THE EFFECT OF COTTONSEED PROTEIN ON THE CALCIUM AND PHOSPHORUS STATUS AND BONE METABOLISM OF CHILDREN

A DISSERTATION

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CHAPTER I

INTRODUCTION

The primary purpose of this research investigation was to analyze the quality of growth in children when fed diets which included liquid cyclone process (LCP) cottonseed. Protein intake of 1 g/kg body weight was designed to allow approximately 30 percent from this source. Maintenance of normal weight and height gains were presumed to be basic indications of a satisfactory balance of amino acid content. Since cottonseed has one of the highest levels of phytin (70) of any of the seed products, it is imperative to look at the interrelationship of calcium, phosphorus, and protein for optimum absorption of each. For more complete analysis of growth, the following items were evaluated:

a. Serum levels of calcium, phosphorus, and protein. Hypoproteinemia may accompany low calcium serum values (3) (56). This would be the first point to study when vegetable protein is being used. Since cottonseed is high in phytin, which tends to bind calcium to render it unavailable, such an investigation would be basic.

b. Hydroxyproline. High amounts of urinary hydroxyproline indicate potential or present growth spurts (8) (35). Markedly lowered excretion of hydroxyproline may indicate malnutrition (75).

c. Skeletal age. Similar skeletal age development would substantiate optimum utilization of calcium, phosphorus, and protein (27).

d. Bone density. Bone mass would show normal mineral deposits indicating not only utilization of each of these, but adequate amounts for storage beyond growth (22).

This research would provide a more precise evaluation of the extent of absorption of calcium, phosphorus, and protein to determine the quality of 30 percent substitution of total protein with liquid cyclone process cottonseed protein.

CHAPTER II

REVIEW OF LITERATURE

The Effect of Cottonseed Protein on Calcium and Phosphorus Absorption in Children 6-18 Years of Age

Prior to the mid-1960's, there were a number of isolated efforts to utilize oilseed proteins in human foods (60) (62). Limited studies with infants have shown cottonseed protein to be satisfactory for growth (26); yet, conflicting research also has been observed. Cottonseed flour, when used as the main source of protein, was ineffective in reversing the acute manifestations of kwashiorkor in Indian studies (69). The cottonseed used contained one percent gossypol, the pigment of glanded cottonseed.

Close correlation exists between protein and calcium serum levels. A depressed concentration of total calcium can be due to hypoproteinemia (3) (56). Therefore, it is important to analyze the absorption of calcium with cottonseed protein intake.

Ionic calcium and phosphorus tend to maintain an equilibrium in the blood. Changes in the calcium ion level often are reflected reciprocally in the phosphorus

level. Therefore, when low serum calcium levels occur they may be accompanied by elevations in the phosphorus levels, or vice versa. Calcium, phosphorus, and protein must be evaluated simultaneously for validity of findings (3).

Goldsmith (24) pointed out that normal serum ranges for calcium are accepted without question too often. This especially may be true of children because so many changes happen and often very quickly.

The level of calcium in plasma is remarkably controlled by means of homeostatic mechanisms. Increases due to intake or decreases due to ossification are quickly adjusted to maintain a constant level of calcium ions in the body fluids. Such ions may stay in the plasma for only a matter of minutes. Hormones from the parathyroid and phosphorus plasma levels are two of the main parts of this mechanism (74).

Great variation of calcium intake has been found among healthy children (4) (7) (77). Adolescent boys' intakes of calcium tend to be higher and to approach the suggested requirements more closely than the intakes of girls. Boys' daily intakes tend to increase from 12 to 17 years of age whereas girls' intakes tend to decline (7) (15) (29) (77).

Herter (32) was one of the first to establish calcium requirements of the school age child at 1 g/day. Similar recommendations have been made by Sherman and Hawley (68).

After Wang et al. (73) compiled data, they suggested that the minimum requirement of an eight-year-old child weighing 20 kg would be 0.64 g CaO (0.457 g calcium) per day supplied by a mixed diet. Petrunkina (56) studied children seven to eight years old. A mixed diet with intakes of calcium between 30 and 40 mg/kg was found to be adequate.

Other calcium requirements have been estimated for children 6 to 12 years old as follows: 0.850 increasing to 1.000 g/day (41); 0.690 increasing to 1.450 g/day for boys and 0.710 increasing to 1.530 g/day for girls. Mitchell and Curzon (54) calculated that the dietary calcium requirement should increase from about 1 g/day at 9 years of age to 2.6 g/day at 13 years for girls and 2.4 g/day at 15 years for boys. Then the requirement would decrease to about 1 g/day at 20 years.

There are also wide differences among children in their ability to utilize calcium (54). Rate of growth as well as calcium intake apparently affects retention especially during the period of steady growth from 4 to 11 years of age. If the intake is ample, the child will

store calcium most heavily during the year or so which precedes the period of rapid growth in height (71). Johnston (37) observed in studies with six girls at puberty that sexual maturation was accompanied by reduced calcium and nitrogen retention.

Children four to eight years old became more alike in their response to their selective retention of food constituents and therefore in their daily retentions of this element, irrespective of body size, when observed by Macy (43).

Retention for growth refers to calcium in addition to any requirement for maintenance. As dietary calcium increased, the efficiency of absorption decreased (41). From infancy to adulthood calcium absorption decreases according to data summarized by Harrison (30). Whereas up to 75 percent of the ingested calcium diet is absorbed in children during periods of skeletal growth, only 30 to 50 percent of the dietary intake may be absorbed in adults (2).

The relationship between intestinal absorption of calcium and phosphorus is not clearly delineated. If the diet contains sufficient calcium, large variations in dietary phosphorus may not influence intestinal calcium absorption (33).

Conflicting research in regard to the effect of the calcium to phosphorus ratio on calcium absorption exists. In the diets in the United States, the intake of phosphorus generally equals or exceeds the intake of calcium (48). In 1960, the Ca:P ratio in the American diet was about 1:2.8. Later it was about 1:4, a striking imbalance. Excess dietary phosphorus or a low Ca:P ratio (less than 2:1) results in a pronounced loss of bone in animals and in man (41).

With diets adequate in both Ca and P, retention of P was not as constant as that of Ca. In the high-P diets the elements were stored in the approximate ratio of 1:1, and in the medium-P diets in the ratio of 2:1. When Ca was adequate but P inadequate, utilization of absorbed Ca was decreased. When there was insufficient Ca but ample P, there was increased retention of absorbed Ca. Such changes in the efficiency of utilization of Ca reflect the need for simultaneous storage of the two elements in the skeleton (76).

About half of the children, aged one-six years, showed adequate growth with a Ca:P ratio averaging 0.77. Thirty percent of the children did not. It was evident that normal maturation of bone may proceed on intakes of calcium and phosphorus of less than 1 gm. Calcium may

be adequate at 0.2 to 0.7 g in some cases but not in others (1).

In general, 1 g per day of calcium with a calcium: phosphorus ratio of 1:1.5 g appears satisfactory (53). Calcium retention is much more closely related to calcium intake than to the Ca/P ratio. Since phosphorus is found in many sources and is easily absorbed, it is not as influential as the amount of calcium. The major problem in the absorption of calcium and phosphorus actually is a problem of calcium absorption alone, for if this is absorbed both are absorbed (22).

One of the primary reasons for this research was to analyze the absorption of calcium and phosphorus with higher phytin intakes. Provision of adequate amounts of calcium was considered as a precaution in total diet balance. Phytin is the principal storage form of phosphorus and inositol in all seeds (19). Not only is part of their phosphorus content nonassimilable; but phytin binds calcium, iron, and zinc and thus interferes with their absorption.

Cottonseed kernels are high in total phosphorus being exceeded only by sesame seed and rice bran. They are at least equivalent to or slightly higher in phosphatide phosphorus than soybeans and are much

higher than the other common oil-seeds and grains in this type of phosphorus compounds. Carbohydrate ester type phosphorus is highest in cottonseed kernels. Inorganic phosphorus is among the lowest, being in the same range as sesame seed and peanut kernels for this type of phosphorus (70).

From available sources in the literature it would appear that the total phosphorus content of the kernel ranges from about 0.8 to 1.0 percent. About 75 percent of this phosphorus has been reported to be phytin phosphorus (70). Phytin P as percent total P is 46.4 percent in whole wheat, 66.0 in rolled oats, and 57.5 percent in peanuts (49). Distribution of phytin in cottonseed kernels has been noted from 6.2 to 10.7 mg/g of moisture-free material (58). So a wide range has been reported.

The importance of a high dietary phytic-acid intake on intestinal calcium absorption in both human and animal nutrition is controversial. Many cereals contain a phytase which destroys phytic acid during the leavening process of bread making. The intestinal tract of many species contains a phytate-splitting enzyme, probably a non-specific alkaline phosphatase, the activity of which depends on cholecalciferol status (79). McCance and

Widdowson (50) observed a marked decrease in calcium absorption accompanying the addition of sodium phytate to the diet.

When breakfasts containing oatmeal also had low calcium intake, calcium absorption decreased. However, the absolute amount of calcium rendered unavailable was very small. When a "moderate-calcium" breakfast was used, no significant difference in calcium utilization was observed between oatmeal (phytate) and farina (no phytate) (6) (9). Cruickshank et al. (9) found the rate of calcium improved when calcium was added to diets based on oatmeal.

The major initial effect of increased dietary phosphorus was to cause a decrease in the intestinal absorption (67). However, 10 year old boys adapted to diets with high phytin content after a 15-day period. Calcium balances that had been negative in the first five days became positive even though they were lower than before the addition of phytin to the diets. It was assumed that absorption would have continued to improve with more time (33). Other investigators (10) (72) also observed that over a period of time, subjects adapted to the high phytin diets.

Two facets of the serum phosphorus concentrations have led to confusion. First, serum phosphorus varies as a function of age and is substantially increased in children as compared to adults. High plasma phosphorus from childhood starts to fall at seven years to the normal adult levels which are reached in girls by the age of 15 and in boys by the age of 17. Normal adult serum phosphorus in that laboratory has a mean of 3.6 mg/100 ml with a range (mean $\pm$ 2 S.D.) of 2.6 - 4.6 mg/100 ml (61). Garn (22) recommended 5.0 mg/100 ml for serum phosphorus in children.

Second, the serum phosphorus displays a substantial circadian variation, in contrast to serum calcium, which varies less during the day. So serum phosphorus can be interpreted only with reference to the normal at each time of the day, usually early morning and fasting (24).

The Effect of Cottonseed Protein on Bone Metabolism

Cottonseed protein concentrate has been considered as a source of protein to alleviate problems associated with malnutrition (45). Infants recover their weight loss, but not their expected height according to Graham (26) in South America. The purpose of this research study was to examine bone mass and growth of children

when liquid cyclone process (LCP) cottonseed product was substituted for approximately 30 percent of the total protein intake. Several investigators have found that the amount of hydroxyproline (HYP) excreted in the urine is correlated with growth rates (8) (35). It may be used for detecting growth failure before changes in body weight occur or as an index of anticipated or existing growth spurts (75).

Urinary excretion of HYP reflects collagen turnover, primarily bone resorption. When collagen is broken down during bone resorption, the HYP containing fragments are excreted in the urine (24).

When 24-hour samples of urinary excretion of hydroxyproline are not available, the total hydroxyproline: creatinine (THY/CR) ratio is used for single urine samples. Whitehead, in 1965, was the first person to use the THP/CR ratio extensively. It was developed particularly as a screening test for marginal malnutrition (75). Markedly lowered excretion of HYP has been noted in the acutely malnourished state. The lowest HYP excretion in children has been found with the greatest deficit in body-weight for height. During recovery periods the HYP index was shown to be closely correlated with the index of thriving based on somatic measurements (51).

A low excretion rate of HYP implies that adolescent growth either has not started or has finished. A high rate implies growth velocity is high and that growth will thereafter continue, though it may not do so at the same rapid rate (81).

The standard deviations for HYP excretion values are quite large during the peak years of adolescence as might be expected due to variations in growth spurts. Standard deviations may be as high as 48.0 mg/24 hr for boys and 30.2 mg/24 hr for girls. The standard deviations increase to a maximum at 14 years in boys and 12 years in girls (80). In adolescent boys, the average level of HYP excretion was significantly higher than in younger or older children of either sex and also than in adolescent girls (56).

The excretion of HYP is probably most useful as an index of growth rate particularly in making comparisons between children studied over the same period of time or in sequential studies of the same child over a short period (78). This has the particular advantage of reflecting growth rate at one point in time whereas anthropometry can show only the end-point which has been reached so that calculations of anthropometric growth rates are necessarily in retrospect.

Skeletal maturation is the best indicator of the total individual physical development and growth status, since it is affected by more extreme variations in nutrition and health. It may be regarded as outside the normal range if the skeletal age differs from the chronological age by more than one year (14).

In malnourished populations where childhood growth is slowed by 20-30 percent, the appearance of ossification centers may be equally delayed 20-30 percent (23). The greater disparity among ossification centers, the more insults the child has suffered (14).

Tunisian boys from poorer classes seemed to be retarded in somatic development as they had many low anthropometric measures (57).

It has been indicated that boys' skeletal maturation is more susceptible to vicissitudes than girls'. Since boys are delayed compared to girls in skeletal maturity, perhaps the timing of the illness in relation to the state of skeletal maturity is important (16). Ossification processes occur earlier in girls than in boys (5). However, skeletal maturation in Central America is equally delayed and to a considerable degree in boys and girls alike. These findings are in apparent contradiction

to the belief that girls are much less affected by unfavorable circumstances (20).

As a rule, the period of most intense growth may be expected between ossification of the sesamoid and the onset of the capping stage (31). Except for the mesophalanx of the fifth digit, the maturities of the phalanges and metacarpals showed a greater tendency to cluster around skeletal age than did the carpals and distal radius and ulna. The selective specificity of middle phalanx V to disturbances in ossification has been noted repeatedly in health and disease by shortening and malformation.

The difference in the ratio of cortical area/weight between boys and girls merits comment since by the age of 18 the ratio is about 20 percent lower in girls than in boys. It has been suggested that the best natural protection against the sequelae of bone loss in later life is the development of a large skeletal mass during childhood (28).

In maturation studies of 740 children, the triquetrum was the most advanced center in both boys and girls. Usually in boys the least advanced center was the lunate, and in girls the middle phalanx (12).

Retardation in skeletal maturation during childhood is not overcome by the adolescent growth spurt. Prolongation of the period of growth does not compensate for the slow rate of growth. It is postulated that the small stature in South America is related to the marked childhood retardation and to the fact that during adolescence, the timing of skeletal maturation is less affected that growth in size (21). It is questionable whether inferior height of children or adults otherwise healthy is essentially a disadvantage.

Deviation from normal skeletal maturation may occur, yet be accompanied by above average bone density values. In such cases, the Ca intake must be considered sufficient for present skeletal needs, but not necessarily for normal needs (78).

A single X-ray film of a child's hand makes it possible to distinguish the poorly from the adequately mineralized skeleton, thus providing an important supplement to the clinical evaluation of nutritional status. Bone density values indicate cumulative nutritional status, whereas nutrient intake data reflect present dietary trends (53). Early investigations of the process of bone mineralization showed that there was a direct relationship between the mineral content of plasma

and extracellular fluids and their ability to induce calcification (60).

Calcium is one of the most important nutrients in regard to bone density according to Garn (22). A diet differing only in calcium content resulted in retarded growth and lower bone density (52). When calcium intake is increased to the recommended allowances, bone density appears to increase accordingly (53). On the other hand, density increased directly with intake of calcium in rats up to a certain level. At that point density did not increase and body weight was retarded. It appears that high dietary calcium may have decreased the growth rate by its interference with the utilization of other nutrients (65).

Replenishment of calcium in the plasma ordinarily comes from the diet. However, the skeleton itself is a depot for reserve supplies. Without sufficient dietary amounts, it may be that calcium is being deposited in one or more places in the skeleton at the same time it is being withdrawn from other places. Bone development may not be optimum under such conditions.

Depressed bone mineralization in Viscos boys was paralleled by an average lag of several years in skeletal maturation. In spite of their growth spurts

after 15 years of age, their total body sizes were ultimately small (64). Malnourished children in Central America had significantly less compact bone than the controls (46). Bones from malnourished Turkish children were shorter, weighed less, and contained more fat and less calcium and phosphorus than the control bones of similar age (11). A 25-30 percent decrease in mineral salts is necessary before decalcification can be diagnosed radiologically in the juvenile skeleton (66).

Age and weight had the greatest effect on bone density of any of the factors analyzed in the 11-20 year old group tested by Odland et al. (53). Bone mineral content increased at an incremental rate of about 8.5 percent each year. Bone density increases slowly between the ages of six to nine years. Both males and females increased more rapidly in bone density after age 15 emphasizing that the tendency for the skeleton to grow is greater than the tendency to achieve greater density (78).

CHAPTER III

METHODS AND MATERIALS

This study represents one portion of the investigation of the effects of inclusion of cottonseed flour, produced by the liquid cyclone process (LCP) as a source of dietary protein. Approximately 30 percent of the total protein intake was in this form. Total food intake provided protein allowances of approximately one gram per kilogram of body weight.

The experimental group included 22 children ranging in age from 8 to 17 years. Thorough medical examinations verified the group to be typically normal without any unusual physical conditions. These subjects resided in a children's home (Home 1). Church and private donations support this facility which is licensed by the State of Texas. A six-months experimental period began November 1, 1972.

A group of 30 subjects, chosen to be the control group, were from another children's home in Texas (Home 2). This home was also church and privately supported. The control subjects were observed for a period of four months, beginning February 1, 1973.

The dietitian of Home 1 and the author planned three-week cycle menus prior to the initiation of the investigation. These were comparable to foods previously consumed; however, they included tested recipes developed by Johnson (36) containing cottonseed flour.

Menus in the control group were reviewed by the residing dietitian and the author. The nutritive content of the food was comparable to that of the experimental group with the omission of cottonseed flour. Special consideration was given to menus preceding collection of urine samples for HYP analyses to assure well-balanced meals without excessive amounts of collagen. For example, gelatin and meat with higher amounts of connective tissue were avoided.

Four to five subjects sat at a table with a staff member for family style meal service. Research team workers observed and recorded food intake at breakfast and the evening dinner meal. School lunch intake was recorded by recall. Snack information also was included. This same procedure was used for the experimental and the control groups. An average one-week dietary pattern was collected for each subject in this manner.

Fasting blood samples were collected at the beginning and upon conclusion of the study. Analyses of the samples were made for serum calcium and phosphorus. Serum calcium was determined by the method of Ferro and Ham (17). The Fiske and Subbarow (18) method as modified by Dryer, Tammes, and Routh (13) was used to determine serum phosphorus. The range of concentration of inorganic phosphorus in the serum of healthy adults is 2.6 to 4.6 mg/100 ml and varies with age (61). For children 5 to 15 years of age normal concentrations are 5.0 mg/100 ml (14).

Analytical techniques included the following procedures:

A modification of the Prockop and Udenfriend (59) method as described by Kivirikko, Litinen, and Prockop (39) was followed by the author for the hydroxyproline analyses. From 2 to 10 years in age the HYP levels range from 34-93 mg per 24 hour urine sample and from 11 to 14 years the values are 40-113 mg per sample (38).

Hand and wrist x-rays were taken at the initial and final stages of the investigation for skeletal maturation evaluation. Comparisons were made to the Gruelich-Pyle (27) standards for rates of skeletal maturation. Radiographs of the finger of each subject

were used for measurement of bone density by the densitometric procedure (42) (47).

All data were compared to the appropriate standards to determine if the experimental feeding of cottonseed protein could have resulted in statistically significant differences.

CHAPTER IV

RESULTS AND DISCUSSION

Similar fluctuations in serum calcium occurred in both the experimental and control populations in this study (Tables 1, 2). For the experimental subjects, only one fell outside the normal serum levels for calcium using the wide range for children through adulthood of 9-12 mg/100 ml (74). A female subject, aged 13, had an initial serum level of 8.4 mg with the final value 11.5 mg/100 ml. Two male subjects, aged 8 and 10, did have values of 9.9 mg and 9.1 mg/100 ml. Another female, 13 years old, had an initial value of 9.4 mg. These levels are low for young children even though they still may be normal.

While only 4 subjects in the experimental group ever fell below 10 mg/100 ml, the lower range for children 5-15 years of age, 17 of the control subjects showed such values. However, none of these fell below 9 mg/100 ml (the lower normal range for adults), so all of them were within the normal ranges. Of the 17 subjects, 3 subjects were older than 15 years, which meant that serum calcium between 9-11 mg/100 ml should

Table 1

Comparison of initial and final serum calcium

Subjects	Calcium	
	Initial	Final
Control	10.0 ± 0.5*	10.7 ± 0.5*
(n = 30)		
Range:	9.1 - 11.1	9.7 - 11.4
Experimental ⊛	10.6 ± 0.9*	10.9 ± 0.6*
(n = 23)		
Range:	8.4 - 12.2	9.1 - 11.7

* mg/100 ml serum mean ± S.D.

⊛ Experimental: diet with cottonseed.

Normal serum calcium concentration:

Annino (3): Adults: 9-11 mg/100 ml.

Watson and Lowry (74): Children: 10-12 mg/100 ml.

Table 2

Summary of analysis of covariance of final serum calcium concentrations adjusted for intake levels

Source of Variance	Degrees Freedom	Sum of Squares	Mean Square	F Ratio	Probability
Total in both groups	51	14.07			
Within each group	50	13.08	0.26		
Difference in experimental and control	1	0.99	0.99	3.79	0.06 N.S.

Subjects:	Raw Mean:	Adjusted Mean:
Control	10.74	10.64
Experimental	10.90	11.03

be the range used. With 14 subjects below the 10 mg serum calcium, even though only slightly, their serum levels must be considered low for children.

The experimental subjects had an initial serum calcium level of 10.6 mg/100 ml and a final value of 10.9 mg/100 ml. The control subjects had an initial mean calcium serum level of 10.0 mg/100 ml and final of 10.7 mg/100 ml. A significant difference was noted in serum calcium levels between the groups initially with the experimental subjects higher, but final serum levels were not significantly different.

All but one of the control subjects with serum calcium levels between 9-10 mg increased, so that they were above 10 mg finally. Of the four experimental children with low concentrations, all but one increased to the acceptable ranges for children. The fourth decreased from a high value of 12.2 mg to 9.9 mg. This last datum is unexplainable.

Even though the increased mean serum calcium values between the initial and final tests do not vary as much for the experimental as for the control group, some factors should be considered. By incorporating cottonseed protein into the diet, chocolate flavoring was added to milk to disguise the LCP. Also, chocolate

pudding became a new item on the menu. So the LCP substitution may have caused some subjects in the experimental group to consume more milk as well as more protein.

The children in the control population drank large quantities of milk with the morning and evening meals where unlimited amounts were available. Several of these subjects also took vitamin pills by personal choice. These higher calcium intake levels, however, did not result respectively in significantly higher serum levels either initially or finally when compared to the experimental subjects.

Significant differences were found between the two populations in calcium intake levels. The mean was 1.38 g for the control and 0.84 g for the experimental group (Table 3). Apparently the control subjects' higher levels of calcium intake did not increase absorption. They may have approached the inverse levels seen as the plateau of calcium intake is reached (44). The experimental group had higher serum calcium mean values than the control subjects in spite of lower calcium intake.

Corresponding balances of serum phosphorus levels to fluctuating calcium did not occur in either

Table 3

Comparison of calcium intake of subjects

Subjects	Calcium intake
Control	
(n = 30)	1.4 ± 0.3*
Range:	0.8 - 2.5
Experimental	
(n = 23)	0.8 ± 0.2*
Range:	0.5 - 1.3

*Mean g/day ± S.D.

population. There was no significant difference between initial or final serum phosphorus either within or between the two groups. Even though the control subjects had higher intake values, the experimental ones had slightly higher, yet still insignificant, final serum phosphorus levels (Tables 4, 5, Figure 1).

All of the subjects in the experimental and control populations had serum phosphorus concentrations within normal ranges. In general, the concentrations gradually decreased as age increased, which is the normal pattern (61).

The experimental group had a better Ca/P intake ratio of 0.83/1.15 rather than the control of 1.38/1.56 mg. This closer correlation to the suggested 1/1.5 mg (53) dietary ratio may be one of the factors involved in higher proportional calcium absorption by the experimental subjects (Table 6).

According to Onley (55), protein intake and serum total protein (25) as well as serum albumin (63) were within normal ranges or slightly higher (34). No statistical differences were found within or between either the control or experimental populations.

At this level of cottonseed fed to children 6-18 years of age, there did not seem to be a marked

Table 4

Comparison of initial and final serum phosphorus of subjects

Subjects	Phosphorus	
	Initial	Final
Control		
(n = 30)	4.4 ± 0.6*	4.1 ± 0.5*
Range:	3.4 - 5.6	3.1 - 5.4
Experimental		
(n = 23)	4.1 ± 0.6*	4.1 ± 0.5*
Range:	3.2 - 5.4	3.0 - 4.9

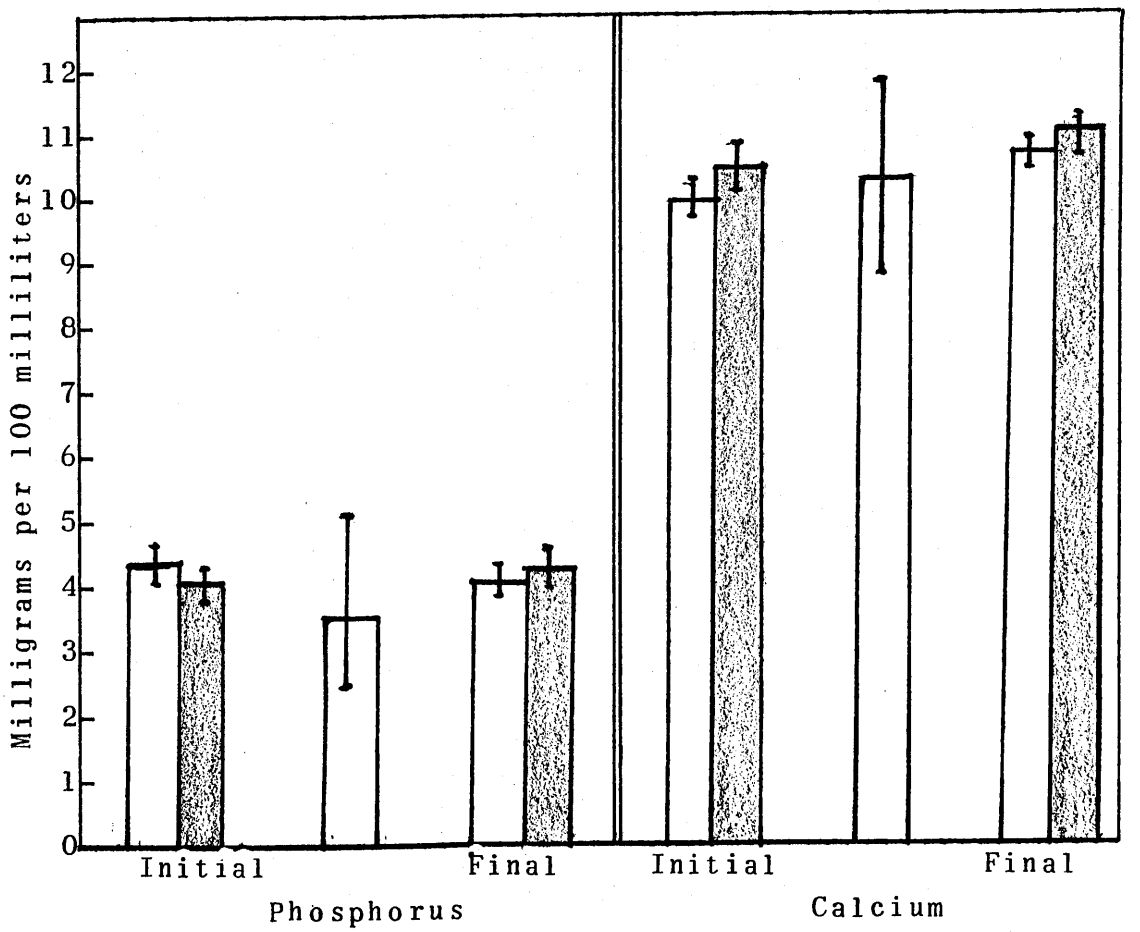
*Mean mg/100 ml serum ± S.D.

Table 5

Summary of analysis of covariance for phosphorus intake and final serum phosphorus

Source of Variance	Degrees Freedom	Sum of Squares	Mean Square	F Ratio	Probability
Total in both groups	51	10.95			
Within each group	50	10.83	0.22		
Difference in experimental and control	1	0.12	0.12	0.54	0.47 N.S.

Subjects:	Raw Mean:	Adjusted Mean:
Control	4.09	4.06
Experimental	4.13	4.17



Control Subjects (Mean with Standard Deviation)

Experimental Subjects (Mean with Standard Deviation)

Normal range for children through adulthood

Fig. 1 Effect of dietary regimens on serum calcium and phosphorus of subjects when cottonseed protein was substituted for 30 percent of total protein intake in the experimental diets.

Table 6

Comparison of phosphorus intake of subjects

Subjects	Phosphorus intake
Control	
(n = 30)	1.6 ± 0.3*
Range:	0.9 - 2.1
Experimental	
(n = 23)	1.2 ± 0.3*
Range:	0.9 - 1.9

*Mean g/day ± S.D.

influence from the phytate present on calcium serum status.

Adolescent males (11-14 years) are reported to have the highest urinary hydroxyproline excretion of any group (56). Data obtained during this study corroborated that report. No significant differences were found between the control and the experimental populations in any age group, including adolescent boys (Tables 7, 8, Figure 2).

Of the two experimental subjects with high hydroxyproline excretion, one had a chronological age of 6.8 years and a skeletal age of approximately 11 years. The second subject had a chronological age of 8.1 years and a skeletal age of 6.0 years. Based on these data an assumption that those with high hydroxyproline levels might be those with lower matching skeletal ages for chronological is not valid. Both children increased in height and weight. The first one with higher skeletal per chronological age also increased in bone density. The second one showed a decrease in bone density.

Of the nine subjects in the control group with high hydroxyproline values, only one had higher chronological

Table 7

Relationships of urinary hydroxyproline excretion of the subjects in this study to normal ranges

Group: Sex/Age	Numbers of Subjects			Total Range
	Above	Below	Within	ml/24 hr
Control 6-10 (male + female)	6	4	6	24.6-149.9
Experimental 6-10 (male + female)	2	1	5	36.3-160.0
Control Male 11-14	2	0	2	86.7-137.5
Experimental Male 11-14	0	0	2	93.9-100.7
Control Female 11-14	0	1	4	31.1- 87.6
Experimental Female 11-14	0	3	4	15.3-111.2
Control 15-18 (male + female)	1	-	4	33.5-141.2
Experimental 15-18 (male + female)	0	-	5	35.1- 90.1

Kivirrkko and Laitinen (1965) (39). Normal ranges for hydroxyproline values (ml/24 hr):

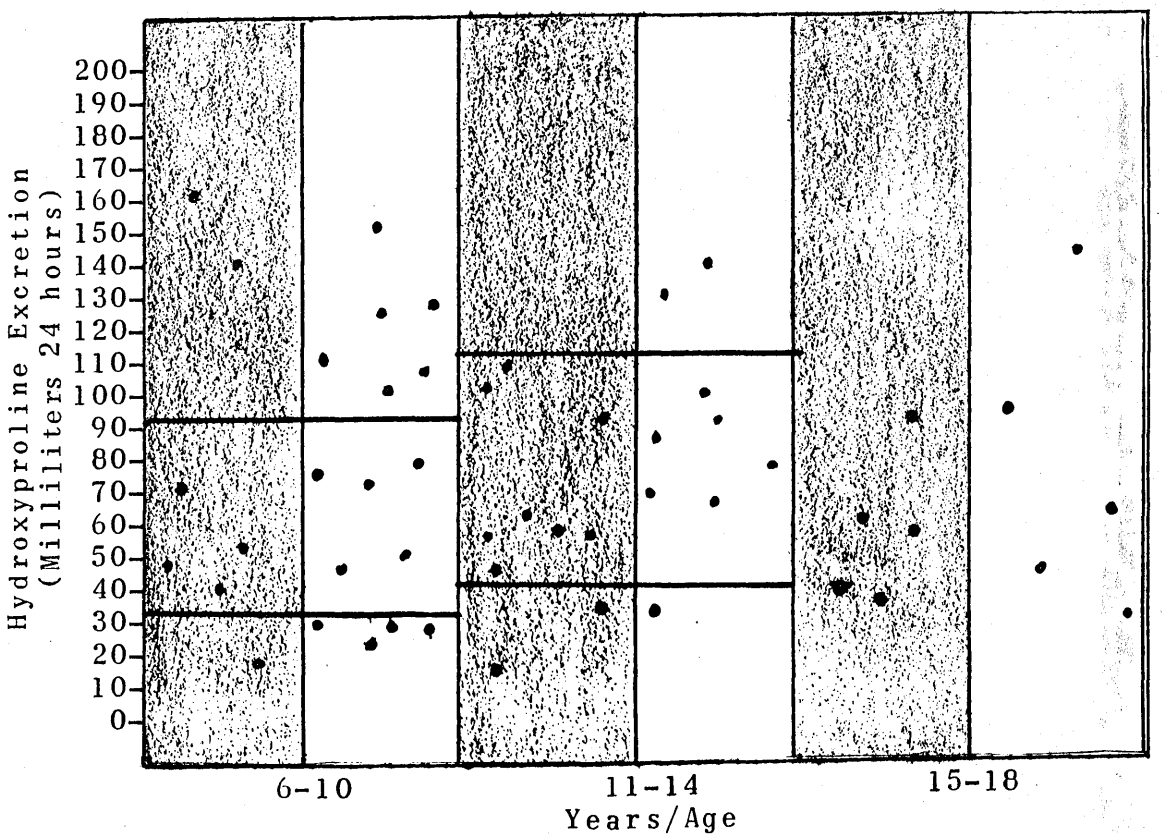
6-10 years: 34-93

11-14 years: 40-113 (no values for older children so 113 considered high)

Table 8

Statistical analysis of urinary hydroxyproline excretion (ml/24 hr) for subjects in the study

Groups Compared by Sex and Age	Mean	Standard Deviation	"t" Value	Probability
Control Males + Females 6-10 (n = 16)	77.1	27.2		
Experimental Males + Females 6-10 (n = 8)	74.0	46.3	0.25	N.S.(P>0.10)
All Males 11-14 (n = 6)	108.1	18.6		
All Females 11-14 (n = 12)	58.0	27.3	6.07	Sig.(P<0.005)
Control Males 11-14 (n = 4)	113.4	20.8		
Experimental Males 11-14 (n = 2)	97.3	3.4	1.53	N.S.(P>0.10)
Control Females 11-14 (n = 5)	65.8	21.9		
Experimental Females 11-14 (n = 7)	52.4	29.3	1.16	N.S.(P>0.10)
Control Males + Females 15-18 (n = 6)	73.4	39.0		
Experimental Males + Females 15-18 (n = 5)	56.7	20.3	1.07	N.S.(P>0.10)



Normal ranges for hydroxyproline values (ml/24 hr):
6-10 years: 34-93
11-14 years: 40-113
No values for older children. Kivirrkko and Laitinen (40).

Experimental

Control

Fig. 2 Urinary hydroxyproline excretion of control and experimental subjects 6-18 years of age when fed diets with cottonseed protein substituted for 30 percent of total protein intake in the experimental group.

than skeletal age (9.0 years; 7.0 years initial, 7.6 years final).

Chronological ages were highly correlated with the skeletal ages among both groups. There was no significant difference between the two populations, and no significant changes occurred during the period of investigation.

Chronological age for both groups is closely correlated with average values for both sexes at 11.95 years for experimental and 11.47 years for the control group. But the skeletal age means indicate greater variance with values of 12.70 years for the experimental and 11.78 years for the control.

Evaluation of bone density reflects the cumulative nutritional status. This may or may not relate to the seven-day dietary records, which indicate current dietary trends of the group under study. Bone density analyses are valuable in assessing mineral deposits. The total amount of bone itself is not as useful since individuals will vary widely in size depending upon their genetic background. So initial and final bone density values serve the primary purpose of indicating normal absorption of calcium and phosphorus.

There was a difference initially between groups in bone density values, so an analysis of covariance was used to analyze these changes within each group initially and finally. Since the largest increases in bone density occur after growth, it is natural for the control group with a lower skeletal age to also have a lower bone density mean. During the research period there were no significant changes within or between these populations (Tables 9, 10, Figure 3).

Table 9

Comparison of the effect on bone density of the inclusion of LCP in the diet of experimental subjects 6-18 years of age

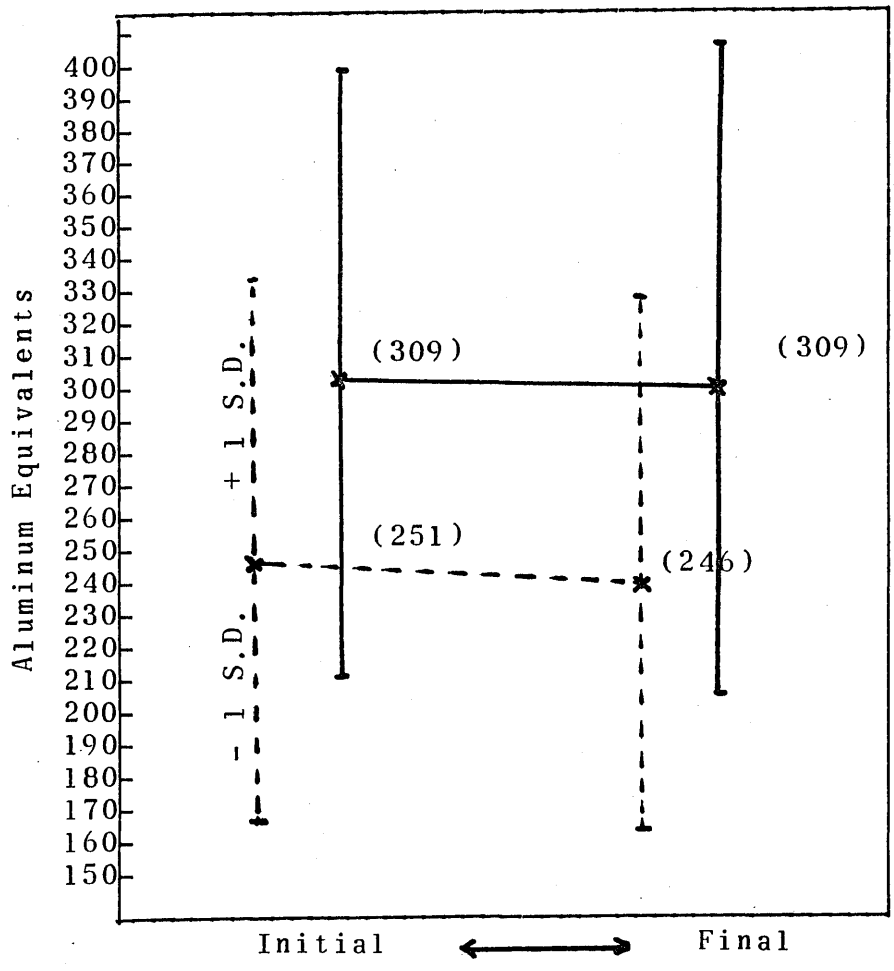
	Bone density	
Subjects	Initial	Final
Control		
(n = 30)	251 ± 83.1*	246 ± 81.4*
Range:	106 - 407	133 - 438
Experimental		
(n = 23)	309 ± 94.5*	308 ± 98.8*
Range:	178 - 494	178 - 486

*Mean aluminum equivalents ± S.D.

Table 10

Summary of analysis of variance for percent change in bone density

Source of Variance	Degrees Freedom	Sum of Squares	Mean Square	F Ratio	Probability
Total in both groups	53	0.51			
Within each Group	52	0.51	0.01		
Differences in experimental and control	1	0.00	0.00	0.04	0.85 N.S.



——— Experimental
----- Control

Fig. 3 Change in bone density for control and experimental subjects 6-18 years of age when fed cottonseed protein substituted for 30 percent of total protein in the experimental population.

CHAPTER V

SUMMARY

Although the initial serum calcium was significantly different with the experimental subjects higher than the control, no significant differences existed between the populations in final serum calcium (Table 2). The initial and final means (Table 1) in the experimental and control subjects were within the normal ranges for children according to Watson and Lowry (74). All of the children, aged 6-18 years, remained within the broad range of combined values for children 10-12 mg/100 ml (74) and those of adults 9-11 mg/100 ml by Annino (3) or 9-12 mg/100 ml.

No significant difference was found in serum phosphorus among the subjects in this study (Table 5). Initial and final means of both groups were 4 4+ mg/ 100 ml (Table 4). High phosphorus concentrations start to decrease after the age of 7 years to normal adult levels by the age of 15 in girls and 17 in boys (61). No individual serum phosphorus fell outside the normal range for adults of 2.6-4.6 mg/100 ml (61), and many were near the concentration for children of 2.6-4.6 mg/100 ml

(61), and many were near the concentration for children of 5.0 mg/100 ml (22).

Significantly higher amounts of calcium and phosphorus were noted among the intake means of the control subjects (Tables 3, 6). Since the serum concentrations were higher in the experimental populations, it may be assumed that these higher intakes were not necessary. The Ca/P ratio of the experimental subjects of 0.83/1.15 was closer to the recommended one of 1/1.5 (53) than that of the control population of 1.38/1.56.

No significant differences were found among these subjects in serum total protein, albumin, or globulin; and all of them were within normal ranges according to Onley (55).

Since the serum levels of calcium, phosphorus, and protein were maintained within normal ranges with no significant differences occurring within or between the experimental and control subjects, these important conclusions may be drawn:

1) It may be assumed in terms of these minerals that the substitution of LCP cottonseed protein at the 30 percent level of total protein was not detrimental.

2) Apparently the phytic acid of cottonseed did not interfere with the final serum concentrations of calcium and phosphorus to the extent to be significantly different from the initial concentrations.

3) Normal elevations of serum calcium, phosphorus, and protein were maintained throughout the investigation, so that the individual serum concentrations of each may be considered accurate.

4) In this study the LCP cottonseed protein was a satisfactory dietary substitution for children 6-18 years of age in regard to calcium and phosphorus.

Since there was no significant difference between populations in urinary hydroxyproline excretion values, it may be assumed that anticipated or present growth spurts were not affected either adversely or positively by cottonseed protein substitution. No significant decreased values were found which would indicate malnutrition.

No statistical differences were noted among the skeletal ages. This, along with Onley's (55) findings

of no significant differences in height or weight, indicate that normal growth did occur with LCP.

Essentially no changes in bone densities occurred in either population during the period of study. Bone density was slightly higher among the experimental subjects initially and finally, which also corresponds to their slightly higher skeletal age mean. These differences were of no statistical significance.

From the analysis of variance for each of these areas, no significant differences were observed. So it may be assumed from this research that substitution with liquid cyclone process cottonseed protein at the 30 percent level did not interfere with normal bone metabolism (Table 11).

Table 11

Summary of physical stature status of children 6-18 years of age when fed diets consisting of 30 percent cottonseed protein substitution of total protein in the experimental population

	Percentage of Subjects with Changes in Physical Stature			
Subjects	Skeletal	Height	Weight	Bone Density
Experimental				
Increased	86.40	90.91	100.0	54.6
Decreased	---	---	---	45.4
No Change	13.60	0.1	---	---
Control				
Increased	50.0	83.4	80.1	26.6
Decreased	---	---	16.6	73.4
No Change	50.0	16.6	3.3	---

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SUMMARY OF DATA: EXPERIMENTAL SUBJECTS

Subject	Sex	Calcium			Phosphorus			Protein		Protein Serum (mg/100 ml)			
		Average intake/g	Serum (mg/100 ml) Init.	Final	Average intake/g	Serum (mg/100 ml) Init.	Final	Average intake/g Animal	Veg.	Total Init.	Final	Albumin Init.	Final
2824	M	0.8	10.5	11.3	1.3	3.6	3.8	69.0	25.8	7.9	8.2	4.9	4.9
2825	M	1.2	12.0	11.5	1.4	3.5	3.6	55.6	21.7	8.1	7.9	4.8	4.9
2827	M	1.0	10.3	10.8	1.9	3.5	3.5	73.5	50.7	7.7	8.0	5.1	5.0
2828	M	0.7	10.6	10.4	1.0	3.6	4.0	42.1	23.3	7.6	7.2	4.4	4.2
2829	F	0.7	9.4	11.5	1.0	3.5	3.0	47.8	16.3	7.2	7.0	4.6	4.6
2830	M	0.5	10.8	10.4	0.9	3.8	4.1	42.4	16.9	7.0	7.4	4.3	4.6
2831	F	0.6	10.5	10.0	0.9	3.2	3.9	42.7	13.7	7.6	7.0	4.3	3.9
2832	F	0.7	8.4	11.5	0.9	4.5	4.3			7.7	7.9	4.8	5.0
2834	F	0.6	10.0	10.7	0.9	4.5	4.4	37.6	20.9	8.4	7.8	4.9	4.4
2835	M	0.8			1.1								
2836	M	0.7	11.1	11.4	1.0	3.9	4.0	35.3	23.1	7.3	7.2	4.6	4.7
2837	F	0.9	11.1	11.3	1.0	4.4	4.1	41.5	20.9	7.7	7.2	4.8	4.6
2838	F	0.7	11.3	11.7	0.9	4.2	4.1	33.9	19.3	8.1	7.5	5.3	4.7
2840	M	0.9	11.0	10.9	1.2	4.0	4.2	47.4	20.7	7.6	7.1	4.6	4.1
2841	F	0.8	11.6	10.8	1.0	3.7	4.2	40.0	21.6	6.8	6.8	4.6	4.3
2842	F	0.9	11.9	11.2	1.3	5.4	4.9	51.6	26.1	7.6	7.4	4.7	4.3
2843	M	0.9	10.9	10.9	1.1	4.2	4.3	40.0	20.7	6.9	6.8	4.9	4.5
2844	M	0.8	12.2	9.1	1.0	4.0	3.7	44.9	21.7	7.8	7.4	4.9	4.2
2846	M	1.0	9.9	11.4	1.2	4.7	4.9	46.1	24.5	7.7	7.3	4.5	4.5
2847	F	0.1	10.7	10.5	0.9	5.0	4.5	39.4	18.4	7.4	6.7	4.7	4.0
2848	F	1.0	10.0	10.8	1.2	4.4	4.1	44.0	23.0		6.8		4.0
2849	M	1.3	10.2	11.2	1.5	4.1	4.5	57.1	28.8	6.8	7.1	4.5	4.5
2850	M	1.3	10.0	10.4	1.5	4.6	4.8	65.0	22.6	7.4	7.1	4.7	4.5

EXPERIMENTAL SUBJECTS, Continued

Subject	Sex	Age (Years)			Height (inches)*		Weight (pounds)*		Bone Density (aluminum equiv.)		Hydroxyproline (mg/24 hr)
		Chron.	Skeletal Initial	Skeletal Final	Initial	Final	Initial	Final	Initial	Final	
2824	M	16.6	15.3	15.9	68.5		134.0		.432	.398	60
2825	M	15.1	14.9	15.6	67.3	68.3	141.0	146.5	.470	.486	96
2827	M	14.2	17.0	17.0	66.0	66.5	148.0	149.5	.494	.462	94
2828	M	16.1	16.6	17.0	70.5	70.8	145.0	148.5	.428	.463	59
2829	F	13.7	14.0	14.9	65.0	65.0	150.0	158.0	.381	.388	51
2830	M	17.1	19.0	19.0	65.0	65.3	126.0	127.5	.356	.436	35
2831	F	15.6	16.0	16.0	65.5	66.0	138.0	144.5	.353	.356	40
2832	F	13.5	13.6	13.9	62.5	62.8	102.0	103.0	.342	.293	35
2834	F	14.5	13.6	13.6	64.3	64.5	95.0	98.5	.305	.279	20
2835	M		17.0	18.0	64.3		120.5		.419	.420	
2836	M	11.0	10.0	10.6	55.0	55.8	73.0	75.0	.243	.228	101
2837	F	11.7	12.0	12.0	59.5	60.8	80.3	86.0	.243	.249	111
2838	F	9.0	9.0	9.3	50.5	51.0	65.0	67.0	.196	.190	71
2840	M	6.8	11.0	11.9	56.3	57.5	95.0	100.0	.247	.256	139
2841	F	12.1	13.6	14.0	62.0	62.8	101.0	106.0	.285	.286	67
2842	F	10.7	11.9	12.6	58.5	60.5	90.3	103.5	.258	.260	53
2843	M	9.3	11.0	11.6	56.3	57.5	87.5	100.0	.279	.289	36
2844	M	10.2	8.6	9.0	52.5	54.5	62.5	63.5	.188	.202	20
2846	M	8.1	6.0	7.0	53.3	53.5	57.0	59.5	.178	.177	160
2847	F	11.5	10.0	11.0	56.5	58.0	68.8	74.0	.224	.233	61
2848	F	11.6	12.0	13.0	58.8	60.0	115.5	117.0	.315	.307	58
2849	M	8.7	8.0	9.0	50.0	51.5	62.0	65.0	.228	.209	64
2850	M	10.3	9.6	10.6	57.3	58.3	80.0	82.0			48

*Protein, height, weight Onley (55).

SUMMARY OF DATA: CONTROL SUBJECTS

Subject	Sex	Calcium			Phosphorus			Protein		Protein Serum (mg/100 ml)			
		Average intake/g	Serum (mg/100 ml) Init.	Final	Average intake/g	Serum (mg/100 ml) Init.	Final	Average intake/g Animal	Veg.	Total Init.	Final	Albumin Init.	Final
2853	M	1.6	9.5	10.3	1.8	3.9	3.9	67.3	26.5	7.5	7.3	4.4	4.4
2854	F	1.3	10.5	9.7	1.4	4.0	3.8	61.8	18.4	7.1	7.3	4.2	4.2
2855	F	1.6	9.5	10.4	1.7	4.0	3.3	76.6	20.4	7.6	7.1	5.1	4.5
2856	F	1.2	10.8		1.3	4.2				7.6		4.6	
2857	M	1.4	9.8	11.0	1.6	4.4	4.3	70.6	21.7	7.8	7.8	5.3	5.0
2858	M	2.5	9.8	11.3	2.6	4.9	4.9	102.2	25.8	7.4	7.1	4.9	4.3
2859	M	1.3	10.3	10.8	1.5	3.9	4.1	67.9	16.9	8.2	7.9	4.7	4.4
2860	M	1.3	10.2	11.4	1.5	3.9	4.1	66.4	18.4	7.9	7.6	5.3	4.7
2863	M	1.9	9.4	11.3	2.1	5.0	4.8	89.0	26.9	7.1	7.1	4.8	4.9
2864	M	1.6	10.3	11.3	2.0	3.7	3.6	88.2	22.5	7.1	7.1	4.6	4.4
2865	F	1.2	11.1	10.9	1.3	4.2	4.1	56.3	15.9	7.5	7.3	4.8	4.7
2870	F	1.5	9.1	10.2	1.7	4.8	4.1	70.7	21.4	7.5	7.1	5.3	4.4
2871	F	1.4	10.0	10.6	1.7	4.8	4.1	82.1	20.8	8.0	7.6	4.8	4.5
2872	M	1.4	9.6	10.6	1.6	5.6	5.4	64.6	21.4	8.2	7.1	4.6	4.2
2873	M	0.8	9.4	10.9	0.9	4.4	4.1	41.3	15.3	7.9	7.3	4.7	4.4
2875	M	1.0	10.8	10.4	1.1	4.5	4.1	46.5	24.8	7.3	7.5	4.3	4.6
2876	M	1.0	9.5	10.4	1.2	4.3	4.0	54.6	16.6	6.8	6.6	4.8	4.4
2877	M	1.2	10.4	10.5	1.4	4.4	4.1			7.1	7.1	4.5	4.6
2878	F	1.1	10.8	10.5	1.2	4.2	4.1			7.5	7.4	4.6	4.7
2880	M	1.7	9.8	10.9	1.9	3.5	4.1	77.1	19.2	7.4	7.6	4.7	4.4
2881	F	1.1	10.8	10.9	1.2	4.7	4.1	51.2	13.7	7.8	7.3	4.7	4.7
2882	M	1.3	10.3	10.5	1.5	4.7	4.4	68.8	18.8	7.4	7.1	4.6	4.4
2883	F	1.4	10.8	10.2	1.5	5.2	5.0	67.1	16.2	7.6	7.3	4.5	4.4
2884	F	1.4	9.8	10.8	1.5	5.1	4.1	76.0	14.2	7.3	7.1	4.9	4.5
2885	F	1.1	9.9	10.9	1.3	5.2	4.3	54.1	16.0	7.4	7.8	4.4	4.8
2886	M	1.3	9.7	10.9	1.5	4.2	3.8	59.3	21.9	7.7	7.8	5.1	5.1
2890	F	1.6	9.8	10.9	1.8	4.4	3.8	79.9	24.1	7.2	7.1	4.9	4.7
2891	M	1.3	10.8	10.8	1.5	4.7	4.2	72.6	18.0	7.2	7.1	4.6	4.7
2892	M	1.4	9.9	10.0	1.8	4.0	3.9	82.2	20.7	6.8	6.8	4.7	4.7
2893	F	1.2	9.5	11.0	1.4	3.4	3.5	54.5	19.3	8.1	7.5	5.1	4.5

CONTROL SUBJECTS, Continued

Subject	Sex	Age (Years)			Height (inches)		Weight (pounds)		Bone Density		Hydroxyproline (mg/24 hr)
		Chron.	Skeletal Initial	Skeletal Final	Initial	Final	Initial	Final	Initial	Final	
2853	M	18.4	18.0	18.0	72.3		150.0		.152	.133	90
2854	F	14.1	14.0	14.6	64.3	64.5	130.0	128.5	.232	.242	70
2855	F	15.1	14.6	15.0	67.8	69.0	120.5	121.5	.269	.268	31
2856	F	14.6	13.0		64.0		113.0				88
2857	M	9.3	5.0	5.6	48.3	48.5	50.5	51.5	.146	.152	61
2858	M	15.0	14.0	14.3	65.3	65.5	146.5	150.5	.338	.337	42
2859	M	8.1	8.0	8.6	48.8	48.8	50.5	51.5	.130	.129	30
2860	M	12.6	12.6	12.9	56.3	57.3	75.5	78.5	.207	.208	137
2863	M	13.1	14.0	14.0	67.3	68.5	142.0	145.0	.471	.438	87
2864	M	15.2	17.0	17.0	67.0	67.3	176.0	174.0	.106	.159	141
2865	F	8.0	8.0	8.0	52.5	53.0	81.0	73.5	.214	.173	80
2870	F	11.3	13.0	13.6	64.0	65.0	96.0	102.5	.265	.264	78
2871	F	10.1	12.0	12.3	62.8	63.5	100.0	102.5	.264	.280	127
2872	M	13.1	13.0	13.3	60.8	62.5	116.0	123.5	.302	.295	129
2873	M	10.6	10.6	10.9	55.8	57.0	79.0	80.0	.236	.228	128
2875	M	10.7	10.6	10.6	52.5	53.3	68.5	70.5	.213	.221	30
2876	M	10.4	10.3	10.9	55.0	55.3	73.5	76.5	.193	.171	113
2877	M	9.3			51.5	52.5	61.5	61.5			25
2878	F	7.4	7.6	7.6	50.0	50.5	74.5	76.5			50
2880	M	18.0	18.0	18.0	66.3	66.5	128.0	134.5	.326	.308	33
2881	F	8.3	8.0	8.0	47.3	47.8	49.0	50.0	.209	.186	33
2882	M	7.3	5.0	5.0	45.5	46.3	52.0	51.0	.233	.176	49
2883	F	10.1	10.6	10.6	51.0	51.8	68.0	71.0	.258	.253	73
2884	F	11.2	12.6	12.6	59.8	60.8	96.0	98.5	.343	.353	62
2885	F	7.2	7.0	7.9	48.3	48.8	60.5	61.0	.172	.164	150
2886	M	11.3	12.6	12.6	58.5	59.8	100.0	102.5	.288	.282	100
2890	F	10.9	10.9	11.0	55.5	56.3	80.0	82.0	.249	.234	105
2891	M	9.1	7.0	7.6	51.8	52.5	71.0	75.0	.208	.231	108
2892	M	8.0	6.0	6.0	48.8		60.0		.238	.233	75
2893	F	16.6	17.0	18.0	63.5	63.5	103.5	101.5	.407	.394	60