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VARIATION IN CALCIUM INTAKE IN RURAL BLACK CHILDREN

Caroline Jane Eyberg

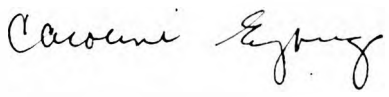
A dissertation submitted to the Faculty of Medicine,
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Johannesburg, 1983

THIS IS TO CERTIFY THAT

this work is my own, except where credit has been acknowledged.

The work has not been presented previously to any other Faculty or University.



Caroline J. Eyberg

ABSTRACT

Two investigations are explored in this dissertation: dietary calcium intake in relation to calcium status in a group of rural Black children and the use of the dietary history and 24-hour dietary recall as a research tool. Children with rachitic bone deformities, members of a community in the Eastern Transvaal, have prompted extensive study. This has shown that the population as a whole has lowered serum calcium levels which appear to be the direct result of calcium deficient diets.

Children in each of three age groups participated in the study: 3-5 years, when the increment in skeletal calcium is at its lowest, 8-10 years when there is a sharp rise in the increment of skeletal calcium and 13-16 years when this increment peaks during the pubertal growth spurt. Although no children with bone deformities were included in the group, the study subjects had lowered serum calcium and raised serum alkaline phosphatase levels, typical of the children with the deformities; they were age/sex matched with subjects who had normal serum biochemistry. Dietary histories including a 24-hour dietary recall were taken from each subject and/or his mother. Information gathered concerned sources and frequency of supply of various foodstuffs including gardening and animal husbandry, personal food preferences, with special reference to foods having a direct bearing on calcium metabolism in man and socio-economic data pertaining particularly to eating habits. Total energy and nutrient value of the 24-hour dietary recall was calculated from standard food tables.

The only statistically significant difference between study and control groups for the age group 3-5 years was dietary calcium intake when expressed per kg body weight. There were no significant relationships

proper training of lay personnel were provided, the 24-hour dietary recall would seem to provide accurate information regarding eating habits and nutritional intake among the rural Black population in South Africa. This could be extremely important in this country where there is a great need for nutritional research but Black nutritionists are rare.

For Eybie Nicholaas Eyberg and John Mathew Dolina

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CHAPTER 1: INTRODUCTION

The classic studies of dietary calcium requirements were completed in the 1920's and 1930's and for nearly forty years little more research was undertaken. The discovery that infantile rickets was due to a deficiency of vitamin D contributed to the halt in calcium research. Although it is generally thought that low calcium diets produce few clinical effects, this may not be true. Recently with the development of more exact scientific techniques for the investigation of the mechanisms of absorption and metabolism of calcium, interest has again grown; and with dwindling food supplies, particularly affecting the developing countries, calcium requirements are being scrutinized and reconsidered.

In many developing countries, populations have an habitually low calcium intake and appear to grow and develop normally; deficient dietary calcium appears to present no problem. However, evidence is accumulating that low dietary calcium intake may produce problems including rickets (Maltz 1970, Kooh et.al. 1977, Pettifor et.al. 1978). Investigations in Driefontein, Eastern Transvaal have shown that the children in the community have significantly lowered serum calcium and raised serum alkaline phosphatase levels than children in two other communities (Pettifor et.al. 1980). Preliminary dietary surveys of select groups of the population have been conducted, however no controlled work has been undertaken. Pettifor (1980) has postulated that low dietary calcium intakes are responsible for the abnormal serum biochemistry and rachitic bone deformities seen in this community. This dissertation is a further investigation into the calcium deficiency problem seen in Driefontein. Its purpose is to show a relationship

between dietary calcium intake and clinical parameters of calcium nutrition using the technique of dietary history and 24-hour dietary recall. In addition, the dissertation will explore the use of this investigative technique, and define the reliability of the dietary history and 24-hour dietary recall in the study of a rural South African Black population. Prior to presenting findings of the dietary studies, calcium metabolism and requirements are discussed.

CHAPTER 2: REVIEW OF CALCIUM METABOLISM

2:1 The function of calcium in the human body

Calcium represents a large proportion of the elemental composition of the human body, ranking fifth in quantity and representing about 20% of the body's dry tissue weight. Calcium, along with phosphorus, is the major mineral content of skeletal tissues; indeed, 99% of the body's calcium is in the skeleton and is responsible for the rigidity and strength of bones and teeth. Calcium is also present in soft tissues, plasma and extravascular fluid. Table 2:1 shows the distribution of calcium in the adult human.

Table 2:1 Distribution of calcium in 70 kg adult human

Organ	Calcium Content	Percentage of Total
Skeleton	1300 grams	99
Teeth	7 grams	0,6
Soft tissues	7 grams	0,6
Plasma	350 milligrams	0,03
Extravascular fluid	750 milligrams	0,06
Total	about 1300 grams	

Calcium in bone is in the form of multiple apatites: $CaF_2 \cdot 3Ca_3(PO_4)_2$, $CaCO_3 \cdot 3CaC_2(PO_4)_2$, $Ca_{10}(PO_4)_6(OH)_2$; various ions and cations arranged in these proportions form a definite crystal lattice. Ions of similar size and charge are, to an extent, interchangable. Bone contains a living cellular matrix composed of 50% mineral, 25% protein, 20% fat and 5% mucopolysaccharides, and is in dynamic equilibrium with plasma and

other tissues; the calcium in this matrix is part of the body's mineral reservoir which can be drawn on through the mediation of parathyroid hormone (PTH), being more easily mobilized than that from the hard tissues of bones and teeth. The rate of exchange between trabeculae and plasma and other soft tissues is much greater than the rate of deposition of new bone, about 700 mg per day. Skeletal growth in length and density occurs until closure of the epiphyses during puberty and from then until about 30 years of age, the skeleton continues to get denser; after this age, skeletal tissue begins to atrophy which can lead to osteoporosis in old age. Hormones and physical activity can affect osteoporosis, but so far there is little or no evidence to support low calcium intake as an influence (Davidson et.al. 1973); however, treating post-menopausal women with calcium supplements has shown evidence of retarding osteoporosis in this population (Nordin et.al. 1974). Bone growth and calcification is a complicated process of mechanisms not completely understood; formation and maintenance are certainly not solely a matter of calcium metabolism.

Calcium outside the skeleton accounts for only about 1% of the total body calcium. It is necessary for the excitability of peripheral nerves and muscles, for blood clotting (with thromboplastin for the formation of thrombin) and for the activity of several enzymes (renin, succinic dehydrogenase, lipase among others) and ATP. Reid (1943) demonstrated that the permeability of membranes is influenced by calcium. Calcium is also essential in the physiological mechanism for absorption and adsorption of vitamin B_{12}.

The normal serum level of calcium is 2.5 mmol/l (10mg/dl) of which approximately half is bound to protein and half is ionized. Lowered serum albumin causes a reduction in protein bound calcium, which is not associated with metabolic disturbances; however, a reduction of ionized calcium causes tetany and loss of muscle tone.

2:2 Digestion and absorption of calcium containing foods

Humans utilize dietary calcium inefficiently, usually absorbing only 20-30% of the amount ingested. Over the years numerous factors have been implicated as either enhancing or interfering with calcium absorption; today most researchers consider many of these to be of little or no influence, as is discussed below.

Preliminary to absorption, calcium containing foods must be digested to liberate the calcium which must be not only soluble but in the ionized form (Schachter et.al. 1960). Calcium availability is influenced by the presence of oxalic acid, phytic acid and phosphates, all of which form insoluble salts with calcium (Bronner 1964, Wiseman 1964, Wills et.al. 1972); however, often large excesses of dietary phytate, oxalate and phosphate are necessary to produce a decrease in calcium absorption. People with diets traditionally high in phytate may develop the ability to split calcium-phytate; and many cereals contain phytase which splits phytic acid so that it no longer binds calcium. Therefore, it is possible that the problems of phytate influencing calcium absorption are overemphasized. Presently the problem of dietary phytate is again important among workers investigating Asian immigrant populations in Britain who include high phytate flour chappaties as a diet staple (Dunnigan 1976, Compston 1979). Most of the investigations are based on clinical observations and also mention lack of vitamin D and low calcium as well as high phytate intakes as possible causes of the clinical abnormalities seen (Robertson et.al. 1977, Singleton 1978, Rudolph et.al. 1980).

Early workers found that increasing dietary protein enhanced calcium absorption (Kunerth and Pittman 1939, McCance et.al. 1942), later investigators failed to corroborate this (Schofield and Morrell 1960, Johnson et.al. 1970, Anand and Linkswiler 1974). Wasserman et.al. (1956) found that certain amino acids (l-lysine, l-arginine) were effective

in increasing calcium absorption, although the mechanism of this phenomenom remains unexplained.

Bergeim (1926) found that adding lactose to the diet increased calcium absorption, an observation confirmed by more recent research (Condon et.al. 1970). The mechanism of this enhancement of calcium absorption is unknown, however Wasserman and Taylor (1969) have put forward two theories: 1) the sugar chelates (complexes) calcium making it more soluble; 2) the sugar increases permeability of the intestinal cell to calcium through an inhibitory effect on the energy-producing mechanism of that cell.

Fatty acids, forming insoluble soaps which may carry calcium and vitamin D into the faeces, can be an important factor in poor calcium absorption in people with malabsorption syndromes or major intestinal disorders and may lead to osteomalacia; however in normal people this factor is of little influence.

Other dietary and digestive factors including intestinal pH, Ca:P ratio, presence of various vitamins (other than vitamin D) and minerals, presence of bile, amount of bulk in the diet probably do not influence calcium absorption under normal circumstances (Irving 1957). Modern researchers feel that although digestion to release calcium from ingested food and conversion to the ionized form is necessary for absorption, the limiting factor influencing calcium utilization is the calcium transport process in the intestinal cells.

Calcium absorption is usually assessed by the metabolic balance technique. This method, however, does not take into account endogenous faecal calcium which is calcium from digestive juices and shed epithelial cells excreted in the faeces and has been found to average 130-140mg/day (Heaney and Skillman 1964, Harrison et.al. 1969). Digestive juice calcium is subject to the same factors governing dietary calcium absorption, although Heaney and Skillman (1964) estimate that normally only

85% of digestive juice calcium is available for absorption with the same efficiency as dietary calcium. Therefore there is both a net (total) calcium absorption and a true calcium absorption figure.

Net calcium absorption is the difference between ingested calcium and faecal calcium; it frequently is expressed as a percentage of dietary calcium intake. Net calcium absorption increases as the level of dietary calcium rises in a curvilinear fashion as shown in Fig 2:1, initially rising steeply with increasing dietary intake, and then more slowly with still further increases in dietary calcium; the curve does not reach a plateau even at the highest intake studied.(Nordin 1976).

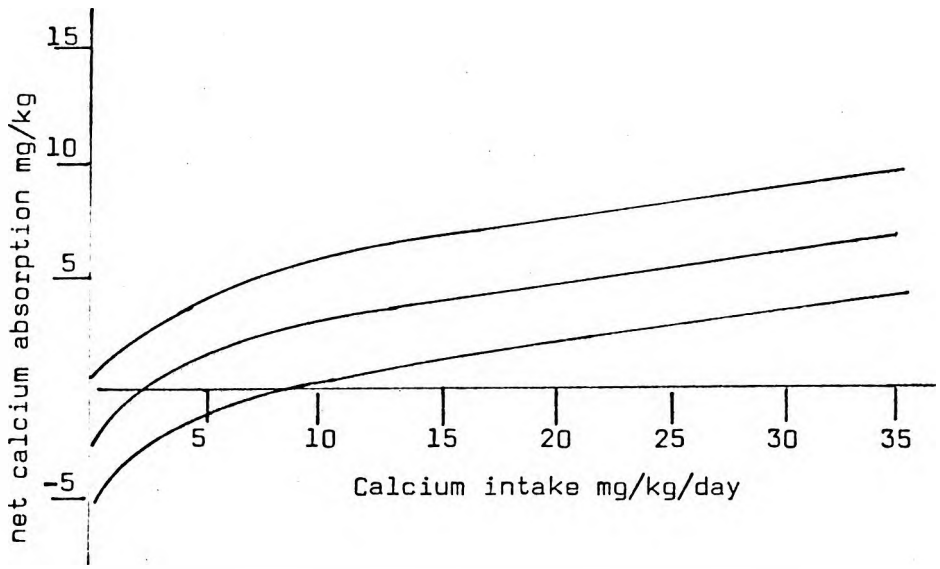


Figure 2:1 Increase in net calcium absorption with increasing levels of dietary calcium intake in normal subjects (compiled from available studies), mean ± 1 SD.

True calcium absorption, on the other hand, is the amount of dietary calcium absorbed and makes allowance for endogenous faecal calcium. The difference between net and true calcium absorption may be important in pateints with coeliac disease and idiopathic steatorrhoea where there is excessive excretion of endogenous faecal calcium (Irving 1957, Harrison et.al. 1969).

The exact mechanism of calcium absorption is not known. Presumably, it takes place by a two component process: a saturable carrier-mediated process and a diffusion process. In vivo studies using an intubation-perfusion technique show that calcium absorption increased as the perfusion solution increased, rising steeply at first, then more slowly; absorption does not saturate and is linearly related to the concentration of the perfusion solution (Ireland and Fordtran 1973, Parker et.al. 1974). This produces a curve similar to that of net calcium absorption at differing dietary calcium intake levels as determined by the balance technique (see Fig. 2:1). Based on perfusion and radio-calcium studies, it is postulated that calcium is absorbed by a saturable carrier-mediated active transport method at lower levels of calcium perfusion and as a diffusion process at higher levels.

The active transport process of calcium across the gut wall might involve a specific intestinal calcium binding protein (CaBP). The exact role of CaBP has been the subject of much recent research; it may be the initial step of a complex transport process; it may affect the permeability of the luminal membrane; it may just protect the cell from high ionic calcium concentration; the ultimate role of CaBP is still to be discovered.

2:3 Factors affecting calcium absorption

Researchers have known for some time that vitamin D enhanced calcium absorption, but until the late 1950's little work was done on the mechanism of the vitamin D action. There is still no accepted agreement by leading workers in the field as to the specific action of vitamin D; however, Wasserman and Taylor (1969) conclude that vitamin D has three effects on calcium absorption: 1) it accelerates the rate of calcium uptake in the absorptive cell; 2) it enlarges the pool of calcium within the cell; 3) it enhances calcium transfer at the basal pole of the absorptive cell. Vitamin D is first metabolised to biologically more

active compounds, but the action of the compounds on calcium absorption and calcium homeostasis is not specificially known. The vitamin D metabolite $1,25(OH)_2D_3$ is probably responsible for man's adaptability to low calcium intakes.

Parathyroid hormone (PTH) also aids in absorption of calcium in the presence of vitamin D and may be involved in the phenomenom of adaptation to changing dietary intakes of calcium (Shah and Draper 1966). The effect of PTH in promoting calcium absorption correlates with the high calcium absorption usually found in patients with hyperparathyroidism and the decreased calcium absorption in those with hypoparathyroidism.

Calcitonin does not seem to have any consistent effect on calcium absorption from the GI tract of man (Gray et.al. 1973, Nordin 1973). However, other hormones have a more consistent influence; corticosteroids and thyroxine both decrease calcium absorption (Bunim et.al. 1958, Slater et.al. 1958, Gallagher et.al. 1973) and pituitary hormones enhance absorption (Henneman et.al. 1960) although the mechanism of these actions has not been completely elucidated.

High dietary intakes of strontium produce skeletal abnormalities which are similar to rickets and it was initially assumed that strontium interfered with calcium absorption. Although the two possibly share a common carrier mechanism, absorption is definitely discriminatory in favour of calcium (Comar et.al. 1956, Wasserman 1963, Lengeman 1967). Large amounts of strontium must be ingested for it to significantly affect calcium absorption.

The effect of various drugs on calcium absorption has been investigated and has procuded somewhat inconsistent results. However the effect of several drugs must be noted as they are frequently used in long term therapy:

Anticonvulsants: Prolonged therapy with phenobarbitone and diphenylhydantoin has been shown to cause decreased calcium absorption,

hypocalcaemia, rickets and osteomalacia (Dent et.al. 1970, Richens and Rowe 1970). This could be by a direct effect on calcium absorption, however it is probably mediated via an effect on vitamin D metabolism (Hahn et.al. 1972). Only a small proportion of patients on anticonvulsant therapy show disturbed calcium metabolism and it may be counteracted by administrering 25 OHD_3 or a larger dose of vitamin D_3 (Stamp et.al. 1972).

Biguanides, cholestyramine, diuretics: Although these drugs are unrelated, they are all frequently used for prolonged therapy. There is sufficient evidence that patients receiving them should be monitored for calcium and vitamin D status although evidence of their effect on this in man is not always known or consistent. Caspary (1971) found that biguanides decrease calcium transport in the rat, although the effect of this drug on diabetic humans has not been investigated. The effect on calcium metabolism of both cholestyramine and diuretics of patients receiving them has produced conflicting results but significantly altered calcium and vitamin D metabolism has been observed in some patients and warrants monitoring (Briscoe and Ragan 1963, Thompson and Thompson 1969, Gursel 1970, Donath et.al. 1971, Runeberg et.al. 1972, Ehrig et.al. 1974, Harrison and Ross 1968).

2:4 Calcium excretion

Calcium is excreted in urine, faeces and sweat. Urinary calcium is usually expressed as mg/24 hours, and on a free diet the range is 50-500 mg/24 hours (Bulusu et.al. 1970). Urine calcium excretion varies by geographical location (probably related to diet), is higher in healthy males than healthy females, and rises during childhood to a fairly steady level in adult life, then decreases with old age. Because of the great variation in urinary calcium excretion, one must establish normals for a population group.

Calcium excretion is regulated by a combination of filtration and partial reabsorption, although the exact nature of calcium reabsorption in the kidney is not clear. Serum calcium level is probably not the primary factor governing this, rather hormonal and other endogenous factors control kidney excretion of calcium. Dietary intake of calcium, however, does influence calcium excretion. Based on studies of changing urinary excretion with reduction of calcium intake, Nordin (1976) has proposed that urinary calcium excretion in an individual decreases by a constant factor of the calcium excretion of the highest dietary intake of calcium. MacFadyen et.al. (1965) maintain that if serum calcium is measured with sufficient precision, changes caused by dietary intake may be detected, which can also be correlated with changes in urinary calcium excretion. Hegsted and Linkswiler (1980) found that high protein diets also caused increased urinary calcium excretion without affecting calcium absorption. There is a correlation between the urinary clearances of sodium and calcium; high dietary sodium intake and some diuretics cause an increase in calcium excretion.

Parathyroid hormone and calcitonin have a major effect on tubular reabsorption of calcium; serum phosphate level (partially a reflection of dietary phosphate intake) also plays a part in calcium excretion.

About 400-800 mg calcium per day (both dietary calcium and endogenous faecal calcium) is lost in the faeces, although this figure depends heavily on dietary intake of calcium. Of this amount, 130-140 mg is endogenous faecal calcium; Heaney and Skillman (1964) found that endogenous faecal calcium tends to rise with increasing dietary calcium.

The amount of calcium lost in sweat is usually insignificant, however, it may be as high as 30% of daily losses when doing heavy labour in excessive heat and must be considered as an important factor in these given conditions (Heaney and Skillman 1964).

2:5 Calcium homeostasis

Figure 2:2 is a schematic drawing of major calcium exchanges in the body. These exchanges are controlled at three sites: bone, kidney and alimentary canal. Both bone and kidneys act via concentration dependent mechanisms. It appears that bone possesses some intrinsic ability to release calcium immediately to sustain a fall in plasma calcium (Rhodan et.al. 1967, Nordin et.al. 1972). There are many factors

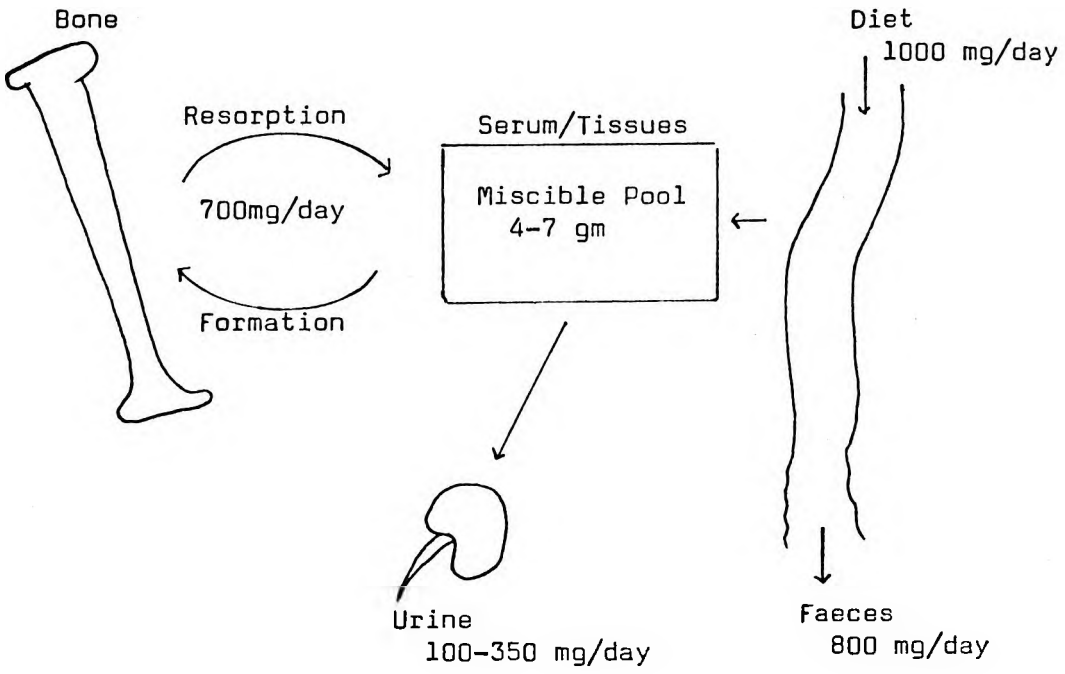


Figure 2:2 Schematic diagram showing the major calcium exchanges in the human body.

involved in calcium homeostasis--growth, vitamin D, hormones, dietary intake. During growth calcium is steadily retained for the formation of new bone. As previously discussed, vitamin D promotes absorption of calcium and PTH indirectly increases active transport across the gut

wall. PTH also influences phosphorus excretion with a concomitant mobilization of bone calcium with rising phosphorus excretion. Calcitonin is important in regulating serum calcium (Davidson 1973). Calcium homeostasis during growth spurts and during pregnancy and lactation seldom causes leeching of calcium from the bones; however, in previously calcium and vitamin D depleted women, lactation may lead to osteomalacia (Davidson 1973).

2:6 Calcium malabsorption

Malabsorption of calcium occurs in a variety of conditions. Vitamin D deficiency causes decreased calcium absorption as does chronic renal disease where patients are unable to metabolise vitamin D to $1,25(OH)_2D_3$ either because of damage to renal tissue or the high plasma phosphate levels which block this metabolic action. Various malabsorption syndromes which impair the intestinal absorptive function and are accompanied by frequent fatty stools are also characterized by decreased calcium absorption.

2:7 Hyperabsorption of calcium

Hyperabsorption of calcium does exist, although for the majority of people in whom it occurs it is not a problem. Overdosage of supplemental calcium plus high intakes of vitamin D, particularly among the overzealous as preventive treatment or as therapy is most frequently the cause of hypercalcaemia. Excessive intake of milk and alkali for peptic ulcers co-existing with renal disease may also lead to hypercalcaemia. Hypercalcaemia occurs secondary to primary hyperparathyroidism; it may also be the result of a hypersensitivity to vitamin D, but this problem is rare.

Hypercalciuria often leads to kidney stones, although the current concept is that is merely increases the 'risk' of stone formation rather than is the cause. Hypercalciuria may be due to high dietary calcium

intake, but is more likely to be caused by high absorption of calcium from the diet (Nordin et.al. 1972).

2:8 Calcium deficiency

Nutritional rickets has been established as a vitamin D deficiency, generally occurring in children under 2 years of age, whose diets may or may not be deficient in calcium and protein. Recently, however, rickets in older children has been reported with the hypothesis that these abnormalities are due solely to calcium deficient diets. Maltz et.al. (1970) reported a child with calcium deficiency rickets, who had a normal vitamin D and phosphorus intake and who responded to a calcium supplemented diet. Pettifor et.al. (1978) investigated nine children with radiological/clinical/biochemical evidence of rickets. The usual causes of rickets (vitamin D deficiency, inherited hypophosphataemic vitamin D resistant abnormalities and renal failure) were excluded and he concluded that low dietary calcium could be the causative factor. Further population studies (of which this thesis is a part) have shown that these children were the most severely affected of a population marked by low serum calcium levels and retarded bone growth (Pettifor 1980). Kooh et.al. (1977) reported a single infant exhibiting calcium deficient rickets; he, too, had no evidence of vitamin D deficiency and healing of the rickets occurred with a change in only the calcium content of the diet. Rudolf et.al.(1980) reported four children with infantile rickets; all had low dietary calcium intakes. Dietary vitamin D was assessed to be inadequate, however the serum vitamin D level was measured in only one child and found to be normal. She did not pinpoint low dietary calcium as the causative factor, but stated that the rickets was "due to factors other than a pure inadequacy of vitamin D".

Osteoporosis is characterized by a reduction in bone mass without a change in percentage composition; studies with radioactive tracers

show that it is an excessive breakdown of bone rather than a lack of new formation. Osteoporosis occurs most commonly in women over 60 years of age; this suggests that the nutrition of bone maintenance, as opposed to bone growth, needs further investigation. Studies of nutritional status have indicated that calcium requirements for the elderly are higher than the standards proposed for adults (Ohlson et.al. 1948, Roberts et.al. 1948, Ackerman and Toro 1953). Opinion is divided about the role dietary calcium plays in osteoporosis; some workers implicate low calcium intake as a cause, others conclude that calcium intakes are not at fault (Albright and Reifenstein 1948, Bogdonoff et.al. 1953, Malm et.al. 1958, Walker 1965, Davidson et.al. 1973). Hormonal deficiencies are also cited as contributing to osteoporosis and studies with calcium supplementation plus hormone therapy have increased calcium retention and delayed osteoporotic activity (Whedon 1957, Nordin 1958, Nordin et.al. 1974).

Osteoporosis occurs less frequently in the Negroid races, but the reason for this ethnic difference is unknown. In the South African Black, osteoporosis associated with siderosis occurs in middle age adult males, but this is probably a special phenomenom; true senile osteoporosis is very rare (Walker 1965).

CHAPTER 3: HUMAN REQUIREMENTS AND RECOMMENDED ALLOWANCES FOR CALCIUM

3:1 Techniques of determining human calcium requirements

The determination of calcium requirements and the setting of dietary allowances is a subject that runs high with strong feelings and much conjecture. Several methods have been employed in an attempt to determine calcium requirements. Total body analysis taking into consideration the relationship between body weight and skeletal weight will estimate calcium deposition at various ages. This figure plus estimates of efficiency of calcium absorption and calcium loss can be used in setting daily calcium requirements.

Balance studies, measuring total intake and excretion of calcium, to determine the dietary calcium level at which intake equals output have been widely used; in fact, the calcium requirements of adults have been determined primarily by this method. Determination of calcium requirements by the balance method has four technical problems: 1) non-random collection errors which tend to increase the apparent retention. 2) adjustment to differing levels of dietary calcium intake may be slow. 3) study periods need to be long enough to overcome irregularities in faecal excretion. 4) no account is taken of dermal loss. Although frequently used, balance studies to determine calcium requirements during childhood are of dubious value because the requirement for optimum growth is unknown.

In recent years isotopic calcium (^{45}calcium or ^{47}calcium) has been used to determine calcium requirements by measuring intestinal absorption, endogenous faecal excretion and urinary losses of calcium from the diet. Measuring dietary calcium intake and relating this figure to growth patterns which are normal for anthropometry, body composition and

bone density has been used to estimate requirements during periods of growth and during aging.

A significant finding with all methods of investigating calcium needs has been the great variation among individuals, further complicating the determination of standard calcium requirements.

3:2 Calcium requirements during childhood

Considering that calcium represents 0,1-0,8% of early foetal fat-free weight, this means, in practical terms, a mean increment in calcium deposition of 160-180 mg per day during the 20 year growth period. Of course, calcium storage does not occur at this even rate, but is retained at rates that vary enormously throughout growth. Relating skeletal growth and the consequent increase in total body calcium to total body weight gain, the estimated daily increment in skeletal calcium at various ages is shown in Fig. 3:1. Balance studies have shown,

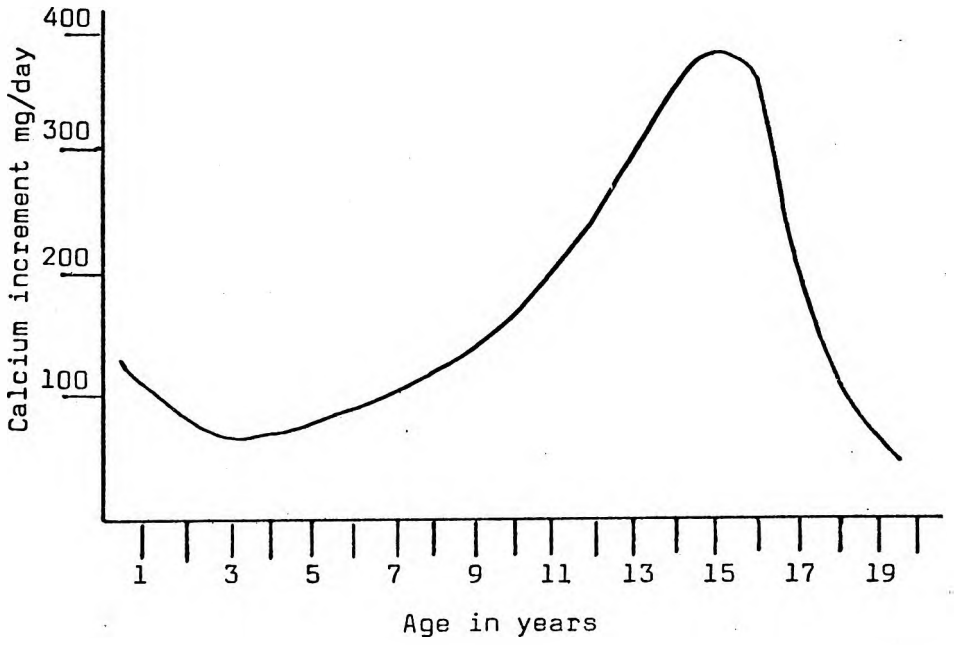


Figure 3:1 The estimated daily increment in skeletal calcium of children at various ages. (Leitch and Aitken)

however, that actual calcium retention may be higher than the theoretical retention estimated as outlined on the previous page, especially during periods of slower growth (Holmes 1945, Sterns 1952).

Children need to be in positive calcium balance, but the degree of positive balance optimal at different ages is unknown. Sherman and Hawley (1922) in a series of balance studies with increasing calcium levels found that calcium retention increased until the dietary intake provided 1 gram of calcium per day; at higher calcium intake levels, calcium retention showed only minimal increases. They concluded that an intake of 1 gram of calcium per day provided for optimum calcium storage from 3 to 13 years of age. Other investigators at this time reported similar results relating to intake and retention of calcium (Wang et.al. 1928, Willard and Blunt 1927). These early studies did not indicate if subjects received an adequate amount of vitamin D; later workers made provision for some form of vitamin D supplementation and found that adequate calcium retentions could be obtained with lower calcium intakes. A summary of the balance studies on children 2 to 18 years is presented in Table 3:1, showing calcium intake per day, calcium intake per kilogram body weight and retention in milligrams calcium per kilogram body weight. All subjects were white children (American or European) unless otherwise noted. As can be seen the studies cover a wide range of calcium retentions reflecting both individual variations in calcium metabolism and differences in balance techniques. The fact that lower calcium intakes will provide positive calcium balance in the Ceylonese, Indian and African studies has been explained several ways: 1) adaptation to constantly low calcium intakes by more efficient absorption of dietary calcium. 2) increased vitamin D from greater exposure to the sun in the tropics enhancing calcium utilization. 3) these children tend to be smaller than their European counterparts and when calcium requirements are

Table 3:1 Summary of findings of calcium balance studies on children

Age Group (years)	No.of Subjects	Calcium Intake		Calcium Retention mg/kg	Author
		mg/day	mg/kg		
2-4	11	200-460		5-13	Yeh & Adolph 1939 (Chinese subjects)
2-6	12	615-1800	95-110	7-8	Outhouse et al 1939
2-6	7	1650-1895	95-110	12-14	Holt & Fails 1923
3-5	28	204	15	7	Begum & Pereira 1969 (Indian subjects)
3-5	10	800-1350		3-10	Daniels et al 1934
3-6	10		35-100	5-14	Daniels et al 1929
3-6	10	700-1100	41-61	11-12	Pierce et al 1940
3-6	7	720	45	9	McLean et al 1940
4-7	4	183-245	12-18	5-11	Nicholls & Nimalasuriya 1939 (Ceylonese subjects)
6-12	17		15-30	7-11	Holemans & Lambrechts 1945 (African subjects)
6-13	9	991	23	7-14	Sherman & Hawley 1922
7-8	12		20-40	2-16	Petrinkina 1934
8-11	8	1295	40	8	Lutwak et al 1964 (Amer. Indians)
12	1	1280	44	13	Willard & Blount 1927
12-13	4	1495-1520	45-55	4-8	Wang et al 1928
12-15	23	1604	42	11	Wang et al 1936
12-13	5	849-1109	32-33	6-10	Herbst 1912/1913

expressed in mg/kg body weight, they are more in line with other studies.

Dietary surveys combined with measurement of growth and skeletal maturation in healthy children should ideally provide a method of establishing calcium requirements by allowing for the study of a large number of children. However, this does not seem to be so, for results of such surveys have shown that there is adequate calcification on a wide range of dietary calcium intakes and that there is no significant correlation between calcium intake and skeletal maturation (Allan et.al. 1953, Cahn and Roche 1961). Epidemiological work of this kind also must take into account optimum potential growth when considering calcium needs.

Determining calcium requirements by the 'factorial method' of figuring estimated calcium accretion and maintenance requirements, endogenous loss and utilization of dietary calcium theoretically should produce values

satisfactory for the general population. However, different workers cannot agree on figures for any of the four factors involved and so produce widely varying estimates of calcium requirements.

Studies estimating calcium requirements for South African Black children have, in addition to the problems besetting all studies, several inherent difficulties: 1) ages of Black children, particularly rural children, may not be accurately known. 2) growth patterns of African children which may be influenced by ethnic and environmental factors have not been determined. 3) the general increase in height characteristic of succeeding generations of European/American/Asian children has not affected African children (Walker 1954). Eighty-five to 90% of the South African Black population has a dietary calcium intake of 250-450 mg per day and although bone growth and mineral content may be less than that in the White population, Black adults are similar in total bone calcium and phosphorus and metacarpal cortical thickness and height to South African Whites and Indians (Walker 1966, Walker 1972, Walker et al 1971). The most well-known worker in this field in South Africa, Professor A.R.P. Walker, concludes, "Finally,... the writer maintains that diets that permit growth and are consistent with everyday good health contain enough calcium for physiological purposes."

Table 3:2 summarizes calcium requirements for different age groups suggested by various workers using the methods cited in the text.

3:3 Calcium requirements in adulthood

Balance studies have been the method primarily used in establishing calcium requirements for adults. Calcium intake in adult life needs to be sufficient to preserve calcium balance and to maintain an intact skeleton. Although, as with calcium balance studies during childhood, there is a wide variation in results, it is generally agreed that a

Table 3:2 Calcium requirements of children as determined by studies employing various criteria.

Age Group (years)	No. of Subjects	No. of Studies	Principle Criteria of Study	Calcium Requirement	Author
2-5	166	1	Roentgenology	0,2-0,7 g	Allen 1953
	69	10	Retention	0,46-1,12 g 43-64mg/kg	See Table 3:1
		3	Calcium accretion	0,5-0,9 g 30-45mg/kg	Holmes 1945 Leitch 1959 Mitchell 1939
6-12	69	7	Retention	0,2-1,3 g 12-40mg/kg	See Table 3:1
		4	Calcium accretion	0,285-1,53 g	Holmes 1945 Leitch 1959 Mitchell 1939 Nicholls 1939
13-16	41	6	Retention	0,9-1,6 g	See Table 3:1
		3	Calcium accretion	1,0-2,6 g	Holmes 1945 Mitchell 1939 Nicholls 1939

calcium intake of 9 mg/kg body weight, 500-600 mg daily, is necessary to meet calcium needs. This requirement may need to be increased with age to compensate for increased bone resorption, basal calcium excretion and decreased absorption of dietary calcium that accompanies aging. Whether these changes are due to hormonal regulation or an alteration in the metabolism of vitamin D rather than an increased need for calcium per se are problems currently being investigated and argued. However, a few studies have shown retarded bone loss with calcium supplementation in the elderly (Albanese et. al. 1973, Nordin et.al. 1974).

That normal young adults can adapt to low calcium intake has been shown most reliably by the classic studies of Malm working with Norwegian prison volunteer subjects (Malm 1953, 1958). Adaptation depends at least in part on the hormonal metabolite of vitamin D, $1,25(OH)_2D$. The probable mechanism involved in adaptation is shown diagramatically in Fig 3:2. Adaptation involves a varying period of

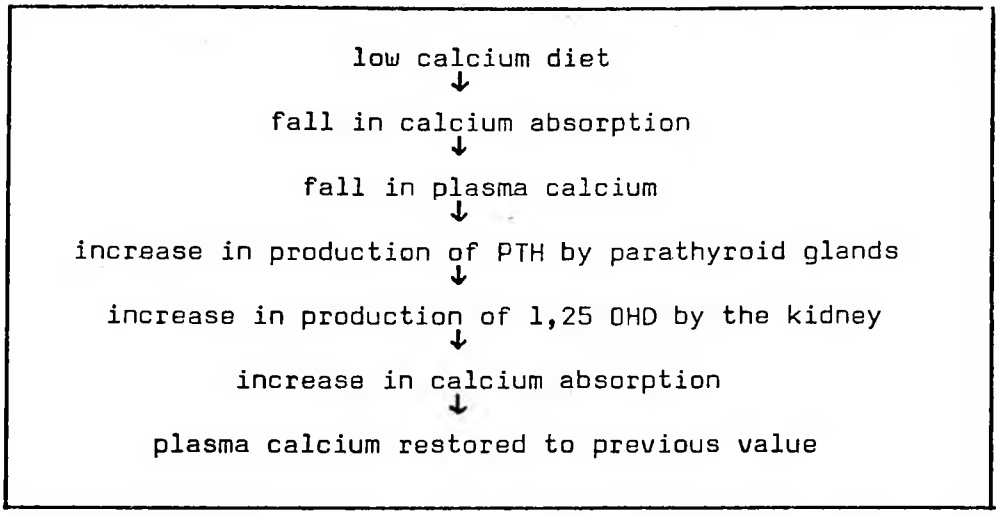


Figure 3:2 Schematic diagram of the probable mechanism involved in adaptation to low dietary calcium intake.

negative calcium balance during which there may possibly be irreplaceable bone loss or other damage suffered by the skeleton. Using these studies as a base for calcium requirements would seem unwise. These limitations are to short term studies of calcium adaptation as opposed to studies among populations whose habitual dietary calcium intake is low.

3:4 Recommended calcium allowances

The foregoing discussion has been concerned with requirements, the mean amount of calcium which will meet the needs of the average person. A recommended allowance should be the amount which when consumed by a population meets the needs of 95% of them. Given the wide range of results of studies of calcium requirements, substantially higher recommended allowances have been made. Table 3:3 lists the daily allowance for calcium for children recommended by various countries and/or organisations.

It has been argued that the recommended daily allowances of the U.S. Department of Agriculture are unreasonably high, but they reflect the standard of optimum nutrition in an affluent society. The FAO/WHO Committee has given substantially lower levels for calcium intake, but

the report of the International Committee (FAO/WHO 1960) used the term 'suggested practical allowances', practical being the determinative word when dealing with underdeveloped nations. The recent recommendations of some European countries have followed the FAO/WHO reports and have lowered their recommended daily allowances.

Table 3:3 Recommended daily allowances for calcium (in milligrams) for children set by various countires/organisations

Country or Organisation Setting Allowance	Recommended Daily Intake of Calcium (mg) Age in Years			
	1	1-9	10-15	16-19
FAO/WHO[1]	500-600	400-500	600-700	500-600
USDA[2]	360-540	800	1200	1200
U.K.[3]	600	500	600-700	600-700
S.Africa[4]		700-800	1200-1300	
Canada[5]	500	500		
India[6]		400-500	600-700	

[1] Food and Agriculture Organisation/World Health Organisation 1973
[2] U.S. Department of Agriculture 1973
[3] U.K. Department of Health and Social Security 1969
[4] Dietary Standards Committee, National Nutritional Council, South Africa 1956
[5] Canadian Council on Nutrition 1964
[6] Indian Council of Medical Research 1968

As absorption of dietary calcium is influenced by vitamin D and other factors, possibly the FAO/WHO recommendations are adequate allowances for underdeveloped countries, most of which are tropical or sub-tropical and have few or no large cities with high buildings and/or smog to screen out sunlight. Too, populations used to constantly low calcium intakes may utilize dietary calcium more efficiently. Quite possibly there may need to be different recommendations for different population groups and/or geographical areas. Many workers seem to think, however, that the FAO/WHO recommendations are inadequate to meet the optimal calcium needs during the pubertal growth spurt (Nordin 1976).

CHAPTER 4: SOURCES OF DIETARY CALCIUM; CALCIUM SUPPLEMENTS

4:1 Dietary Calcium

Calcium intake varies greatly from country to country as well as among individual populations. Table 4:1 lists the daily calcium intake in various parts of the world (Wohl and Goodhard 1960, FAO/WHO 1962).

Table 4:1 Daily dietary calcium intake per capita in various countries

Country	Calcium Intake mg/day
Argentina	651
Australia	833
Brazil	486
Canada	1047
Chile	520
Colombia	240
Denmark	1000
Ecuador	380
Finland	1329
France	930
Holland	980
India	347
Israel	884
Italy	833
Jamaica	345
Japan	368
Lybia	313
New Zealand	1165
Norway	856
South Africa	442
Thailand	266
Turkey	547
U.K.	1000
U.S.A.	1616
Venezuela	870
Vietnam	680

When the subject of dietary sources of calcium is mooted, milk and other dairy products are at the forefront of the discussion. Much of the early work of estimating calcium requirements by balance studies used milk as the main source of calcium because it was a common food among the people participating in the studies and because it made varying the amount of calcium in the diet possible without drastically changing usual eating habits. Thus, when results were published and recommendations made, they usually included the guideline that the daily calcium allowance could be met by the intake of a pint of milk daily. This guideline has been held for 60+ years and comes to us, popularly, as 'drinka pinta' or metrically, 'eata litre'. Indeed, for most Western nations, milk and milk products are the principle dietary sources of calcium. Table 4:2 shows the percentage of calcium in food stuffs supplied by dairy products for various countries. Despite the degree

Table 4:2 Percentage daily intake of calcium supplied by dairy products for various countries

Western Countries		Eastern Countries	
Country	Percentage calcium supplied by dairy products	Country	Percentage calcium supplied by dairy products
Argentina	70,7	India	42,4
Australia	74,4	Ivory Coast	0
Canada	81,6	Japan	15,8
Denmark	74,9	Turkey	43,8
France	66,5	United Arab Republic	31,6
New Zealand	79,5		
South Africa	63,3	Venezuela	34,7
U.K.	58,0	Vietnam	19,7
U.S.A.	76,7		

of optimum calcium nutrition, milk products supply 50% of the total intake in the Western countries. Contrary to this, most Eastern

populations and underdeveloped countries have less of their dietary calcium coming from dairy products.

Of course it is possible to maintain adequate calcium nutrition with a small or no intake of dairy products. Some balance studies have been conducted specifically to test other sources of calcium; Yeh and Adolph (1939) working with children in northern China used vegetables and bone ash; Begum and Pereira (1969) used green vegetables in studies with Indian children and various other studies in India have used indigenously produced plant foods (Karnani et.al. 1948, Sur et.al. 1955, Joseph et.al. 1958, 1959). The calcium content of some representative foods is shown in Table 4:3.

Expressing calcium intake as food needed to provide the recommended daily allowance can be instructive as the following example shows. The FAO/WHO recommended daily allowance for calcium is: 1-9 years, 400-500 mg; 10-15 years, 600-700 mg; 16-19 years, 500-600 mg. Table 4:4 gives three alternate lists of foods that will provide for these daily allowances: 1) milk as the primary source of calcium, 2) mixed diet and milk, 3) mixed diet with a minimum of milk. These lists are solely outlines written specifically to meet calcium requirements; they include foodstuffs commonly eaten by the South African Black. With the addition of stiff mealie meal porridge in the quantity usually consumed by each age group, they would provide adequate energy and protein intakes. The menu patterns written using a minimum amount of milk are cumbersome; the amount of food to be consumed is likely to be greater than the appetite of the younger child and the inclusion of a variety of foodstuffs not produced by the rural Black would make it expensive. The food lists in Table 4:4 were costed and at all three age levels, list 1 (milk as the main source of calcium) was considerably cheaper (33-41 cents as opposed to 38-81 cents for lists 2 and 3); see Appendix A for detailed costing. Much more practical would be a nutrition education program stressing the

Table 4:3 Calcium content of various foods

Food	Calcium mg/100g	Ref
Milk Products		
Whole milk	120	
Skimmed milk	124	1
Condensed milk	290-385	
Dried milk	895-1225	
Butter	15	1
Cheese, soft	362-540	1
hard	810-1200	
Cereals		
Rice, uncooked	24	2
Oatmeal, uncooked	53	
Bread, white	41	
Bread,brown	47	3
Maize meal, sifted, uncooked	3	
Maize meal, unsifted, uncooked	5	
Meat		
Beef	11	
Chicken	12	
Lamb	10	2
Liver	8-12	
Pork	7	
Fish		
Hake	41	2
Kabeljou	11	
Pilchards	33	3
Prawns	100	
Fruit		
Apples	7	
Grapes	21	
Grapefruit	23	3
Pawpaw	11	
Spanspek	6	
Nuts/pulses		
Dried beans	163	2
Peanuts	74	
Vegetables		
Carrots	37	
Cabbage	39	
Potatoes	13	3
Pumpkin	18	
Spinach	58	
Sweet potatoes	24	

[1]Sinclair and Hollingsworth (1969)
[2]United States Department of Agriculture (1950)
[3]Fox (1966)

Table 4:4 Food lists to meet FAO/WHO recommended daily allowance for calcium, using: 1) milk as main source of calcium
2) milk and mixed diet
3) mixed diet, minimum milk

Diet	1-9 years allowance: 400-500 mg/day				10-15 years allowance: 600-700 mg/day				13-16 years allowance: 500-600 mg/day			
	Weight	Measure	Food	Calcium mg	Weight	Measure	Food	Calcium mg	Weight	Measure	Food	Calcium mg
1		500 ml	Whole milk	410		625 ml	Whole milk	510		625 ml	Whole milk	510
	90 g	3 slices	Brown bread	45	120 g	4 slices	Brown bread	60	120 g	4 slices	Brown bread	60
					150 g	1 cup	Potato	40				
					100 g	1 cup	Vegetables	20				
			Daily intake	455			Daily intake	630			Daily intake	570
2		250 ml	Whole milk	205		375 ml	Whole milk	305		375 ml	Whole milk	305
	90 g	3 slices	Brown bread	45	180 g	6 slices	Brown bread	90	180 g	6 slices	Brown bread	90
	75 g	½ cup	Potato	20	150 g	1 cup	Potato	40	75 g	½ cup	Potato	20
	100 g	1 cup	Vegetables	20	100 g	1 cup	Vegetables	20	50 g	½ cup	Vegetables	10
	75 g	½ cup	Cooked rice	10	150 g	1 cup	Cooked rice	20	150 g	1 cup	Cooked rice	20
	100 g	1 cup	Cooked dried beans	100	100 g	1 cup	Cooked dried beans	100	100 g	1 cup	Cooked dried beans	100
	100 g	2 pieces	Fruit	20	150 g	3 pieces	Fruit	30	100 g	2 pieces	Fruit	20
			Daily intake	420			Daily intake	605			Daily intake	565
3		40 ml	Whole milk	25		40 ml	Whole milk	25		40 ml	Whole milk	25
	180 g	6 slices	Brown bread	90	240 g	8 slices	Brown bread	120	240 g	8 slices	Brown bread	120
	150 g	1 cup	Potato	40	300 g	2 cups	Potato	80	300 g	2 cups	Potato	80
	100 g	1 cup	Vegetables	20	200 g	2 cups	Vegetables	40	100 g	1 cup	Vegetables	20
	150 g	1 cup	Cooked rice	20	225 g	1½ cups	Cooked rice	30	150 g	1 cup	Cooked rice	20
	100 g	1 cup	Cooked dried beans	100	150 g	1½ cups	Cooked dried beans	150	100 g	1 cup	Cooked dried beans	100
	50 g		Tinned fish	100	50 g		Tinned fish	100	50 g		Tinned fish	100
	15 g	1 Tbsp	Peanut butter		15 g	1 Tbsp	Peanut butter		15 g	1 Tbsp	Peanut butter	
		OR		15	OR			15		OR		15
	15 g		Meat/chicken		15 g		Meat/chicken		15 g		Meat/chicken	
	100 g	2 pieces	Fruit	20	200 g	4 pieces	Fruit	40	200 g	4 pieces	Fruit	40
					15 g	1 Tbsp	Peanut butter	15	15 g	1 Tbsp	Peanut butter	15
			Daily intake	430			Daily intake	615			Daily intake	535

use of high calcium dairy products. This is particularly pertinent to the South African Black for his diet does not normally contain any food other than milk as a superior source of calcium.

Calcium nutrition in vegetarians has not been widely studied; however adequate calcium nutrition is possible. Dwyers et.al. (1979) investigating children following vegetarian and/or 'macrobiotic' diets found calcium intakes of 300-600 mg per day at ages 1 to 6 years; this overlaps the FAO/WHO recommended allowances.

4:2 Calcium supplements

The necessity and desirability of using calcium supplements for the general population sparks as much discussion as does the supplementation of any vitamin or mineral on an epidemiological basis. Britain introduced calcium supplementation of bread flour (as calcium carbonate) during World War II to counteract the threat of rickets due to the use of high extraction whole wheat flour which had high levels of phytic acid. This reasoning may have been incorrect but the fortification of flour has continued although probably based on the suggestive evidence that increased calcium possibly is protective against cardiovascular disease, rather than to meet calcium requirements per se (Crawford 1968). Presently, there is great controversy about fortifying the chapatty flour used by Asian immigrants to the U.K. as part of a calcium-vitamin D supplementation program to control the increase in the number of cases of rickets among this population (Dunnigan et.al. 1976, Robertson et.al. 1977, Singleton and Tucker 1978, Compston 1979).

The United States fortifies bread with 4% milk solids, providing a six-fold increase in the calcium content, but unfortified bread is also available.

For several years, South Africa supplemented brown bread with a 3-4% premix of skim milk powder, defatted groundnut meal and a calcium salt. This was aimed at the problem of protein-energy malnutrition, but

was discontinued in 1960 because it did not make a significant contribution against the deficiency conditions it was designed to prevent (Walker 1963).

As previously stated, there is little or no evidence that calcium is a limiting factor to growth in humans. There have been reports supporting increased growth with calcium supplementation and studies noting no difference in growth patterns between supplemented and unsupplemented groups (Aykroyd and Kinshnam 1938, 1939, Bansal et.al. 1964, Luyken et.al. 1967, Daraiswamy et.al. 1977). Pettifor et.al. (1981) in a study to determine the effects of calcium supplementation on serum calcium levels of rural Black school children in South Africa found no difference in growth between supplemented and unsupplemented groups over the three months of the study; growth, however, was not one of the primary considerations in planning this short-term study. Investigations into calcium supplementation and the effect on growth would certainly need to be long-term in nature, covering, if possible, a whole generation.

Maize meal, the staple of the rural South African Black's diet is very low in calcium. The practicality of fortifying the meal with calcium is virtually nil with many rural people grinding their own meal or having it done on a community basis. The desirability or necessity of such fortification is also very slight based on experimental scientific evidence. Most rural Blacks are not vegetarian for religious or ethnic reasons and readily include animal products in their daily diet if possible. People usually have cattle and goats and teaching better management of their beasts and the necessity of giving the children the milk produced by the animals would be easier and more economical than considering calcium supplements.

CHAPTER 5: CALCIUM STATUS OF BLACK CHILDREN IN A RURAL S.A. COMMUNITY

Calcium requirements have traditionally been established by the calcium balance technique, but the value of such studies in childhood is doubtful because the requirement for optimum growth is unknown. Both individuals and population groups vary enormously in their need for calcium as established by various techniques. The fact that most people in developing countries appear to be physiologically normal on calcium intakes which may be very low has been explained by long term adaptation, increased vitamin D and smaller stature, but, again, does not take into account the calcium requirement for optimal growth.

Recommended intakes of calcium also vary enormously, depending on the body drawing up the recommendation. The FAO/WHO Committee recommendation is considered to be the minimum to allow for normal growth; it provides very little leeway for individual variation. Many Western nations with more developed economies have recommended a generous intake of calcium, and other countries make recommendations that try to be practical as well as optimal.

In spite of the obvious need for calcium in bone formation, indisputable signs of calcium deficiency in humans are lacking. Although there is little or no evidence that calcium alone is a limiting factor to growth, the doubling and trebling of calcium intakes may be a contributory factor to the increased growth observed over several generations in various countries. However, there is no scientific evidence to support this increase alone as being decisive rather than the combination of it, the increase in energy intakes and the increase in quality and amount of protein intake. Findings are accumulating, however, that low dietary calcium may produce

rickets in infants and children (Maltz et.al. 1970, Kooh et.al. 1977, Pettifor et.al. 1978).

5:1 Calcium deficiency in the rural South African Black

Rachitic bone deformities in Black juveniles admitted to Baragwanath Hospital have been noted over the past 20 years, and it has been thought that the pathogenesis of this condition was not due to vitamin D deficiency (Taitz 1962). However little effort was made to investigate this problem until recently, when Pettifor (1980) proposed that low dietary calcium intakes could be the causative factor. He investigated thirteen children admitted to hospital whose rickets healed on a normal diet with or without clacium supplements. The children were not vitamin D deficient, and renal abnormalities were ruled out as the cause of the condition. Most of the children came from a circumscribed area (Driefontein) in the Eastern Transvaal (near Piet Retief); a further random sampling study undertaken to determine if the problem was confined to this area showed that children in Driefontein had significantly lower serum calcium and higher serum alkaline phosphatase levels than children from either a nearby urban community or from Soweto, near Johannesburg (Pettifor et.al.1980). A further investigation into the aetiology of this problem involved dietary calcium supplementation (Pettifor et.al. 1981). The study group, given a supplement of 500 mg clacium daily, had a significant rise in serum calcium levels after one month and a significant drop in serum alkaline phosphatase levels after 2 months, while the control group, receiving a placebo, showed no significant changes in either serum calcium or serum alkaline phosphatase.

Preliminary work suggested that the children exhibiting the bone deformities accompanied by the biochemical abnormalities (lowered serum calcium, raised serum alkaline phosphatase) had calcium intakes considerably lower than the FAO/WHO recommended allowances (Pettifor 1980).

A further study, planned as a baseline investigation for a socio-logical/health intervention programme, of the whole community of Driefontein allowed for controlled dietary investigations. These investigations were two-fold:

1) to establish if the observed biochemical abnormalities of calcium metabolism were related to dietary calcium intake.
2) to establish the reliability of the dietary history including a 24-hour dietary recall as an investigative technique in a rural South African Black community.

5:2 Selection of subjects

Children were chosen both from randomly selected and volunteer subjects participating in a general health investigation conducted as part of a calcium research project in the Driefontein area (Pettifor, unpublished data). Fifteen children at each age level, 3-5 years, 8-10 years, 13-16 years with lowered serum calcium ($<$9,0mg/dl) and raised serum alkaline phosphatase ($>$300 I.U./l) levels were selected. (N.B.: as serum alkaline phosphatase levels were uniformly within the normal range in the 3-5 years age level, children solely with low serum calcium concentrations were selected for study.) Control subjects with normal serum calcium and alkaline phosphatase concentrations were age/sex matched to the study children as far as possible.

5:3 Biochemical and physical investigation and sampling techniques

Anthropometric data, a blood sample and a random urine specimen were obtained from each child. The measurements recorded were height, weight and triceps skinfold thickness; all measurements were carried out by trained, experienced lay staff members. Bone density was measured on a Norland Bone Mineral Analyser by medical personnel trained in the use of the machine. On all subjects 5 years and older, the average of three separate measurements of bone width and bone mineral content of

the radius measured midway between wrist and elbow was used. An x-ray of the right hand and wrist was taken by a qualified radiographer. Each child was given a general examination by a physician.

Blood was taken by venepuncture and collected in evacuated plain glass tubes with rubber stoppers(Vacutainers): sera was stored at -20°C until the various chemical analyses could be performed. The biochemical investigations were carried out by standard methods and include: serum albumin (Northam et.al. 1967); serum calcium (Duncan 1976); serum phosphorus and creatinine (Technicon Manual 1972); serum alkaline phosphatase (Morgenstern et.al. 1965). As samples were collected as part of a field study, care was taken to ensure subjects had not eaten for at least two hours before sampling in an effort to minimise the effect of dietary intake on the results obtained; food was provided for the subjects following examination.

Random urine samples were collected from subjects and an aliquot stored at -20°C until analysis. Urinary calcium excretion was calculated from the calcium:creatinine ratio (Uca:Ucr). All biochemical tests performed on the children were carried out in the laboratory of the Metabolic and Nutritional Research Unit at Baragwanath Hospital; and analysis of both urine and serum samples was completed within 6 weeks of collection.

Normal values for children for the analyses carried out as part of this study are listed in Table 5:1. As far as possible, these values were obtained from studies carried out in the Metabolic and Nutritional Research Unit using the methods cited.

5:4 Statistical analysis

The data were computerized and analysed by standard methods using the SPSS package programmes (Nie et.al. 1975). Significance limits were determined by the student's one way-t-test unless otherwise noted.

Table 5:1 Normal values for children for the biochemical investigations carried out in the study.

Biochemical Variable	Normal Range	Reference
Serum albumin	3,5-5,0 g/dl	White et al 1970
Serum calcium	9,0-10,4 mg/dl	Jowsey 1976, Pettifor et al 1978
Serum phosphorus	3,6-6,0 mg/dl	Round 1973, Pettifor et al 1978
Serum alkaline phosphatase	100-300 IU/l	Pettifor et al 1978
Uca:Ucr	0,03-0,28	Nordin 1959

5:5 Outline of subject groups

Table 5:2 outlines study and control groups at each age level and lists the results of investigations indicative of general nutritional status. Two study subjects at age level 1 had moved away from Driefontein; their matching control subjects were eliminated (13 in each group remained). At age level 3, one study subject had also moved away from the area and one subject proved to be totally unreliable and was therefore excluded from the study; age level 3 controls were eliminated to make the numbers equal (13 in each group). Although efforts were made to have controls sex matched to study subjects, it was not always possible; sex distribution is listed in the table. There were no significant differences in the general make-up of the study and control groups at any age level ($p > 0,5$).

Table 5:2 Results of anthropometric investigations on children in the dietary survey.

Investigation	Age level 1		Age level 2		Age level 3	
	Study	Control	Study	Control	Study	Control
Number of subjects	13	13	15	15	13	13
Sex	5M,8F	5M,8F	5M,10F	3M,12F	7M,6F	8M,5F
Age in years	4,9 ± 0,7	4,7 ± 0,6	9,3 ± 1,6	9,2 ± 1,3	14,7 ± 1,1	15,0 ± 1,2
Height in cm	99,0 ±15,0	103,7 ± 7,5	126,2 ±10,9	124,2 ± 5,6	151,4 ± 8,6	151,9 ±10,0
Weight in kg	15,2 ± 2,6	15,0 ± 2,0	24,4 ± 6,0	22,5 ± 3,1	40,5 ± 7,2	39,4 ± 9,8
Skinfold thickness in mm	9,3 ± 1,5	8,1 ± 2,2	8,2 ± 1,8	7,5 ± 2,2	9,3 ± 4,6	7,7 ± 3,2
Serum albumin g/dl	3,9 ± 0,4	4,2 ± 0,5	4,2 ± 0,5	4,1 ± 0,4	4,2 ± 0,3	4,4 ± 0,2

Figure 5:1 shows the height and weight for age plotted on the Boston percentile growth charts. There were no significant differences in growth patterns (height and weight for age, $p > 0,5$ for both parameters at all age levels, and standard weight for height, $p > 0,5$ at all age levels) between the study and the control groups at any age level.

There were no visible bone derformities, nor signs of inadequate calcium nutrition (enamel hypoplasia, widening of the epiphyses, Harrison's sulcus) in any of the children.

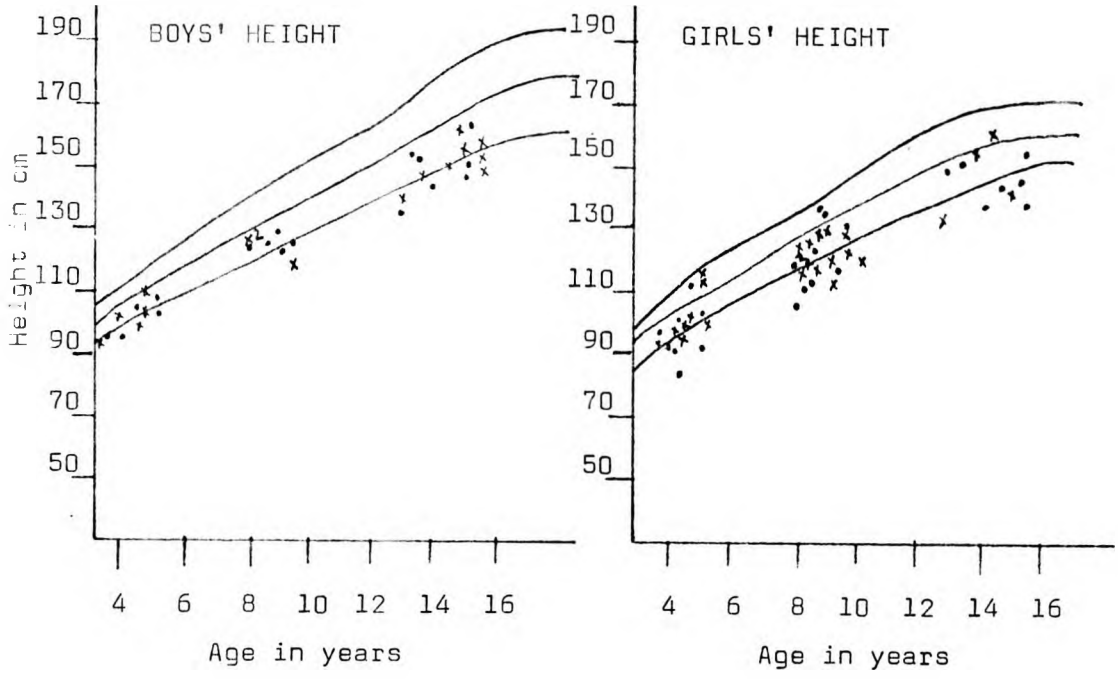
Table 5:3 shows the results of the biochemical and radiological investigations carried out. Serum calcium levels corrected for serum albumin levels (labelled corrected serum calcium) were calculated and the results are included in the table (Payne et.al. 1973).

Table 5:3 Biochemistry and radiology investigations on study and control groups.

Investigation	Age level 1		Age level 2		Age level 3	
	Study	Control	Study	Control	Study	Control
Serum calcium mg/dl[1]	8,86 ±0,26	9,59 ±0,23	8,57 ±0,89	9,76 ±0,26	8,50 ±0,80	9,58 ±0,35
Corrected serum calcium mg/dl	8,97 ±0,32	9,44 ±0,40	8,43 ±0,77	9,70 ±0,46	8,33 ±0,83	9,23 ±0,45
Serum phosphorus mg/dl	4,8 ±0,9	4,5 ±0,7	5,1 ±0,4	4,7 ±0,5	4,5 ±0,9	4,4 ±0,4
Serum alkaline phosphatase IU/l[2]	228 ±130	218 ±120	364 ± 50	186 ± 51	500 ±181	214 ± 84
Calcium excretion Uca:Ucr	0,0125 ±,0086	0,0163 ±,0133	0,0155 ±,0196	0,0151 ±,0127	0,0061* ±,0057	0,0159 ±,0198
Bone mineral density[3] g/cm^2	0,28 ±0,06	0,34 ±0,02	0,34* ±0,10	0,41 ±0,06	0,42** ±0,08	0,54 ±0,06
Metacarpal cortex thickness, mm	1,74 ±0,49	2,09 ±0,89	2,35* ±0,73	2,67 ±0,31	3,09 ±0,87	3,30 ±0,74

[1] Criteria for selection, $p < 0,0005$
[2] Criteria for selection, age levels 2 and 3, $p < 0,0005$
[3] Measured on Norland Bone Mineral Analyser

*$p < 0,05$, compared to control group
**$p < 0,0005$, compared to control group



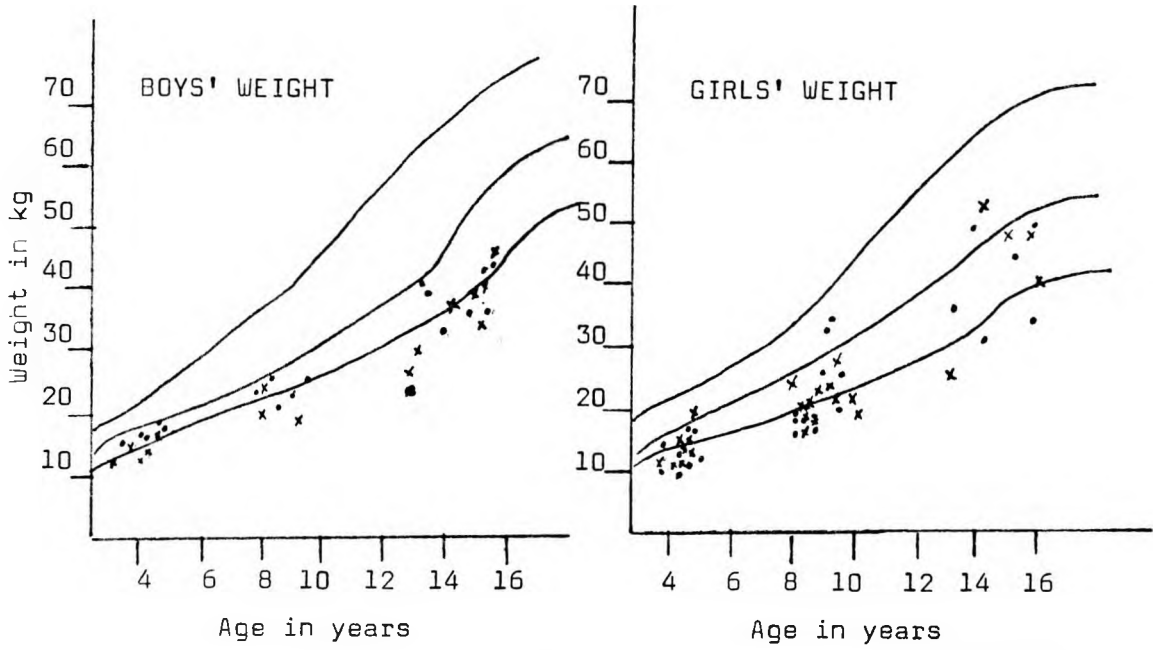


Figure 5:1 Height and weight for age of study children (•) and control children (x).

Because it was not possible to obtain 24-hour urine samples, urinary calcium excretion was calculated from the calcium creatinine ratio of a random urine sample (Uca:Ucr). Nordin (1959) found a good correlation between 24-hour urinary calcium excretion and the Uca:Ucr of random, non-fasting samples. Fellers and Herrera (1972) demonstrated no significant difference between the Uca:Ucr of 24-hour urine collections and non-fasting random urine samples. For South Africans, Walker (1966) has reported calcium excretion (mg/24 hours) in rural Black children to be approximately one-third and in urban Black children to be approximately one-half that of White school children. Mean urine calcium excretion judged by Uca:Ucr is within this range reported by Walker for the control groups at age levels 1 and 2; it is about half the normal level for the study groups at age levels 1 and 2 and for the control group at age level 3. The study group at age level 3 had extremely low urinary calcium excretions; mean Uca:Ucr, plus 2 S.D., is only half that reported by Walker.

Bone mineral density is expressed as gm/cm^2 and was obtained by dividing the results as measured on the Norland Bone Mineral Analyser (mean bone mineral content $\div$ mean bone width). Cortical thickness was measured using vernier calipers at the midway point of the second metacarpal of the right index finger; it is the total breadth of the bone minus the width of the medulla and is expressed in mm.

The results of both these parameters are significantly lower than measurements of White children, and cortical thickness is significantly lower than that of other Black South African children (Mazess and Cameron 1974, Walker 1971). At age levels 2 and 3, when there is a sharp increase and peak in the need for calcium, bone density and x-rays show a correspondingly significant lowering of measurements in the study groups as compared to the control groups.

5:6 Discussion of anthropometric results

Height and weight were below the standards for healthy children of other races in more developed countries; findings are in accord with other work undertaken in developing countries (Schrimshaw and Gordon 1968, Sloan 1969, Fisher and Davidson 1970). Similarly, triceps skinfold measurements are lower than those of healthy children in more developed countries, although they are higher than those measured by Fisher and Davidson in Zambian children.

Forty-five percent of the children were below the third percentile on the Boston weight for age charts; this is comparable to a similar study in Soweto where 40-45% of the children 6-16 years were under the third percentile on the weight charts (Shuenyane et.al. 1977). Fewer of the children in this study were under the third percentile for height than the children in the Soweto study (39% and 58% respectively). Studies in South Africa have reported remarkably similar results over the years; Brock and Latsky (1942) found 56,9% of a group of volunteer Black children 8-16 years of age to be of satisfactory nutritional status. Although nearly half the children were below the third percentile on the Boston weight for age charts, the majority were greater than 90% of standard weight for height. This fact and the finding of mean serum albumin concentrations of 3,9 g/dl or greater would indicate that the children, although small for age, are on the whole adequately nourished. Poor growth of Black children has been judged to be due to environmental factors. A study of 'priviledged' (sic) African children (whose mothers were nursing sisters with the Johannesburg City Health Department) showed growth that was adequate when judged on the Harvard growth for age charts (Kahn and Freidman 1959).

5:7 Discussion of calcium status

Ideally, measurement of ionized serum calcium would most truly reflect the calcium status of an individual as this is the metabolically active portion of measured serum calcium; however, technical problems, particularly in conducting field studies, make this impractical. Calcium-protein interaction has been studied in great detail and studies show that albumin accounts for 90% of the protein bound calcium (Pederson 1971). Measuring total serum calcium and correcting it for differences in serum albumin concentration is an alternative to measuring ionized serum calcium and is used by many researchers (Dent 1962, Parfitt 1969, Berry et.al. 1973, Payne et.al. 1973). Use of this calculation/correction method depends on the uniformity of protein-albumin binding and is disputed by other workers (Pain et.al 1975, Ramsey and Shelton 1976). The formula of Payne (1973), corrected serum calcium (mg/dl) = total serum calcium (mg/dl) - serum albumin (g/dl) + 4,0, has been used in this study to calculate serum calcium corrected for serum albumin and this parameter is used in defining relationships with other factors studied.

Although the corrected serum calcium levels are slightly different to the serum calcium concentrations as measured, the results for the control groups remain within the normal range and those for the study groups are low, and the two groups at every age level are still highly significantly different ($p < 0,00025$, age level 1; $p < 0,0005$, age level 2; $p < 0,0025$, age level 3). There were no significant differences between the measured and corrected serum calcium levels ($p > 0,5$ for each group) indicating that the low serum calcium concentrations are not due to variation in the level of serum albumin.

There was no relationship between corrected serum calcium and calcium excretion as estimated by Uca:Ucr for age levels 1 and 2 ($r = 0,0686$ and $r = 0,0493$ respectively) and a significant relationship

between the two only at age level 3 ($p < 0,05$ (significance limits for $\underline{r}$ when population correlation coefficient is zero)).

There was no significant difference between the study and control groups for cortical thickness at age level 1. Bone mineral density was not measured on children under 5 years of age; however for the few children (11 out of 26) at age level 1 on whom this parameter was measured there were no significant differences between study and control groups. There also was no relationship between corrected serum calcium and either bone mineral density or metacarpal cortical thickness. This group was selected because ages 3-5 years represent the nadir on the graph of daily increment in skeletal calcium and at this time serum calcium concentration does not appear to reflect other parameters of calcium nutrition. Breast feeding often continues for several years in rural African society; possibly, by the age of 3-5 years bone density has not yet been affected by the low calcium diet which follows weaning, if dietary calcium intake proves to be a significant factor. Follow-up of these children as they grow would be most informative; serial studies might show that detection of low serum calcium at an early age indicates those at risk during later growth periods.

However, at age levels 2 and 3, measurements of bone mineral density were significantly lower in the study groups as compared to the control groups ($p < 0,05$ and $p < 0,0005$ respectively for age levels 2 and 3). Table 5:4 shows the comparison of bone mineral density with normal American children. As can be seen, the bone mineral density of both the study and control groups is significantly lower than that of the normal reported by Mazess and Cameron (1972). At present there are no studies on healthy Black children from underdeveloped countries to use for reference. There was a good correlation between bone mineral density and corrected serum calcium for both age level 3 ($r = 0,5164$, $p < 0,01$) and for age level 2 ($r = 0,4368$, $p < 0,05$) (significance limits

Table 5:4 Bone mineral density: comparison of study and control groups, age levels 2 and 3, to normal controls.

Age	Bone Mineral Density (g/cm^2)		
	Normal Controls[1]	Study	Control
8-10 years	0,55 ±0,02	0,34*** ±0,10	0,41**** ±0,06
13-16 years	0,66 ±0,04	0,42**** ±0.08	0,54*** ±0,06

[1] Mazess and Cameron (1972)

*p < 0,05 compared to control group
**p < 0,0005 compared to control group
***p < 0,025 compared to normal controls
****p < 0,0005 compared to normal controls

for r when population correlations coefficient is zero)): see figure 5:2. The relationship between corrected serum calcium and metacarpal cortical thickness was significant for age level 2 ($r = 0,3664$, $p < 0,05$) but not for age level 3 ($r = 0,3539$, $p < 0,1$) (significance limits for r when population correlation coefficient is zero).

5:8 Summary of discussion of subject groups

That the study group differs significantly in calcium status from the control group has been shown by the results presented. The age levels selected represent different stages in the pattern of skeletal growth and calcium retention. Between 3 and 5 years, the daily increment in skeletal calcium is at a minimum; there is a sharp increase in skeletal calcium retention from 8-10 years with a peak in daily increment in skeletal calcium at puberty, 13-16 years (see Fig 3:1). Whether these biochemical and radiological differences are influenced by dietary calcium intake is the subject under study. Investigations were planned to illustrate differences between study and control groups with reference to the relationship of dietary calcium intake and biochemical and radiological parameters.

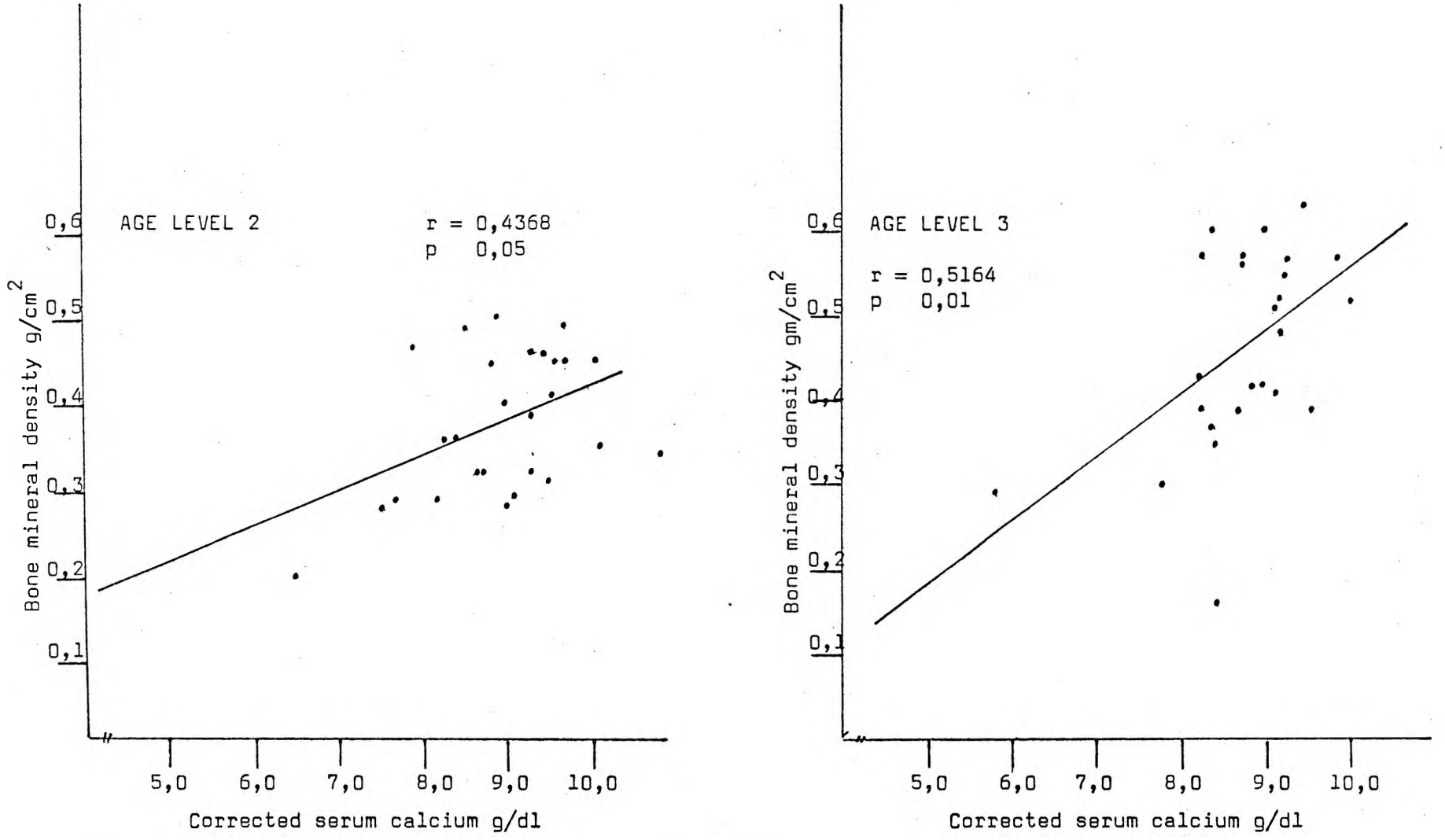


Figure 5:2 Comparison of bone mineral density (g/cm^2) and corrected serum calcium (g/dl) for age levels 2 and 3.

CHAPTER 6: DIETARY CALCIUM INTAKE IN RURAL BLACK CHILDREN

6:1 Method of investigation

The dietary history and recall method for estimating nutrient intake is used in order to obtain information from a greater number of subjects in the field than the more qualitatively and quantatively exact method of duplicate diet analysis. As with any other research tool, the dietary interview and recall must be subject to stringent control measures. The interviewer must be trained to obtain significant information from her subjects and she must be a comfortable part of the community rather than a foreign addition to it. There must be a reliable interview form that includes cross checks of the information collected and the interviewer must be familiar and at ease with the form she uses. With groups of statistically significant numbers, there is general agreement that one day's diet provides valid information on nutritional intake (Emmons and Haynes 1973).

Dietary histories were taken by a Black (Zulu and Swazi speaking) dietician trained in dietary interview techniques using a pre-tested interview form. See Appendix for samples of completed forms: Appendix B, form from study group, age level 2: Appexdix C, form from control group, age level 2. Information was elicited concerning cooking facilities and methods, sources and frequency of supply of various foodstuffs including gardening and animal husbandry and personal food preferences, with special reference to foods having a direct bearing on calcium metabolism in man. Socio-economic data was gathered to supplement information from the larger socio-economic and health surveys, particularly pertaining to dietary habits. The mother or woman who cares for the child was interviewed in her home to obtain information

regarding all aspects of eating habits including a 24-hour dietary recall. At age level 3 the child was also interviewed. Measurements of food intake were recorded in cups and spoons with reference to previously measured standards. Calorie and nutrient composition of the diet was calculated from standard food composition tables, using those compiled in South Africa of South African food products as far as possible (U.S. Dept. of Agriculture 1950, Fox 1966).

The data were computerized and analysed by standard methods using the SPSS package programmes (Nie et.al. 1975). Significance limits were determined by the student's one way t-test unless otherwise noted.

6:2 Results of dietary interviews

Table 6:1 lists the results of the 24-hour dietary recall for calories, calcium and phosphorus; for other nutrients, see Chapter 8. Calcium intake per kg body weight was calculated and is included in Table 6:1, as is calcium intake as a percentage of FAO/WHO recommended allowances. Both study and control children had low calcium intakes, less than 75% of the FAO/WHO recommended allowances for all subjects and less than 40% for the study groups at age levels 2 and 3 (mean intake). Daily dietary intake of calcium was significantly different between study and control groups only at age level 2 (8-10 years), although when calculated as mg calcium intake per kg body weight, all three age levels showed significant differences between study and control groups. Percentage FAO/WHO recommended intakes for calcium were significantly different for age levels 2 and 3.

As would be expected, in the control groups there was a steady rise in daily calcium intake as well as in total calorie intake, reflecting greater food consumption with increasing age. Although calorie intake rose similarly in the three study groups, there was a drop in daily calcium intake between age levels 1 and 2; daily calcium intake rose

Table 6:1 Daily intake of calories, calcium and phosphorus calculated from 24-hour dietary recall.

Investigation	Age level 1		Age level 2		Age level 3	
	Study	Control	Study	Control	Study	Control
Total calorie intake per day	1538 ± 303	1511 ± 367	1863 ± 341	1763 ± 440	2017 ± 449	2052 ± 500
Dietary calcium intake mg/day	249 ± 182	282 ± 144	199* ± 127	338 ± 170	229 ± 175	378 ± 289
Dietary calcium mg/kg body weight	16,4** ±11,0	19,9 ± 9,9	7,1** ± 3,2	14,9 ± 8,0	5,5** ± 4,2	8,6 ± 5,6
Dietary calcium % FAO/WHO recommendations	62,3 ±45,5	70,4 ±36,1	37,7*** ±25,3	62,4 ±30,2	33,7****±24,5	56,4 ±40,7
Dietary phosphorus intake mg/day	1024 ± 291	978 ± 338	1093 ± 237	1081 ± 334	1228 ± 303	1223 ± 312

*p < 0,05 compared to control group
**p < 0,005 compared to control group
***p < 0,01 compared to control group
****p < 0,025 compared to control group

again in the age level 3 study group, although it did not rise to the level of the youngest age study group. There was a consistent drop in calcium intake per kg body weight in both study and control groups over the three age levels as would be expected.

Not all organisations setting nutritional standards specify a daily phosphorus intake; they note it should be equal to calcium intake. On this basis, dietary phosphorus intake was adequate in all cases, meeting more than 100% of FAO/WHO recommendations; indeed, dietary phosphorus intake met 100% of the U.S. recommended allowance, usually acknowledged to be higher standards than are necessary in practical terms for developing countries. Low phosphorus intakes influencing calcium metabolism cannot be responsible for the abnormal serum calcium concentrations in these subjects.

6:3 Discussion of dietary calcium intake

Maintenance of normal serum calcium levels on low dietary calcium intakes is a feature noted in most calcium balance studies carried out in developing countries. This may be due to: 1) long term adaptation to low calcium intakes; 2) more efficient utilization of calcium because of increased vitamin D from the sun in the tropical zones into which most developing countries fall; 3) the fact that children in developing countries are frequently smaller than their counterparts in more developed lands and therefore calcium intake per kg body weight is more in line with higher intakes reported in other countries although total intake is low; 4) some inherent ethnic difference in people. Whatever the explanation, it is a significant factor in the setting of the FAO/WHO standards for calcium intake.

There are many disadvantages in the calcium nutrition situation among South African Black people. The staple, maize meal, has a low calcium content, provides an adverse Ca:P ratio in the diet (although this probably has little bearing on calcium absorption) and because the meal is unrefined in the diets of the majority of rural people, there is an increase in phytic acid intake. Other foods providing phytate or oxalate in the diet in the Driefontein area are brown bread and spinach; both may be eaten in reasonably large quantities. Whether these foods interfere with calcium absorption in this population has not been investigated. Other sources of oxalate and phytate--unrefined breakfast cereals, oatmeal, cocoa/chocolate and rhubarb--are virtually non-existent in the diet in Driefontein. Among South African Blacks there may be a somewhat lowered protein intake, although recent work implies that total protein intake probably does not influence calcium absorption (Schofield and Morrell 1960, Johnson et.al. 1970, Anand and Linkswiler 1974). As milk is not frequently drunk by rural Africans, there is a small intake of lactose, a substance which has been shown to enhance calcium absorption

(Bergeim 1926, Condon et.al. 1970). In addition, calcium loss may be more important with increased loss both in sweat due to hard labour in hot conditions and because there may be a greater number of pregnancies and lactation periods with no calcium supplementation than in most European societies (Walker 1972).

Water, which may make an important contribution to calcium in the diet, is a poor source of calcium in the Driefontein area. Water from twelve different sources in Driefontein (wells and springs) was analysed for calcium content. The average calcium concentration was 23,6 mg/cupful (250 ml) with a range from 3,2 mg/cupful to 40,5 mg/cupful. Note of water source and amount drunk daily was taken and this was included in the 24-hour dietary recall. Most of the children consumed 2 to 4 standard cupsful daily, however the contribution to calcium intake varied greatly. Two cups of water might provide as little as 6 mg calcium or as much as 56 mg, three cups, 10-85 mg calcium and four cups, 12-113 mg calcium depending on the source of the water. Water supplied less than 25 mg calcium in most of the children's daily intakes.

Results from this study show there is a significant relationship at age levels 2 and 3 between dietary calcium intake (mg/kg body weight) and both corrected serum calcium ($r = 0,4081$, $p < 0,01$, significance limits for r when population correlation coefficient is zero) and serum alkaline phosphatase ($r = 0,3292$, $p < 0,05$, significance limits for r when population correlation coefficient is zero); see figure 6:1. Reduced calcium intakes are associated with low serum calcium and high serum alkaline phosphatase concentrations, biochemical parameters which are abnormal in the metabolic disturbances associated with the previously investigated bone deformities peculiar to this geographical area (Pettifor 1980).

Likewise, dietary calcium shows a correlation with calcium excretion (Uca:Ucr) for age levels 2 and 3 ($r = 0,3436$, $p < 0,05$, significance limits

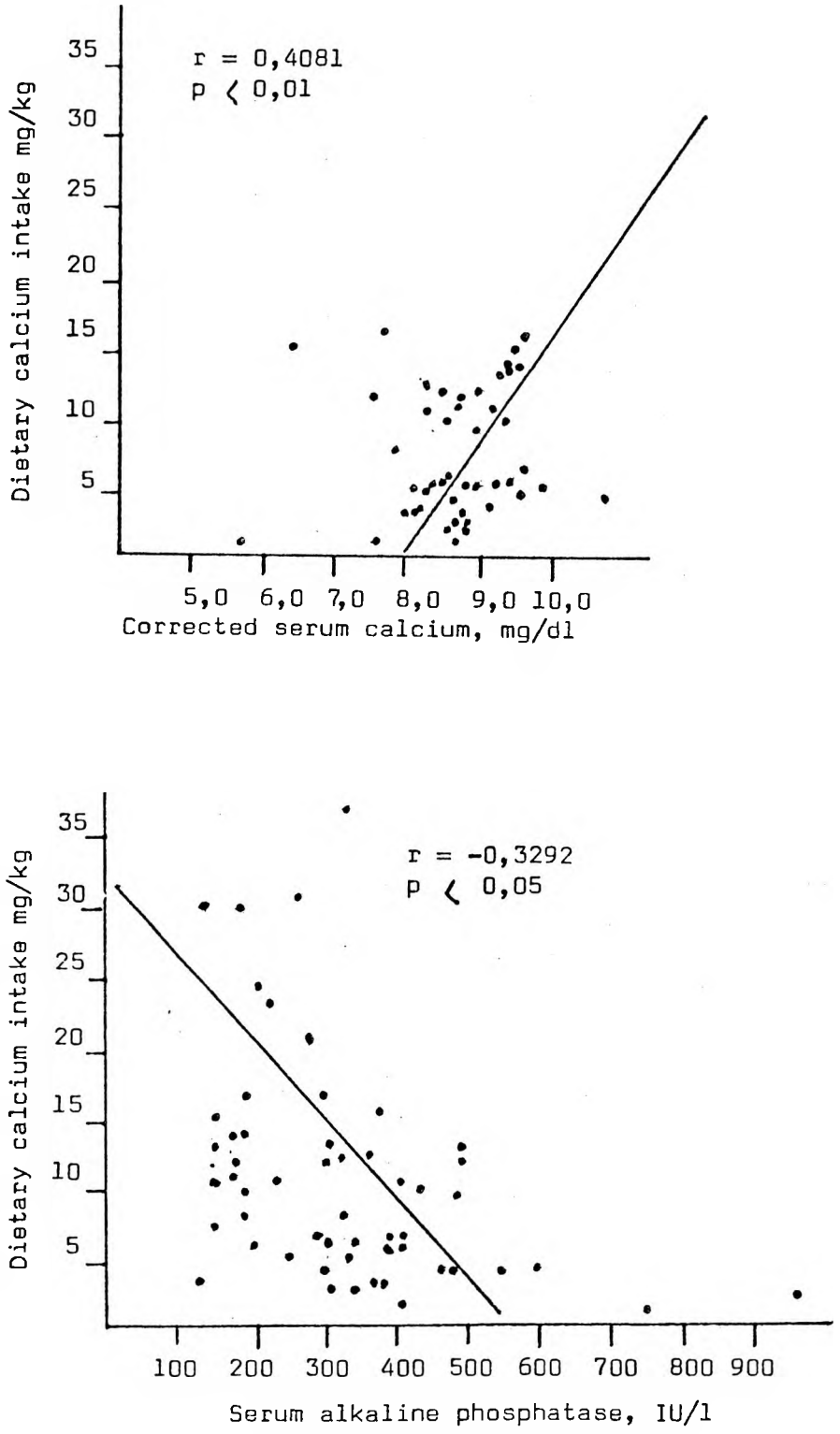


Figure 6:1 Relationship between dietary calcium intake (mg/kg body weight) and corrected serum calcium and serum alkaline phosphatase. (Age levels 2 and 3, all subjects)

for r when population correlation coefficient is zero); figure 6:2. MacFayden (1965) has shown that changes in dietary calcium intake are reflected in serum calcium concentrations and levels of calcium excretion; this would appear to be born out by the present investigation, although there was no relationship between corrected serum calcium and Uca:Ucr.

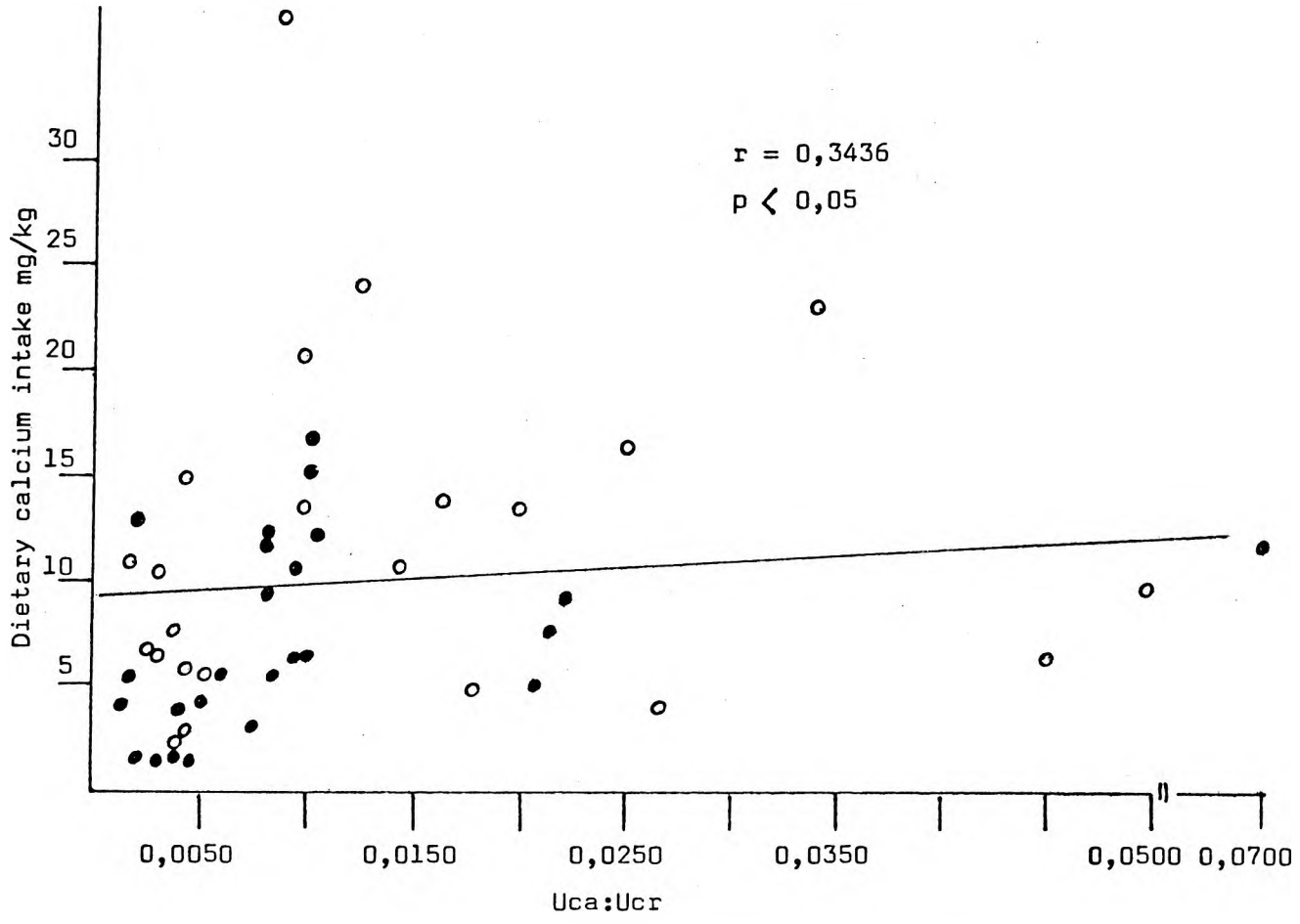


Figure 6:2 Correlation of urinary calcium excretion (Uca:Ucr) with dietary calcium intake (mg/kg body weight) for age levels 2 and 3. (Study ● Control o).

Although there have been no controlled studies showing evidence that calcium intake influences height, in this group there is a significant difference in dietary calcium intake (mg/kg body weight) by percentile height for age when the group is divided as those above the 50th percentile

and those below it ($p < 0,025$). Calorie and protein intakes (per kg body weight) were not significantly different when the children were grouped by height as above ($p > 0,5$ for both calories and protein).

Luykin and Luykin-Koning (1967) working with children in Surinam found no significant difference in the metacarpal cortex thickness of children having dietary calcium intakes of 500 mg and 1130 mg daily. The mean measurement at 9 years, 3,2 mm, is somewhat higher than that for the control group at age level 2 (8-10 years), 2,67 mm; the calcium intake of the control group was considerably lower than the 500 mg of the Luykin group, however. In contrast to the findings of Luykin, there was a significant difference between study and control groups at this age (8-10 years) ($p < 0,05$) with a slight inverse relationship between calcium intake per kg body weight and metacarpal cortex thickness ($r = -0,1680$). Walker (1971) studying rural Black and urban Black and White school children (14 years of age), found that the metacarpal thickness of the White children was significantly greater than that of either the urban or rural Black. Table 6:2 shows the results of Walker's study and age level 3 of the present one. The White children in Wakler's study had significantly higher calcium intakes and significantly greater metacarpal cortex measurements than either group in the present study ($p < 0,0005$ for both factors for both study and control groups). The urban Blacks of Walker's study had significantly greater metacarpal cortical thicknesses than both the study and control groups of age level 3 ($p < 0,005$ and $p < 0,0125$ respectively) on calcium intakes that were in between but not significantly different from either the study or control group. Walker's rural Blacks had significantly greater cortical thickness measurements ($p < 0,025$) on calcium intakes that were not significantly higher ($p > 0,5$) than the study group of age level 3 and similar metacarpal thicknesses ($p < 0,5$) on calcium intakes significantly lower than the control group ($p < 0,025$). However calcium intake was not

Table 6:2 Comparison of metacarpal cortex thickness and daily calcium intake of subjects in two South African studies.

Study	Metacarpal cortex thickness mm	Calcium intake per day mg
Walker (1971)		
Urban Black	3,69 ± 0,43	296 ± 262
Rural Black	3,32 ± 0,46	242 ± 212
Urban White	4,56 ± 0,61	833 ± 410
Present		
Age level 3, Study	3,09 ± 0,87	229 ± 175
Age level 3, Control	3,30 ± 0,74	378 ± 289

reported in mg per kg body weight, the measurement showing the most significant correlation with other indicators of calcium status, so comparison with this factor could not be made.

Pettifor (1980) noted in previous studies in Driefontein that children exhibiting bone deformities could be divided into two groups based on diet. Those with abnormal serum biochemistry (low serum calcium, raised serum alkaline phosphatase) did not include any milk in the diet, whereas those with normal serum calcium and serum alkaline phosphatase consumed at least a cup of milk per day. Particular attention was therefore paid to milk intake in the present dietary assessment. Those subjects who drank no milk or only occasionally included milk in the diet had significantly lower calcium intakes, both per kg body weight and total daily intakes than did those who included milk daily (Table 6:3); indeed, a cup of milk daily at least doubled

Table 6:3 Dietary calcium intake of all children grouped by frequency of milk consumption.

Milk consumption	Calcium intake	
	mg/kg body weight	mg per day
Drinks no milk	6,83* ± 3,58	197,1** ± 148,4
Occasionally drinks milk	7,17* ± 4,30	227,5*** ± 137,5
Drinks milk daily	18,45 ±11,46	413,9 ± 213,7

*p < 0,005 compared to calcium intake with daily milk consumption
**p < 0,0025 compared to calcium intake with daily milk consumption
***p < 0,05 compared to calcium intake with daily milk consumption

calcium intake. Including milk in the diet daily is also reflected in the corrected serum calcium concentrations: corrected serum calcium, 9,31 ± 0,53 mg/dl in children with a daily intake of milk; 8,97±0,74 mg/dl in those who had only occasional or no intake of milk (p < 0,01).

Regrouping the subjects in the study by corrected serum calcium concentrations and using 9,0 mg/dl as the critical level, the mean daily calcium intakes are significantly different for age levels 2 and 3 (p < 0,05 and p < 0,0005 respectively); Table 6:4. The wide range

Table 6:4 Dietary calcium intakes in all subjects when grouped by corrected serum calcium concentration.

Corrected serum calcium	Dietary calcium intake mg/day	
	Age level 2 8-10 years	Age level 3 13-16 years
< 9,0 mg/dl	219,9* ± 128,7	207,3** ± 153,9
≥ 9,0 mg/dl	325,1 ± 187,9	448,0 ± 300,9

*p < 0,05 compared to age level 2, serum calcium ≥ 9,0 mg/dl
**p < 0,0005 compared to age level 3, serum calcium ≥ 9,0 mg/dl

of S.D. indicates the great individual variation which plagues the determination of calcium requirements. As pointed out in Chapter 3, recommended allowances should be the amount which when consumed by a population meets the needs of 95% of them. Accordingly this information would suggest that a minimum daily intake of ±400 mg calcium is necessary for the maintenance of normal serum calcium concentrations, slightly less, but certainly in line with the FAO/WHO recommended allowances.

CHAPTER 7: THE 24-HOUR DIETARY RECALL AS AN INVESTIGATIVE TECHNIQUE

The assessment of dietary intake is of major importance in considering any nutritional research. It is necessary to have a quantitative idea of the existing food intake whether investigating a specific nutrient in relation to a particular health problem, general nutrition in relation to health and socio-economic factors in a community, or planned intervention by dietary supplementation. In spite of the need, little information, particularly from developing countries, is available. The reasons for this are many and diverse, but the main ones are that collection of data is extremely time consuming and accuracy of methodology may be doubtful. Information regarding dietary intakes is not always easily obtained; many problems are inherent in dietary surveys: how to obtain an accurate list of foods eaten without unintentionally affecting the subject's food habits by the mere process of record keeping or interviewing, how to estimate quantities eaten, even when food items areknown and how to translate these quantities accurately into nutritionally useful terms through the use of food tables.

7:1 Method of dietary assessment

The most common methods of measuring dietary intake are: 1) replicate meal analysis, 2) precise weighing of food eaten, 3) dietary recall, 4) diary recording. Each has inherent problems which influence the quality of results in a particular situation.

Chemical analysis of a collection of meals which duplicates exactly, both qualitatively and quantitatively, those put before a subject theoretically is the most accurate method of ivestigating dietary intakes. It makes allowance for variation from the food tables of individual food items; this is especially important when the diet consists mainly

of a single staple such as a cereal porridge which may vary in composition depending on where it is grown and milled and how it is cooked. However this method requires sophisticated equipment and the work load, both in the field and in the laboratory needed for accurate replicate meal analysis would be tremendous if a study is to include statistically significant numbers. Indeed, working with an economically depressed community, duplication of diet introduces 'one more mouth to feed' from available food stuffs and may therefore not be an accurate reproduction of the usual daily food intake.

Precise weighing of food eaten is an accurate method of dietary assessment; however, it must be introduced with great care in order that the normal food pattern of a family does not become atypical during the period of investigation. Whitehead (1977) has used this method of investigation successfully as regular surveillance in the Gambia (now Senegambia) but admits that introduction of the procedure was not easy.

The diary method involves the subject keeping a record for a number of consecutive days of the amount and kinds of food eaten. This is a purely qualitative method, but can be helpful when used in conjunction with other methods. It is important when considering the use of this method that the diary is kept for more than a single day (Schaefer 1966). Although keeping a record involves the assumption of literacy, Rutishauser (1973) working in Uganda has described a modification using photographs of common foods and a pin to denote a single serving of any one food. This requires careful instruction, but is not as intrusive as either weighing or replicate meal analysis.

The 24-hour dietary recall method for estimating nutrient intake is only semi-quantative but it is frequently used in surveys on large numbers of subjects. It is often thought that this method is most suitable for use in sophisticated, educated homes, but indeed, the reverse seems to be true. The more complex the way of life, the greater

the variety of food intake and therefore, the more to recall. The dietary recall method was used with success in Paris during World War II when the diet from necessity was very simple; this is not the case presently with the more complex diets that have come with affluence (Schaefer 1966).

There are arguments as to the numbers of day's recall necessary; three day recalls are sometimes used, although I have found this time period less reliable than one day among Whites in South Africa. Other workers have also reported better results with 1 day records using the precise weighing method rather than a seven day period which causes greater disruption of routine and loss of interest on the part of the participants (Eads and Meredith 1948, Yudkin 1951, Chalmers et.al. 1952, Reed and Burke 1954). Emmons and Haynes (1973) have stated that with groups of statistically significant numbers, a 24-hour dietary recall provides valid information of nutritional intake. When interviews are carefully conducted and techniques refined before use there is a low coefficient of variation of duplicate 24-hour dietary recalls (Frank et.al. 1971). Interview forms must allow space for comments on whether the day is typical of eating habits and what variation can occur. Generally, it is best not to use weekends or public holidays as a day for a 24-hour dietary recall.

7:2 Testing the 24-hour dietary recall as an investigative technique

To test the effectiveness of the interview and 24-hour dietary recall method as an investigative tool in this population, duplicate diet analysis was performed. The dietician recording the dietary interviews also conducted this portion of the field work. Food was collected in a container provided to duplicate exactly that eaten by the subject by the person cooking and serving the meals to the household. The following day, the interviewer collected the container (closed,

with contents unseen by her) and conducted a 24-hour dietary recall. In order to avoid the possible disruptive effect collection of a duplicate diet may have had upon eating habits in the household, this 24-hour dietary recall was not the one used in the general survey; this part of the study was carried out after completion of the dietary assessment. In addition, as the area is poor, duplicate diet analysis was carried out on only 10 subjects (24% of the study participants).

The container with the duplicate diet was refrigerated immediately upon collection. Returned to the laboratory, the diet was homogenised in a Waring blender with a measured amount of distilled water and an aliquot (± 50 grams) was stored at -20°C until analysis was carried out. Diets were analysed for protein, calcium and phosphorus content; laboratory results were correlated with results calculated from the food tables. Acceptable limits of accuracy are ±10% for any nutrient.

7:3 Laboratory methods, duplicate diet analysis

Protein analysis was by the standard Kjeldahl method with the following modifications (Wootton 1964): Homogenate (approximately 5 g) was weighed into a micro-Kejdahl flask; 10 ml concentrated sulphuric acid, 5 ml catalyst solution (50% sulphuric acid containing 1% selenium dioxide), 10 ml 100 volume AnalaR hydrogen peroxide and 4 g potassium persulphate were added and the contents digested by boiling for approximately 1½ hours. The sample was distilled into a 100 ml conical flask containing 5 ml boric acid-indicator solution (2% boric acid with 10 ml Tashiro's indicator (8 mg methyl red, 2 mg methylene blue in 10 ml ethanol), pH 4.8). After the addition of 10 ml 40% sodium hydroxide, distillation continued for 10-15 minutes. The contents (green in colour) were titrated back to the original grey colour with N/70 sulphuric acid using a titration burette. Each sample was analysed in duplicate; a serum sample of known protein concentration as well as an homogenate sample from the previous day were used as a check on method reproducability. Calculation

of results was according to the following formula using these constants:

1 ml N/70 H_2SO_4 = 14/70 mg nitrogen; 1 mg nitrogen = 6,25 mg protein.

Therefore, where: t = titre (ml N/70 H_2SO_4)

wt_A = aliquot weight (grams) homogenised diet

wt_T = total weight (grams) homogenised diet,

then: $$\text{grams protein/day} = 1{,}25 \times \frac{wt_T}{wt_A} \div 1000 \times t.$$

The diet was analysed for calcium and phosphorus as follows: a weighed aliquot of homogenate (approximately 5 g) was ashed at 600°C in a Naber (Naber Industrieofenbau, Lilenthal/Bremen) ashing oven for approximately 12 hours or until ashing was complete. The ash was dissolved in 1 ml concentrated hydrochloric acid and made up to 5 ml with distilled water (Wootten 1964). The calcium and phosphorus content was measured on a Varian 1200 atomic absorption spectrophotometer using lanthanum chloride as a releasing agent and a nitrous oxide-acetylene flame (Duncan 1976). Calculation of results involved the following formula:

$$\text{mM/l} = \frac{\text{mg/l}}{\text{molecular weight}}$$

Therefore, where: Ca_v = uM/l calcium (atomic absorption spectrophotometer)

P_v = uM/l phosphorus (atomic absorption spectrophotometer)

wt_A = aliquot weight (grams) homogenised diet

wt_T = total weight (grams) homogenised diet,

and knowing $Ca_{\text{mol. wt.}} = 40{,}08$

$P_{\text{mol. wt.}} = 30{,}97,$

then: $$\text{mg calcium/day} = 200{,}4Ca_v \times \frac{wt_T}{wt_A}$$

$$\text{mg phosphorus/day} = 154{,}8P_v \times \frac{wt_T}{wt_A}.$$

The results are expressed as grams of protein and mg of calcium and phosphorus per daily intake.

7:4 Results of duplicate diet analysis

The diet of Blacks living in rural communities is generally quite simple; two or three meals of stiff mealie meal porridge accompanied by a 'relish' of stewed vegetables and/or meat available. Bread and tea are usually eaten in the morning and bread may be eaten with each of the other meals. The children at school also had fruit and/or bread or biscuits during a morning break. The 'relish' varied little from meal to meal, usually cabbage, beans or tomatoes with onions supplemented occasionally by meat or chicken.

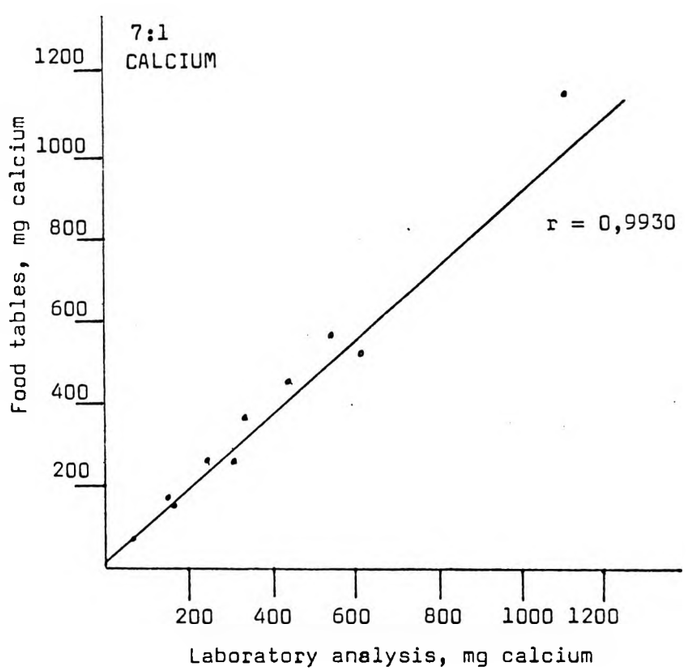
Table 7:1 shows the results of laboratory analysis of duplicate diets and the analysis calculated from the food tables. Although the subjects included in this portion of the study were selected by the interviewer while in the field, there is a wide range of calcium intakes; the protein and phosphorus levels reflect the generally adequate intake of these two nutrients and there is a range in these results, as well. The results are shown graphically in Figures 7:1, 7:2 and 7:3, all of which show a relationship between calculated results and laboratory analysis.

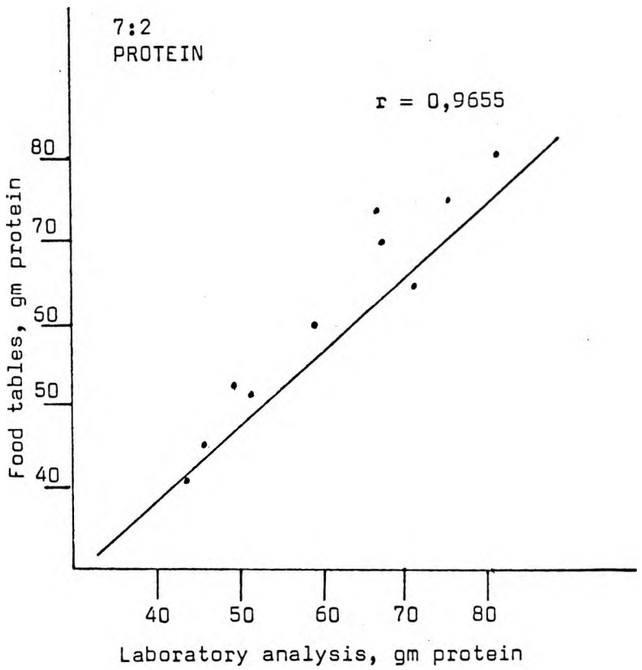
7:5 Discussion of results: 24-hour dietary recall as an investigative tool

As with any other research tool, the dietary interview and 24-hour dietary recall must be subject to review as to effectiveness with the population under study. Young et.al.(1952) found that 24-hour dietary recall gave results ± 10% higher than either dietary history or seven-day diary records; however they were working with a sophisticated population. Lenner et.al. (1977) in Sweden also found that 24-hour dietary recall gave significantly higher results than did dietary histories. On the other hand, Acheson et.al. (1980) found that the 24-hour dietary recall method of investigation underestimated intakes at least 20% as compared to precise weighing of food, although this study was carried out on workers on an Antarctic base and no qualified interviewer

Table 7:1 Comparison of results of 24-hour recall (calculated analysis) and duplicate diet analysis

Diet No.	Protein intake/24 hours			Calcium intake/24 hours			Phosphorus intake/24 hours		
	Tables grams	Laboratory grams	% Diff.	Tables mg	Laboratory mg	% Diff.	Tables mg	Laboratory mg	% Diff.
1	51,1	51,8	+ 1,3	248,0	255,1	+ 2,8	1008	864	- 14,3
2	80,5	81,3	+ 1,0	1154,3	1116,7	- 3,3	1834	1472	- 19,8
3	64,4	81,3	+ 10,6	280,1	318,2	+ 13,6	1267	1634	+ 28,9
4	45,0	46,3	+ 2,9	156,8	160,9	+ 2,6	1040	1014	- 2,6
5	73,7	67,0	- 9,1	170,6	152,9	- 10,4	1490	1241	- 16,7
6	69,9	67,5	- 3,4	569,8	541,6	- 5,0	1276	793	- 37,9
7	40,9	43,7	+ 6,8	526,5	615,2	+ 14,5	824	789	- 4,3
8	52,0	49,8	- 4,2	364,3	340,3	- 6,6	1033	1021	- 1,2
9	75,0	75,8	+ 1,0	73,5	73,5	0	1388	1304	- 6,1
10	59,8	59,3	- 0,9	451,3	451,7	0	1215	1414	+ 16,4





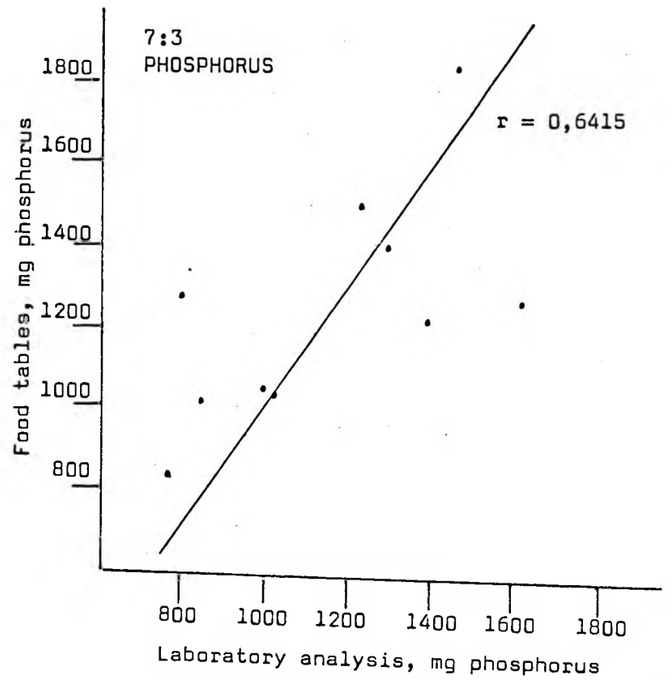


Figure 7: Duplicate diet analysis. Comparison of results of laboratory analysis of duplicate diet with results of 24-hour dietary recall calculated from food tables for calcium (7:1), protein (7:2) and phosphorus (7:3).

conducted the 24-hour recall. Ahrens and Boucher (1973) found good agreement between food table analysis and chemical analysis of diet in the Diet-Heart Review, better than 10% difference for protein, calcium and phosphorus. Lubbe (1968) in a survey of nutritional status of White school children in Pretoria found excellent agreement between precise weighing of food eaten (7 days) and a modified dietary history including a 24-hour dietary recall for protein (54,3 $\pm$ 9,7 g and 55,9 $\pm$ 14,0 g for the two methods respectively) and for total calorie intake (1842 $\pm$ 247 and 1866 $\pm$ 301 respectively). Working with rural Blacks in Uganda, Rutishauser (1973) found good agreement between the recall method of estimating dietary intake and replicate meal analysis: -10,1% for protein and -1,5% for energy intakes.

In the present study, only two results for calcium (Nos. 3 and 7) exceed a 10% difference between calculated and laboratory analysis; however, in neither case does the laboratory result reflect a change from poor to adequate calcium intake over the calculated results. Dietary analysis and 24-hour dietary recall phosphorus intakes varied considerably more than either calcium or protein. The value for phosphorus in the food tables used had a range of 132-210 mg per 100 grams (Fox 1966). Although the table used was for the mill in the area nearest to Driefontein, this may not have accurately reflected locally grown, locally milled maize, and the amount consumed would have compounded the error. Food table values for calcium and protein content of maize meal had a much narrower range (2,3 - 5,4 mg/100 g and 7,6 - 9,0 g/100 g respectively), so that in spite of the large amount of meal eaten, the difference is not so acute. (Fox 1966) It would be more accurate therefore, if dietary phosphorus were the nutrient under study, to analyse samples of locally produced mealie meal and use the mean figure when computing nutrient intake.

The results would indicate that, in this population, the dietary interview and 24-hour dietary recall method of nutritional investigation

when conducted by a selected, trained interviewer accurately reflects nutrient intake. This method, dietary history and 24-hour dietary recall, of nutritional investigation is particularly suitable for use in rural Black communities in South Africa as members of a community--teachers, nursing sisters, social workers--can be trained in dietary interview techniques. A study course in nutritional background and methods of history taking using a professionally constructed interview form circumvents language and cultural problems in a country where professionally trained Black nutritionists or dietetic technicians are scarce.

CHAPTER 8: DIETARY INTAKE OF NUTRIENTS OTHER THAN CALCIUM

8:1 Criteria of an adequate diet

The criteria of an adequate diet is a subject much discussed. As pointed out in Chapter 3, the setting of minimum daily requirements may be done by several methods, each of which has its own adherents. In addition, the influence of social and economic considerations is apparent when comparing recommended dietary standards set by different bodies.

Recommended intakes are now used in six different ways: 1) they provide a standard against which dietary surveys may be evaluated. 2) they provide a guide for dieticians when compiling diets for individuals (both normal and therapeutic diets) and when catering for large numbers in an institutional situation. 3) they are important in economic planning: national agricultural policies, long-term aid to underdeveloped countries, short-term aid in famine and disaster relief. 4) they are the basis for nutritional labelling (as percentage recommended allowance). 5) they are the minimum standard when considering new types of manufactured foods and food enrichment programmes. 6) they are guides for planning parenteral nutrition regimes.

The first comprehensive list of recommended allowances, produced by the League of Nations in 1938, was for 12 nutrients: calories, calcium, phosphorus, iron, iodine, vitamin A, thiamine, riboflavin, vitamin C, vitamin D, protein and fats. Since then nutritional research bodies from various countries have made and reviewed recommended allowances for these same 12 nutrients plus others which have become important with advancing nutritional research. In addition to the usual published tables of recommended daily allowances, most bodies also publish footnotes concerning nutrients for which human needs have been less well defined.

Table 8:1 lists recommended dietary allowances for children 8-10 years of age set by various authorities. As can be seen for almost all nutrients there is a range of recommendations. Maintenance of good health, allowing for a sufficient margin above minimal requirements is a parameter for one set of standards (National Research Council 1974); indication of a 'nutritional floor' beneath which maintenance of health cannot be assumed is the parameter used for others (Pett 1949). Pett's statement, made in 1949, "The ingestion of more of a nutrient than serves a clear physiological purpose is undesirable in the face of the world scarcity of food,...", may be valid more than ever now in light of the growing world population.

8:2 Results and discussion of current investigations

Although the main theme of this dissertation is calcium nutrition, the dietary histories and 24-hour dietary recall records were analysed for the intake of other nutrients as well, using food tables. As there were no significant differences between study and control groups ($p > 0,5$ for all age levels for any nutrient except calcium (discussed separately, see Chapter 6), the results of the two groups are presented together for each age level (Table 8:2). To be more meaningful, the results have been compared with both the South African dietary standards and the FAO/WHO daily allowances and these are used in the following discussion.

The diet of the rural Black in South Africa is quite simple; it consists of the staple, maize meal, cooked to a thick porridge plus a stew of vegetables in season and of meat when available. Traditionally there are only two meals daily, but many people now follow the Western plan of breakfast, lunch and dinner. The majority of the children had 3 meals daily; in addition most of the school age children had a substantial snack of buns during the morning school break. A

Table 8:1 Recommended dietary allowances for children 8-10 years set by various bodies (daily intake)

Committee	Calories		Protein		Thiamine mg	Riboflavin mg	Niacin mg	Vitamin A I.U.	Vitamin C mg	Iron mg
	Daily	Per kg	gm/day	gm/kg						
FAO/WHO[1]	2200	78	25	0,88	0,9	1,0		2400		10
USA[2]	2200	80	40	1,2	1,2	1,2	15	3500	40	10
U.K.[3]	2100	70	53	1,76	0,8	1,0	11	2400	20	10
S.Africa[4]	1900	65	55	1,83	0,7	1,4	10	3000	40	10

[1] Food and Agriculture Organization/World Health Organisation, 1973
[2] U.S. Department of Agriculture 1968
[3] U.K. Department of Health and Social Security, 1969
[4] National Nutrition Council, South Africa, 1956

few of the younger children had four meals daily, the 'extra' one being about 4 P.M. Most meals were stiff maize meal porridge ($\frac{1}{2}$ - 2 cupsful) with an accompaniment of cabbage, spinach, potatoes, soup made from dried beans, or a mixture of tomatoes and onion, or sometimes, meat, chicken or egg. For some children the morning meal would be a thin porridge or just bread and margarine and tea. There was very little eating in between meals, mostly because there was not much extra food, although bread, leftover porridge and occasionally oranges were eaten when available.

There were no clinical signs of protein-energy malnutrition nor of any specific nutrient deficiency among the group studied, although 45% of the children were below the third percentile for weight and 39% below the third percentile for height on the Boston weight and height for age charts.

All the children had high protein intakes, double the FAO/WHO recommended allowances for age levels 1 and 2 and one a half times that for age level 3. For the children up to 10 years, protein intake was adequate by the South African dietary standards, but only about 75% of that recommended for children 13-16 years. Although most of the protein came from vegetable sources, almost all children had at least 10 grams of animal protein daily--meat, egg, milk--or high quality vegetable protein--dried peas or beans. Most households kept chickens and sixty percent of the children had eggs 2-3 times per week. Sixty percent also had meat or poultry 1 or 2 times per week, and another 30% had meat 3 to 4 times per week--admittedly small amounts, but sufficient to provide 7 grams of protein. No estimate of individual amino acids was made, however; low levels of some of the essential amino acids could contribute to the stunting seen in the children.

Calorie intake was over 80% of the FAO/WHO recommended allowances at all age levels and about 100% of the South African dietary standards

Table 8:2 Analysis of 24-hour dietary recalls for study and control groups by age level: 1) Nutrient intake. 2) Nutrient intake as percentage of S.A. dietary standards. 3) Nutrient intake as percentage of FAO/WHO recommended allowances.

Nutrient	Daily intake of nutrient			Percentage S.A. Standards			Percentage FAO/WHO Allowances		
	3-5 yrs	8-10 yrs	13-16 yrs	3-5 yrs	8-10 yrs	13-16 yrs	3-5 yrs	8-10 yrs	13-16 yrs
Calories	1525	1813	2035	101,7*	95,4*	67,8	82,4	82,4	88,5
Protein, gm	46,6	52,3	59,6	103,6*	95,1**	74,5	233,0	209,2	152,8
Fat, gm	24,2	30,0	35,4						
Carbohydrate, gm	282,6	338,9	373,0						
Sodium, mg[1]	1456	1524	1746						
Potassium, mg	819	827	1027						
Vitamin A, IU	59	148	139	2,4	4,9	3,5	3,9	6,2	3,1
Thiamine, mg	1,32	1,42	1,60	264,0*+	202,9****	128,0	146,7	157,8	177,9
Riboflavin mg	0,72	0,75	1,00	65,5***	53,6	50,0	72,0	75,0	76,9
Niacin, mg	5,78	5,86	7,60	72,2*	58,6	50,7			50,0
Vitamin C, mg	14	16	23	35,0	40,0	57,5			
Iron, mg	7,2	9,7	10,4	90,0*	97,0****	69,3			

[1] does not include added salt

*p < 0,0005 compared to age level 3 (13-16 years)
**p < 0,025 compared to age level 3 (13-16 years)
***p < 0,0025 compared to age level 3 (13-16 years)
****p < 0,005 compared to age level 3 (13-16 years)
+p < 0,025 compared to age level 2 (8-10 years)

for the children up to 10 years. There is quite a steep increase in the energy requirements of the South African allowances, presumably to cover the pubertal growth spurt, and at age level 3 (13-16 years), the children were meeting only 67% of the requirement. The greatest portion of the calories was provided by carbohydrates in the diet, approximately 74% at all three age levels; 14% of the calories came from protein and 12% from fats. This is similar to Western diets only in the percentage calories from protein which is 10-20% in the average American diet and was calculated to be 13% in a study on 10 year old children in an economically depressed area of southern USA (Frank et.al. 1977). American diets provide about 50% of the calories from carbohydrates and 40% from fats. American diets include many varied fats whereas the African diet has little fat per se and almost none provided by the main constituent, maize meal. A survey of 7 year old White children in South Africa reported 12-17% of the calories provided by protein sources in the diet (Lubbe 1968).

Unrefined maize meal is rich in thiamine; one cup of stiff porridge provides nearly a third of the recommended allowance. Brown bread, used exclusively by almost all the inhabitants of Driefontein, is also high in thiamine. All the children had high thiamine intakes.

Milk supplies most of the riboflavin in the Western diet, so it is not surprising that riboflavin intakes were low in this community where milk consumption is seriously limited. Riboflavin intakes met at least 70% of the FAO/WHO allowances and 50-60% of the South African dietary standards. Eggs in the diet supply a reasonable amount of riboflavin (0,15 mg/egg); other sources, contributing only small amounts of riboflavin, were fairly evenly spread through the total food intake.

Niacin intakes met approximately 70% of the FAO/WHO recommendations but only 50-65% of the South African dietary standards. Meat and peanut butter are the most important sources of niacin in the foods

usually eaten by the children in the study, and they were eaten in relatively small amounts. Meat, of course, is expensive to buy and chickens or cows are kept for the eggs or milk they produce rather than as a meat supply. Peanut butter is not produced in the area (indeed, peanuts are not grown in Driefontein) and although available, it would be an expensive item for these people. Thick porridge and soup made from dried beans, moderate sources of niacin, supplied most of this vitamin in the diet.

Vitamin C intake was low in all the subjects. Most vegetables are stewed for a long time, destroying a good portion of the heat labile ascorbic acid. Nutrition education could be a remedy for this. Those children who ate oranges more nearly met the allowance for vitamin C, but oranges are not grown in the community and must be purchased.

Likewise, vitamin A was in extremely short supply in the diets of these children. Pumpkin and leafy green vegetables (rape, spinach, imbuya) are virtually the only sources of carotene. Margarine, which has a moderate amount of vitamin A (3,300 I.U./100 gm, $\pm$ 165 I.U./teaspoonful) is not a valuable source of supply because it must be purchased; there seldom is more than 500 g/week/household. With diets low in fat, as these are, small intakes of the fat soluble vitamins may be expected. During winter when pumpkin is more plentiful, it is eaten in larger amounts and provides vitamin A which is stored in the body.

Iron intakes were adequate, judged by the South African dietary standards at age levels 1 and 2, but at age level 3 when the pubertal growth spurt puts up the recommended daily allowance of iron, intake was just under 70%. The mean haemoglobin levels were 11,3 g/dl, 12,9 g/dl and 12,2 g/dl for the age groups 3-5 years, 8-10 years, 13-16 years respectively; although on the lower limits of normal, this does not reflect iron deficiency aenemia.

Sodium intakes were about that of a 'no added salt diet', 1500 mg/day. No assessment was made of salt added to food, although investigating this may prove fruitful in light of the concern over hypertension in adult South African Blacks. All salt used in the households in the survey was plain salt; iodised salt is not available in the local shops. Nutritional goitre is a problem in this area as found in the health survey conducted in Driefontein (Pettifor, unpublished data). This problem, not clinically serious, could be solved through nutritional education of shopkeepers and customers into the exclusive use of iodised salt.

Between age levels 1 and 3 and age levels 2 and 3 there was a decrease in the percentage of South African dietary standards met which was highly significant (see Table 8:2) for nearly all nutrients (only the decrease in percentage requirement met for riboflavin and niacin between age levels 2 and 3 was not significant). This would indicate that the sharply increased nutritional recommendations to cover the pubertal growth spurt are a rather impractical standard in this population. The fact that the difference between the percentage FAO/WHO recommended daily allowances met by each age level is minimal would support this suggestion. However, failure to meet the FAO/WHO recommended daily allowances, even by a small margin, may be responsible for the poor growth exhibited by children in third world countries because these recommendations meet only <u>minimum</u> requirements for adequate development. As discussed in Chapter 3, the argument of adequate physical growth and the definition and achievement of <u>optimum potential</u> development is particularly valid in developing countries.

CHAPTER 9: SOCIO-ECONOMIC FACTORS RELATING TO CALCIUM STATUS OF RURAL BLACK CHILDREN

Driefontein is a Tribal Trust Area in the Walkerstroom distric of the Eastern Transvaal, about 60 km northwest of Piet Retief. Land is freehold, i.e., owned by individual families, although many people live there on rental terms. The community is a fairly homogenous one (57.9% Zulu and 40,5% Swazi) and is served by a chief and a group of elected elders. There are primary, secondary and high schools, several churches and four general stores. Fresh produce is sold by merchants who visit once a week. The ground appears to be reasonably fertile and water is available throughout the year. The nearest hospital is in Piet Retief; private doctors hold clinics several times a week about 8 km away.

9:1 Socio-economic background of Driefontein

A demographic survey was carried out in Driefontein in March 1978 prior to beginning studies investigating the health status of the community and planning intervention programmes (Shuenyane et.al. 1980). There were 1184 households with a total population of 10,142 people; a household is defined as a group of people related by birth or marriage, living in close proximity to each other and acknowledging one person as their head. The survey has elicited a picture typical of many rural Black South African communities; the age structure is representative of a developing country (45,8% of the population is under 16 years of age). Thirty-three percent of the males and 13% of the females between the ages of 21 and 61 work away from Driefontein leaving families behind.

Two months after the demographic survey, in May 1978, a survey of a 10% random sample of households was made collecting social information about these households (Ibid.). Basic amenities appear to be reasonable,

most households had pit latrines and a clean water supply. The community is poor; per capita income is 21-40 cents per day, comparing with other developing countries in Africa, and is about half that of Soweto (61 - 80 cents per capita per day) (Shuenyane et.al. 1977). It is difficult however to assess actual wealth in a community that is relatively self-supporting as Driefontein is.

9:2 Results and discussion of sociological and economic enquiries

Poor growth of African children is generally attributed to environmental factors (Kahn and Freedman 1959). The dietary history included questions designed to elicit socio-economic information that might have a direct bearing on nutritional status, with particular regard to calcium nutrition. Information gathered from subjects in the study was the number of people living in the house, the money spent on food each month, whether the family keeps dairy cows, whether the parents live with the child in Driefontein. Results are shown in Tables 9:1 and 9:2. There were no significant differences between study and control groups or between age groups for any of the results reported.

Table 9:1 Number of people living in the household and money spent monthly on food by the household for each age group

Socio-economic factor	3-5 years		8-10 years		13-16 years	
	Study	Control	Study	Control	Study	Control
No. people in house	10 ± 5	12 ± 6	10 ± 4	9 ± 4	9 ± 5	11 ± 5
Money spent on food monthly (rand)	5.93 ±5.30	8.20 ±5.40	4.50 ±2.30	6.50 ±5.00	7.00 ±6.40	7.80 ±6.60

Lowe (1975) observed among an affluent Western society (USA) that there is little variation in nutrient intake with income. Shuenyane et.al. (1977) similarly reported no correlation between protein-energy malnutrition

and income, number of people living in the house or child-minding in the urban Black community of Soweto. As the per capita income was uniformly low for the entire community, there was no significant relationship between calcium intake (mg/kg body wieght) and money spent monthly on food; see Figure 9:1 ($r = 0,1867$, $p < 0,1$, significance limits for r when population correlation coefficient is zero).

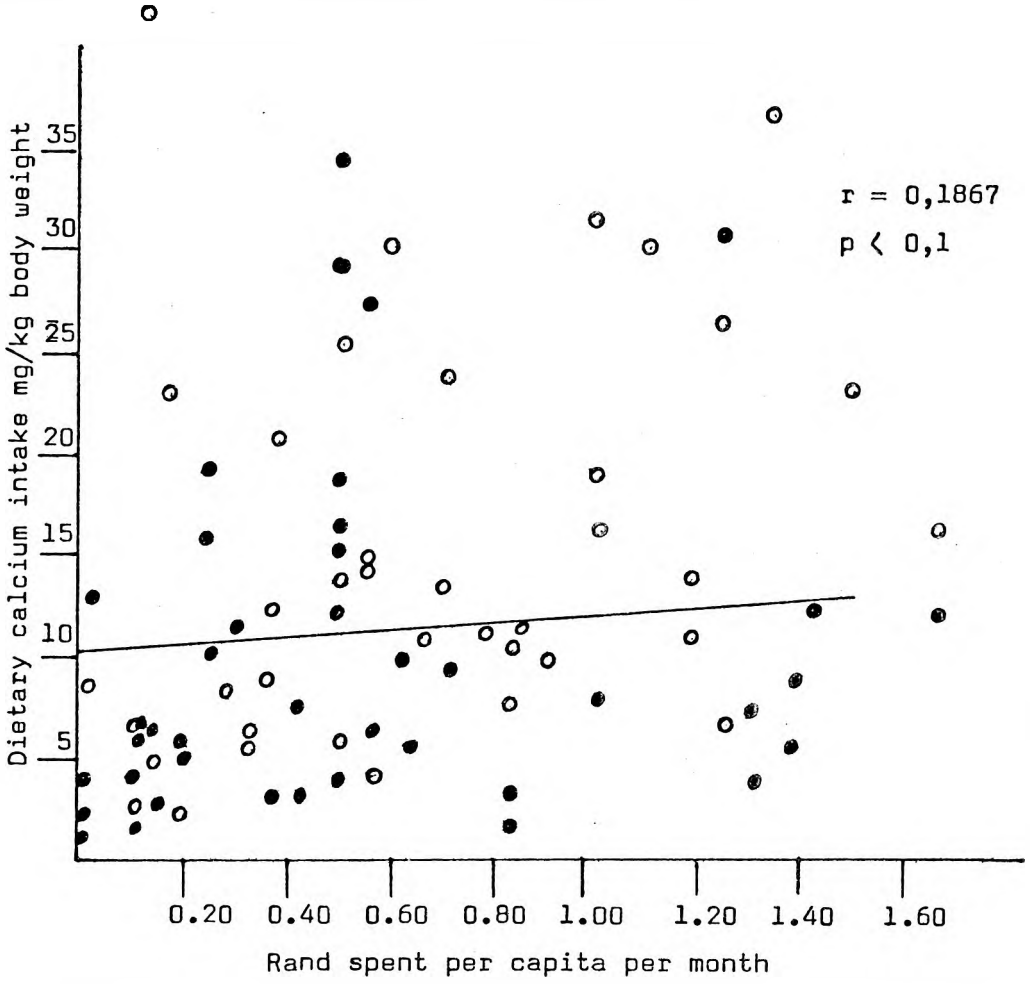


Figure 9:1 Correlation between monthly expenditure on food (rand) and dietary calcium intake (mg/kg body weight)

Food is bought or sent weekly, monthly, quarterly to the people in the community by household members working away from Driefontein, further complicating analysis of income and nutritional status. There was no relationship between the number of people living in the household and the subject's calcium intake. That a dramatic rise in the economic climate of the community might produce an equally dramatic rise in the calcium nutrition of the people is a topic for conjecture; however the

fact that some of the control group were well nourished by any standard points to the fact that there is no correlation between economic factors and calcium nutrition. This, plus knowing that children whose families kept dairy cows did not always consume milk daily (indeed, sometimes did not drink milk at all), emphasizes the need for nutritional education in the community.

Table 9:2 Parents living with child in Driefontein

Parent living with child	3-5 years		8-10 years		13-16 years	
	Study	Control	Study	Control	Study	Control
Both parents	4	3	4	5	5	3
One parent	7	8	6	7	5	6
Neither parent	2	2	5	3	3	4

There was, however, a significant difference in calcium intake per kg body weight between those children who had one or both parents living with them and those who lived away from both parents. Sixty-eight percent of the children in this study were cared for by their mothers, whereas in Soweto, more than 50% of the children were looked after by childminders (Shuenyane et.al. 1977). For only 2 of the children whose parents live away from Driefontein was the calcium intake per kg body weight greater than 15 mg/kg (10% of this group of children), but 33% of the children being cared for by one or both parents had calcium intakes above 15 mg/kg body weight. The mean intakes however were somewhat lower than this: 13,8 $\pm$ 9,5 mg calcium/kg body weight, parent living with child; 10,2 $\pm$ 8,9 mg calcium/kg body weight, both parents living away from child ($p < 0,05$); see Fig. 9:2. The families where at least one parent lived with the child had a significantly greater number of people in the household ($p < 0,0005$), therefore, physically having 'more mouths to feed', but possibly also having more income earners in the household.

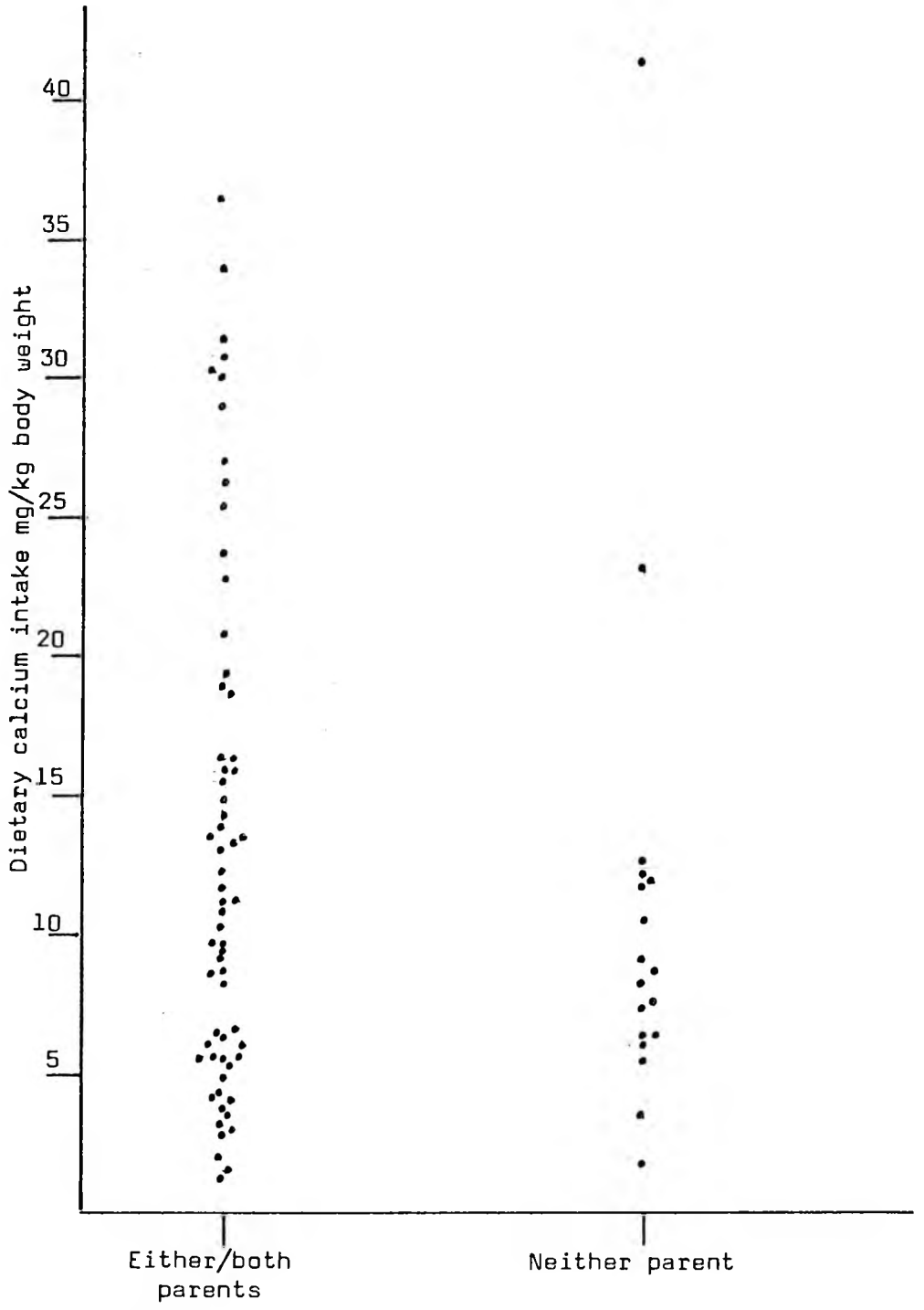


Figure 9:2 Calcium intake per kg body weight grouped by parent living with child

CHAPTER 10: CONCLUSIONS

The investigations reported in this dissertation are two-fold: 1) a detailed examination of dietary calcium intake in Black children living in a rural area whose inhabitants have been shown to have a high prevelance of hypocalcaemia, and 2) a comparison of two methods of dietary investigation used in studying a rural Black population.

10:1 Dietary calcium intake in rural Black children

Pettifor (1980) has suggested that bone deformities accompanied by abnormal serum biochemistry (lowered serum calcium and raised serum alkaline phosphatase levels) he observed were due to a dietary calcium deficiency. This is the first documentation of children in a natural environment with what may be calcium deficiency rickets. Most of these children came from Driefontein, a Tribal Trust Area in the Eastern Transvaal. He also found that children in Driefontein had a higher prevalance of hypocalcaemia, raised serum alkaline phosphatase concentrations and lower urinary calcium excretion that children in either a nearby urban community or Soweto (Pettifor et.al. 1978b).

The children in this study were selected to represent three distinct points in the pattern of skeletal growth and calcium retention based on increase in total body calcium to total body weight gain, as described by Leitch and Aitken (1959). A detailed dietary history and 24-hour dietary recall was taken from each subject and calculated dietary calcium intake was viewed in relation to biochemical and radiological findings.

Children at age level 1, 3 to 5 years, when the increment in skeletal calcium is at its lowest, showed no significant differences in serum biochemistry or in radiology other than lowered serum calcium, the

criteria for selection, between the study and control groups. The study group, however, did have a significantly lower calcium intake per kg body weight than the control group (who had normal serum calcium concentrations), although there was no correlation between calcium intake and serum calcium concentration. Indeed, there were no significant relationships betweeen the hypocalcaemia and the dietary calcium intake and any of the other measurements of calcium status (serum alkaline phosphatase, bone mineral density, metacarpal thickness, calcium excretion). Because breast feeding is usually prolonged in rural African society, it may be that diets have not been deficient in calcium long enough to manifest abnormalities of calcium metabolism other than lowered serum calcium concentrations. Too, because 3 to 5 years is a period of slow bone growth, compensatory mechanisms triggered by hypocalcaemia may provide for increased calcium absorption sufficient to meet the needs at this age.

For the older children in the study, however, there were significant findings highlighting the relationship between dietary calcium intake and measurements of calcium nutrition. Between 8 and 10 years (age level 2) there is a sharp increase in skeletal calcium retention which peaks with the pubertal growth spurt at 13-16 years (age level 3). At age level 2 the children in the study group (serum calcium $<$ 9,0 mg/dl, serum alkaline phosphatase $>$ 300 IU/l) had a mean dietary calcium intake (mg/kg body weight) of less than half that of the control group (serum calcium $>$ 9,0 mg/dl, serum alkaline phosphatase $<$ 300 IU/l). Dietary calcium showed a significant correlation with all other parameters of calcium status measured: serum alkaline phosphatase, urinary calcium excretion, bone mineral density and metacarpal thickness. The difference in calcium intake (mg/kg body weight) at age level 3 was also significant, althought not quite so striking, probably because both the study and control groups had low calcium intakes. There is no evidence that calcium intake alone is responsible for growth, however, in this

study there was a significant difference in calcium intake, but not in energy or protein intakes, between children above the 50th percentile on the Harvard height for age charts and those below it.

Different geographical populations live in apparent physiological health on a wide range of calcium intakes. That there may be ethnic or environmental differences allowing growth and adequate maintenance of calcium nutrition by people in developing countries with a wide range of low (by Western standards) dietary calcium cannot be denied. However, the children in this study, although showing no overt signs of rickets do not represent optimum calcium nutrition. The mean dietary intake (mg/kg body weight) at age levels 2 and 3 is lower than that of the balance studies cited in the text (Nicholls et.al. 1939, Holemans et.al. 1945, Begum and Pereira 1969). It would appear that this method of expressing calcium intake (mg/kg body weight) most truly represents calcium requirements.

Calcification during childhood, growth patterns and calcium metabolism during growth need to be more fully investigated with reference to the population under study. Walker (1972) found that by adulthood, bone mineral calcium showed no significant differences between South African Blacks, Indians, Whites and Ugandan Blacks, and in conjunction with studies of calcium intake in these groups, he concluded, "...a low calcium intake, therefore, does not prejudice bone composition." The present study would show, during childhood certainly, that this statement is not valid. Because of the wide individual and group variation in apparent calcium needs, population studies are not effective in establishing calcium requirements for individuals. Balance studies alone would appear to be inadequate for estimating calcium requirements during growth; new methods involving anthropometry, bone density and body composition need to be devised which, when combined with balance studies may indicate useful guidelines for optimum calcium nutrition (Irwin and

Keinholz 1973). This thesis does not attempt to establish recommended calcium intakes, however the results presented would indicate that ±400 mg calcium per day is the minimum intake to maintain adequate calcium status as an allowance for the general childhood (8-16 years) population.

The possibility of bringing the Driefontein children up to the standard of developed countries as far as dietary calcium intake is probably not possible either economically or culturally. Doubts exist that such a goal is necessary or even desirable (Walker 1972). However, as meeting nutritional standrads bore no relationship to money spent on food, nutritional education into better use of available resources is necessary. This would be possible in a community that is as well motivated as Driefontein appears to be, particularly if plans for social intervention in the community come to fruition. That people have dairy cows and do not use the milk for their children and that in spite of year-round availability of water, there is virtually no cultivation during the winter are two problems which can be remedied and can possibly make a significant contribution to daily calcium intake.

10:2 Dietary investigations in a rural Black population

To test the 24-hour dietary recall method as an investigative technique, duplicate diet analysis was carried out on a 24% sample of the study participants and results compared with those calculated from the food tables. Results show that the 24-hour dietary recall as a method of investigation used in this thesis would seem to be a successful tool in nutrition research in a rural Black population. Both Lubbe (1968) and Rutishauser (1973) working in Africa also found excellant agreement between the 24-hour dietary recall and other, more time and labour-consuming methods. Probably the very simplicity of the diet of the rural African contributes to this for in more sophisticated economies, workers have not found the 24-hour dietary recall to be an

accurate record of dietary intake (Young et.al. 1952, Schaefer 1966, Lenner et.al. 1977). Present results would recommend that a check of the 24-hour dietary recall be made with particular reference to the nutrient(s) under study to be assured of accurate findings. This is particularly relevant when the diet consists of a single staple which is grown and milled locally.

Because the 24-hour dietary recall method of investigation can be used to gather information from a large number of subjects and becuase only one professional nutritionist controlling trained lay workers is necessary, this is a particularly successful method of nutritional investigation.

APPENDIX A: COSTING MENU PATTERNS, TABLE 4:4

Table 4:4 (page 28) lists menu patterns to meet the FAO/WHO recommended daily allowance for calcium for three age groups, 1-9 years, 10-15 years, 16-19 years. Three menu patterns are included for each age group: 1) milk as the main source of calcium, 2) mixed diet and milk, 3) mixed diet with a minimum amount of milk. Prices are from the Witwatersrand in November 1982. Table A:1 lists the cost per food item as purchased and Table A:2 is the costing of the menu patterns.

Table A:1 Cost of food items used in menu patterns to meet FAO/WHO calcium allowances (Witwatersrand, November 1982)

Food	Cost in cents/unit	
	as purchased	as used in menu
Milk	58/litre	5,8/100 ml
Brown bread	39/kg	3,9/100 g
Rice	109/kg	2,0/75 g cooked
Dried beans	56/500 g	5,0/100 g cooked
Fruit (selection)	45/kg	6,0/piece
Tinned fish	54/106 g	27,0/50 g
Peanut butter	64/410 g	2,4/15 g
Meat/chicken OR Peanut butter	264/kg 64/410 g	2,1/15 g

Table A:2 Costing menu patterns, Table 4:4

Menu	Food	1-9 years		10-15 years		16-19 years	
		Cost Cent	Total Cents	Cost Cent	Total Cents	Cost Cent	Total Cents
1) milk as main calcium	Milk	29		36		36	
	Brown bread	04	33	05	41	05	41
2) mixed diet and milk	Milk	15		22		22	
	Brown bread	04		07		07	
	Rice	02		04		04	
	Dried beans	05		05		05	
	Fruit	12	38	18	56	12	50
3) mixed diet, minimum milk	Milk	01		01		01	
	Brown bread	07		10		10	
	Rice	04		06		04	
	Dried beans	05		08		05	
	Fruit	12		24		24	
	Tinned fish	27		27		27	
	Peanut butter			03		03	
	Peanut butter/ Meat	02	58	02	81	02	76

APPENDIX B: FORM FROM STUDY GROUP, AGE LEVEL 2

10/14/78

Study Number ~~5~~ 16
86

INVESTIGATION INTO DIETARY CALCIUM INTAKE

Name NELLIE MABASO Age 10 Sex M (F)

Family history:

Father:			Mother:		
Alive	(yes)	no	Alive	(yes)	no
Living in Driefontein	yes	(no)	Living in Driefontein	(yes)	no
Living with child	yes	(no)	Living with child	(yes)	no
Employed	(yes)	no	Employed	yes	(no)
If so, as what	SALESMAN		If so, as what		

Plural marriage yes (no)

How many people live in the house 20

Siblings' ages:

19 (M) F	8 M (F)	____ M F	____ M F
16 M (F)	5 M (F)	____ M F	____ M F
14 M (F)	3 (M) F	____ M F	____ M F
12 M (F)	1 (M) F	____ M F	____ M F

Does any member of the family living away from home send money or food home regularly? (Money) + (Food) Neither

How much money is available to the household R10 (week)/month

Agricultural Accomplishments:

Does the family have a cow	yes	(no)
Does it give milk	yes	(no)
Approx, size of milking pail	N/A	
How many times a day milked	N/A	
Does it have a calf	yes	(no)
Does the family have cattle (other than a milk cow)	yes	(no)
Are they slaughtered	yes	(no)
How frequently	N/A	

Does the family have other livestock		sheep	(goats)	pigs	none
If they have goats, do they milk the goats		yes (no)			
Approx. how much milk		N/A			
Are any of these animals slaughtered		sheep	goats	pigs	
How frequently		N/A	N/A	N/A	
Does the family have fowls	geese	(chickens)	ducks	turkeys	none
Do they lay eggs	yes no	(yes) no	yes no	yes no	
How many eggs per day	____	2	____	____	
Are they slaughtered	geese	(chickens)	ducks	turkeys	
How frequently	____	Once a week	____	____	

Study Number 16
87

Daily diet (of the child):

Does the child have a good appetite (yes) no

How many meals are eaten per day 1 2 (3) 4

Approximately what time are the meal(s) eaten

B = 7.00 a.m D - 8 00 p.m

L 3.00 p.m

Does he eat other than at mealtime (yes) no

If so, what and how often

Buns at breakfast at school

Tea/(coffee) Do s he use milk yes (no) fresh condensed powdered how much N/A

sugar (yes) no how much 2 teaspoons

Recall of the previous day: fod food and amount the child ate:

Meal	I	II	III	
Mealie meal (thin porridge				
Mealie meal (stiff porridge)		2 cups	2 cups	
Samp				
1/2 Bread (thickness of slice (specify (brown)/white)	2 slices			
Butter/margarine				
Jam				
Milk/sour milk				
Tea/(coffee)	1 cup			
Meat/poultry/fish				
Eggs				
Vegetables: Potato			3/4 cup	
Cabbage		3/4 cup		
Fruit				
Other foods				4 Buns

Study number 98 16

Which day of the week was recalled Monday Tuesday Wednesday Thursday (Friday) 88
Saturday Sunday

Is the day recalled a typical daily intake (yes) no

Does the child ever eat:				how frequently
Milk	fresh	(yes)	no	Once a week
	condensed	(yes)	no	Once a week
	powdered	yes	(no)	
Butter/margarine		(yes)	no	Everyday
Eggs		(yes)	no	2x a week
Chicken/fowls		(yes)	no	Once a week
Lamb/mutton		(yes)	no	Once a month
Beef		(yes)	no	2x a week
Goat		(yes)	no	2x in 4month
Potatoes		(yes)	no	2x a week
Sweet potatoes		(yes)	no	Once a week
Spinach		(yes)	no	Once a week
Cabbage		(yes)	no	2x a week
Dried peas/beans		(yes)	no	Once a week
Peanuts		(yes)	no	Once a week
Rhubarb		yes	(no)	3,
Citrus fruit		yes	no	2x a week
Brown bread		yes	no	—
Breakfast cereals		yes	(no) what kinds	—
Oatmeal		yes	(no)	—
Cocoa powder		yes	(no)	—
Chocolate		yes	(no)	0 —
Sweets		(yes)	no	Once a week
Crisps		(yes)	no	"
Peanut butter		(yes)	no	"

Water supply: (of the whole family)

Where does the water come from (river) spring open well covered well other_____

How much water is collected daily? 2x a day

How much water does the child drink daily? 1 2 3 (4) 5 6 7 8 cupsful

Study Number 88 89 * 16

Shopping facilities: (for the whole family)

Do you go grocery shopping yes (no)

How often do you go grocery shopping In Driefontein daily (weekly) monthly

In Piet Retief weekly monthly (never)

What do you buy In Driefontein In Piet Retief

Mealie rice
Condensed milk
Milk
Fish
Meat
Bread

Approximately how much do you spend R15 per week month

What is the supply source of the following food items (including quantities)

Food item	Produce themselves	Bought How frequently?
Mealie meal	✓	
Samp/mealie rice		Once a week
Bread (brown)/white		Everyday
Butter/margarine		Once a week
Milk powdered (condensed) (fresh)		Once a week
meat/poultry/fish		2x a week
Vegetables (kinds) Tomato, Onion Cabbage, Spinach		Once a week
Fruit (kinds) Apples		Once a week

How much salt do you buy each month? 2lbs.

What kind of salt do you buy (plain) iodized (ask to see the packet)

Do you know what iodized salt is yes (no)

STUDY NO: ~~8~~ 16

COOKING FACILITIES:

Cooking facility available (Stove) Open fire

How big is the cooking facility; one pot two pots (three/more pots.

Approx. size pot used for mealie meal 20 cm

Approx. size pot used for meat/veg/stew. 15 cm

Size drinking cups Homemade mug - 2lbs can

Size table (serving) spoons. Wooden spoon - T. spoon

STORAGE FACILITIES:

Is refrigeration available Yes (No.)

How is meat stored? never really stored

How are vegetables/fruit stored? in the cool area of the house

How is meal stored dishes.

GARDEN:

Is there a garden? (Yes) No.

Who tends the garden MOTHER

What is grown

Summer	Winter
Spinach	Spinach
Cabbage	
Potatoes	

Where do you get the seeds? STORE

INTERVIEWERS NOTES:

Who was interviewed? (Mother) Father Female relative Male relative.

Does this person do the cooking (Yes) No.

Do you think the interviewee was telling the truth? (Yes) No.

House Tidy (Untidy)

Garden tidy untidy

What is growing in the garden?

Potatoes

Did you see evidence of animals (Yes) No.

What animals? (Fowls) Cattle (Goats) Domestic Pets

Dog/s Two

Cat/s One

APPENDIX C: FORM FROM CONTROL GROUP, AGE LEVEL 2

Study Number 39
92

INVESTIGATION INTO DIETARY CALCIUM INTAKE

Name LAEZA NKABINDE Age 9 Sex M (F)

Family history:

Father:			Mother:		
Alive	(yes)	no	Alive	(yes)	no
Living in Driefontein	(yes)	no	Living in Driefontein	(yes)	no
Living with child	(yes)	no	Living with child	(yes)	no
Employed	(yes)	no	Employed	yes	(no)
If so, as what	WOOD FACTORY		If so, as what		

Plural marriage yes (no)

How many people live in the house 17

Siblings' ages:

20 M F	12 M F	3 M F	_____ M F	
18 M F	10 M F	1 M F	_____ M F	
14 M F	9 M F	_____ M F	_____ M F	
14 M F	5 M F	_____ M F	_____ M F	

Does any member of the family living away from home send money or food home regularly? Money Food (Neither)

How much money is available to the household R40 week/(month)

Agricultural Accomplishments:

Does the family have a cow (yes) no

Does it give milk yes (no)

Approx, size of milking pail N/A

How many times a day milked N/A

Does it have a calf yes (no)

Does the family have cattle (other than a milk cow) (yes) no

Are they slaughtered yes (no)

How frequently N/A

Does the family have other livestock sheep goats pigs (none)

If they have goats, do they milk the goats yes (no)

Approx. how much milk N/A

Are any of these animals slaughtered			sheep		goats		pigs		
How frequently			N/A		N/A		N/A		
Does the family have fowls	geese		(chickens)		ducks		turkeys		none
Do they lay eggs	yes	no	(yes)	no	yes	no	yes	no	
How many eggs per day	_____		6 eggs		_____		_____		
Are they slaughtered	geese		(chickens)		ducks		turkeys		
How frequently	_____		Once/week		_____		_____		

1/8/18 LAEZA NKABINDE 9F Study Number 39/93

Daily diet (of the child):

Does the child have a good appetite (yes) no

How many meals are eaten per day 1 2 (3) 4

Approximately what time are the meal(s) eaten

B = 7:30 a.m B = 8:00 p.m

L = 2:00 p.m

Does he eat other than at mealtime (yes) no

If so, what and how often

Fat cakes and achaar at 11:00 a.m

(Tea)/coffee Do s he use milk (yes) no fresh (condensed) powdered how much 1 teaspoon

sugar yes no how much 3 T.spoons

Recall of the previous day: fod fodd and amount the child ate:

Meal	I	II	III	IV
Mealie meal (thin porridge				
Mealie meal (stiff porridge)		2 cups	2 cups	
Samp				
½ Bread (thickness of slice (specify brown/white)	2 slices			
Butter/margarine	1 T.spoon			
Jam				
Milk/sour milk			1 cup	
Tea/coffee	1 cup			
Meat/poultry/fish				
Eggs				
Vegetables: Potato		½ cup		
Cabbage		½ cup.		
Fruit				
Other foods				4 fat cakes + Achaar

Study number TE 39
94

Which day of the week was recalled Monday (Tuesday) Wednesday Thursday Friday Saturday Sunday

Is the day recalled a typical daily intake (yes) no

Does the child ever eat:				how frequently
Milk	fresh	(yes)	no	Once a week
	condensed	(yes)	no	Once a day
	powdered	yes	(no)	
Butter/margarine		(yes)	no	Once a day
Eggs		(yes)	no	2x a week
Chicken/fowls		(yes)	no	Once a week
Lamb/mutton		(yes)	no	Once a year
Beef		(yes)	no	2x a week
Goat		(yes)	no	Once a year
Potatoes		(yes)	no	3x a week
Sweet potatoes		(yes)	no	When available
Spinach		(yes)	no	Once a week
Cabbage		(yes)	no	2x a week
Dried peas/beans (WHITE)		(yes)	no	Once a week
Peanuts		(yes)	no	Once a week
Rhubarb		yes	(no)	
Citrus fruit		(yes)	no	2x a week
Brown bread		(yes)	no	Everyday
Breakfast cereals		yes	(no)	what kinds
Oatmeal		yes	(no)	
Cocoa powder		yes	(no)	
Chocolate		yes	(no)	
Sweets		yes	no	Everyday
Crisps		yes	no	Once a week
[illegible] butter		yes	no	When available at home

Water supply: (of the whole family)

Where does the water come from river spring (open well) covered well other_____

How much water is collected daily? 3x a day

How much water does the child drink daily? 1 2 3 4 (5) 6 7 8 cupsful

Study Number 王-37
95

Shopping facilities: (for the whole family)

Do you go grocery shopping yes (no)

How often do you go grocery shopping In Driefontein daily (weekly) monthly

In Piet Retief (weekly) monthly never

What do you buy In Driefontein

Bread
Tea
Mealie Meal
Meat

In Piet Retief

Condensed milk
Sugar
Tomatoes

Approximately how much do you spend R20 (per week) month

What is the supply source of the following food items (including quantities)

Food item	Produce themselves	Bought How frequently?
Mealie meal		Once a week
Samp/(mealie rice)		Once a week
Bread (brown)/white		Everyday
Butter/(margarine)		Once a week
Milk powdered (condensed) (fresh)		Once a week Once a week
meat/poultry/fish	Poultry	Twice a week - Meat
Vegetables (kinds)	Spinach Cabbage Potatoes	Tomatoes - once a week
Fruit (kinds)		Once a week - Oranges

How much salt do you buy each month? One pound

What kind of salt do you buy (plain) iodized (ask to see the packet)

Do you know what iodized salt is yes (no)

STUDY NO: # 39
98
96

COOKING FACILITIES:

Cooking facility available (Stove) Open fire

How big is the cooking facility; one pot (two pots) three/more pots.

Approx. size pot used for mealie meal 10 litres

Approx. size pot used for meat/veg/stew. 5 litres

Size drinking cups Mug

Size table (serving) spoons. Tablespoon

STORAGE FACILITIES:

Is refrigeration available Yes (No.)

How is meat stored? Cool area of the house

How are vegetables/fruit stored? Usually picked from garden

How is meal stored in Pots.

GARDEN:

Is there a garden? (Yes) No.

Who tends the garden FATHER

What is grown Summer Winter

Spinach
Cabbage
Potatoes

Where do you get the seeds? STORE

INTERVIEWERS NOTES:

Who was interviewed? Mother Father Female relative Male relative. child

Does this person do the cooking (Yes) No.

Do you think the interviewee was telling the truth? (Yes) No.

House (Tidy) Untidy

Garden (tidy) untidy

What is growing in the garden?

Spinach.
Potatoes
Cabbage

Did you see evidence of animals (Yes) No.

What animals? (Fowls) (Cattle) Goats Domestic Pets
Dog/s one
Cat/s

LIST OF REFERENCES

Acheson, K.J., Campbell, I.T., Edholim, O.G., Miller, D.S. and Stock, M.J. (1978) 'The measurement of food and energy intake in man--an evaluation of some techniques.' Amer. J. Clin. Nutr., 35:1147-1154.

Adams, T.H. and Norman, A.W. (1970) 'Studies on the mechanism of action of calciferol. 1. Basic parameters of vitamin D mediated calcium transport.' J. Biol. Chem. 245:4421-4431.

Ahrens, E.H. and Boucher, C.A. (1978) 'The composition of a simulated American diet.' J. Amer. Diet. Assoc. 73:613-620.

Albanese, A.A., Edelson, A.H., Woodhull, M.W., Lorenze, E.J., Wein, E.H. and Orto, L.A. (1973) 'Effect of a calcium supplement on serum cholesterol, calcium, phosphorus and bone density of "normal healthy" elderly females.' Nutr. Rep. Int. 8:119-130

Albright, F. and Reifenstein, E.C. (1948) The Parathyroid Glands and Metabolic Bone Disease. Baltimore: Williams and Wilkins.

Allen, T., MacLeod, A.V. and Young, E.C. (1953) 'On the nutritional requirements of young children with particular reference to calcification.' Can. J. Med. Sci. 31:447-461.

American Academy of Pediatrics, Committee on Nutrition (1978) 'Calcium requirements in infancy and childhood.' Pediatrics 62:826-834.

Anand, C.R. and Linkswiler, H.M. (1974) 'Effect of protein intake on calcium balance of young men given 500 mg calcium daily.' J. Nutr. 104:695-700.

Armbrecht, H.J., Zinser, T.V., Gross, C.J. and Davis, B.B. (1980) 'Adaptation to dietary calcium and phosphorus restriction changes with age in the rat.' Am. J. Physiol. 239:E322-327.

Aykroyd, W.R. and Krishnan, B.G. (1938) 'Effect of calcium lactate on children in a nursery school.' Lancet 2:153-155.

Aykroyd, W.R. and Krishnan, B.C. (1939) 'A further experiment of the value of calcium lactate for Indian children.' Indian J. Med. Res. 27:409-412.

Bansal, P., Rau, P., Venkatachalam, P.S. and Gopalan, C. (1964) 'Effect of calcium supplementation on children in a rural community.' Indian J. Med. Res. 52:219-223.

Bass, M.A. and Wakefield, L.M. (1974) 'Nutrient intake and food patterns of Indians on Standing Rock Reservation.' J. Amer. Diet. Assoc. 64:36-41.

Begum, A. and Pereira, S.M. (1969) 'Calcium balance studies on children accustomed to low calcium intakes.' Brit. J. Nutr. 23:905-911.

Bergeim, O. (1926) 'Carbohydrates and calcium and phosphorus absorption.' J. Biol. Chem. 70:34-45.

Berry, E.M., Gupta, M.M., Turner, S.J. and Burns, R.B. (1973) 'Variation in plasma calcium with induced changes in plasma specific gravity, total protein and albumin.' Brit. Med. J. 4:640-743.

Bogdonoff, N.D., Shock, N.W. and Nichols, M.P. (1953) 'Calcium, phosphorus, nitrogen and potassium balance sludies in the aged male.'J. Gerontol. 8:272-288.

Boyne, A.W. (1960) Human Growth III, Ed. J.M. Tanner. Oxford:Pergamon Press.

Briscoe, A.M. and Ragan, D. (1963) 'Enhancement of calcium absorption in man by a bile acid sequestrant.' Amer. J. Clin. Nutr. 13:277-284.

Brock, J.F. and Autret, M. (1952) 'Kwashiorkor in Africa.' Bull. World Health Organ. 5:1-71

Brock, J.F. and Latskey, J.M. (1942) 'The findings of the Cape Nutrition Study.' S. Afr. Med. J. 16:255-260

Bulusu, L., Hodgkinson, A., Nordin, B.E.C. and Peacock, M. (1970) 'Urinary excretion of calcium and creatinine in relation to age and body weight in normal subjects and pateints with renal calculus.' Clin. Sci. 38:601-612.

Bunim, J.J., Black, R.L., Lutwak, L., Peterson, R.C. and Wheedon, G.D. (1958) 'Studies on desamethasone: a new synthetic steroid in rheumatoid arthritis. A preliminary report.' Arthritis and Rheumatism 1:313-331.

Bureau of Statistics, Office of the Prime Minister (1970) Japanese Statistical Yearbook Tokyo.

Burgess, H.J.L. and Burgess, A. (1975) 'A field worker's guide to a nutritional status survey.' Amer. J. Clin. Nutr. 28:1299-1321.

Cahn, A. and Roche, A.F. (1961) 'The influence of illness and calcium intake on the rate of skeletal maturation in children.' Brit. J. Nutr. 15:411-417.

Caspary, W.F. (1971) 'Effects of biguanides on intestinal transport of sugars, amino acids and calcium.' Arch. Pharmacol. 269:421-422.

Cequeira, M.T., Fry, M.M. and Connor, W.E. (1979) 'The food and nutrient intake of the Tarahumara Indians of Mexico.' Amer. J. Clin. Nutr. 32:905-915.

Chalmers, F.W., Clayton, M.M., Gates, L.O., Ticher, P.E., Weitz, A.W., Young, E.M. and Foster, W.D. (1952) 'Dietary records--how many and which days?' J. Amer. Diet. Assoc. 28:711-717.

Comar, C.L. and Bronner, F. (1964) Mineral Metabolism: An Advanced Treatise. New York:Academic Press.

Comar, C.L., Wasserman, R.H. and Nold, M.M. (1956) 'Strontium-calcium discriminating factors in the rat.' Proc. Soc. Exp. Biol. 92:859-863.

Compston, J. (1979) 'Rickets in Asian immigrants.' Brit. Med. J. 2:619.

Condon, J.R., Nassim, J.R. Millard, F.J.C., Bilbe, A. and Stainthorpe, E.M. (1970) 'Calcium and phosphorus metabolism in relation to lactose tolerance.' Lancet 1:1027-1029,

Crawford, M.D., Gardner, M.J. and Morris, J.N. (1968) 'Mortality and hardness of water supplies.' Lancet 1:827-831.

Cruickshank, E.W.H., Duckworth, J., Kosterlitz, H.W. and Warnock, G.M. (1945) 'The digestibility of the phytate-P of patmeal in adult man.' J. Physiol. 104:41-46.

Daniels, A.L., Hutton, M.K., Knott, E., Everson, G. and Wright, O. (1933) 'Relation of milk ingestion to calcium metabolism in children.' Proc. Soc. Exp. Biol. Med. 30:1062-1063.

_____, (1934) 'Relation of ingestion of milk to calcium metabolism in children.' Amer. J. Dis. Child. 47:499-512.

Daniels, A.L., Hutton, M.K., Knott, E.M., Wright, O.E. and Forman, O. (1935) 'Calcium and phosphorus needs of preschool children.' J. Nutr. 10:373-388.

Davidson, S., Passmore, R. and Brock, J.F. (1973) Human Nutrition and Dietetics, 5th ed. London:Livingstone.

Dent, C.E. (1962) 'Some problems of hyperparathyroidism.' Brit. Med. J. 2:1419-1425, 1495-1500

Dent, C.E., Rowe, D.J.F., Round, J.M. and Stamp, T.C.B. (1970) 'Osteomalacia with longterm anticonvulsant therapy in epilepsy.' Brit. Med. J. 4:69-72.

Department of Health and Social Security (1969) Recommended daily intakes of energy and nutrients for the U.K. London:Her Majesty's Stationery Office.

Department of National Health and Welfare, Committee for Revision of the Canadian Dietary Standard (1975) Dietary Standard for Canada revised edition. Ottowa:Government Printers.

Diem, R. and Letner, C. (1970) Scientific Tables--Documenta Geigy. Basle:J.R. Geigy.

Donath, A., Nordio, S., Macagno, F. and Gatti, R. (1971) 'The effect of hydrochlorathiazide on calcium and strontium transport in intestine and kidney.' Helv. Paediat. Acta. 25:293-300.

Doraiswamy, T.R., Daniel, V.A., Rajalakshmi, D., Swaminathan, M. and Parpia, H.A.B. (1971) 'Effect of supplementing a poor diet based on rice and wheat consumed by school children with vitamins, minerals, lysine and protein-rich foods on their growth and nutritional status.' Nutr. Rep. Int. 3:67-78.

Duncan, L. (1976) Clinical analysis by atomic absorption spectroscopy Springvale:Varian Techtron (Pty.) Ltd.

Dunnigan, M.G., McIntosh, W.B. and Ford, J.A. (1976) 'Rickets in Asian immigrants.' Lancet 1:1346.

Dwyers, J.T., Dietz, W.H., Hass, G. and Suskind, R. (1979) 'Risk of nutritional rickets among vegetarian children.' Amer. J. Dis. Child. 133:134-140.

Eads, M.G. and Meredith, A.R. (1948) 'Dietary surveys: number of days necessary.' Public Health Report 63:777-781.

Edward, D.A.W., Hammond, W.H., Healy, M.J.R., Tanner, J.M. and Whitehouse,R.H. (1955) 'Design and accuracy of calipers for measuring subcutaneous tissue thickness.' Brit. J. Nutr. 9:133-143.

Ehrig, U. Harrison, J.E. and Wilson, D.R. (1974) 'Effect of long term thiazide therapy on intestinal calcium absorption in patients with recurrent renal calculi.' Metabolism 23:139-149.

Emmons, L. and Haynes, M. (1973) 'Accuracy of the 24-hour recalls of young children.' J. Amer. Diet. Assoc. 62:40(-416.

Farquharson, R.F., Salter, W.T. and Aub, J.C. (1931) 'Studies of calcium and phosphorus metabolism. XIII The effect of phosphates on the excretion of calcium.' J. Clin. Invest. 10:251-269.

Fellers, F.X. and Herrera, C. (1972) 'Medical management of simple familial rickets.' Orthop. Clin. North Am. 3:241-249.

Fernández, N.A., Burgos, J.C., Asenjo, C.F. and Rosa, I. (1971) 'Nutritional status of the Puerto Rican population: Master sample survey.' Amer. J. Clin. Nutr. 24:952-965.

Fisher, M. and Davidson,W. (1970) 'Growth curves for some Zambian school children.' J. Trop. Pediat. 16:103-111.

Food and Nutrition Board (1968) 'Recommended daily dietary allowances.' National Academy of Science: National Research Council. Washington, D.C.

Fox, F.W. (1966) Studies on the Chemical Composition of Foods Commonly used in Southern Africa. Johannesburg:South African Institute for Medical Research.

Frank, G.C., Berenson, G.S., Schilling, P.E. and Moore, M.C. (1977) 'Adapting the 24-hour recall for epidemiologic studies of school children.' J. Amer. Diet. Assoc. 71:26-30.

Frank, G.C., Voors, A.W., Schilling, P.E. and Berenson, G.S. (1977) 'Dietary studies on rural school children in a cardiovascular survey.' J. Amer. Diet. Assoc. 71:31-35.

Frank, G.C., Berenson, G.S. and Webber, L.S. (1978) 'Dietary studies and the relationship of diet to cardiovascular disease risk factor variables in 10-year old children: The Bogalusa heart study.' Amer. J. Clin. Nutr. 31:328-340.

Gallagher, J.C., Aaron, J.E., Horsman, A., Marshall, D.H., Wilkinson, R. and Nordin, B.E.C. (1973) 'Corticosteroid osteoporosis.' Clinics in Endocrin. and Met. 2:355-368.

Garn, S.M., Larkin, F.A. and Cole, P.A. (1978) 'The real problem with 1-day diet records.' Amer. J. Clin. Nutr. 31:1114-1116.

Garn, S.M., Pao, E.M. and Rihl, M.E. (1964) 'Compact bone in Chinese and Japanese.' Science 143:1439-1440.

Gray, T.K., Bieberdorf, F.A. and Fordtran, J.S. (1973) 'Thyrocalcitonin and the jejunal absorption of calcium, water and electrolytes in normal subjects.' J. Clin. Invest. 52:3084-3089.

Greaves, J.P. and Hollingsworth, D.H. (1966) 'Trends in food consumption in the U.K.' World Rev. Nutr. Diet. 6:35-89.

Greenhill, A. and Gruskin, A.B. (1976) 'Laboratory evaluation of renal function.' Pediatr. Clin. North Am. 23:661-679.

Gursel, E. (1970) 'Effects of diuretics on renal and intestinal handling of calcium.' N.Y. State J. Med. 70:399-405.

Hahn, R.J., Birge, S.J., Scharp, C.R. and Avioli, L.V. (1972) 'Phenobarbital induced alteration in vitamin D metabolism.' J. Clin. Invest. 51:741-748.

Harland, B.F., Johnson, R.D., Blenderman, E.M., Prosky, L., Vanderveen, J.E., Reed, G.L., Forbes, A.L. and Roberts, H.R. (1980) 'Calcium, phosphorus, iron, iodine and zinc in the "total"diet.' J. Amer. Diet. Assoc. 77:16-20.

Harrison, A.R. and Rose, G.A. (1968) 'The effect of bendrofluozide on urinary and faecal calcium and phosphorus.' Clin. Science 34:343-350.

Harrison, J.E., Hitchman, A.J.W., Finaly, J.M. and McNeil, K.E. (1969) 'Calcium kinetic studies in patients with malabsorption syndrome.' Gastroenterology 56:751-757.

Haeney, R.P. and Skillman, T.G. (1964) 'Secretion and excretion of calcium by the human G.I. tract.' J. Lab. Clin. Med. 64:29-41.

Hegsted, D.M. and Linkswiler, H.M. (1980) 'Long term effects of level of protein intake on calcium metabolism in young adult women.' J. Nutr. 111:244-251.

Hegsted, D.M., Moscoso, I. and Collazos, Ch.C. (1952) 'A study of the minimum calcium requirements of adult men.' J. Nutr. 46:181-201.

Henderson, J. McA. and Kelly, F.C. (1929) 'The influence of certain dietary supplements in relation to the calcium requirements of growing African natives. II.' J. Hyg. 29:439-442.

Henneman, P.H., Forbes, A.P., Moldawer, M., Dempsey, E.F. and Carroll, E.L. (1960) 'Effects of human growth hormone in man.' J. Clin. Invest. 39:1223-1238.

Herbst, O. (1912) 'Beiträge zur Physiologie des Stoffwechsels im Knabenalter mit besonderer Berücksichtigung einiger Mineralstoffe.' Jahrb. Kinderheilk 76:40. Cited by: Sherman, H.C. and Hawley, E. (1922) 'Calcium and phosphorus metabolism in childhood.' J. Biol. Chem. 53:375-399.

Herbst, O. (1913) 'Calcium und Phosphor beim Wachstum am Ende der Kindheit.' Z. Kinderheilk 7:161. Cited: Wang, C.C. et.al. (1936) 'Calcium and phosphorus metabolism in childhood.' J. Biol. Chem. 52:41-53.

Hodges, R.E. and Krehl, W.A. (1965) 'Nutritional status of teenagers in Iowa.' Amer. J. Clin. Nutr. 17:200-210.

Holemans, K. and Lambrechts, A. (1958) 'Calcium and phosphorus balances in African children (Retention and minimum requirements).' J. Trop. Pediat. 4:43-49.

Hollingsworth, D. and Russell, M. (1973) Nutritional Problems in a Changing World. London:Applied Science Publishers.

Holmes, J.O. (1945) 'The requirement for calcium during growth.' Nutr. Abstr. Rev. 14:597-612.

Holt, L.E. and Fales, H.L. (1923) 'Calcium absorption in children on a diet low in fat.' Amer. J. Dis. Child. 25:247-256.

Hunscher, H.A., Hummel, F.C. and Macy, I.G. (1936) 'Variability of metabolic response of different children to a given intake of calcium' Proc. Soc. Exp. Biol. Med. 35:189-197.

Ireland, P. and Fordtran, J.S. (1973) 'Effect of dietary calcium and age on jejunal calcium absorption in humans studied by intestinal perfusion.' J. Clin. Invest. 52:2672-2681.

Irving, J.T. (1957) Calcium Metabolism. London:Methuen.

Irwin, M.I. And Kienholz, E.W. (1973) 'A conspectus of research on calcium requirements of man.' J. Nutr. 103:1019-1095.

Jowsey, J. (1976) Metabolic Diseases of Bone. Philadelphia: W.B. Saunders, Co.

Johnson, N.E., Alcantara, E.N. and Linkswiler, H.M. (1970) 'Effect of level of protein intake on urinary and faecal calcium and calcium retention of young adults.' J. Nutr. 100:1425-1430.

Johnston, F.A., Schlaphoff, D. and McMillan, T. (1950) 'Calcium retained from one level of intake by six adolescent girls.' J. Nutr. 41:137-147.

Joseph, K., Kurien, P.P., Swaminathan, M. and Subrahmanyan, V. (1959) 'The metabolism of nitrogen, calcium and phosphorus in undernourished children. 5. The effect of partial or complete replacement of rice in poor vegetarian diets by ragi (Eleusine coracana) on the metabolism of nitrogen, calcium and phosphorus.' Brit. J. Nutr. 13:213-218.

Joseph, K., Narayanarao, M., Swaminathan, M. and Subrahmanyan, V. (1958) 'The metabolism of nitrogen, calcium and phosphorus in undernourished children. 4. The effect of replacing rice in the diet by tapioca macaroni on the metabolism of nitrogen, calcium and phosphorus.' Brit. J. Nutr. 12:429-432.

Kark, S.L. and LeRiche, H. (1944) 'A health study of South African Bantu school children.' S. Afr. Med. J. 18:100-103.

Karnani, B.T., De, S.S., Subrahmayan, V. and Cartner, D. (1948) 'Relative utilization of calcium from soya milk (fortified with di-calcium phosphate) and cow's milk by growing children.' Indian J. Med. Res. 36:355-360.

Kinsman, G., Sheldon, D., Jensen, E., Bernds, M., Outhouse, J. and Mitchell, H.H. (1939) 'The utilization of the calcium of milk by pre-school children.' J. Nutr. 17:429-441.

Kodicek, E. (1967) 'The absorption of calcium from the intestine.' Proc. Nutr. Soc. 26:67-72.

Kooh, S.W., Fraser, D., Reilly, B.J., Hamilton, J.R., Gall, D.G. and Bell, L. (1977) 'Rickets due to calcium deficiency.' N. Eng. J. Med. 297:1264-1266.

Kugelmass, I.N. (1940) The Newer Nutrition in Pediatric Practice. Philadelphia:Lea and Febiger.

Kunerth, B.L. and Pittman, M.S. (1939) 'A long-time study of nitrogen, calcium and phosphorus metabolism on a low protein diet.' J. Nutr. 17:161-171.

Leichsenring, J.M., Norris, L.M. and Lamison, S.A. (1951) 'The effect of level of intake on calcium and phosphorus metabolism in college women.' J. Nutr. 45:407-418.

Leitch, I. (1937) 'The determination of the calcium requirements of man.' Nutr. Abst. Rev. 6:553-578.

Leitch, I. and Aitken, F.C. (1959) 'The estimation of calcium requirement: a re-examination.' Nutr. Abstr. Rev. 29:393-411.

Lengemann, F.W. (1967) 'Overall aspects of calcium and strontium absorption.' In The Transfer of Calcium and Strontium across Biological Membranes. ed. R.H. Wasserman, New York:Academic Press.

Lenner, R.A., Bingtsson, C. Carlgren, G., Isaksson, B., Lundgren, B.K. Petersson, I. and Tibbin, E. (1977) 'The study of women in Gothenburg 1968-1969.' Acta. Med. Scand. 202: 183-188

Lowe, C.U. (1975) 'Reflections of dietary studies with children in the ten-state nutrition survey of 1968-70.' Pediatrics 56:320-326.

Lubbe, A.M. (1968) 'A survey of the nutritional status of White school children in Pretoria: description and comparative study of two dietary survey techniques.' S. Afr. Med. J. 42:616-622.

Lutwak, I., Laster, L., Gitelman, H.J., Fox, M. and Wheedon, G.D. (1964) 'Effects of high dietary calcium and phosphorus on calcium, phosphorus, nitrogen and fat metabolism in children.' Amer. J. Clin. Nutr. 14:76-82.

Luyken, R. and Luyken-Koning, F.W.M. (1969) 'Studies on physiology of nutrition in Surinam. XII: Nutrition and development of muscular, skeletal and adipose tissue in Surinam children.' Amer. J. Clin. Nutr. 22:519-526.

Luyken, R., Luyken-Koning, F.W.M., Cambridge, T.H., Dohle, T. and Bosch, R. (1967) 'Studies on physiology of nutrition in Surinam. 10: Protein metabolism and influence of extra calcium on the growth of and calcium metabolism in boarding school children.' Amer. J. Clin. Nutr. 20:34-42.

MacFadyen, I.J., Nordin, B.E.C., Smith, D.A., Wayne, D.J. and Rae, S.L. (1965) 'Effect of variation in dietary calcium on plasma concentration and urinary excretion of calcium.' Brit. Med. J. 1:161-164.

Macy, I.G. (1942) Nutrition and Chemical Growth in Childhood, Vol. I: Evaluation. Springfield:Charles C. Thomas.

Malm, O.J. (1953) 'On phosphates and phosphoric acid in the calcium balance of man.' Scan. J. Clin. Lab. Invest. 5:75-84.

Malm, O.J. (1958) 'Calcium requirement and adaptation in adult man.' Scand. J. Clin. Lab. Invest. 10(suppl):1-289.

Maltz, H.E., Fish, M.B. and Holliday, M.A. (1970) 'Calcium deficiency rickets and the renal response to calcium infusion.' Pediatrics 46:865-870.

Mazess, R.B. and Cameron, J.R. (1972) 'Growth of bone in school children: comparison of radiographic morphometry and photon absorptionmetry.' Growth 36:77-91.

McCance, R.A. and Widdowson, E.M. (1960) The Composition of Foods London:Her Majersty's Stationery Office.

McCance, R.A., Widdowson, E.M. and Lehman, H. (1942) 'The effect of protein intake on the absorption of calcium and magnesium.' Biochem. J. 36:686-691.

McLean, D.S., Lewis, G.K., Jensen, E., Hathaway, M. Breiter, H. and Holmes, J.O. (1946) 'Further studies on the calcium requirement of preschool children.' J. Nutr. 31:127-140.

Melvin, K.E.W., Hepner, G.W., Bordier, P., Neale, G. and Joplin, G.F. (1970) 'Calcium metabolism and bone pathology in adult coeliac disease.' Quart. J. Med. 39:83-113.

Meredith, A., Matthews, A. Zichefasse, M. (1951) 'How well do school children recall what they have eaten?' J. Amer. Diet. Assoc. 27:749-751.

'Metabolic bone disease in Asians.' (1976) Brit. Med. J. 2:442.

Ministry of Health and Welfare (1958) Nutrition in Japan Tokyo.

Morgenstern, S., Kessler, G., Auerbach, J. , Flor, R.V. and Klein, B. (1965) 'An automated p-nitrophenyophosphate serum alkaline phosphatase procedure for the autoanalyser.' Clin. Chem. 11:876-888.

Mitchell, J.J. and Curzon, E.G. (1949) 'The dietary requirement of calcium and its significance.' Actualités Scientifiques et Industrielles No. 771 Paris:Herman and Co.

Morley, D. (1976) Paediatric Priorities in the Developing World. London:Butterworth.

Nicholls, L. and Nimalasuriya, A. (1939) 'Adaptation to a low calcium intake in reference to the calcium requirements of a tropical population.' J. Nutr. 18:563-577.

Nicolaysen, R., Eeg-Larsen, N. and Malm, O. (1953) 'Physiology of calcium metabolism.' Physiol. Rev. 33:424-444.

Nie, H.N., Hull, C.H., Jenkins, J.G., Steinbrenner, K. and Bent, D. (1975) Statistical Package for the Social Sciences. 2nd Ed. New York:McGraw-Hill.

Nordin, B.E.C. (1959) 'assessment of the calcium excretion from the urinary calcium/creatinine ratio.' Lancet 2:368-371.

Nordin, B.E.C. (1973) Metabolic Bone and Stone Disease. London: Churchill Livingstone.

Nordin, B.E.C. (1976) Calcium, Phosphorus and Magnesium Metabolism, Clinical Physiology and Diagnotic Procedures. London: Churchill Livingstone.

Nordin, B.E.C., Horsman, A. and Gallagher, J.C. (1974) 'Effect of various therapies on bone loss in women.' Proc. Xth European Symposium on Calcified Tissues. Heidelberg:Springer.

Nordin, B.E.C., Peacock, M. and Wiklinson, R. (1972) 'Hypercalcuria in calcium stone disease.' Clin. Endocr. Metab. 1:169-183.

Northam, B.E. and Widdowson, G.M. (1967) 'Bromocresol geeen method for determination of serum albumin.' Technical Bull. Assoc. Clinical Biochemists. 11.

Ohlson, M.A., Brewer, W.D., Jackson, L., Swanson, P.P., Roberts, P.H., Mangel, M. Leverton, R.M., Chaloupka, M., Gram, M.R., Reynolds, M.S. and Lutz, R. (1952) 'Intakes and retention of nitrogen, calcium and phosphorus by 136 women between 30 and 85 years of age.' Federation Proc. 11:775-783.

Outhouse, J., Kinsman, G., Sheldon, D. Twomey, I., Smith, J. and Mitchell, H.H. (1939) 'The calcium requirements of five pre-school girls.' J. Nutr. 17:199-211.

Pain, R.W., Rowland, K.M., Phillips, P.J. and Duncan, B.McL.)1975) 'Current "corrected" concept challenged.' Brit. Med. J. 4:617-619.

Parfitt, A.M. (1969) 'Chlorothiazide-incuced hypercalcaemia in juvenile osteoporosis and hyperparathyroidism.' New Eng. J. Med. 281:55-59.

Parker, T.F., Vergne-Marini, P., Hull, A.R., Pak, C.Y. and Fordtran, J.S. (1974) 'Jejunal absorption and secretion of calcium in patients with chronic renal disease on haemodialysis.' J. Clin. Invest. 54:358-365.

Patterson, C.R. (1978) 'Calcium requirements in man: a critical review.' Postgrad. Med. J. 54:244-248.

Payne, R.B., Little, A.J., Williams, R.B. and Milner, J.R. (1973) 'Interpretation of serum calcium in patients with abnormal serum protein.' Brit. Med. J. 4:643-646.

Pederson, K.O. (1971) 'Binding of calcium to serum albumin. I. Stoichiometry and intrinsic association constant at physiological pH, ionic strength and temperature.' Scand. J. Clin. Lab. Invest. 28:459-469.

Pento, J.T., Waite, L.C., Tracy, P.J. and Kinney, A.D. (1977) 'Adaptation to calcium deprivation in the rat: effects of parathyroidectomy.' Amer. J. Physiol. 232:E366-342.

Petrunkina, A. (1934) 'Uber die Bilanzen von Stickstoff, Kalk, Magnesium, Phosphor und Eisen bei Kindern von 7-8 Jahren.' Z. Kinderheilk 56:219-226. Cited by: Irwin, M.I. and Kienholz, E.W. (1973) 'A conspectus of research on calcium requirements of man.' J. Nutr. 103:1019-1095.

Pettifor, J.M. (1980) 'Calcium deficiency rickets.' Ph.D. Thesis, University of the Witwatersrand.

Pettifor, J.M., Ross, P., Moodley, G., Shuenyane, E. (1981) 'The effect of dietary calcium supplementation on serum calcium, phosphorus, and alkaline phosphatase concentrations in a rural black population.' Amer. J. Clin. Nutr. 34:2187-2191.

Pettifor, J.M., Ross, F.P., Moodley, G., Wang, J. Margo, G. and Skjolde, C. (1978a) 'Serum calcium, magnesium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D concentrations in children.' S. Afr. Med. J. 53:751-754.

Pettifor, J.M., Ross, P., Wang, J., Moodley, G. and Couper-Smith, J. (1978b) 'Rickets in children of rural origin in South Africa: is low dietary calcium a factor?' Pediatrics 92:320-324.

Pierce, H.B., Daggs, R.G., Merservey, A.B. and Simcox, W.J. (1940) 'The retention of calcium and phosphorus by pre-school children.' J. Nutr. 19:401-414.

'Post-menopausal and senile osteoporosis.' (1966) S. Afr. Med. J. 40:14.

Rakowska, M., Czarnowska-Misztal, E., Brzezińska, Z. and Krupowicz, J. (1969) 'Wyniki badań nad retencja wapnia ze zwczajowo sponzywanej diety w wybranej grupie dzieci w wieku przedszkolnym.' Rocz. Panstwowego Zakl. Hig. 20:533-542. Cited in Nutr. Abst. Rev. (1970) 40:962.

Ramsey, L. and Shelton, J.R. (1976) 'Corrected calcium concept.' Brit. Med. J. 1:219.

Reed, R.B. and Burke, P.S. (1954) 'Collection and analysis of dietary intake data.' Amer. J. Publ. Health 44:1015-1026.

Reid, M.E. (1943) 'Interrelationship of calcium and ascorbic acid to cell surfaces and intercellular substances and to physiological action.' Physiol. Rev. 23:76-99.

Richen, A. and Rowe, D.J.F. (1970) 'Disturbances of calcium metabolism by anticonvulsant drugs.' Brit. Med. J. 4:73-76.

'Rickets in Asian Immigrants.' (1979) Brit. Med. J. 1:1744.

Roberts, P.H., Kerr, C.H. and Ohlson, M.A. (1948) 'Nutritional studies of older women: Nitrogen, calcium, phosphorus retentions of nine women.' J. Amer. Diet. Assoc. 24:292-299.

Robertson, I., Kelman, A. and Dunnigan, M.G. (1977) 'Chupatty intake, vitamin D status and Asian rickets.' Brit. Med. J. 1:229-230.

Rodan, G.A., Liberman, U.A., Paran, M. and Anbar, M. (1967) 'Lack of physiochemical equilibrium between blood and bone calcium in the isolated dog limb.' Israel J. Med. Sci. 3:702-713.

Rost, E., Herbst, O. and Weitzel, A. (1923) 'Die Ernäbrungsverhältnisse der Berliner Waisenhauszöglinge mit besonderer Berücksichtigung des Kalkstoffwechsels.' Arch. Kinderheilk 72:81. Cited by: Wang, C.C., et.al. (1936) 'Metabolism of adolescent girls.' Amer. J. Dis Child. 52:41-53.

Round, J.M. (1973) 'Plasma calcium, magnesium, phosphorus and alkaline phosphatase levels in normal British school children.' Brit. Med. J. 2:137-140.

Rudolf, M., Aruhanantham, K. and Guenstein, R.M. (1980) 'Unsuspected nutritional rickets.' Pediatrics 66:72-76.

Runeberg, L., Miettinen, T.A. and Nikkila, E.A. (1972) 'Effect of cholestyramine on mineral excretion in man.' Acta Med. Scand. 192:71-76.

Rutishauser, I.H.E. and Frood, J.D. (1973) 'The effect of a traditional low fat diet on energy and protein intake, serum albumin concentration and body weight in Ugandan preschool children.' Brit. J. Nutr. 29:261-268.

Samuelsson, G. (1971) 'An epidemiological study of child health and nutrition in a northern Swedish County. I. Food consumption survey.' Acta Paediatr. Scand. 214(suppl.):1-44.

Schachter, D. Dowdle, E.B. and Schenker, H. (1960) 'Active transport of calcium by the small intestine of the rat.' Amer. J. Physiol. 198:263-268.

Schaefer, E. (1966) in Nutrition III: Nutritional Status: Assessment and Application. ed. Beaton, G.H. and McHenry, E.W. NewYork:Academic Press.

Schimshaw, N.S. and Gordeon, J.E. (1968) Malnutrition, Learning and Behavior. Boston:MIT Press.

Schofield, F.A. and Morrell, E. (1960) 'Symposium on metabolic patterns in preadolescent children. Calcium, phosphorous and magnesium.' Fed. Proc. 19:1014-1016.

Shah, B.G. and Draper, H.H. (1966) 'Drepression of calcium absorption in parathyroidectomised rats.' Amer. J. Physiol. 211:963-966.

Sherman, H.C. and Hawley, E. (1922) 'Calcium and phosphorus metabolism in childhood.' J. Biol. Chem. 34:375-399.

Shuenyane, E., Mashigo, S., Eyberg, C., Richardson, B.D., Buchanan, N., Pettifor, J., Macdougall, L. and Hansen, J.D.L. (1977) 'A socio-economic, health and cultural survey in Soweto.' S. Afr. Med. J. 51:495-500.

Shuenyane, E., Saunders, L.D., Mashigo, S., Eyberg, C., Rosen E.U. and Pettifor, J.M. (1980) 'A demographic and socio-economic profile of a rural Black South African community.' S. Afr. Med. J. 57:539-542.

Sinclair, H.M. and Hollingsworth, D.F. (1969) Hutchinson's Food and the Principles of Nutrition, 12th ed. London:Arnold.

Singleton, N. and Tucker, S.M. (1978) 'Vitamin D status of Asian infants.' Brit. Med. J. 1:607-610.

Slater, J.D.H., Heffron, P.F., Vernet, A. and Nabarro, J.D.N. (1959) 'Clinical and metabolic effects of dexamethasone.' Lancet 1:173-177.

Sloan, A.W. and Hansen, J.D.L. (1969) 'Nutrition and physical fitness of White, Coloured and Bantu high-school children.' S. Afr. Med. J. 43:508-511.

South African National Nutritional Council (1956) 'Recommended daily dietary standards.' S. Afr. Med. J. 30:108-111.

Stamp, T.C.B., Round, J.M., Rowe, D.J.F. and Haddad, J.G. (1972) 'Plasma levels and therapeutic effect of 25-hydroxycholecalciferol in epileptic patients taking anticonvulsant drugs.' Brit. Med. J. 4:9-12.

Stearns, G. (1952) 'Nutritional health of infants, children and adolescents.' Proc. National Food and Nutrition Institute U.S.D.A. Agricult. Handbook, No. 56.

Stearns, G. (1956) 'Calcium, phosphorus and vitamin D requirements in infants.' In: Infant Metabolism ed. Scheinberg. New York:MacMillan.

Steggerda, F.R. and Mitchell, H.H. (1941) 'Further experiments on the calcium requirement of adult men and the utilization of the calcium in milk.' J. Nutr. 21:577-588.

Sur, G., Reddy, S.K., Swaminathan, M. and Subrahmanyan, V. (1955) 'The metabolism of nitrogen, calcium and phosphorus in undernourished children. 2. The effect of supplementary groundnut-milk curds on the metabolism of nitrogen, calcium and phosphorus.' Brit. J. Nutr. 9:210-215.

Taitz, L.S. (1962) 'The role of the parathyroid glands in vitamin D deficiency.' M.D. Thesis, University of the Witwatersrand.

Tanner, J.M., Whitehouse, R.H. and Tanaishi, M. (1966) 'Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965.' Arch. Dis. in Child. 41:454-471.

Technicon Operating Manual for the Technicon Autoanalyser II system (1972) Technical publication No. TA1-0170-20.

Thompson, W.E. and Thompson, E.R. (1969) 'Effect of cholestyramine on the absorption of vitamin D_3 and calcium.' Gut 10;717-722.

Truswell, A.S. (1976) 'Symposium on some aspects of diet and health.' Proc. Nutr. Soc. 35:1-14.

United Kingdom Department of Health and Social Security (1969) 'Nutrients for the United Kingdom: Report of the panel on recommended allowances of nutrients.' Rep. Publ. Hlth. Med. No. 120 London:Her Majesty's Stationery Office.

U.S. Department of Agriculture (1950) Composition of Foods, Handbook No. 8. Washington D.C.

United States National Research Council (1974) Committee on Dietary Allowances: Recommended Dietary Allowances. 8th ed. Washington, D.C.: National Academy of Sciences.

Walker, A.R.P. (1948) 'Drinking water as a source of calcium.' J. S. African Chem. Inst. 5:9-12.

_____ (1954) 'Does a low intake of calcium retard growth or conduce to stuntedness?' Amer. J. Clin. Nutr. 2:265-266.

_____ (1955) 'Does a low intake of calcium cause or promote the development of rickets?' Amer. J. Clin. Nutr. 3:114-120.

_____ (1963) 'Problems in nutritional supplementation and enrichment.' Amer. J. Clin. Nutr. 12:157-160.

_____ (1965) 'Osteoporosis and calcium deficiency.' Amer. J. Clin. Nutr. 16:327-336.

_____ (1966) 'Nutritional, biochemical and other studies on South African populations.' S. Afr. Med. J. 40:814-852.

_____ (1972) 'The human requirement of calcium: should low intakes be supplemented?' Amer. J. Clin. Nutr. 25:518-530.

Walker, A.R.P., Fox, F.W. and Irving, J.T. (1948) 'Studies in huamn mineral metabolism. I. The effect of bread rich in phytate phosphorus on the metabolism of certain mineral salts with special reference to calcium.' Biochem. J. 42:452-462.

Walker, A.R.P. and Richardson, B.D. (1964) 'Growth rate in relation to calcium losses through sweat.' Amer. J. Clin. Nutr. 15:

Walker, A.R.P., Walker, B.F. and Richardson, B.D. (1971) 'Metacarpal bone dimensions in young and aged South African Bantu consuming a diet low in calcium.' Post grad. Med. J. 47:320-325.

Walker, A.R.P., Richardson, B.D. and Walker, B.F. (1972) 'The bearing of numerous pregnancies and lactations on bone dimesions in South African Bantu and Caucasian mothers.' Clin. Sci. 42:189-196.

Walker, A.R.P., Walker, B.F., Richardson, B.D. and Christ, H.H. (1970) 'Cortical thickness of bone in underprivileged populations.' Amer. J. Clin. Nutr. 23:244-245.

Wang, C.C., Kaucher, A.M. and Frank, M. (1928) 'Metabolism of undernourished children. IV. Calcium metabolism.' Amer. J. Dis. Child. 35:856-861.

Wang, C.C., Kaucher, M. and Wing, M. (1936) 'Metabolism of adolescent girls. 4. Mineral metabolism.' Amer. J. Dis. Child. 52:41-53.

Wang, C.C., Kern, R. and Kaucher, M. (1930) 'Minimum requirements of calcium and phosphorus in children.' Amer. J. Dis. Child. 39:768-773.

Wasserman, R.H. (1963) The Transfer of Calcium and Strontium across Biological Membranes. New York: Academic Press

Wasserman, R.H., Comar, C.L. and Nold, M.M. (1956) 'The influence of amino acids and other organic compounds on the gastrointestianl absorption of calcium^{45} and strontium^{89} in the rat.' J. Nutr. 59:371-383.

Wasserman, R.H., Kallfelz, F.A. and Comar, C.L. (1961) 'Active transport of calcium by rat duodenum in vivo.' Science 133:883-884.

Wasserman, R.H. and Taylor, A.N. (1964) 'Some aspects of the intestinal absorption of calcium with special reference to vitamin D.' in: Mineral Metabolism: an Advanced Treatise ed. Comar, C.L. and Bronner, F. New York:Academic Press.

Watt, B.K. and Merrill, A.L. (1963) Composition of Foods. Africult. Handbook No. ', Washington D.C.

West, E.S. and Todd, W.R. (1951) Textbook of Biochemistry New York: MacMillan.

White, W.L., Erickson, M.M. and Stevens, S.C. (1970) Chemistry for Medical Technologists 3rd Ed. St. Louis:C.V. Mosby

Whitehead, R.G. (1975) 'Modern considerations in nutritional research.' S. Afr. Med. J. 49:2214-2217.

Whitehead, R.G. (1977) 'Some quantative considerations of importance to the improvement of the nutritional status of rural children.' Proc. R. Soc. Lond. 199:49-60.

Widdowson, E.M. and Dickerson, J.W.T. (1964) 'Chemical composition of the body.' in: Mineral Metabolism II. ed. E.L. Comar and F. Bronner New York:Academic Press.

Willard, A.C. and Blunt, K. (1927) 'A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus and nitrogen.' J. Biol. Chem. 75:251-262.

Wills, M.R., Phillips, J.B., Day, R.C. and Bateman, E.C. (1972) 'Phytic acid and nutritional rickets in immigrants.' Lancet 1:771-773.

Wiseman, G. (1964) Absorption from the Intestine New York:Academic Press.

Wohl, M.G. and Goodhard, R.S. (1960) Modern Nutrition in Health and Disease 2nd ed. Philadelphia:Lea and Febiger.

Wootton, I.D.P. (1964) Micro-analysis in Medical Biochemisty 4th ed. London:J & A Churchill.

Yeh, H.L. and Adolph, W.H. (1939) 'Calcium, phosphorus and nitrogen balances with pre-school children.' Chin. J. Physiol. 14:303-314.

Young, C.M., Hagan, G.C., Tucker, R.E. and Roster, W.D. (1952) 'A comparison of dietary study methods: II: Dietary history versus seven-day records versus 24-hour recall.' J. Amer. Diet. Assoc. 28:218-221.

Yudkin, J. (1951) 'Dietary surveys: variaiton in the weekly intake of nutrients.' Brit. J. Nutr. 5:177-194.

Zemel, M.B. and Linkswiler, H.M. (1980) 'Calcium metabolism in the young adult male as affected by level and form of phosphorus intake and level of calcium intake.' J. Nutr. 111:315-324.