substances to form various salts, called oxalates.

Oxalates occur as the end products of metabolism in a number of plant tissues; some leafy plants and some root crops contain markedly high levels of soluble and
insoluble oxalates. When consumed these oxalates can bind calcium and other minerals. Measurement of oxalate content in vegetables commonly consumed in New Zealand shows that cooking reduces the oxalate content of the food by leaching losses into the cooking water. Roots and brassicas grown in New Zealand appear to contain relatively low levels of oxalates. Leafy vegetables such as silverbeet and NZ spinach appear to approach and exceed levels found in rhubarb stalks, although New Zealand silverbeet stems contain lower levels (Savage, 2000).

Oxalic acid is found in spinach, Swiss chard, beet tops, cocoa and rhubarb. Spinach, for example, contains sufficient calcium to bind the oxalic acid, and none of the calcium in other foods eaten at the same meal would be adversely affected (Johnston et al, 1962).

The mean daily intake of oxalate in English diets has been calculated to be 70-150 mg; tea appears to contribute the greatest proportion in these diets. Thirty-two commercially available teas consisting of green, oolong and black teas were bought from supermarkets in Christchurch, New Zealand in June 2001. Fifteen herbal teas were also purchased at the same time. The mean soluble oxalate contents of black tea in tea bags and loose tea leaves were 4.68 and 5.11 mg/g tea, respectively, while green teas and oolong tea had lower oxalate contents, ranging from 0.23 to 1.15 mg/g tea. The soluble oxalate content of the herbal teas ranged from not detected to 3.00 mg/g tea. A regular tea drinker consuming six cups of tea/day would have an intake of between 26.46 and 98.58 mg soluble oxalate/day from loose black tea, 17.88 and 93.66 mg soluble oxalate/day from black tea in tea bags and a maximum of 18.0 mg/day from herbal teas. (Charrier et al, 2002). Rhubarb, spinach and beet are other common high oxalate-containing foods. Vegetarians who consume greater amounts of vegetables will have a higher intake of oxalates, which may reduce calcium availability. This may be an increased risk factor for women who require greater amounts of calcium in the diet. In humans, diets low in calcium and high in oxalates are not recommended but the occasional consumption of high oxalate foods as part of a balanced diet does not pose any particular problem (Noonan and Savage, 1999).

Pahwa and Kansal (1980) showed that calcium utilization was negatively related to the oxalate content of leafy vegetables. The result also showed that retention of calcium from leafy vegetables was increased when these were consumed with skimmed milk powders which reverse in phosphorus.
The exact correlation between calcium intake and oxalate absorption was assessed by a few workers. They investigated that oxalate absorption in healthy volunteers applying 0.37 mmol of the soluble salt sodium $^{13}$C$_2$ oxalate in the calcium intake range from 5 mmol (200 mg) calcium to 45 mmol (1800 mg) calcium. Within the range of 200 to 1200 mg calcium per day, oxalate absorption depended linearly on the calcium intake. With 200 mg calcium per day, the mean absorption (± SD) was 17% ± 8.3%; with 1200 mg calcium per day, the mean absorption was 2.6% ± 1.5%. Within this range, reduction of calcium supply by 70 mg increased the oxalate absorption by 1% and vice versa. Calcium addition beyond 1200 mg/d reduced the oxalate absorption only one-tenth as effectively. With 1800 mg calcium per day, the mean absorption was 1.7% ± 0.9%. The findings may explain why a low-calcium diet increases the risk of calcium oxalate stone formation (Gerd et al., 2004). Soaking and cooking of foodstuff high in oxalate will reduce the oxalate content by leaching.

Phytate is a storage form of phosphorus which is found in plant seeds and in many roots and tubers. Phytic acid has the potential to bind calcium, zinc, iron and other minerals, thereby reducing their availability in the body (Davis and Olpin, 1979). In addition, complex formation of phytic acid with proteins may inhibit the enzymatic digestion of the protein. Phytates/phytic acids are the storage form of phosphorus bound to inositol in the fiber of raw whole grains, legumes, seeds and nuts. Phytic acid occurs in unspouted grains, seeds, and legumes and is particularly rich in bran. Although these foods have high phosphorus content, the phosphates in phytates are not released through the digestive process.

The effect of phytate would be important only when whole-grain cereals comprised a major part of the diet and when the calcium intake was also low, as is true in some vegetarian diets in which unleavened bread or whole-grain cereals are a major part of the caloric intake. Yeast fermentation in a bread sponge which destroys much of the phytate present in whole meals (Rainhold, 1975). When bread is leavened by yeast, enzymes degrade phytic acid and phytates pose no problem. Phytic acid is also destroyed during baking and food processing. Enzymes, called phytases, destroy phytates during certain food processes such as the yeast-raising of dough, the sprouting of seeds, grains, legumes, the roasting of nuts, presoaking beans, cooking, fermentation as in tempeh, miso and natto, combining acidic foods with zinc-rich foods, etc.
Pearl millet (*pennisetum typhoideum*) was fermented with Lactobacilli or yeasts alone and in combination and with natural microflora after various processing treatments such as grinding, soaking, debranning, dry heat treatment, autoclaving and germination. Fermentation was carried out at 30 degrees C for 48 hours with *Lactobacillus plantarum* (LP) and *Rhodotorula* (R) isolated from naturally fermented pearl millet and *Lactobacillus acidophilus* (LA), *Candida utilis* (CU) and natural microflora (NF). Germination and autoclaving and debranning and autoclaving were the most effective processing treatments to reduce the phytic acid, amylase inhibitors and polyphenols. There was a further reduction in these antinutrients due to fermentation. Phytic acid and amylase inhibitors were completely eliminated after fermentation in some of the samples especially in soaked, debranned and germinated ones. Polyphenols were altered non-significantly in general but fermentation with Lp + R and NF caused a significant increase in polyphenols (Sharma and Kapoor, 1996).

Germination reduced the phytic acid content of chickpea and pigeonpea seeds by over 60% and that of *mung* bean, *urd* bean and soybean by about 40%. Fermentation reduced phytic acid contents by 26-39% in all these legumes with the exception of pigeonpea in which it was reduced by more than 50%. Autoclaving and roasting were more effective in reducing phytic acid in chickpea and pigeonpea than in *urd* bean, *mung* bean and soybean. Germination and fermentation greatly increased the in vitro protein digestibility (IVPD). IVPD was only slightly increased by roasting and autoclaving of all legumes. Germination and fermentation also remarkably decreased the total dietary fiber (TDF) in all legumes. Autoclaving and roasting resulted in slight increases in TDF values. All the processing treatments had studied little effect on calcium, magnesium and iron contents (Chitra, 1996).

The present investigation was conducted to study the concentration of polyphenols, phytic acid and saponins of five high-yielding varieties of rice bean (*vigna umbellata*) and one variety each of green gram and black gram as affected by various domestic processing and cooking methods which included soaking in tap water for 6, 12 and 18 h; sprouting for 40 and 60 h; ordinary cooking of unsoaked and soaked seeds; and autoclaving of unsoaked and soaked seeds. There was a successive and significant reduction in the contents of antinutritional factors with increase in the soaking and sprouting period. A markedly greater reduction in these factors was observed when soaked seeds were cooked and autoclaved than when unsoaked seeds were cooked and autoclaved. Among the various domestic processing and cooking
methods, maximum reduction of antinutritional factors was observed when soaked seeds were autoclaved (Deepinder and Amin, 2003).

Germination significantly altered the nutrient composition of the red gram seed (*cajan L*), causing marked increase in calorific value. Crude protein, soluble carbohydrate, cellular and organic cellular contents, cellulose, lignin, non-nutritive matter, total oxalate and phytic acid contents of the seed were negatively correlated with germination, whereas the reverse was the case with the seed's contents of fat, crude fibre, total ash, soluble ash, acid-insoluble ash, cell wall carbohydrate, hemicellulose, iron, manganese, calcium, magnesium, copper, phosphorus, food energy, digestible energy, tannins, total phenolics and trypsin inhibitory activity. It was concluded that the increased contents of tannins, total phenolics and trypsin inhibitory activity of the seed during progressive germination might limit its nutritive quality (Oloyo, 2004).

Legume seeds (soy bean, lupin and bean seeds) were soaked in 0.5% sodium bicarbonate in an attempt to evaluate their nutritional quality and protein solubility index. Soaking led to an increase in the hydration coefficient, seed weight, total protein, ash, fat, fiber, while non protein nitrogen, total carbohydrates, starch, stachyose, raffinose, reducing sugars and minerals (except Na) were decreased. All antinutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity were decreased during soaking in 0.5% sodium bicarbonate; it was the same for the protein solubility in different solutions, while the in vitro protein digestibility and available lysine were increased (el-Adawy et al, 2000).

Toma *et al* (1979) reported that the phytic acid content was 2.23 % and 2.02 % in red and white sesame, respectively. A positive correlation between total phosphorus and phytic acid, calcium and soluble oxalate were observed in dehusked roasted seeds.

Four tropical fruits and three citrus fruits were analyzed for moisture, ash, antinutritional factors (phytate, oxalate, and polyphenols) and total and available minerals. Moisture contents ranged from 6.00 to 83.17% for tropical fruits while for citrus fruits it ranged from 88.20 to 89.50%. Ash contents ranged from 2.56 to 4.50% and from 3.83 to 4.83%, for tropical and citrus fruits, respectively. All fruits contained no oxalate while phytate and polyphenols ranged from 48.1 to 134.1 mg/100 g and from 0.115 to 0.34%, respectively. For all fruits major mineral contents ranged from 7.7 to 433.3 while trace ones ranged from 0.116 to 1.91 mg/100 g. In vitro
availabilities of major minerals (% of total) varied from 11.1 to 86.2% while for minor ones it ranged from 13 to 72.5% (Malik et al, 2004).

The mineral availability from plant protein containing phytate is complicated by the complex interactions between phytic acid, minerals, proteins, intestinal and plant phytates and the processing treatment which the product has undergone. Phytates interact with proteins to form insoluble complexes (Smith and Rackis, 1957). These phytate-protein complexes cannot be easily dissociated at pH values greater than 9.0, however, it can be dissociated at an acidic pH of 4.4 (O’Dell et al, 1972).

Phytates are also attributed with certain health benefits. Although phytates do bind with minerals, they may actually be preventing the formation of free radicals, thereby keeping the minerals at safe levels in the body. Phytates also have a role to play in cell growth and can move excess minerals out of the body. Phytates shield us from dangerously high levels of minerals such as iron. Some animal studies have suggested that phytates stop the growth of cancerous tumors. Phytates can bind with minerals that may feed tumors. Phytates are generally found in foods high in fiber. Since fiber-rich foods protect against colon and breast cancers, it is now thought that they are the protective agents in the fiber. It appears that by binding minerals in the intestines, phytates inhibit the cancer process, especially when it comes to iron. Iron generates free radicals and phytates may be keeping the mineral balance at a safe level within the body. Phytates act as an antioxidant. Excessive iron is also known to increase the risk of heart disease. Even a small amount of phytates in food can reduce iron absorption by half, but the effect is less marked if a meal is supplemented with ascorbic acid, which can also help the absorption of zinc and calcium. Phytates are also known to help prevent cancer by enhancing the immune system. Phytates may increase the activity of natural killer cells which attack and destroy cancer cells and tumors. By working directly to control cell growth, phytates may be an ideal protective agent against a wide range of cancers, carrying excess minerals out of the body, thereby protecting it from a potential overload. Fiber, along with its associated phytates, also provides benefits by regulating the absorption of glucose from starch (BecomeHealthyNow.com.inc).

The effect of high intakes of dietary fiber has been suspected of reducing calcium absorption. A recent study has shown that in human volunteers, a high-fiber diet increases the dietary calcium requirement slightly (Sandstead et al, 1979). The fibers used in this study were from natural sources such as cereal bran and vegetable
powder rather than a purified fiber such as cellulose; thus, the increased calcium requirement could in part be due to the higher phytate intake as well as the high fiber intake.

Marfo and Oke (1988) have shown that cassava, cocoyam and yam contain 624 mg, 855 mg and 637 mg of phytate per 100 g respectively (Table 1). Fermentation reduced the phytate level by 88 percent, 98 percent and 68 percent, respectively, reduction being rapid within 48 hours but very slow after 72 hours of processing. Thus processing into fermented foods will reduce the phytate level of root crops sufficiently to nullify its adverse effect. The loss of phytate during fermentation is due to the enzyme phytase, naturally present in the tubers or secreted by fermentative microorganisms. Processing into nbo or kokonte resulted in a loss of only 18 percent of phytate in cassava and 30 percent each in cocoyam and yam (Table 1). Oven-drying had only a small reductive effect on the phytate content compared with fermentation. Cooking also had a significant effect, resulting in a decrease of phytate by 62 percent, 65 percent and 68 percent respectively in yam, cocoyam and cassava.

The effect of fats on absorption is reported variously by different investigators. On the one hand, fats reduce intestinal motility so that there is longer contact with the absorbing surfaces. On the other hand, free fatty acids combine with calcium to form insoluble soaps that are excreted. Foods high in unsaturated fatty acids have little effect, whereas those high in saturated fatty acids are more likely to yield some soaps.

Several amino acids particularly, L-lysine and L-arginine raised the percentage of the dose of Ca$^{45}$ deposited in the rat femurs by over 50%. Likins et al (1957) showed the same effect of lysine on the deposition of radioactive calcium in rat skeleton.
Table 1 - Phytate content of some unfermented and fermented tubers (mg/g)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unfermented meal</th>
<th>Fermented meal</th>
<th>% Lose$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hour</td>
<td>48 hour</td>
</tr>
<tr>
<td>Cassava</td>
<td>624</td>
<td>116</td>
<td>99</td>
</tr>
<tr>
<td>Cocoyam</td>
<td>855</td>
<td>180</td>
<td>28</td>
</tr>
<tr>
<td>Yam</td>
<td>637</td>
<td>394</td>
<td>296</td>
</tr>
</tbody>
</table>

$^1$Percentage loss in phytate is the decrease in phytate after 96 hours fermentation expressed as a percentage of total phytate.


Table 2 - Effect of processing on phytate in cassava, cocoyam and yam

<table>
<thead>
<tr>
<th></th>
<th>Fresh and unprocessed</th>
<th>Sliced and cooked (Ampesi)</th>
<th>Flour cooked into a paste (Tug, kokonte)</th>
<th>Dried granular powder (Gari)</th>
<th>Gari made into a paste (Eba)</th>
<th>Fufu (cooked and pounded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava</td>
<td>624</td>
<td>196</td>
<td>411</td>
<td>70</td>
<td>55</td>
<td>188</td>
</tr>
<tr>
<td>% loss$^1$</td>
<td>-</td>
<td>68.5</td>
<td>18.1</td>
<td>86.0</td>
<td>89.0</td>
<td>69.8</td>
</tr>
<tr>
<td>Cocoyam</td>
<td>855</td>
<td>302</td>
<td>592</td>
<td>9</td>
<td>8</td>
<td>281</td>
</tr>
<tr>
<td>% loss$^1$</td>
<td>-</td>
<td>64.6</td>
<td>30.7</td>
<td>98.9</td>
<td>99.0</td>
<td>67.1</td>
</tr>
<tr>
<td>Yam</td>
<td>637</td>
<td>239</td>
<td>412</td>
<td>188</td>
<td>179</td>
<td>209</td>
</tr>
<tr>
<td>% loss$^1$</td>
<td>-</td>
<td>62.4</td>
<td>30.8</td>
<td>70.4</td>
<td>71.8</td>
<td>67.1</td>
</tr>
</tbody>
</table>

$^1$Percentage loss in phytate is the decrease in phytate resulting from each processing method expressed as a percentage of total phytate content.

Conway et al (1981) reported that when calcium deficient or calcium adequate diet with a sucrose solution was fed to rats for four weeks, the calcium absorption and excretion, as well as retention and density of the femur increased.

Waltner (1933) tried to see the significance of calcium and phosphorus in nutrition. The result showed that in all experimental groups’ body weight declined during the period of high phosphorus intake and during those on high calcium intake.

Bernhart et al (1969) showed that young male rats required 22 mg calcium and 20 mg phosphorus for minimal growth rate and 48 mg calcium and 30 mg phosphorus for maximal growth rate.

Stearns (1931) showed that in the first year of life the retention ratio of Ca : P lies normally between 1.5:1 and 2.1:1. A ratio lower than 1.5:1 indicates a more rapid growth of soft tissues and if over 2.1:1 the correction of previous calcium shortage. Pelegano et al (1991) concluded that 1.7:1 ratio was best for the delivery of calcium and phosphorus.

Herta et al (1978) showed that intestinal calcium absorption did not differ significantly during phosphorus supplementation. Only urinary and stool phosphorus increased with increasing phosphorus supplementation.

Hummel et al (1936) studied the retention of calcium in the last 145 days of the pregnancy in healthy women. The average daily intake was 3.04 g of calcium. The result of a balance study conducted indicated that about 66-90% of intake of calcium was rejected in the fecal and only 12 % of the total intake was retained. The amount was roughly twice the calcium of an average term infant.

Distribution:

Of the approximately 1200 gm of calcium in the adult body, 99% is combined as salts that give hardness to bones and teeth. Bones not only provide a rigid framework for the body, but they also furnish reserves of calcium to the circulation so that the concentration in the plasma can be kept constant at all times. The remaining 1% of the calcium in the adult, about 10 to 12 gm – is distributed throughout the extra cellular and intracellular fluids of the body.

Walser (1961) studied the distribution of calcium in plasma. According to him calcium present in bound form is 3.28 mg, in soluble complex 1.2 mg and ionized 5.32 mg. The total amount of calcium present in plasma is 10 mg%. The total plasma contains about 200-300 mg calcium. According to Chapin and Smith (1967) blood cells are almost or entirely devoid of calcium but the plasma contains from 9 to 12 mg.
per 100 ml in most species. Plasma calcium occurs in two forms; soluble and complex. The soluble, ionized form makes up about 60% of the total. The other fraction is bound with protein, primarily albumin and plasma proteins. The level of blood calcium is not readily influenced by dietary intake though there are species differences in this respect.

In contrast, all or almost all the inorganic phosphorus in serum is ultrafilterable and ionized. Walser (1961) reported a mean for his 20 normal subjects of 3.6 mg % and a range of 2.6 to 4.7 mg %. Total phosphorus in blood accounts for less than 2 g.

The concentration of calcium in the plasma is kept within the narrow range of 9 to 11 mg per 100 ml (4.5 to 5.5 mEq per liter). About 40 per cent of the calcium is bound to plasma protein and 60 per cent is diffusible. The plasma level is regulated by (1) vitamin D hormone synthesized by the kidney (2) parathormone and (3) Calcitonin, a hormone secreted by the thyroid gland.

Calcitonin is a peptide hormone produced by the C cells of the thyroid gland. The secretion increases with rising serum calcium and helps to maintain the blood level by preventing calcium release from the bones. Estrogen raises the level of calcitonin and prevents bone loss. Nakatsuka et al. (1990) observed that daily injections of calcitonin significantly reduce loss of bone mass and significantly elevated serum alkaline phosphatase. Selvension et al. (1981) reported that estrogen raises the level of calcitonin and prevents bone loss. Postmenopausal bone loss is ascribed to a decreased estrogen level that decreases plasma calcitonin. Therefore, calcitonin rather than estrogen therapy is suggested for the prevention of postmenopausal bone loss as well as for the treatment of osteoporosis.

Calcitonin is antagonistic to parathormone and lowers the blood calcium when it becomes abnormally high. It does this by inhibiting bone resorption.

Most of the 4 or 5 g of calcium, upto 8 g according to Heany and Whedon (1958), in soft tissues is in striated muscle with a concentration of about 0.015%. Total phosphorus present in soft tissues is 65 to 80 g, nearly half of it in striated muscle with a concentration of about 0.15%.

Though somewhat variable according to age, state of nutrition and species, the normal adult bone is composed of approximately 45% water, 25% ash, 20% protein and 10% fat. In mammals the ash is made up of 36% calcium, 17% phosphorus and 0.8% magnesium. The major constituents of bone are collagen, which comprises the
bulk of the inorganic matrix, and calcium phosphate, largely in the form of small crystals of hydroxyapatite. Other components include glucoproteins, acid mucopolysachharides, lipid, calcium, phosphate salts other than hydroxyapatite and ions, which are associated with the mineral phase (Raisz, 1977).

Bone consists of organic and inorganic substance. Protein collagen and the ground substance consists of small amounts of mucoproteins and mucopolysaccharide especially chondroitin sulfate. The formation of bone is initiated early in fetal life with the development of the cartilaginous matrix.

Early in fetal development this strong but flexible protein matrix or pattern for bone begins to form. It bears the same general shape as the mature bone but lacks in strength and rigidity. The matrix remains rather flexible until after birth, possibly to facilitate the birth process. This matrix, which accounts for 30% of the bone, is composed of fibres of the protein collagen embedded in a gelatinous ground substance composed of a carbohydrate, mucopolysaccharide and a glucoprotein. Shortly after birth this matrix gains strength and rigidity, primarily as a result of the deposition and growth of mineral crystals within the matrix in a process known as ossification or calcification. These crystals are either calcium phosphate or a combination of calcium phosphate and calcium hydroxide, which makes up a physiologically stable compound called hydroxyapatite \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \). The deposition of calcium compounds in the mature collagen matrix appears to begin ten days after the collagen has been laid down by the osteocytes, or living bone cells.

During growth the addition of mineral to bone exceeds the amounts that are removed. The bone hardness consists of a gradual addition of minerals by the process referred to as mineralization or ossification. During fetal development and the first few months after birth the bones achieve sufficient mineralization so that the skeleton can support the weight of the baby when he or she walks. Throughout childhood and adolescence the bones increase in length and diameter. This increase in size is dependent upon adequate protein as well as mineral elements. The hardness of bones increases throughout the first 20 years- sometimes longer. About 165 mg calcium are added to the skeleton daily during the early growing years. At adolescence the retention is as high as 300 mg a day, with a yearly increase as high as 90 gm.

Bones do not serve only as structural elements but also as a storehouse of calcium and phosphorus which may be modified at times when the assimilation of these minerals is inadequate to meet body needs. Thus the mineral metabolism of
bone involves not only the deposition of calcium and phosphorus during growth but also involves the process of storage and mobilization throughout the life span. Contrary to popular belief, bones are continuously remodelled and reshaped by osteoblasts (bone-forming cells) and osteoclasts (bone destroying cells). About 250 to 1,000 mg calcium enter and leave the bone each day in the adult (Avioli, 1980).

Fresh skeleton accounts for about 16% of the body weight and contains about 30% of water. But there is clear divergence of opinion about the location of the water. Robinson (1952) said, “that portion of water which is not in the cells seem to be largely held in the amorphous cement substance, water rises as high as 60% in forming bone and it drops as low as 10% in senile cortical bone”.

The water content of bone is affected by a number of factors, such as (i) species, (ii) age and (iii) nutritional factor. Water content of bone decreases with age and the fat is variable according to the nutritive state, since the bone marrow serves as a fat depot, thus ash content is expressed most frequently on the basis of the moisture free, fat free bone.

The importance of lipids in the maturation of the bone is suggested by the observation that the proliferation and calcification of cartilages is associated with a 4-6 fold increase in the lipid of the epiphyses (Wuthier, 1968).

Bone is the principal reserve of calcium and phosphorus in the body. About 700 mg calcium enter and leave the bone each day in the adult. Because of the turnover of calcium within the bone, widely varying intakes of calcium have no direct effect on the blood calcium.

In the well-nourished individual the readily available stores of calcium are in the ends of the long bones, and are known as trabeculae. In the absence of trabeculae calcium is withdrawn from the shaft of the long bone.

The calcium content of the human body is estimated as about 28 g in the infant bone at term, 1200 g in the young adult male and 1120 g in the young adult female according to Leitch and Aitlen (1959) and Newman and Newman (1960). Corresponding estimates of phosphorus content are 18 g at birth and about 670 g for men and 630 g for women.

Bone contains calcium and phosphorus approximately in the ratio of 2:1 while in the whole body being 1.8:1. The Ca/P ratio in the rat femur epiphyses was found to be 1.0 at birth. It increases sharply to 1.9 at 21 days. When the animals were weaned
the ratio continued to rise slowly to a value of 2.2 till 150 days after birth (Dickerson, 1962).

The amount of calcium present in bone depends on the level of calcium present in the diet. In this regard, a series of experiments were conducted by Thomas et al (1988) on young growing rats to study the development of bone and growth. On a diet containing 0.1% calcium diet, rats grew at the same rate as rats given 0.5% calcium. Measurement of femur length indicated that long bone length was the same for all rats but the 0.1% calcium group had mild hypocalcaemia. Bones from 0.1% calcium group contained less than half as much calcium as bone from the 0.5% calcium group, similar observations are reported by Peterson, Eurella and Erdman (1992).

Dietary protein is one of the most important factors involved in normal bone development. Frandsen et al (1954) have investigated that in protein deficient animals the growth of long bones was retarded. Histologically the bones showed a decreased width of the epiphyseal plate, diminution in the number and size of cartilage cells and an increase in the amount of cartilaginous ground substance.

**Excretion:**

Feces and urine are main paths of calcium and phosphorus excretion. The distribution between urine and feces vary with the species and is somewhat influenced by dietary and age factors. In all species, the feces is a primary path for calcium excretion. Some species, such as the equine and rabbit, may also excrete considerable amounts of calcium in the urine when high levels of calcium are fed. The feces is the primary path for phosphorus excretion in the case of herbivore, but the urine is the principal path for carnivore, and the output is about equally divided between the two channels in the case of humans.

Fecal calcium includes endogenous calcium that is not reabsorbed from the digestive juices and dietary calcium. Fecal calcium varies directly with dietary calcium. Under normal conditions the skin loses very small amounts. When people only work strenuously at very high temperatures and perspire profusely, the calcium losses could be considerable.

Urinary excretion for a given individual remains relatively constant regardless of calcium intake, but varies widely from one individual to another. The effect of dietary protein on urinary calcium excretion is greater than that of dietary calcium.
Urinary calcium losses are increased as the protein intake is increased according to Walker and Linkswiler (1972); Allen et al. (1979).

Walker and Linkswiler (1972) reported that when adults were fed a diet containing 800 mg calcium and 47, 95 and 142 g protein, the urinary calcium excretion on the three levels of protein intake was 217, 303 and 426 mg, respectively. By contrast, little effects on calcium excretion were found in another study, in which meat was the primary source of a high-protein intake (Spencer et al., 1983). The high phosphorus content of the meat appears to be responsible for the differences in results (Zemmel and Linkswiler, 1981).

Urinary excretion for a given individual remains relatively constant regardless of calcium intake, but varies widely from one individual to another. Calcium excretion is increased as the protein intake increases (Walker and Linkswiler, 1972; Anand and Linkswiler, 1974; Margen et al., 1974). In one investigation adults were fed a diet containing 800 mg calcium and 47, 95 and 142 gm protein (Walker and Linkswiler, 1972). The urinary calcium excretion on the three levels of protein was 217, 303 and 426 mg, respectively. The calcium balances were +12, +1 and -85 mg, respectively. Thus, high protein diets used in osteoporosis, weight reduction, and other clinical situations could lead to negative calcium balances.

Breslow et al. (1988) concluded that urinary calcium excretion increased from 103 ± 15 mg / day on the vegetarian diet to 150 ± 13 mg / day on the animal protein diet. According to them the increased urinary calcium excretion and inability to compensate for the animal protein the calcuiic responses may be a risk factor for the development of Osteoporosis. In this regard Spencer, Kramer and Osis (1988) carried out a widespread survey and examined the calcium loss with varying amount of protein and phosphorus and concluded that diets low in protein and phosphorus may have adverse effects on calcium balance in the elderly. Studies with adults suggest that high protein foods do not cause calcium loss.

Kitano, Esashi and Azmi (1988) concluded from their experiment that a diet high in protein increased urinary calcium and caused a significant change in urinary calcium and phosphorus or in calcium and phosphorus balance during physical exercise.

Fecal calcium includes endogenous calcium that is not reabsorbed from the digestive juices and dietary calcium. Fecal calcium varies directly with dietary calcium. Under normal conditions skin losses are small. When people work
strenuously at very high temperatures and perspire profusely, calcium losses could be considerable.

Ramarao (1981) reported that excretion of calcium is mainly through feces and reflects the unabsorbed portion of dietary calcium. While phosphorus excretion is mainly through urine. Only small amounts are excreted in feces.

Hock et al (1988) concluded from their study that the fecal outputs of calcium, phosphorus and magnesium were significantly lower after feeding a low calcium diet. Urinary excretion of phosphorus decreased with increasing dietary calcium and increased with increasing phosphorus intake.

Fernandez and Hill (1989) studied four healthy persons for normal diets supplemented with calcium carbonate (500 mg) 4 times daily for 1 week. Mean fecal calcium excretion in controls was $26.7 \pm 7.3 \text{ mg/g}$ which increased to $34.9 \pm 6.7 \text{ mg/g}$ during supplementation which suggested that as the concentration of calcium contributed in the diet increased, calcium excretion in the fecal also increased.

**Calcium deficiency:**

One of the major symptoms of a deficiency of this important mineral are skeletal abnormalities. Osteopenic, osteomalacia, osteoporosis and rickets may all be caused by calcium deficiency. Osteomalacia is a failure to mineralize the bone matrix, resulting in a reduction of the mineral content of the bone. In children, osteomalacia is known as rickets. When children have rickets, their bones become soft and flexible, bending in ways normal bones would not. Features of rickets include bowed legs, beaded ribs, large foreheads, sunken chests (pectus excavatum), protruding chests (pectus carinatum) and hyperextandable joints.

Failure to provide vitamin D by exposure to sunshine or in the diet reduces the absorption and utilization of calcium. Eventually this leads to rickets in the young or osteomalacia in adults. Osteomalacia is a reduction in the mineral content of the bone without reduction in bone size. Apart from a deficiency of vitamin D, a diet deficient in calcium and phosphorus, can lead to the development of rickets in children. In normal children serum inorganic phosphate levels vary from 4-7 mg / 100 ml, but in rickets it may fall to as low as 1-2 mg%.

Osteopenia is the presence of less than the normal amount of bone. Osteopenic, if not treated, may result in osteoporosis. Osteoporosis occurs when the composition of the bone is normal, but the mass is so reduced that the skeleton loses its strength and becomes unable to perform its supporting role in the body. In this
case, fractures may occur due to minor falls and bumps, or bones may even break under their own weight. People with osteoporosis may have a hump in their backs, scoliosis (curvature of the spine), kyphosis (rounded shoulders) or lose height. These conditions may be caused by the buckling of their weakened spines, no longer being strong enough to hold the body upright.

Other symptoms of calcium deficiency include insomnia, tetany, premenstrual cramps, hypertension (high blood pressure).

Osteomalacia involves a reduction in the mineral content of the bone without reduction in bone size.

Osteoporosis is a reduction in the total bone mass. It occurs in millions of American women after age 50 and to a somewhat lesser extent in men. According to Lutwak, a low-calcium diet for 20 to 40 years is an important etiologic factor in periodontal disease and 5 to 10 years later in osteoporosis (Lutwak, 1974). The loss of mineral from bone is not detected by radiography until 30 to 40 per cent has disappeared. Lutwak believes that a daily intake of 800 to 1000 mg calcium would reduce the incidence of osteoporosis.

In malabsorption diseases such as sprue large amounts of fat are excreted. The fat combines with calcium in the intestinal lumen to form soaps and the absorption of calcium as well as fat soluble vitamins is greatly decreased. Hypocalcemia, tetany, and osteoporosis are frequently seen in these cases.

Chronic renal disease has long been recognized as contributing to hypocalcemia, osteitis, and osteomalacia. The cause of the metabolic disorder is the failure of the malfunctioning kidney to synthesize the metabolically active vitamin D3. When the synthetic hormone is given, the calcium absorption and utilization are improved.

Osteoporosis:

The term "Osteoporosis" is commonly used without a clear indication of its meaning (Kanis1990). It was first coined in the 19th century in France and Germany (Consensus Development Conference Report, 1991). Anon (1993) defined osteoporosis as a "systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue with a consequent disease in bone fragility and susceptibility to fracture risk" (Arden and Cooper, 1998). It is thus a silent killer leading to a reduction in bone mass per unit volume without any qualitative changes (Rao, 1998).
Osteoporosis is characterized by a reduction in bone mass to a level below that required for adequate mechanical support function. There is no significant ratio of the mineral to organic phase. Histologically, osteoporosis is characterized by a decrease in cortical thickness and a decrease in the number and size of trabeculae of cancellous bone with normal width of osteoid stem (Krane and Holick, 1990). It mainly occurs when the rate of bone formation is slower than the rate of bone resorption (Robinson et al, 1986).

In osteoporosis, there is reduction in bone mass that renders an individual susceptible to fracture with a moderate degree of trauma (Shils et al, 1999). Bones affected by this disease lose calcium and phosphate salts, thus becoming porous, brittle and abnormally vulnerable to fracture. It is one of the critical diseases facing the ageing population and indeed, is one of the most important disorders encountered in clinical practice (Riggs and Melton, 1995).

Osteoporosis is a disease in which the density and quality of bone are reduced, leading to weakness of the skeleton and increased risk of fracture, particularly of the spine, wrist, hip, pelvis and upper arm. Osteoporosis and associated fractures are an important cause of mortality and morbidity.

In many affected people, bone loss is gradual and without symptoms or warning signs until the disease is advanced. Osteoporosis is a global problem which is increasing in significance as the population of the world both grows and ages. For these reasons, osteoporosis is often referred to as the "silent epidemic".

The WHO (1994) has proposed that both low bone mineral density (BMD) and fracture be combined in a stratified definition of osteoporosis. Thus, there are four categories of osteoporosis.

Normal: BMD not more than 1 standard deviation (S.D) below young adult mean.

Osteopenic: BMD between 1 and 2.5 S. D. below young adult mean.

Osteoporosis: BMD more than 2.5 S.D. below young mean.

Established (Or severe) Osteoporosis: BMD more than 2.5 S.D. below young adult mean in the presence of one or more fragility fractures.

Epidemiology:

Osteoporosis is a disease affecting over 6.1 crore people in India (Rao 1998). Fractures occurring from osteoporosis each year, throughout the world, were projected to increase from 1.7 million in 1990 to 6.3 million in 2050 (Riggs and
In U.K., U.S.A. as well as in India, the number of osteoporosis related fractures have increased (Minihane and Fairweather – Tait 1998). There is no clear statistical data available regarding the incidence of osteoporosis in India, in spite of it being a well recognized entity by the Gynecologist, Physician, Orthopaedician and the Family Physician. It is presumed that by 2000, the population of India, which is expected to touch one thousand million, will have at least 2 million fractures attributed to osteoporosis (Rao 1998).

Worldwide over 30 million people are affected by osteoporosis; it’s incidence is more common in women, especially after menopause (McIlwain et al 1993).

The vast majority of osteoporotic fractures occur in elderly women and incidence increases markedly with age. It is very often called the “silent killer” or “silent epidemic” as there is no evidence of postmenopausal bone loss until the patient experiences a fracture (Cummings, 1985 and Melton, 1995). The incidence of fractures in women is twice that in men (Melton 1995). The reason for this is related to the lower bone mass of the women at the time of maturity (peak bone mass), the accelerated bone loss that occurs after the menopause and the greater likelihood of falling among women over the age 70 years (Winners et al, 1989). Women also live significantly longer than men do so that the frequency of osteoporotic fractures among elderly women is six times that among elderly men (Melton, 1992 and Lauritzen, 1993).

In men, the incidence of fracture increases substantially after the age of 45 years. In women, fractures of the forearm occur predominantly upto the age of 65 years. These fractures are caused generally by a fall on the outstretched hand (Lauritzen 1993). Such fractures of the distal forearm called Collie’s fracture are much less common in men.

Hip fractures assume greater importance in both men and women after an age of 65 years. It is the most serious complication of osteoporosis and is associated with considerable morbidity and mortality. The increased incidence results from a combination of declining BMD and increased incidence of falls with advancing age. With age, the incidence of hip fracture increases exponentially in both sexes (Cooper et al, 1993).

The epidemiology of vertebral fractures is less well characterized. They are asymptomatic, or at least cause too few symptoms to prompt investigation of the cause of back pain (McClaskey et al, 1993). The incidence of vertebral fracture is
greater in women than in men and increases with age (Melton, 1989). Between the ages 60 and 90 years, the incidence of vertebral fractures raises 20 fold in women but only 10 fold in men (Kanis and McClowskey, 1992).

**Risk Factors:**

Although osteoporosis can have serious consequences in all, some people are at a much higher risk for osteoporosis and related fractures than others are. Risk factors for osteoporosis can be easily detected.

The more risk factors present, greater the chances of developing osteoporosis. Once identified, these risk factors can be reduced and the chances of having osteoporosis and fractures in the future can be lessened (McIlwain et al, 1993). The table below expresses the uncontrollable and controllable risk factors for osteoporosis (Vergis, 1997).

* Risk Factors for Osteoporosis (Vergis, 1997)

<table>
<thead>
<tr>
<th>Uncontrollable Factors</th>
<th>Controllable Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Low calcium intake</td>
</tr>
<tr>
<td>Caucasians or Asian</td>
<td>Low vitamin D intake</td>
</tr>
<tr>
<td>Family history of osteoporosis</td>
<td>Lack of exercise</td>
</tr>
<tr>
<td>Small frame</td>
<td>Immobilization, Long term bed rest</td>
</tr>
<tr>
<td>Being female</td>
<td>Excessive caffeine intake</td>
</tr>
<tr>
<td>Early menopause</td>
<td>Concomitant diseases / health problems (anorexia, bulimia, GI reaction or malabsorption, liver or renal disease, hyperthyroidism, hyperparathyroidism, diabetes, various malignancy, Cushing syndrome)</td>
</tr>
<tr>
<td>Low weight / underweight</td>
<td>Long-term use of certain drugs (Aluminium containing antacids, anticonvulsants, glucocorticoid, excessive levothyroxine, heparin)</td>
</tr>
<tr>
<td></td>
<td>Alcohol / Smoking abuse</td>
</tr>
</tbody>
</table>

**Uncontrollable Factors:**

It is known that bone mass declines with age in all people and is related to sex, race and ethnicity, menopause, family history and body weight. The age at which bone loss starts is uncertain but on the basis of cross sectional studies, it is generally believed to start during thirties in both sexes (Buchanan, 1988).

It is widely believed that, after reaching peak bone mass (i.e. the maximum bone obtained at skeletal maturity), men are exposed to a small annual loss of bone mass (Thomsen et al, 1986). This rate of bone loss in men is low, probably about 3-
5% per decade while in women it averages to 2% per year. This partly explains the comparatively low incidence of osteoporotic fractures in men (Gallagher et al., 1987).

Whites and Asians suffer more osteoporotic fractures than Blacks and Hispanics, who have greater bone density. Data now suggests that differences exist between Blacks and Whites in bone metabolism, but more studies are needed to explain the differences especially for the intake of lactose, vitamin D and calcium. Hypovitaminosis D with secondary hyperparathyroidism (Figure-2) occurs more often in the black population.

Petite or thin women, particularly of Northern European extraction, are more susceptible to osteoporosis (Krause et al., 2000). There is much higher risk of osteoporosis after menopause in women. The degree of estrogen deficiency is an important factor and oopherectomised women lose bone mass more rapidly than women who undergo natural menopause. This is due to the lack of estrogen, a female hormone, which is important for bone formation. The menopausal ovaries produce less of this protective hormone resulting in gradual thinning of the bones. Some but not all studies suggests that women who undergo a premature menopause, for any reason are at high risk for osteoporosis (Seeman, 1999; Christiansen and Riis, 1990).

**Controllable Factors:**

It is found the regular exercise helps to delay or even reverse the process of osteoporosis probably by stimulating osteoporosis. Adolescents with a diet low in calcium for a number of years have less bone formed. Thus, when the rate of bone loss becomes greater than the rate of bone formation, osteoporosis occurs more quickly (Sandler et al., 1985; Leighton and Clark, 1992).

Certain medication taken for other reasons may increase the risk of osteoporosis. One of the most common is the group of cortisone—like drugs. These medications if taken regularly over a long period lower the absorption of calcium from the intestine. They also seem to increase bone loss and decrease bone formation (Riggs and Melton, 1995).

It is therefore of utmost importance to identify the risk factors so that adequate measures can be taken for reducing them, thereby minimizing the risk of developing osteoporosis.

**Causes of Osteoporosis:**

Osteoporosis occurs when the body fails to form enough new bone, or when too much old bone is reabsorbed by the body.
Food Calcium

Small Intestine

- Body needs Gastric Acidity, Vitamin D, Lactose, Ascorbic acid, certain amino acids
- Too little vitamin D, Phytic acid, Oxalic acid

Fecal Calcium

Intestinal juices

Calcium in Blood Plasma

- Vitamin D, Ascorbic acid, Calcitonin
- Parathyroid hormone, Excess vitamin D, Bone immobilization

Teeth
Bones
Urinary Calcium

Source: Robinson et al. (1986)

Figure 1: The Utilization of Calcium
Calcium and phosphate are two minerals essential for normal bone formation. Throughout youth, the body uses these minerals to produce bones. If calcium intake is not sufficient or if body does not absorb enough calcium from the diet, bone production and bone tissues may suffer.

As people age, calcium and phosphate may be reabsorbed back into the body from the bones, which makes the bone tissues weakened.

The leading causes are a drop in estrogen in women at the time of menopause and a drop in testosterone in men.

Falls are a primary cause of osteoporosis related injury.

The other causes include corticosteroid excess from Cushing’s syndrome, hyperthyroidism, hyperparathyroidism.

(http://www.nim.nih.gov/medlineplus/ency/imagepages/17287.htm)

**Determinates of Bone Mass:**

In addition to age related bone mass in both men and women and specific diseases that cause osteoporosis in some individuals, the most important cause of osteoporosis is the bone loss that occurs after menopause. A great deal of evidence indicates that postmenopausal bone loss is causally related to menopause (WHO, 1994).

The skeletal mass increases progressively during growth. In absolute terms, this represents a rise in calcium content from about 25 grams at birth to 900 to 1300 grams at maturity. In the first seven years of life, the daily calcium increment in the skeleton is about 100 mg, rising to about 350 mg in puberty. After longitudinal skeletal growth ceases, calcium retention is about 15 mg per day (Kanis and Passmore, 1989). For many years after the cessation of growth, bone mass increases to consolidate the skeleton, this increase varies in extent and rate with sex and site (Gilsanz, 1988). At skeletal maturity, depending on site, men have 10-15% greater bone mass than women (Bonjour, 1991).

Generally, age related bone loss is believed to begin during the thirties in both sexes, but the rate of bone loss in men is slower as compared to that in women (Buchanan 1988). In women, the process of bone loss is more complicated. Bone loss before menopause is small and probably parallels that in men. Irrespective of premenopausal losses, bone loss accelerates around the menopause and averages 2% per year over the next 5-10 years (Gallagher et al, 1987). During this period the bone loss follows an exponential decline. Loss is greatest in the early menopausal
years, levels off thereafter and finally reaches the premenopausal level. This pattern of bone loss has been derived from population studies. However, the accelerated postmenopausal bone loss and relatively lower peak bone mass at least partly explains why osteoporosis is much more common in women than in men (WHO, 1994).

Bone loss occurs at all sites including the head, arms, hands, chest, spine, pelvis and legs (Godfredsen, 1986). During the early postmenopausal years, the proportion of bone lost from the peripheral skeleton (largely cortical bones) differs from that lost from the axial skeleton (cortical and cancellous bones); for e.g. the rate of bone loss is more rapid in the spine than in the forearm. By the age of 75 years, however, women have lost about the same amount of bone from the peripheral and axial skeleton, which suggests that relatively high rate of spinal bone loss that occurred in early postmenopausal years, slows by this time. The more rapid bone losses from the axial skeleton may account in part for the earlier presentation of vertebral fractures while hip fractures characteristically occur in later life (Christiansen et al, 1987).

Postmenopausal bone loss is probably modulated by a variety of nutritional and environmental factors, but it cannot be prevented merely by eliminating the known environmental factors (WHO, 1994).

Types of Osteoporosis:

Osteoporosis is most commonly classified as type I, type II or type III based on the causes of development.

Type I—Post Menopausal Osteoporosis:

It occurs to osteoclasts mediated bone loss primarily of trabecular bone formed in the vertebrae (spine) and distal extremities (wrists). Type I osteoporosis is most common in women 51 to 75 years of age and has a gender ratio of 6:1 (women to men) (Vergis, 1997). It is thought that the lower level of estrogen after menopause is the basic problem and causes of more rapid removal of the bone. The intestine also seems less able to absorb the calcium, which is necessary for bone formation. As a result, more bone is removed and less is formed (McIlwain et al, 1993).

Type II—Senile Osteoporosis:

Senile osteoporosis occurs in men and women at an older age, usually 70 to 75 years of age. It results when osteoblast activity and formation decrease. As people age, body produces less vitamin D. Vitamin D is necessary for calcium absorption
from the intestine. Again, when the intestine is less able to absorb calcium, less bone is formed while bone removal continues (Mcllwain et al., 1993 and Vergis, 1997).

**Characteristics of Primary Osteoporosis: Type I and II**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female; rare in males</td>
<td>Female and male</td>
</tr>
<tr>
<td>Age/Period of life cycle</td>
<td>Menopause (~ 50 years)</td>
<td>Beyond 65 years of age</td>
</tr>
<tr>
<td>Bone tissue</td>
<td>Trabecular</td>
<td>Trabecular and cortical</td>
</tr>
<tr>
<td>Fracture sites</td>
<td>Lumber vertebrae and wrists</td>
<td>Hips and vertebrae; any other bone in the skeleton</td>
</tr>
<tr>
<td>Etiology</td>
<td>Loss of estrogen and androgens</td>
<td>Aging – otherwise poorly understood</td>
</tr>
</tbody>
</table>


**Type III Osteoporosis:**

It occurs secondary to certain disease states (like chronic renal failure or liver damage, hyperthyroidism, hyperparathyroidism, diabetes, Cushings syndrome, GI reaction) or medications used (example: corticosteroids, anticonvulsants, furosemide, insulin, aluminum containing antacids). Women and men are equally at risk for type III osteoporosis. Vertebrae, hips and the ends of long bones are primary fracture sites (Vergis, 1997).

**Stages of Osteoporosis:**

Osteoporosis can be divided into four stages – from stage 1 in which osteoporosis is not detectable to stage 4 with chronic fractures and deformities. The table below provides a review of the course of the stages of osteoporosis (Mcllwain et al. 1993).

- The course of Osteoporosis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Begins</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Sometime after reaching young adult age: 30-40 years</td>
<td>Before osteoporosis is detectable</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Age: 35 – 55 years</td>
<td>Osteoporosis becomes detectable</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Age: 45 years and older</td>
<td>Osteoporosis results in fractures of bones</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Age: 55 years and older</td>
<td>Osteoporosis with chronic pain and deformity</td>
</tr>
</tbody>
</table>

Source: Mcllwain et al. 1993
Stage 1:

It is the period before which osteoporosis becomes detectable. At some point after the age of a young adult (30-40 years), the gradual decrease in total amount or density of bone in the body begins but is not yet possible to detect. During this time, body is still constantly building and removing bone with the balance of the two processes slowly shifting such that gradually more bone is removed than is formed. The bones are still strong, exhibiting no pain and no unusual fractures. The denser the bones present at the beginning of this stage, the longer the density of the bone will last (Gaby, 1994).

Stage 2:

It gradually begins sometime after age 35, bone density has reduced to a detectable extent by radiographic examinations. The very gradual loss of bone mass has continued for a number of years, but the remaining bone is still strong enough so that unusual fractures do not happen. Of course, with severe injury, fractures can occur, but there are still no fractures following minor injuries. Activities that have been done over many years can still be performed (Riggs and Melton, 1995).

Stage 3:

It is the stage of osteoporosis with fractures. It often begins around age 55 or older. At this stage, bones become thin enough so that minor injuries now can cause a fracture of the bones in spine, hips and wrists on the slightest pressure. Other areas can also be affected by fractures in this stage (McIlwain et al, 1993).

Stage 4:

If the process of osteoporosis continues, the bone becomes thinner and fragile each year. This stage begins after age 55 or can happen earlier if many risk factors are present. More fractures occur, usually with more and more pain. The most common areas affected are still the spine, especially the middle and the lower back areas called thoracic and lumber spine. Since every fracture of vertebrae in the spine cause it to become a little shorter, as more fractures occur, the person experiences loss of height. This results in the familiar ‘Dowager’s Hump’ (figure-3) and eventually leads to a stooped appearance (Christiansen and Riis, 1990).

Symptoms of Osteoporosis:

Osteoporosis is a silent disease that may not be noticed until a broken bone occurs, signs may include diminished height, rounded shoulders, Dowager’s hump
Figure 3: Normal spine age 40 and osteoporotic changes at ages 60 and 70. These changes can cause a loss of as much as 6 to 9 inches in height and result in the so called ‘Dowager’s Hump’ (far right) in the upper thoracic vertebrae (Mellwain, 1993).
and evidence of bone loss from diagnostic tests. The symptoms may include acute
backache caused by pathologic vertebral compression fracture, or an episode of groin
or thigh pain caused by a pathologic hip fracture. When as much as 30% or more i.e.
1/3 or more of original bone mass is lost, bone will fracture under the slightest stress
and heal poorly. In advanced stages of osteoporosis, any activity can cause fractures
of the vertebrae (backbone), wrists, hips, 75% of individuals who fracture a bone due
to osteoporosis will suffer deformities and permanent disabilities which greatly
decrease their quality of life (Glaser and Kaplan, 1999).

Symptoms occurring late in the disease include:

- Fractures of the vertebrae or hips
- Low back pain
- Neck pain
- Bone pain or tenderness
- Loss of height over time
- Stooping posture called kyphosis.

**Diagnosis and Treatment**

Rapid progress is being made in diagnosis, treatment evaluation and prognosis
of osteoporosis. Early detection of bone loss is key to the prevention of suffering and
escalation of health care costs. Bone mineral density (BMD) measurements are
effective in assessing fracture risk, confirming a diagnosis of osteoporosis and
monitoring the effect of treatment. A major concern is that access to measuring
equipment and qualified technical personnel – and reimbursement by medical
insurance schemes – remain inadequate in a great many countries.

People with osteoporosis suffer from a reduction in their bone mass and bone
quality- put simply, their bones become fragile, leading to an increased risk of
fractures. Bone density loss is usually gradual and without noticeable symptoms. The
only reliable way to determine loss of bone mass is to have a bone mineral density
(BMD) test.

**Diagnosis:**

People with osteoporosis suffer from a reduction in their bone mass and bone
quality. Put simply, their bones become fragile, leading to and increased risk of
fractures. Bone density loss is usually gradual and without noticeable symptoms. The
only reliable way to determine loss of bone mass is to have a bone mineral density
(BMD) test.
Techniques used for measuring Bone Density:

A variety of methods is available to assess bone density. All are painless and noninvasive. The most common types of tests are:

(1) Bone Densitometry
(2) Ultrasound
(3) Quantitative Computed Tomography
(4) X-Ray (Radiographic Technique)

(1) Bone Densitometry:

Currently, the standard technique for determining bone density is a form of bone densitometry called Dual-Energy X-ray Absorptiometry (DEXA). DEXA is simple and painless and takes two to four minutes. Measurements of bone mineral density are generally given as the average concentrations of calcium in areas that are scanned.

(2) Ultrasound:

This technique measures bone density in the heels, fingers and leg bones. In early studies, advanced Ultrasound techniques, such as quantitative ultrasound (QUS) are promising features when used with DEXA. Ultrasound itself is less expensive than DEXA and uses no radiation.

(3) Quantitative Computed Tomography:

QCT scans, a form of CT scans, can provide highly detailed information about spinal density. Radiation close-ups from this technique are higher than the others. Whether QCT predicts fracture risk accurately is however unknown.

(4) X-Ray Radiography:

The use of radiographic technique is most important in detecting BMD. The trabecular pattern in the pelvis correlates with bone density. High resolution radiography has also been used to assess cortical porosity.

In this background the present investigation was planned to study the bone mineral status of women from the age group 26 years and above upto 60 years and above.

BMD results from a study of 50 pre-menopausal women, divided among vegan (n=17), lacto-ovo vegetarians (n=18), and omnivorous (n=15) subjects was reported by Vegetarians had followed their respective diets for at least four years. All subjects were within 20% of ideal body weight, had regular menstrual cycles, engaged
in <5hrs/wk of aerobic or strength building exercise, did not consume calcium supplements or take any medications known to affect bone metabolism, including oral contraceptives. The three groups did not differ with respect to age, height, weight and BMI. Percent of expected BMD of the spine as determined by quantitative computed tomography (QCT) was significantly less in vegans (mean±SE) (88 ± 2.7%) compared to LOV (107 ± 3.7%, p<.001) or omnivores (101 ±4.9%, p<.05), but not different between LOV and omnivores (Johnston, 1962).

Through early detection, people with osteopenia (low bone mass) or osteoporosis, can take action to stop the progressive loss of bone mass. By making positive lifestyle changes and following appropriate treatment strategies in consultation with a doctor, osteoporosis can be prevented and treated.

**Treatment of Osteoporosis:**

The aim of treatment is to prevent the development of osteoporosis and to prevent further bone loss in order to decrease the risk of osteoporotic fracture. Today there is a wide range of therapeutic options and several safe and effective pharmacological treatments that have been shown to act quickly (within one year) and to reduce the risk of fracture by upto 50%. It is important that the choice of treatment be tailored to a patient's specific medical needs and lifestyle.

Evidence that high calcium consumption increases peak bone mass at skeletal maturity and mitigated bone loss in later life, has resulted in public awareness to increase the consumption of calcium supplements for both prevention and treatment of osteoporosis. Calcium supplements are available throughout the world and are a major non-HRT intervention used in osteoporosis (Minihane and Fairweather-Tait, 1998).

After menopause, bone loss accelerates. This reduction in bone mass is related to a decline in ovarian function and consistent with this; a similar loss of bone density is observed in those who undergo a premature menopause.

Thus, the major thrust of treatment has been directed towards preventing bone loss that occurs in association with the menopause. The intervention used are largely pharmacological and include Hormone Replacement Therapy (HRT), calcitriol, fluoride, calcitonin, anabolic steroids, parathyroid hormone (PTH), growth hormone, insulin like growth factors and most commonly used calcium supplements (Mcllwain et al 1993).
Types of therapy

Antiresorptive drugs, already available, slow the progressive thinning of bone. Bone-building agents help to rebuild the skeleton and are now becoming available or are in the developmental pipeline. Non-pharmacological interventions are also very important in reducing the risk of fracture.

❖ Antiresorptive drugs

Hormone Replacement Therapy: Hormone replacement therapy (HRT) remains the method of choice for the prevention of osteoporosis in postmenopausal women at risk of osteoporosis if some loss of bone density is already evident. In addition to its beneficial effects on menopausal symptoms, estrogen replacement dramatically slows the rate of postmenopausal bone loss. Estrogen is available as tablets, transdermal patches or creams. Clinical trials have shown positive results for an intranasally delivered estrogen. However, for women with a uterus, estrogen plus progestin combination therapy is recommended to prevent endometrial cancer which can result from estrogen therapy alone. Combination therapy is available orally or transdermally. Newer formulations of estrogen and progestin provide greater dosage flexibility, allowing the physician to tailor the therapy according to the individual woman’s needs and age. Newer progestins also have fewer unwanted side effects. Additional benefits of HRT are being investigated including possible protection against heart disease and senile dementia.

Bone density has been shown to increase by up to 30% in postmenopausal women treated with a year of HRT and fracture risk is reduced by as much as 60% if HRT is taken for 6 years or longer (Spencer et al, 1998). HRT initiated soon after menopause slows or reverses the loss of bone that occurs normally during those years (Eiken et al, 1997).

HRT has also been shown to reduce the risk of atherosclerosis and myocardial interactions by 50% or more in some studies and relieve the symptoms of menopause. The current practice is to commence HRT at menopause and continue it for 10 years (Harold, 1977). There is some evidence that Estrogen Replacement Therapy (ERT), combined with high calcium supplementation may even result in increased bone mineral density (Aloia et al, 1994).

However, the use of HRT in the treatment of osteoporosis has been associated with certain hazards. This includes the possible risk of endometrial cancer (Collins et
al, 1980), however adding progestin lowers this risk (Whitehead et al, 1981). Return of the menses discourages its use for some women. Some studies have also shown an increase in breast cancer associated with the use of HRT (Steinberg et al, 1991). Ultimately, the patients and physicians will have to arrive at their own decision as to whether the risk outweighs the benefits (Harold, 1997).

Calcitriol: It is the active form of vitamin D i.e. cholecalciferol. Calcitriol has been shown to decrease loss of bone mass associated with estrogen deficiency in both animal and human models (Ott and Chestnut, 1989 and Wardlow, 1989).

In women with established postmenopausal osteoporosis, calcitriol therapy (>0.06 mg/day) for two years increased spinal bone density by 0.18% and 1.94% in separate studies, compared with a decrease in bone density in placebo recipients. However, there is a risk of hypercalcaemia especially if patients are on a calcium and vitamin D supplementation (Harold, 1997). Maintenance of an adequate dietary intake of vitamin D (100 I.U. or 5 μg of cholecalciferol) is important for the many housebound elderly who fail to get adequate exposure to sunlight (Riggs and Melton, 1995).

Bisphosphonates: Bisphosphonates inhibit bone resorption. They are currently the first choice of treatment in a variety of bone metabolism disorders characterised by high bone resorption. They bring about an increase in bone mass and a decrease in fracture incidence in osteoporosis.

Estrogen Analogs: Selective estrogen receptor modulators (SERMS) mimic estrogens in some tissues and anti-estrogens in others, and ideally provide the bone-retaining effects of estrogen without its unwanted side effects. Currently, the only marketed SERM is raloxifene. Raloxifene prevents bone loss and is indicated for the prevention and treatment of vertebral fractures in postmenopausal women.

Fluoride: Fluoride was first recognized as a bone growth stimulator over 30 years ago; sodium fluoride and sodium monophosphate have currently been licensed for the treatment has been showed to produce a linear increase in vertebral bone mass of 4-8% per year, reduce vertebral fracture incidence and increases bone density (Keen, 1997).

Tibolone: Tibolone is a synthetic analog of the gonadal steroids with combined estrogenic, progestogenic and androgenic properties. Its effects on bone density are
comparable to those of hormone replacement therapy. Its efficacy on fracture risk has not yet been assessed.

Calcitonin: Calcitonin therapy decreases the rate of bone loss in osteoporotic women; however, it is most effective if given early. It must be administered by subcutaneous injection, which limits its clinical usefulness, although other forms are being developed. Calcitonin was known to bind to high-affinity receptors on osteoclasts with pronounced inhibition of activity, both in vitro and in vivo data have given support to the hypothesis that calcitonin might have direct or indirect effect on osteoclast activity. It is postulated that estrogen deficiency in postmenopausal women resulted in a reduction in circulating calcitonin levels (Stevenson and Whitehead, 1982).

Anabolic Steroids: They are derived from 19-non testosterone. Early studies carried out in osteoporotic patients showed a markedly positive skeletal balance induced by testosterone treatment. However, the masculinizing effect of androgens in women made them unacceptable as a treatment for postmenopausal osteoporosis. Anabolic steroids have been developed from androgens to decrease the virilizing effect and retain the anabolic action.

❖ Bone-forming drugs

Parathyroid Hormone: The bone-forming effects of parathyroid hormone (PTH) have been known to exist for more than 70 years. However, it is only in the last 5-10 years that data has emerged that provides consistent and encouraging results in animals and humans. A recent multinational study on postmenopausal women with prior vertebral fractures demonstrates that a synthetic fragment of PTH will be useful in the management of osteoporosis. The results showed that the risk of vertebral fracture was reduced by 70% within 18 months of treatment. Nonvertebral fracture risk was reduced by 50%. It is expected that a form of injectable PTH will be available in some countries in the near future.

PTH is a single chain polypeptide of 84 amino acids. This hormone stimulates the release of calcium and phosphate from bone, stimulates reabsorption of calcium and inhibits reabsorption of phosphate from the glomerular filtrate and stimulates the renal synthesis of 1, 25 (OH) 2D. The 1-34 fragments of PTH have been evaluated as an anabolic agent in osteoporosis (Gennari and Nuti, 1998). High plasma concentration of PTH has been found to stimulate bone resorption; however, PTH may stimulate bone resorption when given intermittently at low doses. PTH has also
been found to have an anabolic effect on the central skeleton in patients on HRT. At present no long-term data on PTH therapy and vertebral fracture incidence are available (Lindsay et al, 1990).

Growth Hormone and Insulin like Growth Factors: Growth Hormone (GH) promotes both direct and indirect action on bone. GH improves both muscle mass and strength, and leads to increased physical activity, with the resultant beneficial effects on bone mass. The hormone also potentiates the gonadal secretion of the sex steroids. In postmenopausal women with osteopenia, short-term GH therapy promoted stimulation in bone formation and bone resorption (Papadakis et al, 1996).

Statins: Statins, drugs used to lower cholesterol, may also have a bone-forming effect. It has been reported that statins increase bone formation by enhanced osteoblast differentiation.

Strontium Ranelate: Strontium ranelate is a compound that has been shown in animal models to decrease bone resorption and increase bone formation.

**Nonpharmacological Interventions**

Nutrition and lifestyle play an important role in osteoporosis prevention and treatment. Other factors, like fall prevention techniques or hip protectors to reduce the impact in case of a fall, are also very important.

**Calcium:** Calcium supplements (0.5-1 g/day) and low doses of vitamin D (800 IU/day) have been shown to reduce the risk of hip fracture in elderly women living in nursing homes (who are often vitamin D deficient). In addition, calcium and vitamin D supplementation is often part of the treatment regimen for osteoporosis in younger patients. Sufficient protein intake is mandatory to help maintain muscle function and bone mass.

Although heredity and environmental conditions are usually thought of separately, both influence the skeleton through a common mechanism. It has been suggested that various life style factors viz; calcium, vitamin D, protein intake, smoking, alcohol use, body weight and physical activity influence bone mass, rate of bone loss and fracture rates in adults. Modifying these factors to prevent osteoporosis is especially important, since this disease is not reversible. The prevention of this major public health problem is the only cost-effective approach (Dawson-Hughes 1998).

To assess the effect of dietary calcium intake or risk of hip fracture, a 14 year prospective population study was carried out in 947 men and women aged 50 to 79
years. It was found that a daily calcium intake of over 750 mg/day was associated with 60% reduction in risk of hip fractures (Halbrook et al., 1988). It was also demonstrated that healthy older postmenopausal women with daily calcium intake of less than 400 mg can significantly reduce bone loss by increasing their calcium intake to 800 mg per day (Dawson–Hughes et al., 1990).

A high calcium diet over a lifetime, especially in the developing years, seems more likely to increase the amount of bone formed as the skeleton matures. This would allow more bone to be present before the process of osteoporosis begins. Thus the idea is to build maximum bone strength before osteoporosis slowly decreases the amount of bone present (McIlwain et al., 1993).

Most epidemiologic studies have shown that the usual dietary calcium intake of postmenopausal women is lower than the RDA for calcium. The benefits of calcium supplementation have been debated, however, several studies have prove it's positive effects in elderly women in the prevention of bone loss and fractures (Fardellone et al., 1998).

Adequate calcium retention is important for building and maintaining peak bone mass. Calcium sources must be evaluated for both calcium content and bioavailability for their role in the diet. Calcium bioavailability from a variety of plant sources have been compared to milk using hydroponically grown vegetables and intrinsic labeling techniques. Generally, plants which contain oxalic acid have poor calcium bioavailability except for soybeans. Plant sources which are low in both oxalic acid and phytic acid typically have better calcium bioavailability than milk although the amount per serving is lower. Some dietary constituents increase urinary calcium loss, and thus, impact calcium retention even though they do not affect calcium absorption. For every gram of dietary salt consumed, approximately 26 mg calcium is lost in the urine. For every gram of metabolically protein, approximately 1 mg additional calcium in the urine is lost. Thus, choices present themselves to meet individual needs. Diets can be constructed which are higher in calcium or lower in salt, protein, caffeine, and other constituents which lead to calcium loss.

The most thoroughly studied segment of the population is women in the first several years after menopause, the time when bone-loss occurs at a rate of 2% to 3% per year (Dawson-Hughes, 1995). In a 1997 study, 72 postmenopausal women were given either calcium supplements (800 mg/day) estrogen or no treatment. Untreated
women continued to lose bone, while those given estrogens did not. Bone loss in the calcium-treated group was intermediate (Horsman 1997).

The value of calcium supplementation in the prevention of bone loss was confirmed by further studies. In a randomized, placebo-controlled double blind trial in women with high (estimated at 1000 mg) usual dietary intakes, supplementation with 2000 mg calcium as the carbonate for 2 years has a modest but significantly favorable effect on bone loss from the proximal radius (Rijffiet al 1997). However, there was no effect of calcium on bone loss from the spine in a second, controlled but not randomized or double-blind trial Ettinger et al (1987) also found that in early postmenopausal women with mean dietary calcium intake of 650-850 mg/day, supplementation with 1000 mg calcium as carbonate had a favorable effect at the radius but not at the spine. Another randomized trial by elders et al 1989, reported that supplementation with 1000 or 2000 mg calcium as carbonate retarded bone loss from the spine for the first year of treatment. During the second year, however spine loss was similar in the control and treated group.

In another study, in a group of normal women at least 3 years after menopause with a mean dietary calcium intake of 750 mg/day, calcium supplementation (1000 mg/day) for 2 years significantly lowered axial and appendicular bone loss in comparison to a controlled placebo-group; the rate of loss was reduced by one-third to one-half in the calcium group (Reid et al 1993).

From these trials it appears that bone loss from at least part of the appendicular skeleton can be retarded (not prevented) by calcium supplementation and that the response of the radius to added calcium is maximal at a supplement dosage of approximately 1000 mg elemental calcium/day. Supplementation with calcium even in high amounts does not provide any substantial benefits to the spine in early, postmenopausal women (Dawson-Hughes 199D).

However, a number of other studies have shown that calcium has little or no value in the prevention or treatment of osteoporosis. In a study carried out on 106 healthy women, between the ages 23 to 84 years, they were observed for a period of 2 to 6 years. During this time, there was no correlation between dietary calcium intake (which ranged from 260 to 2035 mg/day) and the rate of bone loss (Riggs, 1997). In another study, women in the early postmenopausal period who received 2,000 mg/day of supplemental calcium had approximately the same rate of bone loss as those given a placebo (Rijff et al, 1997).
These conflicting results are consistent with the viewpoint that calcium deficiency is only one of the many causes of osteoporosis and that not everyone who had osteoporosis is actually deficient in calcium (Gaby, 1994). However, calcium supplementation could be helpful only in osteoporotic patients who have a very low calcium intake (Gennari and Nuti, 1998).

Selection of a calcium source: The effectiveness of calcium supplements varies to some extent with the supplement chosen, the dosage schedule and the time of administration. Although all recommendations specify that calcium is best utilized if obtained from food, many women who are making anabolic effort to increase their intakes are taking calcium supplements (Krause et al., 1996).

It has been reported that intestinal absorption of calcium from a variety of salts and from milk is generally similar; some differences among sources have been observed (Miller et al., 1998). Calcium is better absorbed from citrate than from carbonate. In young adults, calcium was absorbed better from calcium citrate-malate than from carbonate or milk. Heaney et al. 1997 demonstrated that the absorbability of calcium from different salts is only weakly related to the solubility of the salt at neutral pH. Overall, variation in the absorption of calcium from different sources is fairly small when compared with variation in other indices affecting fractional absorption of calcium (Dawson-Hughes, 1996).

Vitamin D: Vitamin D is important in the prevention of osteoporosis because the active metabolite 1, 25-dihydroxy vitamin D [1, 25(OH)₂ D], regulates calcium transport across the intestinal mucosa and because 1, 25(OH)₂D stimulates bone formation. Vitamin D is derived from diet, and this fat soluble vitamin is absorbed in the distal ileum by a process that requires the presence of bile salts. Vitamin D is also synthesized in the skin from the precursor 7-dehydrocholesterol, after exposure to U.V. radiation (Devlin et al., 1998).

The ability to absorb and synthesize vitamin D appears to decline with age. Absorption of vitamin D from a test meal was reduced by 40% in healthy women aged 68 to 98 years compared with those aged 30 to 58 years. Decreased skin synthesis of vitamin D with age probably results from local substrate deficiency. The concentration of 7-dehydrocholesterol in the epidermis declines by about 50% between the ages 20 and 80 (Gennari and Nuti, 1998).

Low vitamin D status in elderly patients can become apparent either as muscle weakness, which may lead to a fall and subsequent fractures or as secondary...
hyperparathyroidism. To increase the absorption of calcium from the intestine, vitamin D intake can be taken in through diet or as a supplement (McIlwain et al 1993).

Protein: Positive correlation between dietary protein intake and urinary calcium excretion have raised the question of whether the high protein diets commonly consumed in Western countries to bone loss. Protein may affect calcium metabolism in several ways. Protein is metabolized to organic acids and these may be buffered at the expense of bone. The observation that simultaneous administration of bicarbonate with protein reduces the calciurea caused by dietary protein alone supports this possibility (Lutz, 1984). Protein may also enhance calcium excretion because it increases the glomerular filtration rate (Dawson – Hughes 1990).

In balance studies of Heaney et al 195% a 50% increase in nitrogen intake, from 10.9 to 16.4 grams daily, resulted in a loss of additional 28 grams of calcium in the urine. Despite the metabolic studies cited, evidence that a high protein intake adversely affects the skeleton is mixed. A large cross sectional study in Japanese men found no correlation between protein intake and bone mass.

Exercise: Regular weight-bearing exercise has been shown to help maintain and build up bone mass. The stronger muscles, better balance and agility to which exercise contributes can also help in fall prevention. The type of exercise should be tailored to the individual’s needs and abilities. People with osteoporosis must take special care when exercising to reduce the risk of fracture due to impact or falls.

Body Weight: Body weight and bone density are positively correlated in men and women. The influence of weight on rates of bone loss has been studied less extensively; however, there is evidence that loss of mineral from the forearm is reduced in postmenopausal women who are heavier (Christiansen et al, 1978). Hassager et al (1989) found that osteoporotic women had similar amounts of lean tissue but less fat tissue than non-osteoporotic women. It is often proposed that enhanced peripheral conversion of andostenedione to estrogen in fat tissue is a means by which fat tissue benefits the skeleton of postmenopausal women. The indication that fat tissue may be just as important in postmenopausal women implies that mechanisms other than peripheral estrone production are involved (Ried et al, 1993). Additional work is needed to identify the mechanism by which fat tissue influences bone.
Physical activity: Immobility in varying degrees is recognized as a cause of bone loss. Maintenance of healthy bone requires exposure to weight bearing pressure. Stresses from muscle contraction and maintaining the body in an upright position against the pull of gravity stimulates osteoblast function. Bones not subjected to normal use rapidly lose mass. To a lesser degree, lack of exercise and a sedentary mode of living that continues over lifetime also contribute significantly to bone loss (Krause et al, 2006).

In a study, postmenopausal women gained 5% in spinal bone density over the course of a one-year exercise program and lost 4% over the ensuing year, during which they reverted to their usual sedentary life style. In postmenopausal women, both aerobic exercise and strength training increased radius density and total body calcium in randomized trials (Chow et al, 1987).

Exercises like walking, skipping, jogging, hiking, dancing, cycling and weight lifting are associated with increased bone density. Strenuous forms of exercise are inappropriate for the elderly particularly those already suffering from osteoporosis. However, moderate walking is beneficial and swimming is a no traumatic form of exercise that can aid bone density to some degree. In addition to arresting the loss of bone mass, exercise leads to increased fitness with an improvement in muscle control that can prevent fall or at least make them less traumatic (Krause et al, 1996).

Soybean:

The nutritional importance of legumes extends far beyond the realm of protein, and beyond the obvious fact that legumes, grains and starchy vegetables have provided the human race's staple sources of complex carbohydrates.

The one nutritional attribute of legumes that has not remained a secret in this century has been their abundant protein content. All legumes seem to derive 20 to 44 percent of their calories from protein (except for the peanut, at 16.3% due to its uniquely high fat content).

Soybeans (Glycine max) contain more calcium than do other legumes; their calcium-to-calorie, calcium-to-phosphorus and calcium-to-protein ratios are similar to those of chickpeas or navy beans. Despite their phytate and unusual calcium-bindable oxalate content, they show, in some studies, about twice as much calcium absorbability as other common unsprouted beans.

Cultivation of Soya: Soya is a frost-sensitive summer annual, and it takes about 75-80 days for the beans to fully mature, plants may reach 1 metre high. Seeds are borne
in hairy pods which grow in clusters of three to five, each pod contains two or three seeds, which resemble peas.

Soya is now a global staple food and about 110 million tones of beans are produced, mainly in the United States (50%+), Brazil (20%), Argentina (10%) and China (8%). European oil mills process about 15 metre soy beans annually, mainly imported from the USA.

**Soya as a food ingredient:** About two-thirds of all manufactured food products contain derivatives or ingredients made from soya. Before they can be used in food products the soya beans have to be cleansed, cracked, dehulled and rolled into flakes, which ruptures the oil cells for easy extraction. The oil is extracted using a food-grade solvent, n-hexane – mostly for production of vegetable oil and margarine.

Soya flour was developed in the 1940s by grinding and screening defatted flakes, these are used to increase the shelf-life of many products and improve the colour of pastry crusts, the flour is free of gluten, so cannot replace all the wheat or rye flour in bread-making but can be used at about 15% to give a dense bread with a nutty flavour and moist quality. Texturised soy protein (TSP or TVP) is made from soya flour that is compressed until the fibres change in structure. It is available to home cooks as a dried, granular product and in chunk-sized pieces for rehydrating and use as a meat-replacer. Following the development of methods to produce isolated soya proteins in the 1950s, it is also processed for use as soya protein in biscuits, sweets, diet drinks, pasta and frozen foods; it also improves the consistency of meat products.

**Feeding and Handling Characteristics:** Processing is critical when utilization of nutrients contained in soybeans is trying to be maximized. Heat processing is required to inactivate many of the anti-nutritional factors, but excessive heating will reduce nutrient utilization of the nutrients contained in soybean meal. Several methods are used for achieving the proper heat processing of soybeans. In order to properly process soybeans the moisture content, temperature and length of processing time need to be precisely controlled. Soybeans can be roasted in a rotating drum to which heat is being applied. Another method is to pressure-cook the soybeans at 115°C for ten minutes or at atmospheric pressure for two hours. Excessive heating wastes energy and reduces the nutrient availability, especially when being fed to monogastric animals. Testing the urease levels in soybean meal is a good indicator of if the soybeans have been properly heat processed, because urease is inactivated at about
the same temperature as the trypsin inhibitors. Urease values are normally around 2 in raw soybeans and 0.05 to 0.2 in properly processed soybean meal. It is important to assure that the moisture content of soybeans that are going to be stored is below 15 %. Often when soybeans are harvested their moisture content is above 15 % and they must be dried prior to being stored. It is best to store soybeans in their whole form, because ground soybeans will deteriorate rapidly when stored, especially in hot humid climate. Soybean meal can range in CP from 41 to 50 % (dry matter basis), depending upon its hull and oil content. The hulls of soybeans can be used as a low quality roughage source for ruminant animals. Various other products can be prepared from soybeans. Soy flour is derived from soybean meal by screening it and collecting the fine powder. Soy protein concentrate is prepared from dehulled beans that have had the fat extracted, then extracted with water and is often used as extender in various food products.

**Soya in nutrition:** Soy beans are the only plant source of protein that provides a "complete" protein – a protein that contains all the amino acids essential to human growth and development. Soya bean protein quality is comparable to meat and eggs. While fermented soy products contain protein, vitamins, anti-carcinogenic substances and important fatty acids, they can under no circumstances be called nutritionally complete. Like all pulses, the soybean lacks vital sulfur-containing amino acids cystine and methionine. These are usually supplied by rice and other grains in areas where the soybean is traditionally consumed. Soy should never be considered as a substitute for animal products like meat or milk.

The vegetable oil is poly-unsaturated, has a low level of saturated fatty acids and is free from cholesterol, but contains both essential fatty acids – linoleic and linolenic.

Soybean contains large quantities of a number of harmful a substances. First among them are potent enzyme inhibitors which block the action of trypsin and other enzymes needed for protein digestion. **Protease inhibitors (or trypsin inhibitors)** block the enzymes that the body uses to break down protein. This is only a problem if people eat raw, unprocessed soy, since protease inhibitors are mostly destroyed upon cooking. Low levels of protease inhibitors in the diet are generally considered to be safe.

Soybeans are high in protein but also contain a number of minor constituents traditionally considered to be antinutritional factors. These include trypsin inhibitors,
phytic acid, saponins and isoflavones. These compounds are now thought to have beneficial biological effects in the diet, such as lowering blood cholesterol or preventing cancer.

Phytates are generally found in foods that are high in fiber such as whole grains and beans. They are chemical substances that encourage metal ions to bind together, including calcium, copper, iron, manganese and zinc. High phytate intake can prevent the body from absorbing these essential minerals from our intestinal tract. Therefore, phytates are also known as antinutrients. Soybeans contain high levels of phytates; some researchers say more than other beans. Additionally, soy's phytates are so stable that many survive phytate-reducing techniques such as cooking. (The phytates in whole grains can be deactivated simply by soaking or fermenting). It is possible that only long periods of soaking and fermenting - as are used in making miso, natto, shoyu, tamari, and tempeh (but not tofu, soymilk, texturized soy protein, or soy protein isolate) - significantly reduce the phytate content of soybeans. It has also been reported that tempeh has lower phytate levels than unfermented soyfoods. Eating too much unfermented soy may lead to a shortage of crucial minerals (Anderson and Wolf, 1995). However, it is the binding of iron (a producer of free radicals), that seems to be one of the mechanisms by which phytate may inhibit cancer.

Soybeans are also high in phytic acid or phytates. This is an organic acid, present in the bran or hulls of all seeds, which blocks the uptake of essential minerals-calcium, magnesium, iron and especially zinc-in the intestinal tract. Although not a household word, phytates have been extensively studied. Scientists are in general agreement that grain and legume based diets high in phytates contribute to widespread mineral deficiencies in third world countries.

These "antinutrients" are not completely deactivated during ordinary cooking and can produce serious gastric distress, reduced protein digestion and chronic deficiencies in amino acid uptake. In test animals, diets high in trypsin inhibitors cause enlargement and pathological conditions of the pancreas, including cancer. The soybean also contains hemagglutinin, a clot promoting substance that causes red blood cells to clump together. Trypsin inhibitors and hemagglutinin have been rightly labeled growth depressant substances. Fortunately they are deactivated during the process of fermentation. However, in precipitated products, enzyme inhibitors
concentrate in the soaking liquid rather than in the curd. Thus in tofu and bean curd, these enzyme inhibitors are reduced in quantity, but not completely eliminated.

Proximate nutrients, calcium, and some anti-nutrients in 16 varieties of whole horsegram and their dehulled seeds were estimated. The protein, fat, and carbohydrate contents were higher in the dehulled samples than in the corresponding whole horsegram. However, the moisture, fibre, ash, and calcium contents of the dehulled samples were lower. A significant portion of the anti-nutrients studied were removed by dehulling.

**Health benefits of soy:** Soya protein is said to have the effect of reducing cholesterol levels in hypercholesterolaemic people. The Food and Drug Administration (FDA) is proposing to authorize the use of a health claim for foods which contain soya protein which will allow them to state that they can reduce the risk of heart disease. If such a claim is allowed soy will join oats as a food allowed to claim on packaging and labels that it “may reduce the risk of heart disease, as part of a diet low in saturated fat and cholesterol”. Foods that would be able to carry the claim include soy milk, vegetable burgers and tofu (New Nutrition Business, 1999).

Soya is an important source of a group of non-nutrients known as the phytoestrogens; compounds with structural and functional similarities to the natural oestrogenic hormones present in the body. Examples are daidzein and genistein, present at levels around 3 mg/100 g wet weight in raw beans. In certain situations these chemicals can behave like a very weak form of oestrogen.

Epidemiological studies (primarily from Japan, where soya consumption is high) suggest a beneficial, protective effect for the phytoestrogens against certain sex hormone-dependent cancers — including breast and prostate cancers. Phytoestrogens present in a wide range of food plants (including soya) may have deleterious effects on reproductive efficiency when consumed by animals; there is no evidence for a parallel effect in man.

Soybeans are also the only food source with nutritionally significant amounts of *isoflavones*, a type of phytoestrogen (plant estrogen) chemically similar to human estrogen that may cause weak estrogen-like effects on the body. Some examples of isoflavones are *daidzein*, *genistein*, and *glycitein*.

Soybeans are a natural dietary source of isoflavones, which have estrogen-like properties. Therefore, it is worthwhile to consider the implications for soy of the recently published findings of the Heart and Estrogen/Progestin Replacement Study.
HERS I/II and the Women's Health Initiative (WHI). The WHI found coronary heart disease (CHD) risk to be increased in women receiving hormone replacement therapy, and both studies found increases in venous thromboembolic disease in such women. Additionally, stroke and breast cancer risk were increased in the WHI, although the risk of colorectal cancer and fracture was decreased. Because research suggests that it is the combination of estrogen plus progestin, and not estrogen alone, that increases breast cancer risk, soy seems unlikely to increase risk because it has no progestin activity. Similarly, there is no evidence to suggest that soy will increase venous thromboembolic disease or stroke; however, only limited data are available in this area. There are promising data suggesting that soy may decrease CHD risk, although studies conducted thus far have examined only markers of risk and not actual CHD events. Similarly, short-term studies generally suggest that soy reduces bone loss in postmenopausal women; however, such effects have been noted primarily only at the spine, and longer-term studies are needed. Finally, very limited human research suggests that soy may decrease colon cancer risk, but this is highly speculative. The results of HERS I/II and WHI suggest that soy may have some of the advantages, but not the disadvantages, of combined hormone replacement therapy (at least with respect to the specific hormones and doses used in the HERS I/II and WHI), but that large, long-term intervention studies examining disease outcome are needed before definitive conclusions can be drawn. Nevertheless, the evidence warrants recommendations that menopausal women include soy in their diets (Messina, 2002).

Isoflavones, in the presence of too much estrogen, compete for receptor sites and minimize the negative effects of excessive estrogens. In addition isoflavones have many other non-estrogen health effects that scientists are just beginning to uncover.

Body fat accumulation and bone loss are both often associated with estrogen deficiency following menopause. In this study, we examined whether soy isoflavone, one of the phytoestrogens, and moderate exercise interventions exhibit cooperative effects on body composition and bone mass in ovariectomized (OVX) mice. Eight-week-old female mice were assigned to 6 groups: (1) sham-operated (sham); (2) OVX; (3) OVX with received a soy isoflavone diet (OVX+ISO); (4) OVX with exercised on a treadmill (OVX+EX); (5) OVX with given both isoflavone and exercise (OVX+ISOandEX ); and (6) OVX with treated with 17 beta-estradiol subcutaneously (OVX+E2). The combined intervention of soybean isoflavone and exercise prevented body fat accumulation in the whole body with an increase in lean
body mass and restoration of bone mass, and reduced high serum cholesterol in OVX mice (Wu et al., 2004).

The study assessed the dose-dependent effects of daily soybean isoflavone (IF) consumption in reversing bone loss in adult ovariectomized rats. On d 0, female Wistar rats (7 mo old; n = 55) were either sham-operated (SH; n = 14) or ovariectomized (n = 41). On d 80, intermediate rats (SH: n = 5; ovariectomized: n = 5) were killed to confirm the ovariectomy-induced bone loss. The remaining ovariectomized rats were randomly assigned to one of four groups of nine rats each and fed soybean IF (mixed with a soy protein-free semipurified diet) at 0 (OVX), 20 (IF20), 40 (IF40) or 80 (IF80) mg/(kg body, d) for 84 d. Simultaneously, SH rats were fed the semipurified diet without any additional compound and killed on d 164, as were the other rats. As expected, both bone mineral density in the total femur and in its diaphyseal and metaphyseal subregions and cancellous bone area/measured surface in the distal femur metaphysis were lower in OVX than in SH rats (P < 0.05). Results indicate that in adult ovariectomized rats, daily soybean IF consumption decreased bone turnover but did not reverse established osteopenia (Picherit et al., 2001).

A double-blind, 15-month pilot study was designed to investigate the effect of soy protein isolate with varying concentrations of isoflavones on early postmenopausal bone loss and lipids. A total of 65 women, with a mean age of 55 years and 7.5 years since menopause, were randomized to one of three groups; soy protein with 96 mg isoflavones/day, soy with 52 mg isoflavones/day, or soy without isoflavones (< 4 mg isoflavones/day). Soy was given for 9 months and then discontinued; participants were followed for an additional 6 months. There was no significant effect of the soy supplements on BMD of the spine or femoral neck in any of the three groups. There was no significant effect of soy on lipid metabolism at the end of the intervention (Gallagher et al., 2004).

To study reported were the effect of soy extract on energy metabolism in ovariectomized rats, 90 Wistar rats were randomly divided into 8 groups: control group, sham group, model group, estrogen group, soy isoflavone group of high dose, soy isoflavone of low dose, soy extract of high dose, soy extract of low dose, 10 rats each group. Beside of control and sham groups, the rest rats were ovariectomized. One week after operation, the rats were treated with different drugs, measurement of body weight and feed weight each week. Six week after operation, the rats were killed, serum was taken, abdomen lipid was removed and weighed. Ovariectomized
rats have imbalance of energy metabolism showed weight gain and accumulation of abdominal lipid; administration of estrogen, soy extracts or soy isoflavone could attenuate these changes induced by ovariectomizing (Wang et al, 2002).

The effect of jump exercise on middle-aged osteopenic rats was investigated. Forty-two 9-mo-old female rats were either sham-operated (Sham) or ovariectomized (OVX). Three months after surgery, the rats were divided into the following groups: Sham sedentary, Sham exercised, OVX sedentary, and OVX exercised. Rats in the exercise groups jumped 10 times/day, 5 days/wk, for 8 wk, with a jumping height of 40 cm. Less than 1 min was required for the jump training. Data suggests that jump exercise has beneficial effects on lower limb bone mass, strength, bone mineral density, and morphometry in middle-aged osteopenic rats, as well as in Sham rats (Honda et al, 2003).

Pomegranate is known to contain estrogens (estradiol, estrone, and estriol) and show estrogenic activities in mice. In this study, the researchers investigated whether pomegranate extract was effective on experimental menopausal syndrome in ovariectomized mice. Prolongation of the immobility time in forced swimming test, an index of depression, was measured 14 days after ovariectomy. The bone mineral density (BMD) of the tibia was measured by X-ray absorptiometry and the structure and metabolism of bone were also analyzed by bone histomorphometry. Administration of pomegranate extract (juice and seed extract) for 2 weeks to ovariectomized mice prevented the loss of uterus weight and shortened the immobility time compared with 5% glucose-dosed mice (control). In addition, ovariectomy-induced decrease of BMD was normalized by administration of the pomegranate extract. The bone volume and the trabecular number were significantly increased and the trabecular separation was decreased in the pomegranate-dosed group compared with the control group. Some histological bone formation/resorption parameters were significantly increased by ovariectomy but were normalized by administration of the pomegranate extract. These changes suggest that the pomegranate extract inhibits ovariectomy-stimulated bone turnover. It is thus conceivable that pomegranate is clinically effective on a depressive state and bone loss in menopausal syndrome in women (Mori-Okamoto et al, 2004).

There are many different components in soybeans that are being studied for their possible health effects. These components and their proposed health benefits are reviewed in the following chart:
### Substances found in soy foods

<table>
<thead>
<tr>
<th>Soybean components</th>
<th>Proposed health benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy protein</td>
<td>May lower blood cholesterol levels when consumed in place of animal proteins, May have a modest effect in preventing bone loss (positive effect on calcium absorption from the gut)</td>
</tr>
<tr>
<td>Essential fatty acids</td>
<td>An essential nutrient for human health, may have cardioprotective effects such as lowering blood triglyceride levels, decrease the risk of arrhythmia or sudden death cardiac</td>
</tr>
<tr>
<td>Oligosaccharides (short chains of simple carbohydrates)</td>
<td>Nondigestible substances that promote the growth of healthful bacteria in our colon helping to improve gastrointestinal health, Possibly reduce blood pressure and blood cholesterol levels</td>
</tr>
<tr>
<td>Minerals</td>
<td>May help to maintain bone density and protect bone health</td>
</tr>
<tr>
<td>Vitamins (soybeans contain vitamins such as Vitamin E and the B Vitamins – thiamin, riboflavin, niacin and folacin)</td>
<td>As an antioxidant may prevent oxidative damage to body cells, May improve immune function and perhaps lower risk of infection and cancer</td>
</tr>
<tr>
<td>Fiber</td>
<td>Nondigestible plant component that may help to lower blood cholesterol levels, May promote the growth of healthful bacteria in our colon helping to improve gastrointestinal health</td>
</tr>
<tr>
<td>Phytosterols (fat–like substances found in plants)</td>
<td>May help lower blood cholesterol levels, by inhibiting cholesterol absorption</td>
</tr>
<tr>
<td>Isoflavones (Plant estrogens or phytoestrogens that have a chemical structure very similar to estrogen, such as daidzein, genistein, and glycitein).</td>
<td>Reduce blood cholesterol levels, May increases vasodilation, May reduce menopausal symptoms and act as selective estrogen receptor modulators (SERM), May protect bone health after menopause, May improve immune function, May have anticancer activity</td>
</tr>
<tr>
<td>Saponins (A type of steroid or triterpene glycosides widely found in plant and marine animals. Saponins are characterized by their ability to “foam” upon shaking in water.)</td>
<td>May inhibit cholesterol absorption and therefore help to lower cholesterol, May have an anticancer activity and antiinflammatory activity.</td>
</tr>
<tr>
<td>Lecithin (A type of fat or phospholipid, lecithin is a byproduct produced when soy is processed. It is used in foods as an emulsifying agent to keep fat from separating.)</td>
<td>May help to reduce heart disease risk, May act as an antioxidant and also may help the immune system</td>
</tr>
<tr>
<td>Phytates (Also known as phytic acid, binds minerals helping to prevent free radicals from forming)</td>
<td>May help control blood sugar, cholesterol, and triglycerides, May reduce cancer risk</td>
</tr>
</tbody>
</table>
In this background the present study was planned to assess the effect of varied dietary calcium levels on the tissue mineral status of animals (albino rats) as well as human subjects.

**Objectives of the Study:**

The present investigation focuses upon nutritional status with respect to calcium in the animal model and in human beings.

The objectives of the study may be expressed as follows:-

- To study the effect of feeding low, medium and high levels of calcium on a short-term and a long-term basis on the growth and metabolic aspects of weanling animals and also the reproductive performance of these animals. Further comparison of body calcium status of first generation and second generation animals after long term feeding will also be studied.

- To study the bone mineral status of pre and post menopausal women using the radiographic (x-ray) technique of the hand and the pelvis will be conducted and then assessed by two different methods namely the manual method and the software method and compared with control subjects aged 20-30 years belonging to middle SES, residing in Anand and Vallabh Vidyanagar. Further the effect of factors such as age, literacy, family size, general dietary pattern, lifestyle and exercise on the bone mineral status of pre and post menopausal women and control subjects will also be assessed.

- To assess the effect of different processing techniques such as soaking, cooking drying, dehulling, fermentation, germination etc. on the calcium, phosphorus, oxalic acid and phytic acid content from selected foodstuff.

- To develop soya based food products as a measure of increasing available calcium in the daily diet and to assess the effect of the most acceptable food product developed on the calcium status of young women.