SUMMARY AND CONCLUSION
Chapter 4

Summary and Conclusion

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

The objectives of the study may be expressed as follows:

(i) 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, blood calcium and bone calcium levels of albino rats.

(ii) To study the bone mineral status of pre and post menopausal women using the radiographic (x-ray) technique of the hand and the pelvis and to compare this with control subjects aged 20-30 years belonging to middle SES and residing in Anand and Vallabh Vidyanagar. Further the effect of factors such as age, literacy, family size, general dietary pattern, lifestyle and exercise was observed on the bone mineral status of pre and post menopausal women and control subjects.

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

(iv) To develop soy 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.

Part I

Two animal experiments in three phases were planned to study the effect of varying dietary calcium levels on the growth and metabolic aspects of weanling animals.

Experiment I

Phase I: In the first experiment the effects of varying levels of dietary calcium on the growth of weanling animals was studied after a four week feeding period.

Phase II: Further on a long term basis the reproductive performance of these animals as well as blood and bone calcium levels were studied in the second experiment after experimental feeding for twenty four weeks.
Experiment II

The progeny obtained in the second phase was continued on the same diet and the reproductive performance as well as blood and bone calcium levels were studied after a feeding trial of twenty four weeks.

Further, comparison of body calcium status of the first and second generation animals were also studied, after long term feeding.

Part II

(iii) The bone mineral status of pre and postmenopausal women, x-ray studies of the hand and pelvis were conducted. They were 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 and residing in Anand and Vallabh Vidyanagar.

(iv) The effect of influencing factors such as age, literacy, family size, general dietary pattern, lifestyle and exercise status was studied on the bone mineral status for pre and post menopausal women and control subjects.

Part III

The effect of different processing techniques such as soaking followed by drying / fermentation / germination etc. was studied on the calcium, phosphorus, oxalic acid and phytic acid content from selected foodstuff.

Part IV

Processed soy flour was used for food product development in order to increase available calcium in the daily diet. The effect of the most acceptable food product developed was studied on the urinary calcium status of young women.

In the first part of the experiment weanling albino rats were fed varying levels of calcium for four weeks to study the effect on growth and development. After four weeks of feeding different dietary levels of calcium no significant differences were observed for final body weights. As initial weights were higher the low calcium group showed higher body weight throughout the study period. Feeding low calcium produced lower weekly weight gain whereas feeding normal or high calcium produced similar weight gain which was higher than the low calcium group.

Animals were bred twice to observe the effect of different dietary calcium levels on the reproductive performance and tissue calcium status. During the first reproductive cycle at the end of 12 weeks, the level of dietary calcium did not influence conception or the delivery of pups to a great extent. But when the animals
were kept for breeding for the second time at the end of 20 weeks the groups fed low
calcium and high calcium were not able to conceive indicating that both a deficiency
and an excess of calcium interfered with the reproductive process.

Animals were sacrificed at the end of 24 weeks and tissue was analysed. Liver
weight, liver: body weight ratio and liver moisture did not show any significant
differences on feeding different levels of dietary calcium. Femoral weight was higher
in the normal calcium group while femoral length did not show any major differences.
Bone moisture levels for both tibia and femur showed no significant differences due
to feeding different levels of dietary calcium. Bone fat levels were higher in the low
calcium fed groups and lower in the normal calcium fed group. The high calcium
group showed values in between these two groups, although no significant difference
were observed between any of the groups.

In the present study, alkaline phosphatase levels in the serum varied from
23.34 to 26.40 KA. Alkaline phosphatase activity was slightly higher in the low
calcium group when compared to the normal calcium and high calcium groups but
there were no significant differences between any of the groups. Serum calcium level
varied from 9.70 to 10.15 mg%. There was not much variation between the three
groups and no significant differences were observed.

Bone analysis indicated that the least bone calcium level was observed in the
low calcium fed group and the highest level was observed in the high calcium fed
group. The two groups fed low and normal calcium showed significantly (p < 0.05)
lower values compared to the bone calcium level of the high calcium group. Bone
phosphorus level varied from 10.23 to 11.44 g%. Both the low calcium and the
normal calcium fed groups showed lower bone phosphorus levels ranging from 10.23
to 10.28 g% whereas the high calcium fed group showed significantly higher values
compared to the other two groups. The group fed a high calcium diet showed higher
bone hydroxyproline levels.

The progeny (2nd generation animals) were taken for the second experiment of
the study. They were continued on the experimental diets for 24 weeks and bred twice
during this period.

At the end of 24 weeks final body weight ranged from 160.75 to 178.25 g.
Body weight was lowest in the low calcium fed group which was significantly
different from the normal and high calcium fed groups both of which showed values
closer to each other i.e. 177.00 to 178.25 g. In the second generation animals fed low
calcium, growth of the animals was affected whereas in the normal and high calcium
groups, the animals were not affected by the two different levels of calcium feeding.

None of the females of the experimental groups were able to conceive during
the first breeding cycle whereas in the second breeding cycle only one female from
the high calcium group was able to conceive and deliver pups (7 pups in number).

At the end of 24 weeks of feeding the animals were sacrificed. Tissue and
bone parameters related to calcium were estimated. Only the high calcium fed group
showed a value within the normal range for liver: body weight ratio. Both the low
calcium and normal calcium groups showed higher values beyond the normal range
and among the two groups, the low calcium fed group showed the highest value.
Feeding high dietary calcium levels led to lowering of the liver moisture level. Bone
weight was lower in the normal calcium group and higher in the high and low calcium
groups. Again in the second generation animals, bone length was lower in the normal
calcium group and slightly higher in the low and high calcium groups. Femoral
moisture ranged from 21.11 to 38.46 g%. Moisture content was significantly higher in
the normal calcium group and lower in the low and high calcium groups. Bone fat
content was lower in the high calcium group whereas the low calcium and normal
calcium groups showed higher and nearly similar values.

Alkaline phosphatase values ranged from 10.19 to 20.52 KA. Enzyme activity
was significantly higher in the low calcium group compared to the normal and high
calcium groups. Serum calcium values ranged from 5.71 to 8.85 mg%. Values were
significantly different between groups and was highest in the normal calcium group.

Bone analysis indicated that feeding normal calcium levels in the diet led to
maximum mineral deposition as seen from the present data. Feeding low calcium in
the diet led to minimum mineral deposition as seen from the ash content.

Bone calcium levels ranged from 21.44 to 24.38 g%. Calcium deposition was
least in the low calcium group as expected and higher in the normal and high calcium
groups. Although the normal calcium group showed the highest deposition of
calcium, no significant differences were observed between the normal and the high
calcium groups. Both groups showed significant differences when compared to the
low calcium group. The data on both these groups indicates that although these are 2nd
generation animals, altering the dietary calcium levels did not seem to alter the
calcium deposition in the bone. Feeding normal calcium seemed to show higher
phosphorus deposition. Data also indicates that feeding normal calcium levels led to the highest hydroxyproline values.

Comparing the bone calcium levels in the 1st and 2nd generation animals, in the 1st generation animals a clear difference was observed between the low calcium, normal calcium and high calcium fed groups. In the 2nd generation animals the low calcium and normal calcium groups showed values similar to their respective 1st generation animals but in the high calcium fed group calcium values in the bone decreased in the 2nd generation animals.

The data from x-ray studies conducted on 240 subjects namely 40 controls and 200 pre and post menopausal women based on the palm and pelvic x-rays, indicates that majority of the subjects in the 21 to 50 year age group fell in the normal category. About 5% of the subjects in the 21 to 30 year age group were osteoporotic whereas no osteoporosis was found in the 31 to 50 year age group. In the 51 to 60 plus age group only 50% of the subjects were normal, of the remaining, majority (37.5%) were osteopenic whereas about 12.5% were osteoporotic. The data also indicates that osteopenia is prevalent in all the age groups studied, the prevalence ranging from 4 to 17% in the 21 to 50 year age group and 31.5% in the 51 to 60 plus age group.

Data on anthropometric measurements indicated higher BMI as age increased. Highest values were observed in the 51 to 60 plus age group.

Looking at the data on x-ray studies of the palm measured by the manual method, bone length, bone width and marrow width did not show any major differences. Cortical thickness and metacarpal index showed significant differences between the normal, osteopenic and osteoporotic groups for all the age groups studied. When the same parameters were analysed by the software method a similar trend was observed for cortical thickness and metacarpal index. Data also indicated that cortical thickness and metacarpal index when measured by both methods showed no variation in values for the younger age groups but between 51 to 60 plus years both cortical thickness and metacarpal index showed a decrease.

BMD values ranged from 0.56 to 0.58 in the normal subjects, from 0.47 to 0.49 in the osteopenic subjects and from 0.39 to 0.30 g / cm² in the osteoporotic subjects, values were as expected significantly higher in the normal subjects compared to the osteopenic and osteoporotic subjects. Again the osteoporotic subjects showed the lowest BMD which was significantly different from the osteopenic subjects. T score and Z score values were significantly higher in the osteoporotic
groups compared to the normal and osteopenic groups. The osteopenic group also showed significantly higher standard deviation compared to the normal subjects.

Looking at several influencing factors which affect the BMD it was seen that none of the osteopenic and osteoporotic subjects belonged to the heavy worker category. Again none of the osteoporotic subjects were brisk walkers or yoga performers. Most of them were either non-exercisers or slow walkers.

The data on dietary habits indicated that among the normal subjects, those who consumed 300 ml milk per day showed higher BMD than those consuming lesser quantities of milk. Data on tea drinking indicated that among the two older age groups when tea drinking increased from 2 cups / day to 3 cups / day, BMD values decreased for the osteopenic subjects. Similarly for the osteoporotic subjects, subjects consuming tea whether 1 cup or 2 cups per day showed lower BMD compared to subjects who did not consume tea or coffee.

Looking at the data on the number of children, in the normal subjects, it was observed that for the age groups 31 to 40, 41 to 50 and 51 to 60 plus years, as the number of children increased from 2 onwards the BMD value showed a decrease.

Qualitative grading of subjects on the basis of pelvic x-rays indicated that majority of subjects fell in the -0.5 category (50 – 71.82%). Very few subjects belonged to the +1.0 category.

In the next part of the study change in the mineral and antinutrient content was assessed for selected foodstuff given various treatments. For cereals (wheat, bajra, maize and rice flakes) calcium values increased during germination and fermentation. Germination produced higher increases for bajra and maize. Among all the four pulses studied (whole bengalgram, greengram dhal, redgram dhal and soybean) whole bengalgram and soybean showed a decrease after fermentation while greengram dhal and redgram dhal showed an increase for calcium levels. A three fold increase in calcium was observed in soybean after germination. Phosphorus content decreased after fermentation for all the cereals and pulses studied.

Oxalic acid content did not show any differences after fermentation for wheat, bajra, rice flakes or redgram dhal. Soybean on giving different processing treatments showed a significant lowering in the oxalic acid content. Lowest value was seen in the soaked, dehulled, cooked and dried sample. Phytic acid content showed a decrease with increasing hours of fermentation for the two cereals i.e. wheat and rice flakes and the three pulses i.e. bengalgram, greengram dhal and redgram dhal. Soybean given
different processing treatments and then fermented showed the least phytic acid content for the soaked, dehulled, cooked and dried samples.

For the three different nuts and oilseeds studied namely cashewnut, almond and gingelly seeds, highest calcium content was seen in gingelly seeds and for the three spices studied namely fenugreek seeds, cumin seeds and coriander seeds, cumin seeds shows the higher value. Phosphorus values ranged between 344 to 393 mg% for all the nuts and oilseeds as well as spices mentioned above except for cumin seeds which showed a very high value for phosphorus content (449.30 mg%). Oxalic acid content was highest in gingelly seeds among the three nuts and oilseeds studied and ranged from 4.50 to 13.72 mg % for the three spices studied.

The procedure for preparation of various soy based products were standardized and evaluated through sensory scores. From the different preparations three products were found to be higher in acceptability compared to the others.

These three different soy fortified products namely Chapati (levels of supplementation: 0, 25, 30, 35 and 40%), Sev (0, 25, 30, 35 and 40%) and Muthiya (0, 20, 30 and 40%) were prepared and evaluated through two sensory evaluation tests namely numerical scoring test and hedonic rating test.

Scores for overall acceptability of chapati incorporating different levels of processed soy flour ranged from 5.89 to 8.11. The lowest score was observed for the highest level of incorporation. For muthiya the scores ranged from 6.22 to 6.56 and for sev it ranged from 5.61 to 8.61.

Hedonic scores for the three products showed higher values for muthiya at 30 % and 40 % level of incorporation compared to chapati and sev. Although muthiya showed higher scores, but based on the ease of preparation and the increased shelf life of sev and also because no major variation were seen in the scores for all the three products, sev was selected for the human feeding trial.

Thus soy fortified sev was fed to 10 young females aged 20-22 years and urinary calcium level was estimated at the beginning and at the end of a feeding trial of 21 days. The data shows a decrease in urinary calcium excretion at the end of the experimental feeding period indicating that increased calcium was available during this period, which was retained. Individual excretion also indicates that about 5 subjects showed a decrease in calcium excretion due to experimental feeding, the decrease ranging from 60.55 to 470.92 mg/g of creatinine. This indicates that feeding processed soy flour supplemented sev led to increased calcium retention in the body.
The data indicates that feeding experimental sev led to a decrease in urinary calcium excretion thus it promoted calcium retention in the body.

Summarising, the study indicates that feeding normal calcium in the diet promoted a normal and healthy picture for all the nutritional parameters studied in the animals. The prevalence study on adult women indicated that 2.97% of the total subjects were osteoporotic while 17.5% were osteopenic. About 80% of the population showed normal bone mineral density. Various processing treatments given to soy flour indicated maximum decrease in the oxalic acid and phytic acid content in the soaked, dehulled, cooked and dried soy flour. Feeding 25% processed soy flour incorporated sev to adult young females aged 20 to 22 years indicated a decrease in urinary calcium excretion indicating increased retention.