THE EFFECTS OF HIGH FLUORIDE INTAKE ON SCHOOL CHILDREN IN KWANDEBELE, SOUTH AFRICA

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The Effects of High Fluoride Intake on School Children in KwaNdebele, South Africa

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A Thesis Submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree Doctor of Philosophy

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Declaration

I, Ruth Margarete Greeff, declare that this thesis is my own work, except where assistance has been acknowledged. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been previously submitted to any other Department or University.

This study was approved by the Committee on Research in Human Subjects of the University of the Witwatersrand (No 41/4/87).

Ruth M Greeff

1st day of June, 1997.

This dissertation is dedicated to my parents and brother

Rev Trygvar and Mrs Anne-Marie Brauteseth
Karl J Brauteseth

and

my supportive and understanding husband and daughter

Dr Michael C Greeff
Carly Marie Greeff

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The children, parents and teachers of KwaNdebele
for opening their schools and communities.

Abstract

The Effects of High Fluoride Intake on School Children in KwaNdebele, South Africa.

Long-standing high fluoride (F) intake results in fluorosis as manifested by mottled dental enamel and diffuse osteosclerosis in adults. The effects of high F intake on bone and mineral metabolism in rural school children were studied in an endemically high fluoride area of KwaNdebele. Water fluoride levels in the study area were 9.4-9.9 ppm and in the control area 0.6 and 1.6 ppm. Scholars from 6 to 22 years of age were randomly selected from the study (n=636) and control areas (n=626). Anthropometric, biochemical and bone densitometry investigations were carried out. Dietary recalls were conducted on a random sample from each group (study, n=63 ; control, n=62).

Calcium intakes ranged from 16 to 1 078 mg/day (mean 240 mg/day). Both groups had similar dietary intakes and growth patterns. In both groups almost twice the number of males as females fell below the 5th percentile for height (35-40% vs. 20-23%) and weight (42-46% vs. 24%). Biochemical data in the study group showed higher alkaline phosphatase (p=0.0001) and higher serum phosphate (p=0.000 1), raised urine fluoride (p=0.000 1) and lower urine calcium (p=0.001). Evidence of hypocalcemia was seen in 10% of the study group compared with 3% in the control group (p=0.000 1). The study group had a higher prevalence of genu valgum (24.7% vs. 13.8% p= 0.0001) with a significantly higher prevalence in females (p=0.001). Children in the study group had increased radial bone width and decreased radial bone density (p< 0.001). Bone mineral content and density in both rural communities were lower than values in a well-nourished urban group.

The findings of this absorptiometric study are that children in the endemic fluoride area have wider, less dense bone in conjunction with elevated alkaline phosphatase, low urinary calcium excretion and evidence of hypocalcemia. It is suggested that fluoride increases the calcium requirements of growing bone and exacerbates the prevalence of hypocalcemia. This, along with elevated levels of alkaline phosphatase (suggestive of elevated PTH), results in wider, less dense bone - a picture consistent with secondary hyperparathyroidism. Thus, children living in an endemically high fluoride area show evidence of altered bone mineral homeostasis and reduced dietary calcium intake. The role of low dietary calcium in the pathogenesis of this condition requires further investigation.

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Chapter 1

1.0 Fluorine - The Element

Fluorine is the most electronegative of all chemical elements. It is virtually never encountered in nature in its free gaseous form. Fluoride is the term for the ionised form of the element fluorine, and it is mainly found in this form. It is the 17th in the order of abundance of elements in the earth's crust, and it is present in small but widely varying concentrations in practically all soils, water supplies, plants and animals. It is therefore a constituent of all normal diets.

Fluoride occurs most commonly as fluorite or fluorspar (CaF_2). It also occurs as cryolite (Na_3AlF_6) and fluoroapatite ($Ca_{10}(PO_4)_6\ F_2$). The occurrence of fluoride in various rock types has been extensively reviewed by Correns (1956). Granite rock has been shown to be high in fluoride. In different localities in the USA it varies from 20 - 2 300 ppm, and similar values are found in Germany (WHO, 1970).

Granite is the predominant rock type in KwaNdebele (the research area of this study), and water fluoride levels vary from 6 - 22 ppm (CSIR, Water Research, personal communication). Fluorspar (CaF_2) is the principal fluoride-bearing mineral, and it is mined approximately 20 km away from the research area (CSIR, Water Research, personal communication). Fluoroapatite is a constituent of rock phosphate and is unimportant as a commercial source of fluoride (WHO, 1984). Water in contact with rock containing fluoride, as in the case of ground water, has higher fluoride concentrations than surface water, due to the solvent action of water on rocks and soil. The effects of fluoride in man stem largely from dissolved fluoride present in drinking water, although industrial exposure to fluoride also plays a role (Correns, 1956).

1.1 Historical Perspective of Endemic Fluorosis Research

The effects of excessive fluoride intake in the aetiology of dental mottling was known in the mid-thirties. Dean published the first authoritative paper on dental fluorosis in which he classified the dental changes into seven stages (Dean, 1934). Soon after, the skeletal effects of excess fluoride in man were well documented (Roholm, 1937; Short et al., 1937). Chronic toxic effects of fluoride on the skeletal system were first described in India from the state of Madras in 1937 (Short et al., 1937). Subsequently cases have been reported from Ceylon, South Africa, Japan, Saudi Arabia, USA, Canada and Europe (WHO, 1974). During the forties, extensive epidemiological data were collected on the nature of the disease and its clinical presentation. In the years that followed, reports of new areas of endemic skeletal fluorosis were featured (Siddiqui, 1955; Singh and Jolly, 1961; Teotia et al., 1969). In the original description, endemic fluorosis was believed to present in a similar manner to that of industrial fluorosis as reported by Roholm (1937). However, Czerwinski and Lankosz showed significant differences in the presentation of endemic and industrial fluorosis (Czerwinski and Lankosz, 1978). The clinical picture of endemic fluorosis as characterised by the above authors was of new bone formation, musculo-skeletal dysfunction, arthritis and fixed flexion deformities. The disease was considered chronic in nature with a very slow progression to clinical signs. For skeletal changes to occur it was accepted one would need exposure to high levels of waterborne fluoride for at least four decades (today it is known that far less time is required for the presentation of skeletal fluorosis). Primary radiological features were described as osteosclerosis and calcification of membranes and ligaments. Nutritional deficiency in general was an associated feature whose precise role was not clear. Investigation into the metabolic and biochemical nature of the disease was highlighted in the next two decades. In the seventies, different manifestations of fluorosis were recognised, including secondary hyperparathyroidism and genu valgum in children (Christie, 1980; Teotia et al., 1978; Krishnamachari and Krishnaswamy, 1974). Fluoride was

thought to be permanently deposited in the bone, but studies have shown that fluoride is excreted in urine for years after removal from fluoride areas (Bagga et al., 1979). Fluorosis research peaked in the late 1970s and early 1980s. Less emphasis has been given to endemic fluorosis recently, and research has moved to the role of fluoride in the treatment of osteoporosis. Interaction between fluoride, nutritional status, dietary habits, environmental factors and the body's response to ingested or inhaled fluoride seems to be equally important in understanding the nature of this disease.

1.2 Recommended Intake of Fluoride

Fluoride is one of the few trace elements whose beneficial and toxic doses are not widely separated. Due to the beneficial effects of fluoride in substantially decreasing dental caries, a number of medical bodies have supported fluoridation of water or fluoride supplements to be given orally to children throughout the period of tooth development. The Royal College of Physicians, London, recommends fluoridation of water to a level of approximately 1 mg/litre (1 ppm) where natural levels are below that level (Royal College of Physicians, 1976). The dose of 1 ppm of fluoride represents a compromise between maximal tooth hardening and minimal fluorosis.

The US Recommended Daily Allowance (RDA) for adults ranges from 1.5 to 4 mg/day, and for children the range is reduced to a maximal level of 2.5 mg in order to avoid dental mottling. These suggested ranges are obtained without difficulty in areas with a water supply of at least 1 mg/litre of fluoride (RDA, 1989). The American Dietetic Association, The American Medical Association and The American Dental Association all endorse fluoride supplementation in optimal amounts (ADA Report, 1989).

The World Health Organisation recommends an upper limit of 1.5 mg/litre (1.5 ppm) in drinking water; above this level objectionable mottling may occur (WHO, 1984). The Council for Scientific and Industrial Research (CSIR) in South Africa has established optimal (1 mg/litre) and critical (> 2.0 mg/litre) levels of fluoride in drinking water. Critical levels are those that are high enough to raise concern of possible toxic effects (Kempster and Smith, 1985).

1.2.1 Fluoride Sources

Fluoride will be found in most potable water in varying concentrations. The amount of fluoride which is ingested with water depends on the fluoride content of the water and on the amount consumed daily. Water consumption is dependent on ambient temperatures, dryness and individual activity. The fluoride content of water can be determined fairly readily, but precise determination of the daily water intake of the individual is considerably more difficult.

The richest dietary sources are tea and marine fish that are consumed with their bones (Kumpulainen and Koivistoinen, 1977). Despite every food containing at least traces of fluoride, the total intake from solid food is relatively small and amounts to only about 0.5 to 1.0 mg/day in Britain (Burt, 1982). The major factor influencing the fluoride content of any diet in man is the fluoride concentration of the water supply.

Tea contains more fluoride than any other dietary item in the United Kingdom. Dry tea leaves can contain up to 200 mg/kg, much of which is extracted during infusions (WHO, 1970). In the United Kingdom, Walters et al., (1983) found that tea was the main source of dietary fluoride for adults, where tea accounted for 1.3 mg of the total daily intake of 1.8 mg of fluoride.

Food processing has a stong influence on the fluoride content of foods. The fluoride content of various foods can be increased significantly by cooking them in fluoridated water (Marier and Rose, 1966; Martin, 1951). Even the type of cooking vessel can be important. Cooking in utensils treated with Teflon, a fluoride-containing polymer, can increase the fluoride content, whereas an aluminium surface can reduce it (Full and Parkins, 1975).

1.2.2 Estimation of Intake

The early literature dealing with water intake has been reported in the WHO publication "Fluorides and Human Health" (WHO, 1970). Adolph, and later McClure, attempted to estimate the daily fluoride intake from water (Adolph, 1933; McClure, 1941). Adolph assessed the daily water requirement as equal to 1 ml per calorie of energy in the daily diet. McClure determined the daily fluoride intake of children aged 1 - 12 years from water containing 1 ppm fluoride. He estimated that daily intake of water was equal to 25% of the daily energy requirement. He allowed for variations in individual drinking habits. His results showed the consumption of fluoride from water containing 1 ppm fluoride for children aged 1 - 3 years was 0.39 - 0.56 mg fluoride daily (390 - 560 ml water consumed), rising to 0.81 - 1.16 mg daily in children aged 10 - 12 years (812 - 1166 ml) (WHO, 1970). These intakes were comparable to those of Hodge and Smith (1970) who reported 0.3 - 0.8 mg fluoride/day. Barbakow reports mean daily intakes varying from 1.7 to 3.4 mg fluoride/day in fluoridated areas and 0.78 - 1.03 in non-fluoridated areas (Barbakow, 1983). Although these estimates are a guide, they do not adequately allow for fluctuations of water intake under different climatic conditions. These estimations also exclude the fluoride content of foods and beverages, which is significantly affected by the concentration of water used during processing.

1.3 Physiology of Fluoride Action

1.3.1 Fluoride Absorption

In the light of current knowledge fluoride, either as F^- or HF, is passively absorbed across the gastric and small intestinal mucosa with no apparent active transport mechanism. Fluoride absorption depends not only on the absolute quantity of the element present, but also upon the effect of other dietary constituents. Any substance able to bind with fluoride and render it insoluble hinders fluoride absorption. Calcium affects absorption in this way, reducing the uptake of fluoride and increasing faecal excretion of the element (Ekstrand and Ehrnebo, 1979; WHO, 1984).

Fluoride absorption has primarily been studied in the rat with few human experiments. Although some of the literature supports active mechanisms that homeostatically regulate plasma fluoride concentrations and the absorption of the ion from the gastrointestinal tract of the rat, Whitford and Williams (1986) dispute the validity of these theories due to problems with the experimental design. Whitford and Williams used four different methods to evaluate the effect of plasma fluoride levels on the absorption of the ion in rats. They concluded that unless extremely high plasma fluoride levels are involved (pharmacological or toxic doses), the absorption of the ion is independent of plasma levels, and that plasma fluoride concentrations are not homeostatically regulated (Whitford and Williams, 1986).

In vitro studies of segments of rat gastrointestinal tracts carried out by Stookey et al. (1964) showed that the mechanism of removal of fluoride from the gastrointestinal tract is by simple diffusion along the entire gastrointestinal tract. There appears to be no active regulatory homeostatic mechanism in the classical sense. Instead fluoride plasma levels, usually in the range of 0.01 - 0.03 ppm, are

regulated both by bone acting as a storage depot and by the kidneys which are the main excretory organs.

Guy et al. (1976) showed that a linear relationship existed between plasma and drinking water fluoride levels with a slope of 0.015. Radioactive tracer experiments have shown that maximum plasma fluoride levels are reached within an hour of ingesting 1 mg of fluoride. After giving a loading dose of ^{18}F to rats and judging by the rapidity of distribution, absorption was assumed to be rapid. Using a portable crystal scintillation counter placed over the abdominal area, radioactivity after ingestion of 1 mg of fluoride labelled with ^{18}F showed a rapid decline after a few minutes, and maximum plasma radiofluoride concentration was reached within 60 minutes (Carlson et al., 1960). More recently Nopakun et al. showed that 90% of the fluoride dose in rats was absorbed within 120 minutes and that the major area of absorption was the small intestine although the stomach played a significant role (Nopakun et al., 1989). Although quantitative data on fluoride absorption in humans from specific segments of the gastrointestinal tract are lacking, a similar site of fluoride absorption in man may be present. Most of the ingested fluoride is in the form of hydrogen fluoride (HF), which has a pKa of 3.4 and readily passes through biological membranes (Ekstrand and Spak, 1990).

Calcium had a protective effect in relation to fluoride absorption, as was shown by Harrison et al. (1984) where they looked at the effect of calcium on fluoride toxicity in growing rats. The rats showed a decrease in intestinal fluoride absorption from 76% to 47% when dietary Ca was increased from adequate to excessive levels (from 0.5% to 2.0% of the diet) (Harrison et al., 1984).

1.3.2 Fluoride in Plasma

There are two general forms of fluoride in human plasma. The ionic form, detectable by the ion-specific electrode, is the one of interest in dentistry, medicine and public health. Ionic fluoride is not bound to proteins, to other components of plasma, or to soft tissues. The other form consists of several fat-soluble organic fluorocompounds, which can be contaminants derived from food processing and packaging. The concentration of ionic fluoride in soft and hard tissues is directly related to the amount of ionic fluoride intake, but that of the organic fluorocompounds is not (WHO, 1994).

Provided that water is the major source of fluoride intake, fasting plasma fluoride concentrations of healthy young or middle-aged adults, expressed as micromoles per litre (μmol/l), are roughly equal numerically to the fluoride concentrations in drinking water, expressed as milligrams per litre (mg/l) or parts per million (ppm). Plasma fluoride concentrations tend to increase slowly over the years with continual exposure (WHO, 1994).

1.3.3 Excretion of Fluoride

About 10% to 25% of the daily intake of fluoride is not absorbed and is excreted in the faeces. Fluoride removal from plasma occurs via two processes: uptake and accumulation in the bone, and excretion via the kidneys. Fluoride is eliminated predominantly via the renal route. Fluoride excretion depends on several factors: 1) the total daily consumption of the element, 2) the degree of renal efficiency, 3) age, 4) physiological state, and 5) interaction of fluoride with other factors such as binders. In a normal person in a non-endemic area, excretion is generally 0.1 to 0.5 mg of fluoride within 24 hours (WHO, 1994).

Hodge and Smith (1970) in their review considered urinary fluoride to be the best indicator of intake of the element. According to their studies, between 20%

and 30% of an ingested fluoride dose was found in the urine within 3 to 4 hours. In endemic fluorosis, urinary fluoride varies between 1.2 and 10 mg per 24 hours. Measurements of this nature can be regarded as an indicator of exposure to high levels of fluoride in a community (Krishnamachari, 1986).

Studies on renal clearance of fluoride in children and adolescents showed that 60% of filtered fluoride was reabsorbed, and no evidence for tubular secretion could be found. The results suggest that children have lower renal fluoride clearance rates than adults and indicate that a moderate impairment of the renal function could lead to increased retention of fluoride (Spak et al., 1985).

Bagga et al. had the opportunity to study an endemic area several years after the village had changed its water source to one containing low amounts of fluoride. The normal control group had an average daily excretion of 1.63 mg of fluoride, residents of the endemic area excreted 10.49 mg fluoride. However, residents of the village with altered water sources, who had consumed high fluoride water until nine years prior to the study, were also excreting fluoride in higher than normal amounts, an average of 6.89 mg. This study suggests that the elimination process continues long after the high fluoride source is removed (Bagga et al., 1979).

Faecal fluoride, which forms between 10% to 30% of the amount ingested, has been traditionally believed to be unabsorbed fluoride. However, Whitford showed that elevated plasma fluoride concentrations increased faecal fluoride excretion and thereby reduced the net absorption balance of ingested fluoride. He also showed that fluoride does not move passively and unidirectionally from the gastrointestinal tract, but could actually be secreted into the gut when plasma fluoride concentrations are elevated. He showed that fluoride in faeces is that which re-enters the distal gastrointestinal tract along the diffusion gradient

between the interstitial and luminal fluid and subsequently becomes associated with unabsorbed cations like calcium and magnesium (Whitford, 1994).

1.4. Distribution of Fluoride in Bone

The main known target organs of fluoride in man are bones and the enamel of teeth, where the fluoride ion is incorporated into the hydroxyapatite, resulting in increased caries resistance of the teeth (Sognnaes, 1965). In the bones, fluoride enters the lattice structure of the bone crystal. There it increases the stability of the apatite lattice and decreases the solubility of the apatite crystals. Primary involvement of teeth and bones in chronic fluorosis is attributed to the strong affinity between fluoride and biological apatite to substitute the hydroxyl component of calcium hydroxyapatite by fluoride (Krishnamachari, 1986).

Pure fluoroapatite contains approximately 3.7% fluoride; up to about one-third of the total hydroxyl ions in enamel can be replaced by fluoride ions. Normal human apatite tissue, i.e. bone and tooth tissue, never approximate to pure fluoroapatite, although fluoride substitution varies considerably, being dependent on the tissue-fluoride environment at the the time of calcification. Once formed, the apatite/fluorhydroxyapatite remains chemically stable until the tissue is resorbed, remodelled, or otherwise metabolised (WHO, 1984). Bone mineral deposition is delayed by fluoride, which it does not diffuse into bone already formed, but incorporates during mineralisation. Consequently fluoride tends to accumulate in the bone undergoing mineralisation (Grynpas, 1990). A small amount of fluoride increase is possible by diffusion and adsorption of fluoride to the crystal structure (WHO, 1984). Once fluoride attaches itself to the bone crystal it is not easily eliminated.

1.4.1 Bone Fluoride Levels

Deposition of fluoride in the skeleton is a major factor in the metabolism of absorbed fluoride. Experimental studies using radiofluorine demonstrate that the skeletal distribution pattern of fluoride is the same as that of calcium - in long bones there is a preferential deposition in the primary spongiosa under the cartilage, a generalised patchy distribution in trabeculae and a linear distribution on endosteal and periosteal surfaces (Faccini, 1969).

The quantity of fluoride absorbed by the skeleton is usually more closely related to the amount present in the drinking water than to that contained in the diet. Zipkin et al. (1958) showed a linear relationship between the fluoride level of the water supply and the average concentration in human bone. At levels of intake between 1 and 20 ppm, up to half of the ingested fluoride appears to be retained by the skeleton. Most of the remainder is rapidly excreted via the urine (Weidmann and Weatherell, 1970). Little remains for any length of time in the blood or the soft tissues. The fluoride retained by the body is largely combined with mineralised tissue.

The predictably high uptake of fluoride by the skeleton during growth is documented by considerable experimental data. The rapidly growing bones of young animals absorb fluoride more readily than those of older individuals. Sipkin and McClure (1952) fed 20 mg fluoride to rats of different ages and showed that the younger the animal, the more fluoride accumulated in its skeleton. Suttie and Phillips (1959) also demonstrated this when they compared fluoride uptake by the femora of weanling, half-grown and mature rats. Weidmann and Weatherell (1970) found that under conditions of ad libitum ingestion, the overall uptake of fluoride into bones of young, half-grown and mature rabbits likewise decreased with advancing age.

Early human research in fluorosis tended to look at adult fluorosis and not until the 1970s did children become a topic of investigation for skeletal fluorosis. This exclusion of children from fluorosis research could account for the previously held theory that it took decades for fluorosis to present clinically.

The concentration of fluoride in bone undoubtedly increases with time, and a number of surveys have been carried out to establish the extent to which the element accumulates throughout life. Armstrong et al. (1970) report on the first investigations into human bone of different ages done in the early 1950s, where the fluoride content of ground samples from 14 whole ribs taken post mortem from persons in two London hospitals was estimated. A rise in fluoride content with age was clearly demonstrated, but the rate of the accumulation could not be established. These results were considered incomplete as whole ribs were analysed not allowing for the wide disparity between the fluoride levels of tissue surfaces compared with interior regions and between compact and cancellous bone. To avoid the error of faulty sampling, Smith et al. (1953) compared samples of the rib cortex, and Jackson and Weidmann (1958) restricted their work to rib cancellum. Both groups demonstrated that the fluoride content of bone rose with age. However, there was no conclusive opinion on whether a plateau level of fluoride in bone is reached.

The loss of fluoride from the skeleton following a change from a high to a low fluoride intake is dependent on two aspects of bone turnover: 1) the extent to which fluoride is mobilised as a result of bone resorption, and 2) the extent to which released fluoride is reincorporated into adjacent mineralising bone.
In rats, Guo et al. showed, by labelling with tetracycline, that approximately 60% of fluoride mobilised during bone resorption was redeposited in the skeleton (humeri and vertebrae). Vertebrae showed a greater loss than the humerus,

reflecting the differences between cancellous and cortical bone as cancellous bone has a greater turnover rate than cortical bone. Dietary calcium deficiency further increased the extent of fluoride loss. The redeposition of fluoride showed a positive correlation with mineral deposition. Thus Guo et al. concluded that the retention of fluoride in the skeleton of growing rats results predominantly from redeposition of resorbed fluoride rather than passive retention associated with low bone turnover (Guo et al., 1988). They calculated that the half life for bone fluoride retention in the adult human is approximately 8-9 years (Guo et al., 1988). Removal of individuals with skeletal fluorosis from high fluoride areas results in a gradual improvement in clinical symptoms and radiological improvement (Song, 1989).

1.5 Osteoporosis and Fluoride

Osteoporosis is defined as a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk, osteoporosis is considered present when bone mineral density falls to -2,5 SD or lower. below the mean peak bone mass value (WHO, 1994).

Early diagnosis of osteoporosis is difficult because osteoporosis is asymptomatic until it has advanced far enough to cause structural failure of the bone. Severe clinical manifestations of osteoporosis are: loss of cortical bone, which leads to fracture of long bones, and loss of trabecular bone, which may cause fractures in the spine. In treating osteoporosis the aim is to decrease the fracture rate by increasing bone mass. Thus the ideal therapeutic agent for osteoporosis should not only prevent bone loss but also increase mass to a level that exceeds the fracture threshold. The therapeutic agents currently available (oestrogen, calcium, calcitonin and bisphosphonates) act by reducing bone resorption rather than by increasing bone mass. In contrast, fluoride has attracted attention

as a promising agent in the treatment of osteoporosis because of its effectiveness in stimulating new bone growth. This unique role of fluoride has led to a growing interest, and there have been numerous clinical studies on the therapeutic value of fluoride in osteoporosis (Dixon, 1983; Riggs, 1983; Kleerekoper and Mendlovic 1993; Bovin et al., 1993).

Sodium fluoride was first used in the treatment of osteoporosis by Rich and Ensinck (1961). It improved the mineralisation of bone but did not reduce the number of bone fractures. In later studies, it was realised that it was necessary to combine the fluoride therapy with a supplementation of calcium to counteract fluoride-related induction of osteomalacia. Combinations with or without vitamin D gave beneficial effects (Jowsey et al., 1972; Hansson and Roos, 1978; Riggs et al., 1980, 1982). Different combinations containing fluoride were tested in the therapy of osteoporosis by Riggs et al. (1980). Most effective in post-menopausal osteoporosis appeared to be a combination of calcium, fluoride, vitamin D and oestrogen. It was noted that the most beneficial effects were seen in the second year of treatment.

1.5.1 Dose

Two different fluoride salts have been used for the treatment of vertebral osteoporosis: sodium fluoride (NaF), which was used in early treatment, and disodium mono-fluorophosphate (MFP), which is used more recently. Both these salts have been shown, in vitro, to stimulate osteoblast proliferation (Bovin et al., 1993). In a comparative study between NaF and MFP using a similar dose of fluoride-ion, Delmas et al. (1990) found a progressive increase of lumbar bone mineral density with both compounds. However, the increase was higher in the MFP group than the NaF group after 6, 12, and 18 months of treatment. Urinary fluoride was 50% higher in the MFP group than in the NaF group, suggesting a better absorption of fluoride with MFP than NaF (Delmas et al., 1990).

Sodium fluoride in dosages ranging from 20 mg/day to as much as 100 mg/day have been shown to have long-term beneficial effects on bone mineral content and fracture incidence (Riggs et al., 1982). However, inappropriate dosage regimens and inadequate information on the pharmacokinetic properties of fluoride, have produced conflicting clinical results (Ekstrand and Spak, 1990). Bone biopsy studies have shown that there is a narrow therapeutic window for NaF dosage. Dosages of 50 mg/day or less do not consistently stimulate bone formation, and dosages of 90 mg/day or more result in the formation of incompletely mineralised bone (Riggs et al., 1994). Most therapeutic regimens include calcium supplements as a component of treatment.

Fluoride therapy is also known to produce side-effects, in particular pain and swelling in the region of large joints of the lower limbs in roughly a third of patients, as well as gastric irritation and nausea (Schnitzler et al., 1990). Side-effects of fluoride therapy are common and their incidence is clearly dose-dependent. A complication of fluoride therapy is the risk of developing stress fractures in osteoporotic patients. A stress fracture is a break in normal bone that develops insidiously as a result of unusual, repetitive physiological loading. Schnitzler et al. (1990) investigated the characteristics of patients who spontaneously developed peripheral stress fractures during NaF therapy to determine the risk factors. They concluded that stress fracture patients had more severe trabecular and cortical osteoporosis and possibly a poorer bone-forming capacity before therapy than patients without stress fractures. It was suspected that fluoride therapy may weaken bone temporarily and so lead to stress fractures in severely osteoporotic patients. They recommend that fluoride therapy should be started before osteoporosis is profound and that lower fluoride dosage, or intermittent therapy, should be used in patients with cortical as well as trabecular osteoporosis (Schnitzler et al., 1990).

1.5.2 Effects of Therapeutic Fluoride Treatment on Bone Mass

Studies in humans and several animal models have shown that sodium fluoride stimulates bone formation (Marie and Hott, 1986). In patients with osteoporosis, sodium fluoride (NaF) therapy has been shown to increase bone mass, especially in the spine. The increase appears to be linear for up to 4 years and amounts to between 5% and 10% per year, as measured by dual-photon absorptiometry of the lumbar spine (Heaney et al., 1990).

Kleerekoper and Mendlovic (1993) concluded after a review of the literature that the basic mechanism whereby sodium fluoride (NaF) increases bone mass is by an increase in the proliferation of active osteoblasts rather than the increased function per osteoblast. Another effect of NaF therapy is the apparent potential of fluoride to bypass the normal stages of bone remodelling and coupling. Normally osteoclastic activity is followed by osteoblastic activity resulting in zero or slightly negative skeletal balance throughout adult life. In NaF therapy the osteoclastic phase seems to be bypassed and osteoblastic bone formation is directly initiated. For this reason NaF demonstrates great potential in treatment of osteoporosis (Kleerekoper and Mendlovic, 1993).

New bone formed due to NaF treatment (doses above 80 mg/day) is often histologically abnormal. The bone matrix is irregularly fibrous, woven rather than lamellar and has a mottled appearance. The matrix is made up of irregularly distributed osteocytes lying in enlarged lacunae and surrounded by halos of low mineral density (Kleerekoper and Mendlovic, 1993).

Questions have been raised about whether the new bone laid down during fluoride therapy is mechanically sound. Severely fluorotic bone is stronger in compression but weaker in tension than normal bone. Fluoride in high enough

concentrations is known to slow mineralisation of new bone, and this effect may also alter bone strength. Although the details of net fluoride effect on bone mechanics are still uncertain, it is clear that aggregate strength at the organ level will result from increased density on the one hand and altered mechanical properties of the bone on the other. It appears from the available studies that the fluoride therapy effect has reduced the incidence of spinal fractures (Heaney et al., 1990).

Riggs (1990) and Kleerekoper and Balena (1991) conducted placebo-controlled double-masked studies based on the same design. The widely publicised results raised doubts concerning fluoride therapy. In a 4 year clinical trial, Riggs et al. treated and observed 202 post-menopausal osteoporotic women. They were either on NaF 75 mg/day or placebo while both groups received supplementary calcium at 1 500 mg/day. They found that NaF substantially increased bone mineral density in the lumbar spine, but did not decrease the vertebral fracture rate, and increased non-vertebral fracture rate (Riggs, 1990). These findings have been revisited by Riggs. Additional analyses and extended observations on 50 women from NaF groups with up to 6 years of treatment addressed questions from the previous report (Riggs et al., 1994). They found that:

1) NaF is at present, the only therapeutic regimen to result in increases in cancellous bone that continue for more than 1 or 2 years.
2) Women receiving placebo and calcium 1 500 mg/day did not lose bone from the lumbar spine over the 4 year period, thus these data support the recommendation that calcium supplements should be included in treatment regimens.
3) An interpretation of the data implies that NaF therapy decreases vertebral fracture rate moderately in women whose serum fluoride level and lumbar spine bone mineral density increase moderately. By allowing prudent increases in serum fluoride levels and lumbar spine bone mineral density it

may be possible to achieve decreases in vertebral fracture rates (VFR) of 30%-35%. Studies using antiresorptive drugs report a 50% decrease in VFR after treatment despite only small increases in lumbar spine bone mineral density. According to Riggs et al., this may suggest that antiresorptive drugs may decrease VFR by a mechanism that is different from that of NaF and other formation-stimulating drugs. If this is true, then Riggs et al. suggest NaF treatment regimens may provide better therapeutic effects with an antiresorptive compound added than either regimen would achieve alone.

4) In conclusion, because of the increased incidence of fractures of the hip (a threefold increase at NaF 75 mg/day, and at NaF 50 mg/day a twofold increase) and the serious consequences of this type of fracture, Riggs et. al. recommend that NaF therapy under the conditions of this study is not appropriate for the treatment of osteoporosis. However, they recommend that further clinical trials should proceed to establish lower serum fluoride levels and lower rates of increase in lumbar spine bone mineral density, with evaluation of the effect of NaF on the incidence of hip fractures (Riggs et al. 1994).

Kanis (1993) in his article draws conclusions which are in keeping with those of Riggs et al. (1994). He reviewed the use of fluoride in osteoporosis and concluded that the balance of probabilities is that fluoride significantly decreases the risk of vertebral fracture. However, in the clinical use of fluoride it would seem prudent to avoid the use of fluoride in patients with marked cortical osteoporosis because of the risk of hip fracture. In order to minimize the risk the dose should be appropriate, taking account of the absorption of the preparation. Kanis suggests that a regimen that supplies 10-20 mg/day would be reasonable (Kanis, 1993).

Although fluoride has been used for 30 years in the treatment of osteoporosis both the efficacy and risks of fluoride regimens continue to be the subject of controversy. Although impressive increases in bone mineral density of the vertebra have been consistently reported during treatment, the findings in some studies of loss of cortical bone from the appendicular skeleton, of modest or no decreases in vertebral fracture rates, and of increases in the incidence of non-vertebral fractures during treatment, have been major concerns (Riggs, 1993).

Although therapeutic doses of fluoride are not the focus of attention of this research, the fluoride action at these levels provides understanding of its effect on bone. It must be noted that the osteoporosis treatment doses are much higher than the hydric fluoride doses of endemic areas (± 30 - 90 mg/day vs. 4 - 20 mg/day) and that the effect of fluoride on bone at endemic levels may differ to that of therapeutic outcomes. It is also important to note that the endemic fluoride areas are often in rural and economically poor areas resulting in diets with low calcium intakes which may alter the outcome of fluorosis.

1.5.3 The Effect of Fluoride on Bone: Animal Studies

The effects of fluoride on bone in various animal models were reviewed by Chavassieux (1990). She found that the doses of fluoride geiven in animal studies varied from those equivalent to therapeutic doses given in osteoporosis (1 mg NaF/kg in humans) to toxic doses (5 mg NaF/kg in animals) (the smallest dose was 0.7 mg NaF/kg/day in the beagle and the highest was 125 mg/l fluoride in the rat). The duration of the treatment was from 15 days to 33 months. No significant modification in serum calcium, phosphorus and alkaline phosphatase was reported. Evidence for hyperparathyroidism was found in some but not all animal models. Increases in osteoid parameters and in the number of osteoblasts were noted in mouse, cat, pig and ewe. Most authors reported a mineralisation defect either due to a direct effect on the osteoblast or due to a low calcium

intake - both show a modification in the composition of the bone matrix . The formation period was augmented during fluoride treatment, but at a fluoride dose equivalent to therapeutic doses, the augmentation was mainly due to an increased active formation period. But when a fivefold dose was given, there was an inactive formation period. The increase of bone volume after fluoride treatment was attributed to an unbalanced coupling between resorption and formation in favour of formation. All these experimental studies support the conclusion that fluoride induces a stimulation of the birth-rate of osteoblasts, but at high doses decreases their activity (Chavassieux, 1990).

Marie and Hott (1986) tested the early effects of sodium fluoride (0.8 mg/kg/d) in the mouse and found that short-term treatment produced a rapid stimulatory effect on bone formation. Four weeks of fluoride supplementation induced a rapid 21% increase in the osteoblastic surface and a 26% stimulation of the bone matrix apposition rate.

Burnell et al. (1986) fed 384 growing pigs supplemented fluoride (NaF) at different levels by supplementing a Milo and soya bean meal base (7, 132, 257, 382, 507 and 632 ppm per day in experiment 1 and 7, 25, 43, 61, 79, 97, 115, and 133 ppm in experiment 2). The duration of the experiment was not reported. As the level of fluoride increased in the diet, serum fluoride and alkaline phosphatase concentrations increased as well. Bone fluoride also increased with increased dietary fluoride, metatarsal stress and modulus of elasticity decreased (capacity of the bone to return to its original shape after being deformed by a force), and cortical bone thickness decreased. Burnell et al. suggested that dietary fluoride levels of greater than 7 ppm are detrimental to bone integrity in growing pigs and, on the basis of their findings cautioned against the use of fluoride in the treatment of osteoporosis (Burnell et al., 1986).

1.6 The Effect of Endemic Fluoride on Bone

Bone changes observed in chronic skeletal fluorosis in man can be grouped into the following distinct categories; 1) Osteosclerosis, 2) Osteomalacia, 3) Secondary hyperparathyroidism and 4) Osteoporosis. A combination of the above features to varying extents can occur in a given case (Krishnamachari, 1986). The extent to which a particular histological type predominates depends mainly on the dose and duration of exposure to fluoride, although nutritional status, hormonal responses, age, and perhaps sex, and bone type (cortical or trabecular) all influence the effect of fluoride on bone changes.

1.6.1 Clinical Presentation

The clinical picture of endemic fluorosis is characterised by 1) dental fluorosis and 2) skeletal fluorosis. The early stages of endemic skeletal fluorosis may be asymptomatic but as the disease advances the typical picture reveals new bone formation, musculo-skeletal dysfunction leading to arthralgia, arthritis, flixed flexion deformitites, peripheral neuropathy and incapacitation in the advanced stages. Bone deformities of the lower legs such as genu valgum and genu varum can occur, as well as kyphosis and scoliosis of the spine. Endemic genu valgum, a manifestation of fluorosis, is characterised by the simultaneous presence of exostoses and compression features along with clinically discernible osteomalacia (Krishnamachari, 1986).

1.6.2 Radiological and Gross Changes

The most significant effect of long-term fluoride ingestion is seen in the skeletal system. Radiological investigations offer one of the best methods for diagnostic confirmation of the condition. Radiological features in fluorosis include four types of changes: osteosclerosis, osteoporosis, osteomalacia and suggestive features suggestive of hyperparathyroidism. These features are seen in varying

combinations in different parts of the skeleton. There is also variation between individuals regarding the severity and extent of these skeletal changes. Short et al. (1937) mention that all patients with fluorosis had evidence of osteosclerosis, interosseous membrane calcification, calcification of the ligaments and the muscular attachment to bone.

Wang et al. (1994) studied radiographic features in 127 patients and concluded that fluorosis has various radiological appearances and none of them alone is characteristic. A history of exposure is essential for early diagnosis. It is generally thought that several factors influence the type of bone change seen in fluoride intoxication. These include nature or source, dose and duration of fluoride exposure; nutritional status; hormonal responses; age; sex; type of bone affected (cortical or trabecular) and dietary habits (Wang et al., 1994).

Endemic genu valgum is a condition which presents in areas where ground waters are high in fluoride. In patients with endemic genu valgum, Krishnamachari described the occurrence of lines of growth arrest, subperiosteal resorption of phalanges, cystic expansion of short long bones and expansion of lower ends of long bones (Krishnamachari and Kamla, 1973). The latter three features are typical of hyperparathyroidism.

Singh et al. (1961) had the opportunity of studying a complete skeleton of an individual who had lived in an endemic area with a water fluoride level of 9.5 ppm. All the bones were observed to be heavy and irregular and to have a dull colour due to irregular deposition of fluoride. There was an increase in the weight of fluorotic bones, the coefficient of weight to length of fluorotic bones generally showed a doubling of the normal weight, but the vertebral column, pelvis and scapulae were many times heavier.

> "The sites where muscles and tendons attached were abnormally prominent by excessive periosteal reaction with development of multiple exostoses. Irregular bone was laid down along the attachment of muscles and tendons in the extremities as well as in joint capsules and interosseous membranes. Maximum changes were detected in the spine with calcification of various ligaments, particularly the inter-transverse and interspinous ligaments, resulting in marked osteophytes." (Singh et al. 1961)

They also reported that skeletal changes result in limitation of movements, particularly of the cervical spine, lumbardorsal spine, joints of the lower limbs and joints of the upper limbs, in that order. The vertebrae were also fused at many places - this could explain the limitation of movements (Singh et al. 1961).

Although fluorosis of the bone can be a severe skeletal condition with clinical manifestations that affect lifestyle, it has been shown in China to be reversible. Skeletal fluorosis was diagnosed in 495 clinical cases and 25 radiographic cases. Six years later, after treatment with low fluoride drinking water, 140 cases had been completely cured, 105 had marked clinical improvement and 172 had moderate improvement. Of the x-ray diagnosed cases, 15 showed marked improvement, namely reappearance of evenly distributed fine bone trabeculae, improvement in the grade of osteosclerosis and in one osteosclerotic case a return to normality (Song, 1989). This demonstrates fluorosis of the bone to be reversible.

1.6.3 Histopathological Changes

New bone formation with irregular mineralisation has been the hallmark of fluorosis. Roholm described the occurrence of new bone when fluoride was fed in small amounts over a long period. Large doses, however, led to the formation of osteomalacia with uncalcified osteoid (Roholm, 1937). Pandit and Rao observed new bone formation and hypermineralisation of bone in monkeys fed

fluoride (Pandit and Rao, 1940). Teotia et al. (1976) investigated 100 patients with proven endemic fluorosis and reported an increase in the percentage of the trabecular bone surface covered with osteoid as well as an increase in the proportion of total bone surface which was covered with resorption lacunae (Teotia et al., 1976).

A detailed description of autopsy bone findings in workers at an aluminium smelter revealed increased turnover rate and subperiosteal fibrous bone formation, as well as coarsening of spongy bone, irregular matrix formation, enlarged osteocytic cavities and irregular arrangement of lamellae and osteons in 25 iliac crest biopsies (Franke and Horn, 1976).

In general Singh and Jolly (1961) found that compact bone showed disordered lamellar orientation and an enlarged, poorly formed Haversian system. In the spongy bone, areas of osteoid tissue were found within well-formed trabeculae. Some of the irregular deposits of osteoid tissue extended into the attached muscle. The bone trabeculae were very dense in places and contained a considerable amount of calcium. The extensive production of new bone in the fluorotic skeleton was usually accompanied by increased bone resorption (Singh and Jolly, 1961).

Similar trends were seen in a histomorphometric study of skeletal fluorosis in 29 patients. Boivin et al. (1990) found high bone fluoride content in all cases in comparisons with control values. The origin of exposure to fluoride was known in 20 cases and was either hydric, industrial or iatrogenic. Radiologically each patient showed an increase of cancellous bone volume compared with controls. Significant increases in cortical width and porosity were noted without a reduction of cortical bone mass. Osteoid parameters were significantly increased in fluorotic patients. There was a threefold increase in the cancellous osteoid

perimeter when compared with cancellous eroded perimeter. The fluorotic group had a higher number of osteoblasts than controls with a high proportion of flat osteoblasts. In 15 patients doubly labelled with tetracycline, the mineral apposition rate was significantly decreased while the mineralisation lag time significantly increased (Boivin et al., 1990).

Radiological osteosclerosis observed in each patient was confirmed by the significant increase in cancellous bone volume in fluorosis patients compared with controls (p=0.000 1). Bone formation and adjusted apposition rates were significantly decreased in skeletal fluorosis. Skeletal fluorosis in this study was characterised by an unbalanced coupling in favour of bone formation. Bovin stated that these osteoblasts have an increased formation period mainly due to a prolonged inactive formation period and that these results support the view that fluoride may have a dual effect on osteoblasts: an increased birth-rate at the tissue level, and a toxic effect at the individual cell level. The addition of these two effects represents, however, a marked increase of bone formation at the organ level (Boivin et al., 1990).

1.7 The Role of Calcium in Fluorosis

The fluoride ion has great affinity for the calcium ion. Biological interactions between these two elements are important factors in determining the clinical and metabolic course of fluorosis. The extent to which a particular histological type of bone predominates depends on several factors, of which the dose and duration of exposure to fluoride are the most important, although the calcium nutritional status seems to play a determining role. Roholm observed the heterogeneous character of bone changes that accompany fluoride intoxication. He proposed that while smaller doses of fluoride given over a long period would cause osteosclerosis, large doses could lead to osteomalacia and osteoporosis. Growing children exposed to high levels of fluoride seem to respond differently

than adults who had achieved maximum bone growth. The role of calcium was raised by Roholm. Low calcium nutritional status appears to induce a rachitic or osteomalacic picture of the bone (Roholm, 1937).

Calcium-fluoride interactions can be studied in two ways: 1) metabolic balance studies and 2) calcium turnover studies using radioactive calcium. Calcium balance studies indicate the net retention of calcium in the body, but radioactive studies facilitate understanding of the kinetics of calcium in different target areas. Either method can highlight the influence of fluoride on calcium metabolism in fluorotic patients.

Calcium retention is higher in patients with fluorosis than in controls.
Calcium balance studies were carried out by Srikantia and Siddiqui (1965) in six adults with skeletal fluorotic lesions and four matched controls. Both groups were given 800 mg of calcium per day in their diets. They observed a significantly higher retention of calcium in fluorosis patients, retention being 50% of intake. Only 18% calcium retention was seen in the controls. Narasinga Rao et al. observed a similar trend in calcium retention. Fluorotic patients retained 53% of calcium on a 800 mg per day diet (Narasinga Rao et al., 1968).

Teotia et al. (1969) reported that when skeletal fluorosis patients were provided with 900 mg of calcium per day they retained 32% of intake and reported a negative calcium balance of 30% (42.6mg) when given 140 mg of calcium per day, highlighting the need for a high calcium intake in fluorosis (Teotia et al., 1969).

Reddy and Narasinga Rao induced experimental fluorosis in monkeys by intragastric feeding of sodium fluoride for several months. In addition to other studies, ^{45}Ca turnover was investigated in these animals. It was observed that irrespective of the administration of fluoride, animals maintained on a low

protein diet retained less ^{45}Ca, and those on the high protein diet retained more ^{45}Ca. Calcium deficiency caused increased retention of calcium. Fluoride given to animals on an adequate diet was found to increase the cumulative retention of ^{45}Ca, and those animals fed on low-calcium and low-vitamin-C diets were found to further increase the cumlative retention of labeled calcium. This study suggests that in fluoride-fed animals, avidity for calcium increases, particularly when fed a low calcium diet (Reddy and Narasinga Rao, 1971).

In the treatment of osteoporosis using fluoride, calcium supplements are prescribed in an attempt to reduce the severity of histological osteomalacia. Calcium supplements have been reported to cause improvement in the osteomalacic bone picture (Jowsey et al., 1972), but this beneficial effect may be due to reduced fluoride absorption, an effect which would be equivalent to a lower dose of ingested fluoride. Harrison et al. studied the effect of dietary calcium on fluoride toxicity in growing rats. Rats were maintained on either adequate (0.5%) or high (2.0%) dietary calcium and given NaF in drinking water for 5 weeks (Harrison et al., 1984). Their results show a decrease in intestinal fluoride absorption from 76% to 47% when dietary calcium is increased from adequate to excessive levels (0.5 to 2.0). This effect of dietary calcium is reported to be associated with alterations in the intestinal cell membrane affecting transport of fluoride (Harrison et al., 1984). Although the mechanism by which dietary calcium inhibits fluoride absorption is unclear, this inhibition appears to be independent of luminal calcium at the time of fluoride ingestion. Harrison et al. concluded that in their rat model additional dietary calcium reduced fluoride toxicity, but that this effect appeared to be related, at least in part, to decreased fluoride absorption. In humans, Ekstrand et al. found intestinal fluoride absorption to exceed 90%. This high level of fluoride absorption may be related to the relatively low Ca/P ratio of 0.5 in North American diets examined in this study (Ekstrand et al., 1978).

Briancon et al. (1990) looked at the fluoride absorbability following the administration of NaF alone and in combination with different calcium salts. The pharmacokinetic studies were carried out in 12 healthy fasting volunteer subjects. In the first study NaF, was administered orally as Osteofluor which contains 25 mg of NaF. The second and third studies used the same NaF dose, but in combination with 500 mg of elemental calcium as Calcium Sandoz F (2.94 g calcium gluconolactate, 0.800 g calcium carbonate per tablet) or Calcifort (1.050 g calcium gluconolactate, 0.960 g calcium lactate, 0.930 g calcium glucoheptonate, 0.544 g calcium chloride and 0.070 calcium cyclarate per ampule).

Results showed that: 1) when NaF is accompanied by calcium, the peak fluoride level in plasma is lower and delayed, and 2) the areas under the curves obtained with each of the preparations were not significantly different, but the 24-hour urinary fluoride was significantly lower in volunteers receiving simultaneous NaF and calcium salts (35% of dose being excreted) than in volunteers receiving only NaF (50% of dose being excreted). These results support the conclusion that intestinal fluoride bioavailability, evaluated from the area under curves, is not significantly impaired by the concomitant intake of calcium salts. However, contrary to the above findings, the significant reduction in 24-hour urinary fluoride in volunteers who received sodium fluoride and calcium salts suggests a reduction in bioavailability of fluoride due to the presence of calcium. Briancon et al. do not discuss the possible uptake of fluoride by bone contributing to the decreased urinary levels.

In skeletal fluorosis varying degrees of osteomalacia are present. The spectrum is wide with softening of bones with clinically evident deformities at one end, to mild grades of osteomalacia not evident except following infusion tests using calcium on the other. Rao et al. (1978) investigated 16 clinically confirmed

patients with skeletal fluorosis who were maintained on a hospital-based diet supplying 800 mg of calcium, 400 mg of phosphorus and 150 mg of magnesium per day. A single infusion of calcium gluconate (15 mg/kg body weight) was given in 500 ml normal saline over a four hour period. Total output of calcium during the following 12 hours was expressed as a percentage of the administered dose. In all patients a low output was observed ranging from 2.9% - 14%. The mean value for the group was 5.5% of the dose administered. Although Roa et al. do not state what a normal calcium output should be, they note that the mean value of 5.5% was considerably lower than they would expect in fluorotic conditions. They make the assumption that "a decreased avidity of bones for calcium should be expected and the calcium infusion in the endemic patients should have elicited greater excretion of calcium than in normal persons" (Rao et al., 1978). However, contrary to what they expected, they report that bone showed an enhanced avididty for calcium. In the light of current literature it is consistent that endemic fluorosis shows the increased uptake of calcium by bone, suggesting osteomalacia or increased bone formation. This confirms the view that even in patients with osteosclerotic-type fluorosis, avidity for calcium exists (Rao et al., 1978). This is also in line with the observation that in fluorosis, urinary calcium is reduced.

In an attempt to determine calcium turnover in patients with either endemic fluorosis or genu valgum, Narasinga Rao et al. (1979) gave intravenous injections of radioactive ^{47}Ca to patients and age-matched controls. Total Ca turnover in the body, loss of Ca from the body in urine, faeces and sweat and bone mineralisation rate were calculated from whole-body retention of ^{47}Ca and specific activity of ^{47}Ca in serum. They found that:

1) total Ca turnover was significantly higher in younger subjects than in older ones,

2) total Ca turnover was significantly higher in patients with fluorosis and in those with endemic genu valgum than in age-matched controls, but the external losses (urine, faecal and sweat loss) of Ca were lower in both groups of patients than in the controls,

3) bone mineralisation rate was significantly higher in patients with fluorosis and genu valgum than in age-matched controls,

4) an increase in calcium accretion rate, which may be attributable to fluoride toxicity, is of a greater magnitude in genu valgum patients than fluorotic ones.

These results highlight that there is an altered calcium metabolism in the presence of fluoride resulting from increased bone mineralisation.

Narasinga Rao et al. (1979) make the case that endemic genu valgum is a different condition to that of endemic fluorosis. However, endemic genu valgum is most probably a condition which results from endemic fluorosis occurring during childhood. Thus the patients investigated in this study are more likely to be those coming from endemic fluorosis areas with or without genu valgum than patients with two different conditions.

Griffiths et al. showed that dietary calcium plays an important role in determining the type of bone lesions which would emerge during the process of experimental fluorosis in monkeys. They demonstrated that while a low calcium diet alone produces osteoporosis, addition of dietary fluoride causes osteomalacia, while protecting from osteoporosis (Griffiths et al. 1975).

In human populations fluorosis has been observed in those living in endemic areas. Krishnamachari and Krishnasamy conducted a demographic study, which included a dietary survey of patients who presented with fluorosis and lived in endemic areas, in an attempt to better understand the contributing factors. They

reported a very low intake of calcium in the population. Adults in the study had an average intake of 268.5 mg calcium per day and children 145 mg calcium per day (Krishnamachari and Krishnaswamy, 1974). Dietary calcium intakes may be low due to poor socio-economic factors in these areas. Although this does not clarify the role of calcium intake in relation to fluoride effect, it raises the question of a possible relationship between calcium and fluorosis.

Krishnamachari proposed that dietary calcium intake influences the manner of fluorosis presentation. Figs 1.1 and 1.2 depict the effect of normal and low calcium intake on bone metabolism in endemic fluorosis (Krishnamachari, 1986).

The above studies emphasise the important role of dietary calcium in determining the nature and severity of fluorosis. However, the model proposed by Krishnamachari does not distinguish between adults and children, nor does it address the fluoride dose or calcium intake at which these bone changes are seen other than to qualify them as "excessive", "adequate" or "low".

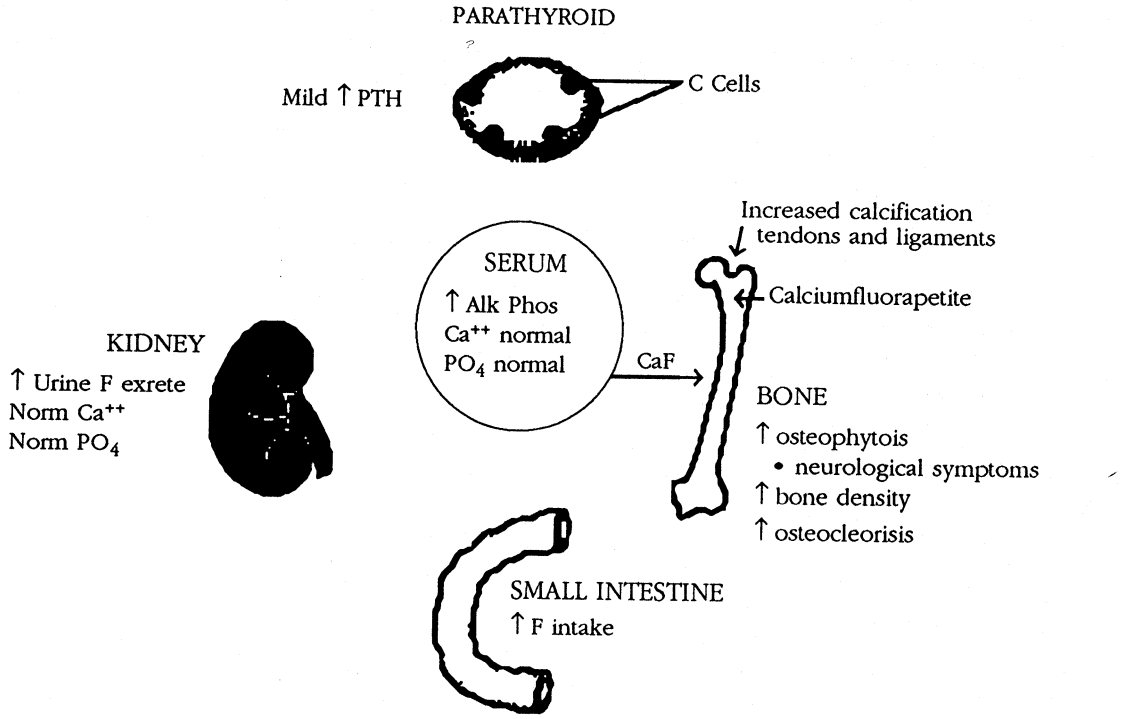


Fig 1.1 Adequate Calcium and Excess Fluoride Intake

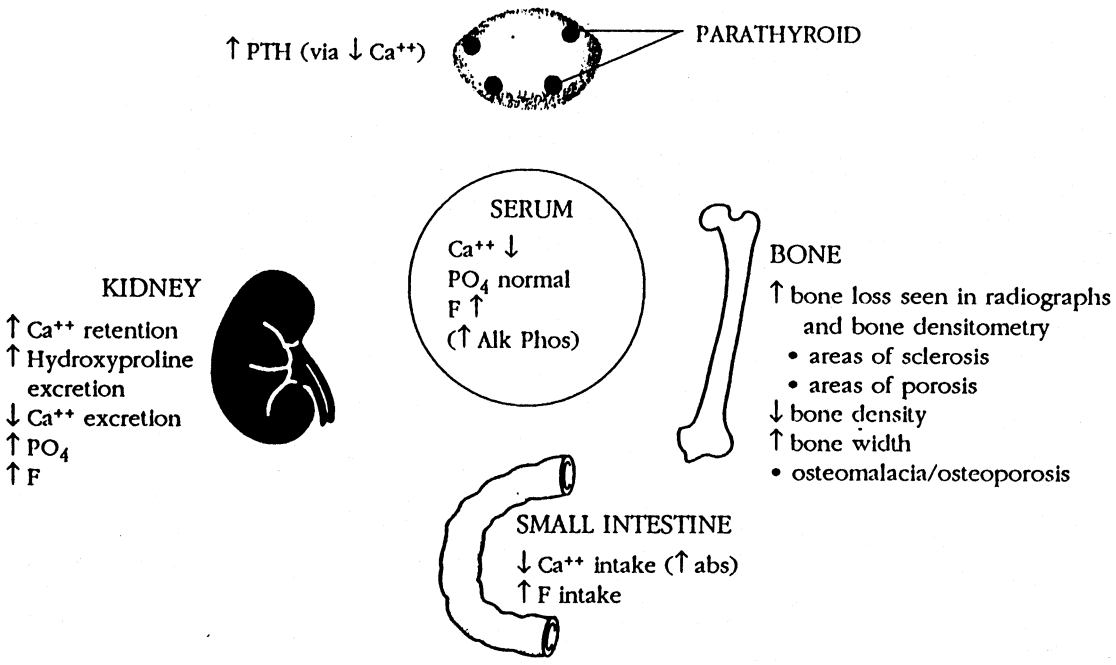


Fig 1.2 Low Calcium and Excess Fluoride Intake

1.8 Biochemical Changes in Endemic Fluorosis

In endemic skeletal fluorosis, whole blood fluoride concentration is elevated 10- to over 100- fold, and this has been documented by Sing and Jolly (1961) (0.5-5.6 ppm), Srikantia and Siddiqui (1965) (0.9-7.5 ppm) and Susheela et al. (1981) (mean = 0.48 ppm; normal =0.04 ppm). Metabolism of bone-related minerals is also altered to varying extents in endemic fluorosis. These metabolic alterations are reflected to some extent in serum levels of the minerals concerned. Alkaline phosphatase is a sensitive indicator of altered bone metabolism in fluorosis. Serum changes, however, are not real indicators of tissue status of minerals such as calcium in this disease since compensatory mechanisms of the parathyroid-thyrocalcitonin axis come into operation at different stages of the disease (Krishnamachari, 1986).

Typically, endemic fluorosis presents a picture of raised serum alkaline phosphatase (14 - 80 K.A units), normal serum phosphorus, calcium and magnesium levels. High urinary fluoride (3 - 4.8 ppm) and phosphate excretion and low urinary calcium excretion is characteristic (Teotia et al., 1971, 1979; Siddiqui, 1955). Serum 25-hydroxyvitamin D (25-OHD) values are reported to be normal (Teotia et al., 1978).

There are contradictions in the literature regarding endemic fluorosis and its effect on serum calcium levels. Teotia et al. (1971, 1979) found serum levels to be normal to elevated, however Pettifor et al. (1989) found serum calcium levels to be normal to low (23% hypocalcemic $\leq$ 2.25 mmol/l). The reason for these differences is unclear but may be related to different dietary calcium intakes.

Fluoride is an element which is known to interfere with metabolism in bone, which is rich in collagen. When fluoride enters bone structure in toxic amounts,

it modifies not only the mineral metabolism but also the collagen component of bone matrix (Krishnamachari, 1986). Collagen, when broken down, is a rich source of hydroxyproline. Secondary hyperparathyroidism, which occurs in fluorosis, causes breakdown of bone collagen to varying extent. Hydroxyproline is excreted in urine and is correlated to bone resorption. In endemic genu valgum, a manifestation of fluoride toxicity associated with secondary hyperparathyroidism in children, total hydroxyproline in urine is significantly increased (Krishnamachari, 1986). Kinetic data obtained from fluorosis patients indicate that the bone mineralisation rate is increased (Narasinga Rao et al., 1968). Also ^{47}Ca turnover studies conducted in fluorosis patients and endemic genu valgum patients confirm the inference that the observed increase in urinary hydroxyproline in the osteoporotic form of fluorosis is due to an increased rate of bone resorption (Narasinga Rao et al., 1979).

1.9 Hormonal Changes in Fluorosis

Fluoride-induced parathyroid alterations have been observed in animals and man. Faccini found elevated levels of serum parathyroid hormone (PTH) in young sheep reared on water containing 100 ppm fluoride. He demonstrated that daily doses of fluoride given for a week stimulate production of PTH and thereby cause an increase in the circulating levels of the hormone (Faccini, 1969). Yates and Talmye (1964) and Nicholas et al. (1965) explained the observed elevation of PTH as the possible consequence of fluoride-induced reduction of serum calcium accompanying fluoride-induced depression of bone mineral solubility.

Changes in hormonal profile are believed to be related to chronic exposure to fluoride. Teotia et al. (1972) demonstrated the occurrence of secondary hyperparathyroidism in patients with endemic skeletal fluorosis. The same authors demonstrated hyperparathyroidism in children who developed endemic

skeletal fluorosis. Sivakumar and Krishnamachari (1976) observed a significant increase in circulating PTH levels in patients with skeletal fluorosis and also in those with endemic genu valgum. Mean values for PTH were 2.67 ng/ml and 5.39 ng/ml in the two groups respectively, while in the control group a mean value of 1.24 ng/ml was recorded. Teotia et al. (1978) reported elevation of both PTH and calcitonin in their fluorosis patient group and not in their control group.

Table 1.1 Summary of Fluoride Effect

Ingested Fluoride
↓
Almost complete absorption
↓

Bone Compartment ←	**Serum/Blood Pool** →	**Urinary**
Bone Ca/P not altered	Serum F ↑	F ↑
	Serum Ca unaltered	Ca ↓
Bone Density ↑ or ↓	Serum P unaltered	PO_4 unaltered or ↑
Sclerosis ↑	Alkaline Phosphatase ↑	Hydroxy-
Osteoporosis ↑ or ↓	PTH ↑	proline ↑
Osteomalacia ↑		
Features of hyperparathyroidism ↑		

Chapter II

2.0 Endemic Fluorosis in Children

Skeletal fluorosis in adults has been known for many years. Short et al. (1937) described endemic fluorosis as manifest by mottled dental enamel and by osteosclerosis of the skeleton in adults, and they were under the impression that residence of 30 to 40 years in endemic areas was necessary for skeletal fluorosis to develop. Jolly et al. (1969) reported that advanced stages of fluorosis usually resulted from continuous exposure to a water supply of 10 ppm for a period of 10 to 20 years. Mottled dental enamel in children in endemic fluoride areas has always been obvious and well documented. However, there are few reports of skeletal changes in children. Siddiqui (1955) reported that it took one to four years to show marked skeletal symptoms after moving into an endemic fluoride area. He also reported that:

> "Children, apart from dental changes, do not suffer from the ill effects of fluorine. Probably the growing tissues are somehow able to deal with fluorine ingested." (Siddiqui, 1955).

Jackson was puzzled over the cause of skeletal changes in children as he had read reports where it took 20 - 30 years of exposure to high fluoride water for bone disease to occur and then it was of the sclerotic type (Jackson, 1962). However, Steyn and Jackson in South Africa found children affected with skeletal changes and deformities with as little as 2.6 ppm fluoride in the water (Steyn, 1938; Jackson, 1962).

Later Teotia et al. described "skeletal fluorosis" in children in India (Teotia et al., 1969). The minimum number of years needed to live in an endemic area before fluorotic changes occur is not known. The fluoride level of the drinking water and the amount consumed are crucial factors in determining that time interval. Children as young as 4 years old showed skeletal changes in Tanzania (21 ppm F) (Christie, 1980). Teotia et al. found children 4 years and older showing skeletal

changes but most commonly children were at least 10 - 11 years of age when they began to show marked changes (Teotia et al., 1971, 1979).

2.1 Dental Fluorosis

The factors which govern the incorporation of fluoride into dental structures are essentially similar to those in bone. Fluoride is taken up most rapidly during the phases of growth and development. Dental tissues differ from bones in that once formed there is no remodelling and, in the enamel, there is no cellular activity. In the early stages of formation and calcification, the overall uptake of fluoride in dentine and enamel is at its highest. The much lower permeability of mature dentine, especially enamel, decreases the effect of fluoride in developed teeth (WHO, 1970; Richmond, 1985). Thus dental mottling (fluorosis) is an indication of having been in a high fluoride area at the time of tooth formation and can indicate when fluoride was introduced by the part of the tooth enamel affected.

In dental fluorosis, the enamel-forming cells, the ameloblasts, are affected, the maturation of the enamel is delayed and the general mineralisation processes may be inhibited, perhaps through interference with nucleation and crystal growth. In addition, calcium homeostatic mechanisms may be affected. Histological changes are found in the enamel and, in severe fluorosis, also in the dentine (Fejerskov et al., 1977, 1979).

Dental fluorosis is easily recognisable by mottled tooth enamel. In mild stages of dental fluorosis, lustreless, opaque white patches are seen in the enamel which may become striated, mottled and/or pitted. The opaque areas may progress to yellow or dark brown "stains" in severe cases of the condition. Classification of dental fluorosis was pioneered by Dean using a 7-point scale of fluorosis (Dean 1934). As a consequence of higher water consumption, the frequency and

severity of dental fluorosis increases with increasing mean maximum temperature. As the community index of fluorosis increases, caries prevalence decreases until the destructive forms of fluorosis (scores of 4 and 5 on Dean's index) become prevalent (WHO, 1984).

In a Tanzanian study, Wenzel et al. (1982a) attempted to define the relationship between dental fluorosis and skeletal maturity (measured by radiographs of the hand) within a high fluoride area (3.6 ppm). Girls 11 - 14 years of age were investigated. They found no relationship between hydric fluoride levels and skeletal maturity, but did find a significant relationship between dental fluorosis and skeletal maturity suggesting that increased fluoride ingestion retards maturation. This relationship was less obvious in areas with < 2.5 ppm in drinking water. To evaluate the significance of these findings in temperate climates a similar study was carried out in two Danish areas containing < 0.2 and 2.4 ppm fluoride in the drinking water. No differences in skeletal maturity could be detected between the two Danish groups (Wenzel et al., 1982b).

2.2 Signs and Symptoms of Fluorosis in Children

The dental and skeletal changes in endemic fluorosis provide important clinical diagnostic criteria. Dental fluorosis is easily recognisable by mottled tooth enamel, however skeletal involvement is not clinically obvious until the advanced stage of crippling fluorosis. Early endemic fluorosis cases are usually in young adults whose complaints are vague pains in the hands, feet, knees and spinal joints. In later stages there is an obvious stiffness of the spine with limitations of movement followed by the development of kyphosis (Singh et al., 1961; Teotia et al., 1979).

Teotia et al. (1969, 1971, 1979) described fluorosis in children ranging in age from 11 to 13 years, 6 to 14 years and 4 to 15 years of age respectively. The problem

was described in areas where fluoride levels in water varied from 10.5 to 26 parts per million (ppm). Steyn et al. (1962) described fluorotic signs and symptoms in Kenhardt children from age 4 where fluoride levels were 3.6 - 13 ppm.

Some researchers (Jolly et al., 1969; Pandit and Rao, 1940) have taken dental fluorosis as an index of the endemicity of fluorosis. Bagga et al. reports that dental fluorosis is a sensitive index of chronic fluoride toxicity and may be its earliest and only visible manifestation in children (Bagga et al., 1979). However, Jolly et al. reports that dental fluorosis has no relationship with the incidence of skeletal fluorosis (Jolly et al., 1969). Tamboli and Mathur found that pain and restricted movement were reliable predictors of fluorosis. He presented clinical criteria for screening in endemic fluorosis with water fluoride levels of 13.9 and 14.3 ppm. When screening for endemic fluorosis, it was found that 80% of the population complaining of bone pain, painful and/or restricted movement at various joints had radiological features of fluorosis (Tamboli and Mathur, 1982).

Teotia et al. (1979) investigated 550 children aged 4 - 15 years living in an area with high water fluoride levels. Diagnostic radiological findings were observed in only 200 of these children. The following signs and symptoms pertain only to the cases with skeletal fluorosis (Table 2.1) (Teotia et al., 1979).

Table 2.1 Signs and symptoms in children with skeletal fluorosis.
The mean daily intake was 16 ppm F. All the patients lived in the endemic area since birth and the average age of onset of symptoms was 11.4 years. (Teotia et al., 1979)

Percent of cases n = 200	Sign/symptoms
6.0%	thoracic kyphosis
16.5%	flexion deformity; hips, knees, chest
22.5%	difficulty in walking or unable to walk
23.5%	constipation
62.5%	severe skeletal pain
85.0%	inability to close fists
95.0%	spinal stiffness, cervical flexion
100%	mottled discoloration of teeth

2.3 Deformities of the Lower Limbs

Children in Kenhardt (Northern Cape, South Africa) presented with pain in the shins and later bowing of the femora and tibiae. Ground water from various areas in Kenhardt varied in fluoride content from 2.6 - 13.0 ppm. At the time, 1932, Stein and Jackson were unable to find any literature describing similar skeletal changes in children. Severe genu valgum (knock-knees) became known as the "Kenhardt Bone Disease" after the geographical area where it was studied (Jackson, 1962). A Tanzanian study of 251 children below the age of 16 years living in an area with 21 ppm fluoride in the drinking water showed 23% had knock-knees, 17% had bow-legs and 12% had sabre shins (Christie, 1980). Deformities were more prevalent in boys than girls and this trend has also been noted by other researchers (Christie, 1980; Krishnamachari and Sivakumar, 1976; Teotia et al., 1979; Pettifor et al., 1989).

Krishnamachari and Kamla (1973) and Krishnamachari and Sivakumar (1976) documented 4% of residents in Indian endemic fluorotic areas with the syndrome of genu valgum. Villages were selected on the percentage prevalence of dental fluorosis (80% incidence). Some of the fluoride levels were reported and were over 3 ppm. It was observed that invariably the poorest segment of the village was the one affected most, and more males than females were affected, with a sex ratio of 10:1 (Krishnamachari and Kamla, 1973; Krishnamachari and Sivakumar, 1976). They attempt to distinguish between skeletal fluorosis and endemic genu valgum. They note that in a study where there was skeletal fluorosis present but not genu valgum, the urinary hydroxyproline levels remain normal. However in endemic genu valgum these levels are raised and circulating PTH (parathyroid hormone) levels were higher than in the endemic fluorosis group. Krishnamachari and Sivakumar also note that onset of the condition is always over 7 years of age, and onset does not occur in adulthood (Krishnamachari and Sivakumar, 1976). Endemic genu valgum is reported to have increased total body calcium turnover and bone mineralisation rates compared to endemic fluorosis (Rao et al., 1978).

2.4 Radiological Findings

Steyn et al. reported on the radiographic changes of the anterior and posterior view of one femur and lateral view of one tibia of children with deformity. They found that the changes in the bones of the leg included thinning of the cortex with widening of the medullary cavity in the lower part of the femur and upper end of the tibia, with anterior thickening (buttressing) of the cortex in that area. The normally clear demarcation between the cortex and medulla was obliterated. Lateral view of the spine showed transverse (Harris) lines, and the bones of the hands of the affected children were clearly demineralised, with coarse trabeculation (Steyn et al., 1962).

In a study of 200 children with skeletal fluorosis (Teotia et al., 1979), x-rays of children showed osteosclerosis of the spine, pelvis and thorax in 192 cases, as well as periosteal bone formation, irregular exostoses, and calcification of the interosseous membranes and muscular attachments (Teotia et al., 1971; 1979). Forty-three patients (21.5%) showed changes characteristic of hyperparathyroidism, namely: 1) subperiosteal resorption in the phalanges, 2) erosion or loss of the lamina dura, 3) coarse cystic trabeculations, metaphyseal erosions, and thinning of the cortices particularly in the pelvis, knees and hands, and 4) para-haversian canal resorption.

Christie (1980) found many of the same radiological features as Teotia et al. However, he pointed out that these changes did not always develop in a progressive, predictable pattern to the adult stage of osteofluorosis. In some young children, early osteosclerosis developed without significant deformities. In some families, only one child presented with severe genu valgum while others showed no gross deformities. The degree of dental mottling did not always correlate with the severity of skeletal involvement, a finding supported by Jolly et al. (1969).

Patients who had lived in an endemic area (drinking water 8 - 12 ppm) for longer than 8 years and presented with progressive deformities of the legs, particularly knock-knees, were investigated by Pettifor et al. (1989). Radiological findings revealed sclerosis and coarsening of the trabeculae of the pelvises, with osteopenia of the long bones and Harris's growth arrest lines. Osteopenic hands with scanty coarse trabeculae in the metacarpals and widening of metacarpal width were also observed. On examination of the skull the diploë had a fine salt and pepper appearance. The lamina dura around the teeth was absent. Although leg deformities appeared rachitic-like, active rickets at the metaphyses were absent except in one child (Pettifor et al., 1989).

Teotia et al. suggest that x-rays revealing osteosclerosis of the spine and showing periosteal bone formation and interosseous membrane ossification of the forearms are accurate enough for the diagnosis of skeletal fluorosis in children (Teotia et al., 1971).

Although different authors have used different methodologies when examining skeletal fluorosis in children, some of the more common radiological findings are tabulated below.

Table 2.2 General Radiological Findings by Different Researchers

Characteristics	Jackson	Teotia	Pettifor	Christie	Krishnama-chari and Sivakumar
Osteosclerosis (spine, pelvis and thorax)		X	X	X	X
Calcification of ligaments and interosseous membrane		X			X
Periosteal bone formation		X			
Erosion/ loss of lamina dura		X	X	X	
Coarse cystic trabeculations (osteoporosis)	X	X		X	X
Metaphyseal erosion/ rarefaction		X			X
Widening and rarefaction of lower end of long bones	X	X	X	X	X
Subperiosteal resorption of phalanges		X		X	X
Cystic expansion and thinning of cortex	X	X		X	X
Rackitic metaphyses (knees, wrist and hip)		X	X	X	
Demineralised bone	X			X	
Harris/stress lines (spine, femur and tibia)	X		X	X	

Jackson (1962), Teotia et al. (1976), Pettifor et al. (1989), Christie (1980), Krishnamachari and Sivakumar (1976)

2.5 Histopathological Changes

Histopathological studies performed specifically in children are not common. Bone taken from the iliac crest by open biopsy was studied in 20 children (Teotia et al., 1979). The chemical composition of the iliac crest bone in the 20

children showed excess calcium and fluoride. The compact bone showed poorly formed Haversian systems and disordered lamellar orientation of the bone. Irregular deposits of osteoid tissue were seen in the trabecular bone. Osteoclastic resorption and the irregular erosion of the bone trabeculae were observed in all the cases. Pettifor et al. questioned the validity of these results reported by Teotia, as identical results to these were presented in a study with 44 patients (Teotia et al., 1972) of which only 6 were children (Pettifor et al., 1989).

Hyperosteoidosis with an increase in osteoid volume, osteoid surface and osteoid seam thickness was observed by Pettifor et al. (1989) on histomorphometric analysis of iliac crest bone biopisies from 9 fluorotic children. Erosion surfaces were increased in the majority of patients. Bone fluoride levels were elevated tenfold in the 7 patients in whom it was measured (Pettifor et al., 1989).

In an interesting study by Chongwan et al. (1986), light microscopic and scanning electron microscopic observations were made on human foetal bones from an endemic fluorosis area of Guizhou, China. Five human foetuses were delivered during the eighth month of gestation by means of termination of pregnancy. Bone samples of humerus and femur were studied under light and scanning electron microscopes. New bone formation was more active in foetuses from the endemic area than in those from a non-endemic area. In those from the endemic area, calcification was inadequate and development of epiphyseal chondrocytes was abnormal. Chondrocytes were arranged in clusters instead of parallel rows. Light microscopy revealed that beneath the zone of provisional calcification, many trabeculae were poorly calcified. Between the trabeculae, hemopoietic tissue was distributed unevenly. In the cortex, many Haversian canals were filled with osteoblasts and fibrous tissue. This change was more prominent in the humerus than in the femur. Electron microscopy revealed that

in the endemic area samples, the diaphyseal endosteal surface was rough, uneven and granular, indicative of inadequate calcification, while samples of the same bone area from non-endemic fluorosis samples were not as rough; prominent resorption depressions of Howship's lacunae were noted, which were sharply demarcated and subdivided by ridges into compartments. Areas of woven bone, characterised by an increased number of osteocyte lacunae of uneven size and depth were noted on the periosteal surface in samples from the endemic area, but not in samples from the non-endemic area (Chongwan et al., 1986).
The higher level of fluoride in bone samples from the endemic area was ascribed to fluoride ion deposited in foetal bones via placental circulation. It was therefore concluded by the authors that skeletal fluorosis begins during the foetal period (Chongwan et al., 1986).

2.6 Serum Biochemistry in Children Living in Endemic Areas

In skeletal fluorosis, metabolism of bone-related minerals is altered. These metabolic alterations are reflected, to a degree, in serum levels. In Teotia's 1971 and 1979 studies serum phosphate and magnesium concentrations were normal. Serum 25(OH)D levels were also reported to be normal, but PTH concentrations were elevated (Teotia et al., 1978). Serum alkaline phosphatase was elevated in most cases, while serum calcium concentrations were normal (Siddiqui, 1955; Teotia et al., 1979). In 2 of 6 cases, studied by Teotia et al. in 1971, raised serum calcium values were noted (Teotia et al., 1971). All cases showed increased urinary fluoride and phosphate clearance and decreased tubular reabsorption of phosphate. Low urinary excretion of calcium was found.

Christie also found that serum alkaline phosphatase values were considerably elevated and serum calcium values at normal to low normal levels (Christie, 1980). Krishnamachari and Sivakumar found serum calcium, phosphorus and alkaline phosphatase concentrations were within normal limits in genu valgum patients,

but they had increased PTH levels and increased hydroxyproline in urine (Krishnamachari and Sivakumar, 1976). He explained this phenomenon with the hypothesis that in the osteosclerotic form of the disease, elevation of alkaline phosphatase occurs, while in the osteomalacic type of the disease it does not (Krishnamachari, 1986).

In South Africa, Pettifor et al. (1989) conducted a prevalence study in 260 children between the ages of 6 and 16 years. This study population was living in an endemic fluoride area of KwaNdebele, in close geographic proximity to the current study. Of the 260 children, 3.8 % were hypoalbuminemic (< 35g/l), suggesting the presence of protein energy malnutrition. Serum calcium values were low in 23 % of the children (< 2.25 mmol/l) and 15 % were hypophosphatemic (< 1.3 mmol/l). Serum phosphate concentrations were significantly correlated with serum calcium concentrations (r=0.46). Alkaline phosphatase values were elevated (> 300 IU/l) in 24.5 % of boys and 27.7 % of girls. Serum 25(OH)D concentrations were measured in 40 children and were found to be within normal range (9.1 - 36.0 ng/ml). These serum changes seen in children with endemic fluorosis reflect a picture of altered bone homeostasis with increased osteoblastic activity.

2.7 Secondary Hyperparathyroidism

Secondary hyperparathyroidism occurs when serum calcium is low. High levels of PTH in turn stimulate calcium absorption from the intestine via 1,25-$(OH)_2D$, reabsorption of calcium in the renal tubules as well as release of calcium from the bone. Increased osteoblastic activity is reflected by high levels of alkaline phosphatase, confirmed by findings of researchers in endemic areas (Siddiqui, 1955; Krishnamachari and Sivakumar, 1976; Teotia et al., 1978; Christie, 1980).

Secondary hyperparathyroidism has been reported by a number of authors working in endemic fluorosis. Teotia et al. tested for the occurrence of secondary hyperparathyroidism in 20 patients with skeletal fluorosis. In 5 adult male patients, they showed clear evidence of secondary hyperparathyroidism with raised serum PTH (Teotia et al., 1978). Krishnamachari and Sivakumar (1976) observed a significant increase in circulating PTH levels in patients with skeletal fluorosis and endemic genu valgum.

Pettifor et al. (1989) investigated 9 children from KwaNdebele. They were admitted to the Mineral Metabolism Research Unit for progressive limb deformities or skeletal pain. On admission to the unit, 6 of the 9 children were hypocalcemic with levels as low as 1.55 mmol/l. Hypophosphatemia was not present in any of the children but, rather, hyperphosphatemia was found in 4 of the children. Alkaline phosphatase was elevated in all but one child. The histological picture was one of severe hyperosteoidosis associated with secondary hyperparathyroidism and a mineralisation defect (Pettifor et al., 1989). Pettifor et al. conclude that the degree of hypocalcemia (which could have been due to low dietary calcium intakes, estimated at approximately 200 mg/day) plays an important role in determining the severity of osteomalacia present in endemic skeletal fluorosis and influences the renal response to hyperparathyroidism, which was reflected in the variable serum phosphate found in this study (Pettifor et al., 1989).

2.8 Renal Clearance of Fluoride in Children

Collins and Segreto (1984) investigated fluoride levels of children residing in areas with naturally occurring fluoride in the drinking water. The median water fluoride levels in the different areas ranged from 0.2 ppm to 3.4 ppm. They found a direct and proportional relationship between a community's water fluoride level and average fluoride excretion. Urinary fluoride levels of children and adults

were investigated after assessing water fluoride levels (Gedalia, 1958). His report stated that the younger the child, the lower the urinary fluoride level and the greater the storage, thus indicating that children store fluoride more than adults (Gedalia, 1958).

Spak et al. (1985) examined renal clearance of fluoride in children. Due to ethical limitations this study could not be carried out in healthy children, however it was carried out on children who were suspected of renal disease but whose glomerular filtration rates (GFR) were within the range for healthy children. These children had normal findings on IV urograms, and all had kidneys with normal concentrating capacities and were used as the control group. The study group of children was divided into three groups according to GFR rate: normal (92 - 136 ml/min/1.73 m^2 body surface area {BSA}), low < 92 ml/min/1.73 m^2 BSA, and supernormal >136 ml/min/1.73 m^2 BSA. The mean renal fluoride clearance was 45.0 ml/min ± 9.8 (SD) in children with normal GFRs and 31,4 ml/min ± 8.8 (SD) in the group with low GFRs. There was a close linear relationship between renal fluoride clearance and GFR rate. The authors suggest that children have a lower renal fluoride clearance rate than adults and that a moderate impairment of renal function could lead to increased retention of fluoride (Spak et al. 1985).

2.9 Summary

Endemic skeletal fluorosis does occur in children as evidenced by clinical, radiological and histopathological indicators. Bone and serum mineral homeostasis is altered and is associated with secondary hyperparathyroidism. In the above reviewed studies investigating children with endemic skeletal fluorosis, the following areas are not well defined and further investigation in these areas would be helpful:

1) *Growth patterns of children in endemic areas*

Apart from Pettifor et al. (1989), the pattern of growth of children in endemic areas is not detailed. It would be interesting to establish whether children from endemic fluorosis areas follow normal growth curves and if puberty has any influence on skeletal fluorosis.

2) *Base line dietary analysis, specifically calcium intakes*

The endemic fluorosis literature does not give adequate dietary analysis of the patients living in the area. Given the increasing evidence that calcium intake plays an important role in the outcome of skeletal fluorosis (Krishnamachari, 1986, Pettifor et al. 1989), detailed information on daily calcium intakes would be an important contribution to future studies.

3) *Control populations*

The literature investigating children did not have age-matched controls in low fluoride areas. This would be an important component of research in endemic areas - controlling for local trends and highlighting the effect of fluoride in a growing population.

4) *Fluoride dose*

It is important that investigators try to establish and report the mean fluoride dose in a research population. Many studies do not even state clearly the fluoride concentration in the water source. Although it is difficult to ascertain accurate fluoride doses in endemic areas, it is of paramount importance as fluoride intake is the most critical factor in the development of skeletal fluorosis.

Chapter III

3.0 Methods: Statement of the Problem

Investigation of abnormal bone and mineral homeostasis with specific reference to calcium deficiencies has been a major research thrust of the Paediatric Mineral Metabolism Research Unit at Baragwanath Hospital. Over the past years, a number of children presenting with marked lower limb deformities and rickets have been cared for and investigated by the unit. On closer demographic investigation a number of these patients came from the same geographical area of KwaNdebele. In an attempt to better understand the causes of this geographical clustering of patients, a survey of the local school children was conducted in 1984. Results of this study revealed that 23% of the sampled population were hypocalcemic. It was also noted that the majority of children investigated showed marked dental mottling, and water samples taken from the local water source, borehole water, revealed a fluoride level of 8.4 ppm (Pettifor et al., 1989). It was unclear whether fluorosis was responsible for the observed hypocalcemia, as no control population was included. Furthermore, dietary calcium intakes were not obtained, nor were measurements of appendicular bone mass made.

The hypothesis tested in the current thesis is that prolonged high fluoride intakes result in alterations in bone mineral concentration, increase in bone deformities and alteration in appendicular bone mass.

3.1 Research Aim

The aim of this study was to determine the effects of high water fluoride intake on children living in an endemic area, the study population, and to compare these children with a control group living in a control area with lower water fluoride concentrations.

This was achieved by examining:

1) anthropometric measurements;
2) dietary intakes of calcium and protein;
3) biochemical measurements of serum and urine calcium, phosphorus, magnesium, albumin, creatinine and serum alkaline phosphatase;
4) differences in appendicular bone mass measurements of
    - bone width,
    - bone mineral content,
    - bone density in both groups.

3.2 Historical Information

The KwaNdebele area is located in the Eastern Province (Mpumalanga), 90 kilometres north-east of Pretoria (See Fig 3.1). At the time this research was undertaken KwaNdebele was a self-governing homeland. The area achieved self-governing territory status in 1981 and aspired to independence in 1986; however this was never achieved.

The Southern Ndebele-speaking people are the dominant population group although over the years there has been a decline in the Ndebele portion, from 74% in 1979 to 69% in 1984. The remainder include Swazi (10%), Zulu (7%) and Pedi (6%) (McCaul, 1987).

There is a high degree of peri-urban (concentrations of people significantly dependent on commuting to a town for employment and services) and semi-urban (more than 5 000 residents) settlement in KwaNdebele, with very few of the working population (8%) employed within its borders. At the time this study was conducted the population was reported to be 450 000 residents with at least 5 000 new residents per month (McCaul, 1987). The average annual income

per household (1.36 persons earning) in KwaNdebele in 1984 was R3 578.90, (approximately $1 200 at the time) which is high in comparison with other homelands. There are approximately 6 people per dwelling (of which 53% are four-roomed or smaller) (McCaul, 1987).

Shopping facilities in the homeland consisted of 127 trading stores, 104 cafes and fresh produce dealers, 50 butchers, 24 bottle stores, 20 licensed restaurants, 20 unlicensed restaurants and 1 hotel in an area of 92 000 ha (McCaul, 1987). None of this land fell within the 750 mm rainfall curve, thus effective agriculture is a problem.

The question of "independence" was a source of much political instability and violence in the region until the recent democratisation of South Africa. Poor living conditions, lack of employment near to home, high costs of commuting and many other economic and health factors made the KwaNdebele residents unhappy and disgruntled with both the South African and KwaNdebele governments. The unrest had direct implications on this research. Prolonged delays resulted from the violence, and changes in study design were necessary beause of concerns for the safety of the research staff.

3.3 Community Beliefs About Fluorosis

The occurrence of dental mottling in children, or "rusted teeth" as the condition is referred to locally, has been of concern to the residents for many years. Those individuals suffering with "rusted teeth" freely admitted to the use of battery acid, Jik (bleach) and other corrosive agents to try to clean their teeth. The fluorosis problems were unknown before resettlement to the area of Klipplatdrift (see Fig 3.2) approximately 30 years previously. In discussions with the community leaders about the fluorosis in the area, certain beliefs were expressed. Beliefs varied as to the cause of this condition. Some believed the

soil was "bad", others that the air was contaminated and that the locally grown food was "bad". Residents had noticed that only those individuals under 25-30 years of age were affected by dental mottling and had concluded that some vector in this specific area was to blame. The idea that water had high levels of fluoride was not understood or well accepted (it was stated that the water was clear and clean), although they were willing to agree that the problem be investigated.

3.4 Research Approval

This study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand: Protocol # 41/4/87.

3.5 Community Consent

Permission was obtained from the then Ministers of Health and Education of KwaNdebele to investigate the effect of high fluoride intake on school children in the area. All schools in the affected areas were open for investigation.

Community meetings were held with residents and headmen , to explain the purpose of the study and request their consent. Signed consent was obtained for all children participating in the research programme by distributing consent forms at these meetings to the parents of children attending schools in the area.

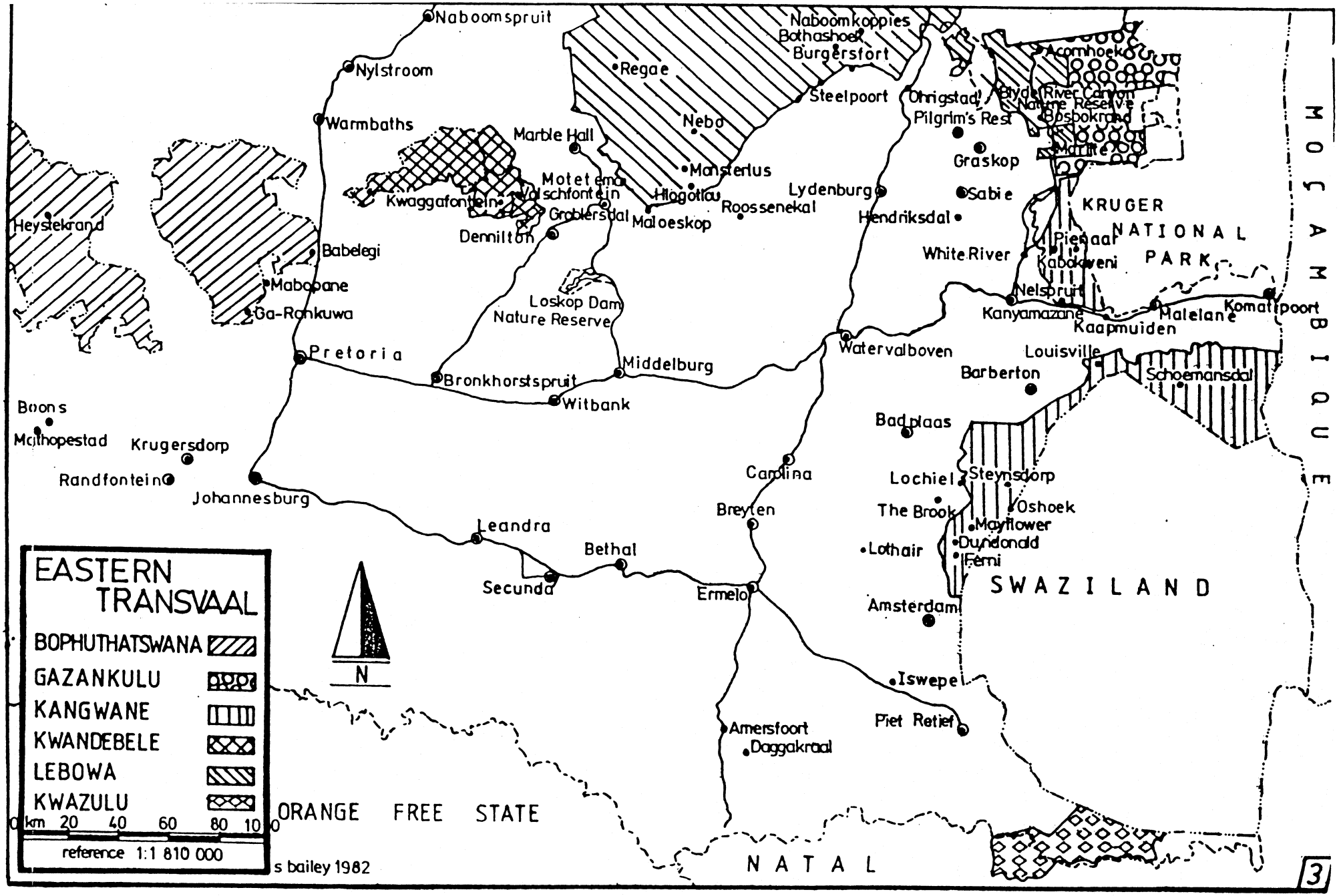


Fig 3.1 Geographical Location of KwaNdebele

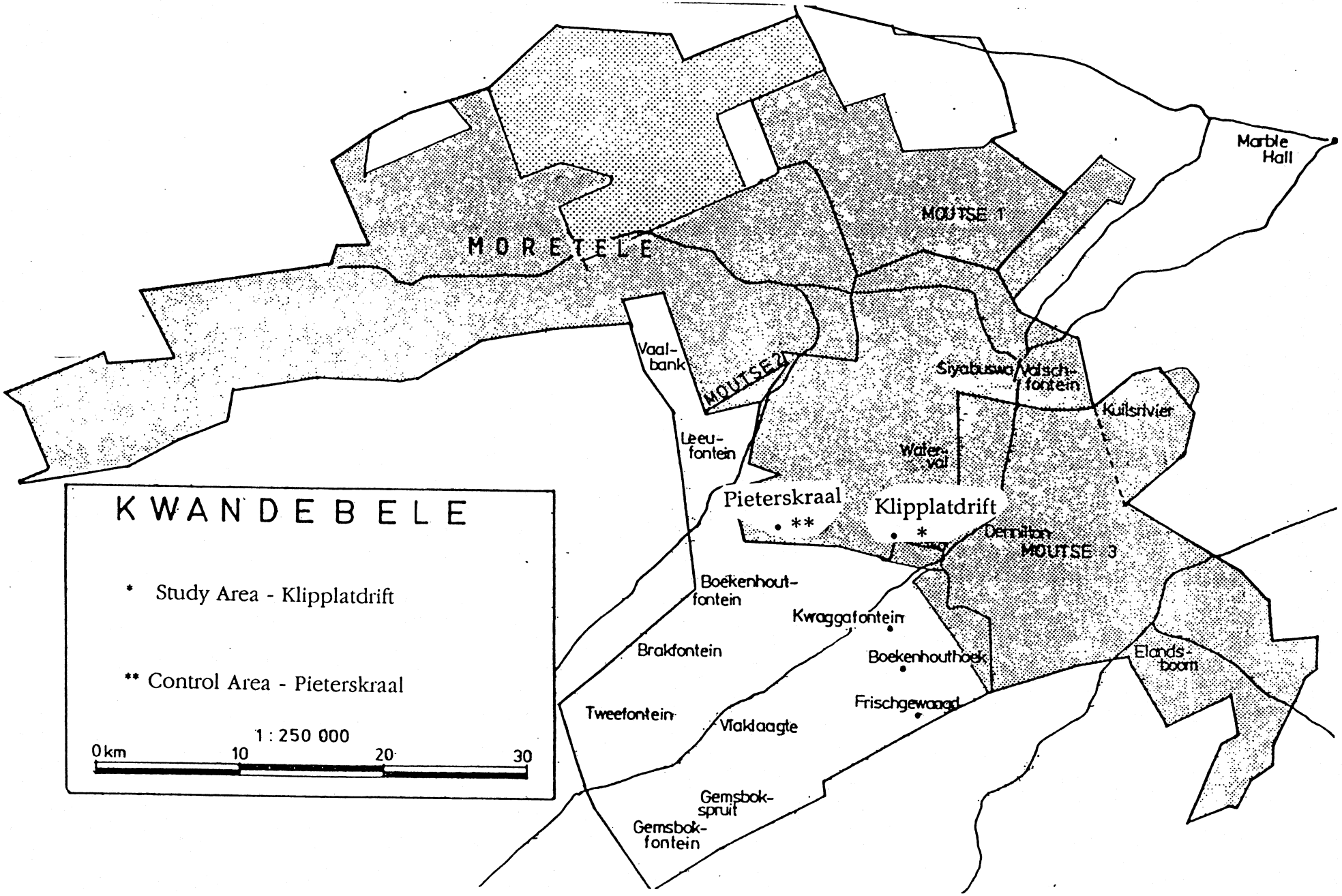


Fig 3.2 Geographical Location of Study and Control Areas

3.6 Water Sources

Accessibility to water has been one of the most serious problems for KwaNdebele residents. In 1984, there were only 900 taps in the entire homeland (McCaul, 1987). KwaNdebele is an arid and hot area with a mean annual rainfall of 676 mm and a mean maximum annual summer temperature of 27 °C. This is a summer rainfall area with arid winters. Surface water in the area is limited and boreholes are the major source of water. These underground supplies are frequently very high in fluoride content. Sampling in 1986 showed levels ranging from less than 1 ppm to 18 ppm in different areas. This water is pumped into reservoirs and then piped to the community. Taps are located at strategic points in the community, usually in the street. Residents collect water in buckets for home use. Borehole water was the major source of water for the homeland until the phasing in of a R43 million surface water scheme in 1986 for certain areas.

Water samples at specific sites were taken and fluoride levels tested by the National Institute for Water Research at the CSIR (Council for Scientific and Industrial Research). These data were used as baseline data for the purposes of this study. Communities were classified as either users of, 1) piped-in surface water or 2) borehole water, according to records at the CSIR. Communities using borehole water where levels of fluoride were higher than the recommended limit of 1.5 mg/l fluoride (Kempster and Smith, 1985) were evaluated for possible research sites.

After identification of high fluoride levels in ground waters, communities were selected for inclusion in the study on the following criteria:

- High ground water fluoride levels.
- An established community using this water source for 22 years or more.

- A community which did not have access to piped-in water as a supplementary source.
- A school-going population living in the community.

Table 3.1 Water Fluoride Levels in KwaNdebele

LOCATION	Fl LEVEL	Month/Year	Map Ref.
Tested			
Borehole Water			
Allemansdrift C •	18.8	7/1981	a*
Kameelriver •	10.4	7/1981	b*
Renosterkop •	12.2	1/1984	c*
- (source changed to piped water)	0.4	7/1985	
Klipplatdrift - reservoir •	9.4	7/1985	d*
- reservoir •	8.8	10/1986	
- Sothembani school pump •	5.0	10/1986	e*
- Pond near headman's hut	0.4	10/1986	
Bundu Inn Hotel •	9.5	10/1986	f*
- Youth Camp	3.5	10/1986	
Surface Water			
Waterval A ('86)	2.5	7/1981	
Waterval B	1.0	7/1981	
	1.6	1/1982	
Enkeldoring	0.2	10/1986	
Leeufontein	4.0	1/1982	
Matjiesgoedkuil	2.8	7/1981	
	2.4	7/1985	
	2.5	10/1986	
Nuwe Pieterskraal	1.6	7/1981	
Ou Valschfontein	3.4	7/1981	
Vlaklaagte	0.1	7/1981	
Wolvenkraal	3.8	7/1981	
	4.5	1/1984	

• indicates a borehole
* indicated on map (Fig 3.3) as borehole high in fluoride

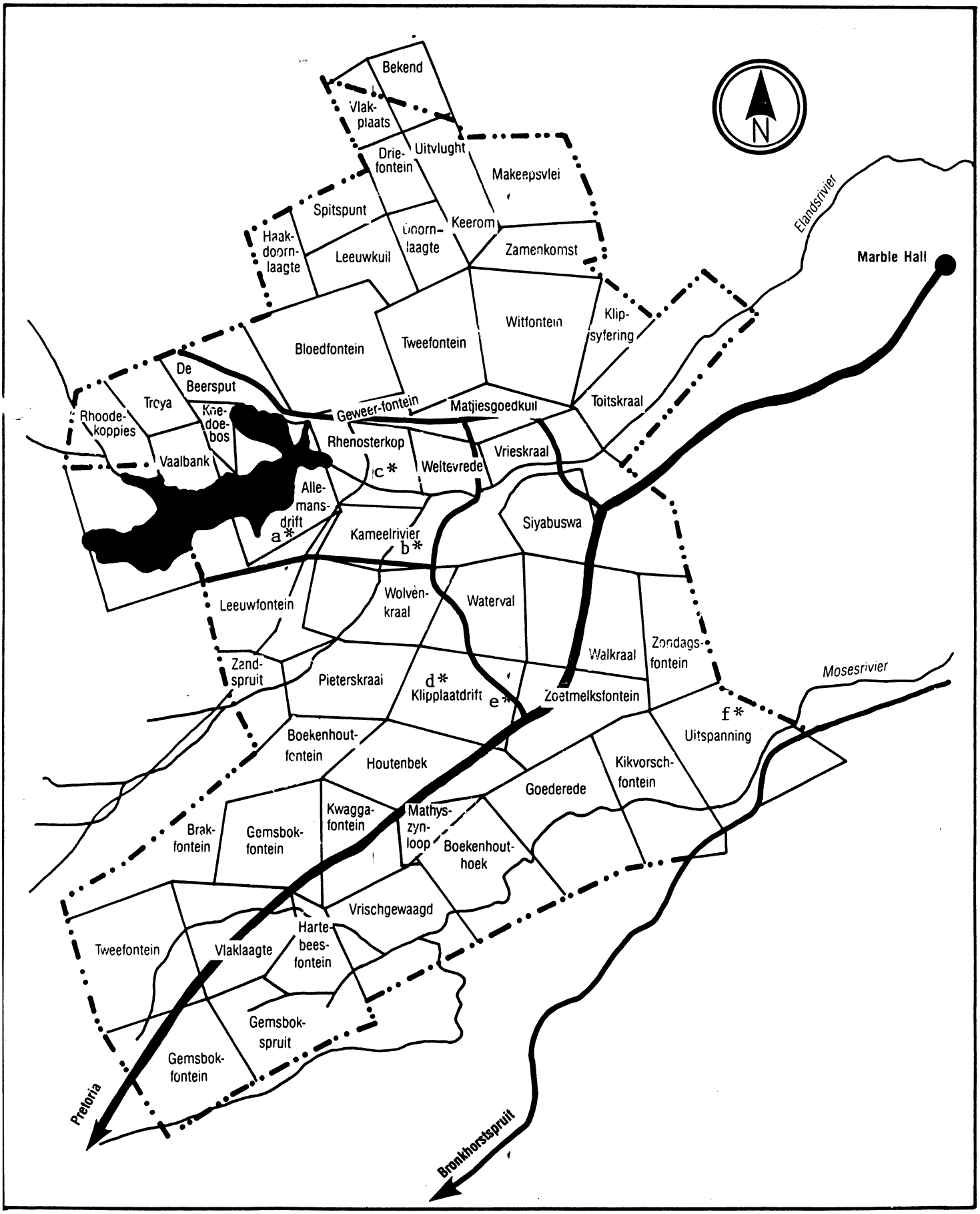


Fig. 3.3 Local Communities in "KwaNdebele" using Borehole Water High in Fluoride

3.7 Research Area

3.7.1 Study Population

The study population chosen was situated in Klipplatdrift, a community that was established in 1960. The community consists of 430 households and approximately 6 000 people. Before the declaration of the KwaNdebele homeland, a small number of farm workers lived in this area on the farm Klipplatdrift. It is therefore one of the older communities in KwaNdebele and showed evidence of more established homes, gardens, and a few mature trees. In June 1984 a large vegetable gardening project was established in Klipplatdrift. The research area, Klipplatdrift, had used borehole water for the last 25 years. These borehole water fluoride levels ranged from 8.8 ppm in 1984 to 9.4 ppm in 1986, however there may be even greater seasonal variations in fluoride concentrations. To the best of our knowledge, the only time there had been any deviation in the use of this high fluoride borehole water, was in the 1981-1982 drought when the borehole water dried up and water was trucked in from local farms by "water merchants". The fluoride content of the farm water was not established. The community used the trucked-in water as a supplementary source for a period of approximately 7-9 months.

This population had prolonged exposure to high fluoride levels. The sample to be investigated were young males and females aged from 6 to 22 years attending the local schools, who had lived in the area for the majority of their lives and had used borehole water with high fluoride levels as their primary water source.

3.7.2 Control Population

An area with lower fluoride levels in the drinking water was used as a comparative group. Pieterskraal, the adjacent zoned area, 15 kilometres from the study area was selected as the control population. Fluoride levels over the

past years had ranged from 1.6 to 2.0 ppm. Drinking water was piped from a surface water source into the local reservoir for domestic use. Demographic and geographic qualities were similar to those of the study area. The total population of the community was estimated at 3 600 by the Bureau of Market Research, University of South Africa (communication with KwaNdebele government). The control group consisted of an age-matched sample with the study group selected from this area.

3.7.3 Exclusion Criteria

All students with signed consent forms were included in the study and control groups except:

1. Those individuals who had not lived in the specific area for the majority of their lives (a minimum of 6 years for primary school children and 12 years for high school children), and/or did not use the local water source.
2. Those who were younger than 6 years of age or older than 22 years.

3.8 Sample Selection

3.8.1 Study Group - Klipplatdrift

In the initial stages of the study design, it was proposed that investigation of the groups be by home visits, but due to changes in the political climate and outbreaks of violence, the research was conducted through the local schools. Three of the four schools in the study area were used for sample selection. The total number of enrolled scholars at the time of the study was as follows:

Makozana Secondary School	686 pupils
Sothembani Senior Primary	664 pupils
Nkonsiphile Junior Primary	601 pupils
Total	1 951 pupils in study area

A meeting of students and parents was called at the Sothembani School. All families who had lived in the area before or near the birth of their children were invited to take part in the study. All students who were eligible, after the exclusion criteria were applied, were given consent forms. Those with signed consent forms were assigned to the relevant age category. Students' ages were determined from the birth dates on class registers. A total of 1 571 (80.5%) of the school population returned signed consent forms.

Sample size was determined by the following consideration: The study and control groups would be regarded as clinically different within age and sex subgroups if their means lay one standard deviation or more from each other. The sample size to detect such differences when the levels of significance set at $\alpha = 0.05$ and the power was determined to be 90%, was 23 subjects per sex and age subgroup. In view of the lack of information on normal values for bone parameters, it was decided to increase the sample size for this study to approximately 40 per subgroup.

Consent forms were grouped according to the two-year age categories. Scholars were chosen randomly from each age group. The age category was considered full when 40 students or as close to that as possible were included. In the younger ages (6-7 years) and the older ages (males of the 18-19 and 20-22 year groups and females in the 20-22 year old group) it was difficult to find students to meet the category quota. In these cases all students with signed consent were included in the study.

The total number of students in the sample of the study population was 614. This represents a research sample of 31.5% of the school-going population in the three schools living in the high fluoride area.

3.8.2 Control Group - Pieterskraal

The same methods of gaining community/parental consent and scholar selection and the same exclusion criteria that were used in the study area were applied in the control community. All three schools in the study area were used for sample selection. The total number of enrolled scholars at the time of the study was as follows:

Madlayedwa Secondary School	596 pupils
Vusa Combined Primary	678 pupils
Buthlelani Primary	334 pupils
Total	1 608 pupils in control area

The meeting for obtaining consent from students and parents was called at the Vusa Combined Primary School. A total of 1 394 (86.7 %) of the school population returned signed consent forms.

In the younger ages (6-7 and 10-11 years) and the older ages (20-22 years) it was difficult to find students to meet the category quota. In these cases all students with signed consent were included in the study. The total of number of students in the control sample was 613 subjects, representing 38.1% of the school-going population in the control area.

3.8.3 Dental Cohort

A sample of the research population was investigated by researchers from the Community Dentistry Programme at the University of the Witwatersrand. They investigated the dental effects of naturally occurring high fluoride (study group)

and lower fluoride (control group) on school children in a subgroup 6 - 18 years of age. Their findings are reported separately (Lewis et al., 1992).

3.9 Anthropometric Methods

3.9.1 Anthropometric Recorders and Forms

All anthropometric measurements were made by trained staff from the Mineral Metabolism Research Unit at Baragwanath Hospital. The staff person measuring a specific index, e.g. height, continued to do so throughout the study to minimise inter-recorder errors due to different measurement techniques.

A simple data form was designed to record all relevant information. This included subject's name, school, age, date and specific anthropometric measurements (Addendum A).

3.9.2 Anthropometric Measurements

Anthropometric measurements were made on all subjects in the sample. Data collection on the study group was completed in August 1987 and the control group in October 1987.

3.9.2.1 Height

Measurements of height are assessments of linear skeletal growth.

An anthropometer, consisting of a vertical measuring rod attached to a standing platform with a movable headboard which was lowered to the head, was used to make these measurements (Jelliffe, 1966). The anthropometer was placed on a flat surface, against the classroom wall. Subjects were measured barefoot with heels touching the back of the foot board. Feet were parallel, with heels, buttocks, shoulders and back touching the upright measuring rod. The head was held erect with eyes looking straight ahead. The headpiece was lowered against

the head, compressing the hair. The subject stepped off the platform and the reading was taken and recorded.

3.9.2.2 Weight

Measurements of weight are the most sensitive of the anthropometric measurements to changes in nutritional status. Isolated weight measurements can be used in conjunction with other anthropometric data to give an accurate nutritional profile.

Spring scales which were periodically checked for accuracy were used for these measurements. Weighing was done to the nearest 0.5 kg. Subjects were weighed wearing their summer school uniforms and no shoes. Girls wore a blouse and skirt and boys wore shorts and a shirt. Readings were taken while the subjects stood still and were free standing.

3.9.2.3 Body Mass Index

Body mass index (BMI) was calculated for subjects in the study and control group. Body mass index is used as a measure of body fat. Cronke and Roche (1982) examined data from subjects in the NHANES-I study (United States Health and Nutrition Examination Survey) and found that this measure was the best indicator of body fat for females of all ages as well as for adult males over the age of 19 years. Killeen et al. (1978) applied four indices to the data of young men (6-17 years) in the NHANES-II and III. They found that the BMI correlated best with skinfold thickness for all ages, sexes and ethnic groups.

BMI is calculated from the formula :

$$BMI = \frac{wt\,(kg)}{ht^2\,(m^2)} \times 100$$

3.9.3 Lower Leg Measurements

When the legs were measured for evidence of genu varum (bow-legs) and genu valgum (knock-knees), the subjects assumed a comfortable standing position, namely feet parallel, ankles touching where possible and knees pressed back comfortably. Subjects were measured without shoes.

3.9.3.1 Intermalleolar Distance

The distance between the medial malleoli of the tibia was measured with adjustable callipers. The callipers were placed next to a ruler and measured in centimetres to the nearest 1 mm. Distances greater than 5 cm were considered to indicate genu valgum (knock-knees) (Richardson, 1974).

3.9.3.2 Interpatellar Distance

The distance between the medial femoral condyles, which for practical purposes is approximately level with the mid-patella when the quadricep femoris muscle is relaxed, was measured with adjustable callipers. The callipers were placed next to a ruler and measured in centimetres to the nearest 1 mm. Distances greater than 5 cm indicated genu varum (bow-legs). This would indicate greater than normal population variation, which includes from straight legs to measurements up to 3.5 cm (Richardson, 1974).

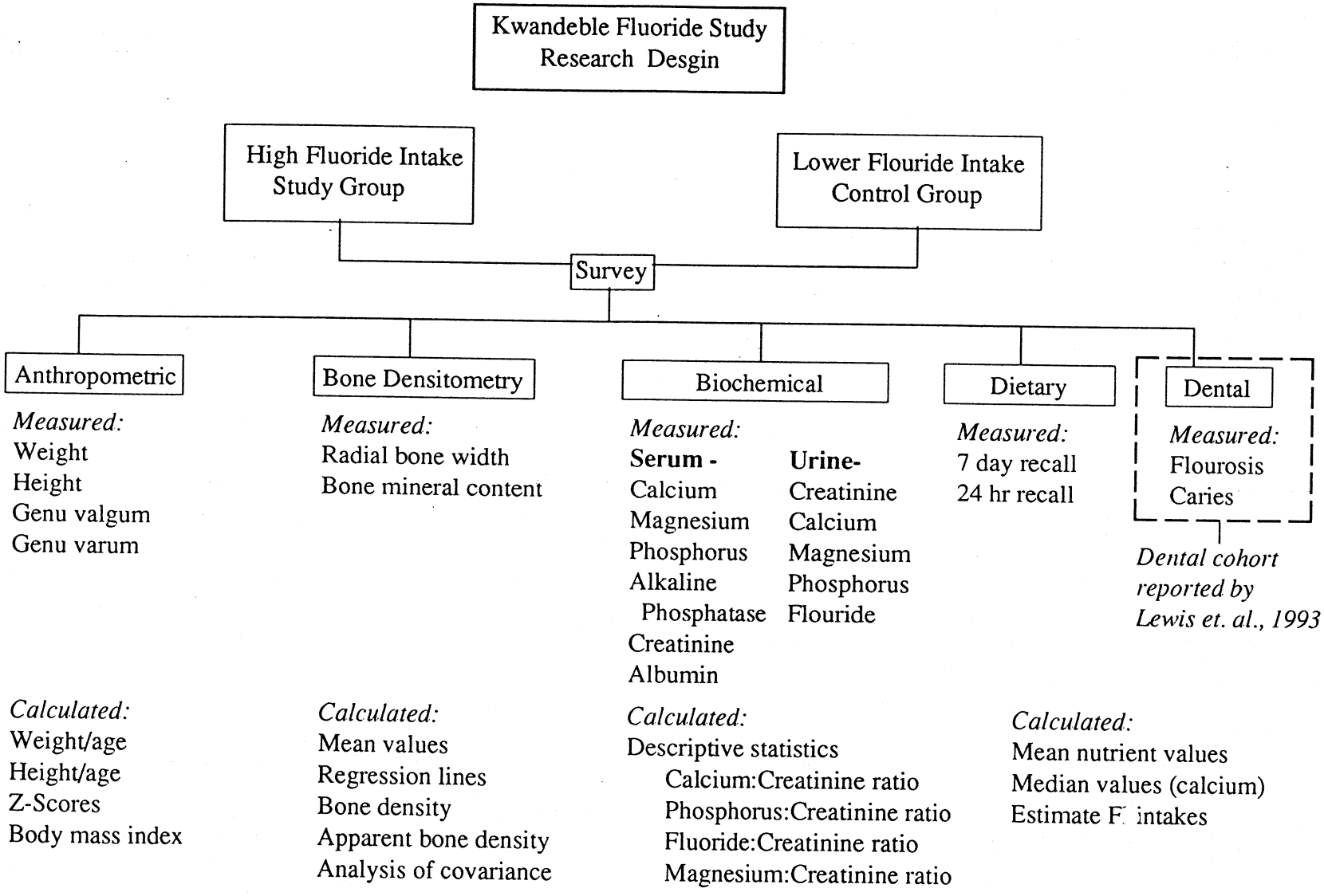


Fig 3.4 Research Design : Schematic Representation

3.10 Biochemical Methods

3.10.1 Blood Sample Collection

The research team included medical doctors who were responsible for the drawing of blood. Blood samples on all subjects were carried out where possible. Blood samples were collected from the antecubital vein in plain Vacutainers®, allowed to settle, spun down and separated within 2 hours. Serum samples were transported in cooler boxes packed with dry ice and frozen at the Mineral Metabolism Research Unit at -20 °C until assayed.

3.10.2 Biochemical Techniques - Serum

All biochemical testing was carried out under research laboratory conditions by the medical technician at the Mineral Metabolism Research Unit at Baragwanath Hospital. These methods of analysis were periodically checked on the Bayers Miles Quality Control Programme: Protocol No T03-1221 and T03-1220.

3.10.2.1 Calcium and Magnesium

Serum and urine calcium and magnesium levels were analysed using the atomic absorption spectrophotometry. The Spectra AA10 - Varian machine was used and the units of measurement were millimoles/litre (mmol/l). Reference values obtained from children of similar age groups and tested in the same laboratory were: serum calcium 2.43 ± 0.15 mmol/l (coefficient of variation 1.473 %) and magnesium at 0.85 ± 0.012 mmol/l (coefficient of variation 3.397 %) (Pettifor et al, 1978).

3.10.2.2 Inorganic Phosphorus

The inorganic phosphorus method is based on the formation of phosphomolybdic acid which is then reduced by stannous chloride-hydrazine (Hurst, 1967). This procedure was carried out on the Technician AutoAnalyzer II. Units of measurement were mmol/l. The reference serum value for females 6 to ≤ 13 years is 1.44 mmol/l (range 0.96 - 2.16 mmol/l) and for males 6 to ≤ 16 is 1.49 mmol/l (range 0.90 - 2.18 mmol/l) (coefficient of variation 1.1%) (Pettifor et al., 1978). Prepubertal values less than 1.3 mmol/l are considered abnormal.

3.10.2.3 Alkaline Phosphatase

The method for analysing alkaline phosphatase was that of Morgenstern et al. (1965) on the Technicon AutoAnalyzer . The procedure is based on the enzymatic hydrolysis of p-nitrophenyl phosphate during incubation at 37.5 °C. After incubation the free p-nitrophenyl is dialysed into a 2-amino-2methyl-1propanol buffer analytical stream. The absorbency of the analytical stream is measured at 410 nm in a 15 mm flowcell. Units of measurement were International Units/litre (IU/l). Children 6 - 16 years of age had a concentration range from 51-425 IU/l (coefficient of variation 2.88%) (Pettifor et al., 1978). Whitby et al. give a reference range in childhood of 125-400 IU/l (Whitby et al., 1993) while Pettifor et al. interpreted concentrations of 300 IU/l or greater as elevated (Pettifor et al., 1978).

3.10.2.4 Creatinine

The creatinine assay was based on the reaction between alkaline picrate and creatinine, the Jaffe reaction. The procedure was carried out on the Technicon AutoAnalyser II by the automated method (Chasson et al., 1961). The sample stream is segmented with air and diluted with 1.8% NaCl. It is then dialysed into segmented distilled water. After being dialysed the diluted creatinine is mixed with 0.5 NaOH. Picric acid is added to the stream and the three components are

mixed. The absorbency of the analytical stream is measured at 505 nm in a 15 µm flowcell. Units of measurement were micromoles/litre (µmol/l). Reference values for children range from 30 to 80 µmol/l (coefficient of variation 0.7%) (Whitby et al., 1993).

3.10.2.5 Albumin

Serum albumin levels were assayed by the BCG Method - (Bromocresol Green), described by Doumas et al. (1971). Units of measurement were grams/litre (g/l), and the reference range was 36 - 47 g/l (coefficient of variation 1.2%) (Whitby et al., 1993).

3.10.3 Urine Sample Collection

Single random urine samples were collected on all subjects. Scholars were given sterile plastic bottles with screw-tops and asked to void into them. Urine specimens were labelled, handed in and ticked off on the research sheet before anthropometric data were collected. The urine specimens were kept at room temperature during collection at the schools and repacked and refrigerated for transport to and storage at the Mineral Metabolism Research Unit.

3.10.3.1 Biochemical Techniques - Urine

Urine calcium, creatinine, phosphate and magnesium levels were measured in the same manner as the serum samples for the respective chemicals and reported a ratio of the respective chemical to creatinine concentration. Units of measurement were millimoles/millimoles (mmol/mmol).

3.10.3.2 Fluoride

Urinary fluoride samples were analysed using the Fluoride Selectrode, type F1052F Radiometer. This is a solid-state membrane electrode which uses a single crystal of lanthanum fluoride. The electrode has a high degree of selectivity for

fluoride ions against a background of other ions, and is primarily intended for fluoride determinations. Units of measurement were milligram/millimole (mg/mmol).

3.11 Bone Densitometry

The Norland single energy (^{125}I) photon absorptiometer (Model 278A) was used in this study. The distal 1/3 of the left radius was measured for bone mineral content, bone width and bone density.

Bone mineral content is determined by measuring the transmission of a single photon beam from ^{125}I through bone and soft tissue (Cameron and Sorensen, 1963). The beam moves transversely across the forearm and the intensity of the beam as it crosses the forearm is plotted. The transmitted counts are transformed logarithmically, and beam attenuation through bone is compared with beam attenuation in surrounding soft tissue. The integral area under the absorption curve is proportional to the bone mineral mass in the cross-sectional area of the scanned bone. The mineral mass (g) is expressed for a piece of bone 1 cm in axial length to give the bone mineral content in grams per centimetre. The bone mineral content is assumed to be directly proportional to the amount of photon energy absorbed by the bone being studied. Bone width (BW) is measured in centimetres (cm). Bone mineral content (BMC) normalised for bone width (BW) is expressed as bone mineral density (BMC/BW=BD) measured in grams per square centimetre (Wahner and Rigs, 1986).

Bone density measures bone mass/cm^2 and does not take into account the volumetric changes of bone. Apparent bone density is a mathematical

correction for the changes in volume of bone and has units of measurement in g/cm^3. Apparent Bone Density (ABD) is calculated from the equation:

$$ABD = BMC / (22/7 \times (BW/2 \times BW/2))$$

as it is assumed that the radius is cylindrical at the point of measurement.

3.11.1 Bone Densitometry Measurements

The point of measurement of the radial bone mass was determined by measuring the distances between the proximal end of the olecronon and the distal tip of the ulna styloid process, dividing the measured distance by 3 and then positioning the plate so that the beam passed through the junction of the middle and distal thirds. Subjects stood or sat parallel to the bone densitometer and placed their left arm on the steel plate. After correct positioning of the left arm (by either raising or lowering the subject's chair), the arm was secured with weighted straps (straps provided on the densitometer). Subjects were cautioned not to move during the bone densitometry readings. Bone mineral content (BMC) and bone width (BW) values were read and bone density (BD) calculated. Three readings per child were taken and the mean values entered onto the data form.

3.12 Dietary Methods

3.12.1 Development of the Dietary Questionnaire

3.12.1.1 The 24-Hour Recall Tool

The 24-hour recall tool was the method of choice and was selected for the following reasons:

Group mean dietary intakes were being investigated, not individual intakes, and this is a simple, practical and quick method to use. The 24-hour recall method of interviewing could easily be conducted at the school without needing to observe the home eating environment. School attendance in the area was sporadic and

the diet history could be completed while the student was with the interviewer. Finally, this method does not require written records by the students.

The literature is in agreement that where groups are concerned, the 24-hour recall can provide valid information although it is not as accurate for individual analysis (Young et al., 1952, Beaton et al., 1979, Rider et al., 1984). Young et al. (1952) recommend that at least 50 persons need to be included in the dietary sample to tolerate a 10% error.

3.12.1.2 The 24-Hour Recall Method

The 24-hour recall method has been used in many nutritional surveys. The specific tool developed by Steyn (1986) was suitable for our use for the following reasons:

1). It included food options available in the community.

2). It had appropriate quantities used by the local residents included in food descriptions.

3). It had six intervals of the day in which to record dietary intakes, namely breakfast, morning snack, midday meal, afternoon snack, dinner, and after dinner snack.

4). Foods were clearly described with regard to the local cooking methods.

5). Food portions and their weights were included making computer coding and calculations easier to carry out.

3.12.2 Local Food Survey

Preliminary visits to the local shops and vendors were carried out to identify common brands of foods available and patterns of consumption in the study and control areas. The information collected from these visits identified the foods not already included in the detailed 24-hour recall form and the types of food models needed.

Three areas where foods were available locally were observed: the community shops, shops near the schools and the school vendors.

1. Local food surveys

Surveys were conducted in the most frequently used grocery shops in the study and control areas. Brands of foods available were noted and in some cases the most common size of a brand sold was also recorded. There were three shops in the study area. The two most frequented were visited and observed. The third shop was on the far side of the community with little customer traffic and limited goods available. Although this shop was visited, stock and buying patterns were not observed. There were two shops in the control area and both shops were visited and observed.

2. School lunches: shops and vendors

There was a shop located close to the high school in the study area where some scholars bought their lunches. Observation of food items bought only during the lunch break was carried out in this shop. Food items chosen, quantity sizes, and brands were noted. These foods were included in the 24-hour recall tool.

3. Vendors

Mothers who set up "kitchens" outside the school gates were also well supported, especially in the primary schools as they were further away from the local shops. The variety of foods sold, the methods of preparing foods, common quantities dished up and the utensils used were observed and noted. All foods available here, especially ingredients in "stews" and "soups", were included in the 24-hour recall tool.

3.12.3 Pilot of the 24-Hour Recall Questionnaire

24-Hour Recall Tool : foods verification. (See Addendum B)

Sample interviews were conducted to identify foods used locally and to verify that they were included on the questionnaire. In both the study and control areas twelve students, 6 male and 6 female, 14 years and older, were randomly selected for interviewing. Typical 24-hour dietary recalls were carried out on these students, however no food models were used. Interviews were conducted in Ndebele and were carried out by the research team nurse and nutritionist. The questionnaire was amended accordingly.

Following the verification of the types of foods eaten in the first survey, a pilot 24-hour recall tool based on the design of Steyn (1986) was condensed to include only foods common to the research area. It was tested on 10 randomly selected students in the study and control areas (n=20) to assess if the tool was comprehensive for categories of local foods available and reflective of commonly used quantities and portions.

This trial revealed the need to:

1) expand the food categories in specific areas and,
2) develop an Ndebele questionnaire standardising the interviewer techniques so as to minimise inter-interviewer differences.

A second trial was undertaken to establish the correct use of the 24-hour recall tool and its appropriateness, and to compare interviewer reproducibility. A revised 24-hour recall tool was retested in 12 interviews in each area (n=24). The same 24-hour period was recorded by both interviewers and results were compared for food categories and quantities recorded. Students were interviewed using the standardised Ndebele questionnaire.

The results of this second trial revealed that inter-interviewer results for food categories and quantities were comparable using the revised tool over the same

24-hour period. When total energy intakes reported for the scholars (n=12 matched pairs) were correlated using the Spearman ranked correlation technique, there was a high correlation between the two interviewers (r=0.98 ; p=0.000 1).

A third pilot study was performed to compare weekend and weekday food intakes as significant differences have been reported in an individual's diet for different days of the week, with absolute intakes for many nutrients being higher on weekend days than on weekdays (McGee et al., 1982). In the USA, data in the Nation-wide Food Consumption Survey (NFCS) were analysed for dietary patterns between weekends and weekdays. There were significant differences between weekends and weekdays in the number of meals and snacks eaten, the distribution of energy intake throughout the day and the nutrient intake (Thompson et al., 1986).

Thus the pilot study was undertaken to check the reliability of the 24-hour recall to adequately reflect a typical daily diet of high school students in the two areas. High school students in the study (n=20) and control area (n=20) were asked to keep a 7-day food record . They were given a folder with pre-printed diary sheets and a pen which they could keep. Careful instructions were given on how to fill in the food diary.

Twenty-four-hour recalls were conducted on these students on the second day of the 7-day food recording period and were to be correlated with the first day of the food diaries. These correlations would hopefully reveal the monotony of the daily diets in the area. It could be argued that the strength of a 24-hour recall method would be greater in a group with a monotonous diet as the recall method would be more reflective of the population's eating habits and norms due to smaller variations in the daily diet.

Unfortunately the 7-day food diaries received a poor response. Only 17 of the 20 students in the study area and 14 of the 20 in the control area completed the diary. The diaries were not kept as instructed and food intakes were impossible to quantify. It was therefore not possible to correlate the 24-hour recall to the diary. In an attempt to salvage some information from the diaries and examine variations in the diet, food frequency lists were compiled from the 7-day food diaries instead (see Dietary Results chapter for these results).

3.12.4 Pilot for Sample Selection

Steyn (1986) in her study found that children under the ages of 11 years were not reliable when it came to accurate food recall information. To establish the truth of this finding in the current study a small sample of children younger than 11 years of age was interviewed using the 24-hour recall form (n=12 matched pairs). The same interviewer interviewed the mother or childminder for the identical 24- hour period and the two records were correlated. The Spearman ranked correlation was used ($r=0.266$; $p=0.404$) indicating that there was a low correlation between the reported diet from the mother and child. On the basis of this result and Steyn's findings children 12 years and older were used in the dietary survey.

3.12.5 Interview Language

All interviews during the pilot and research surveys were conducted in the local vernacular, Ndebele or Sotho.

3.12.6 The Dietary Survey

The dietary survey was conducted 10 months after the last of the clinical data were collected. This delay was due to political unrest (violence in the community and burning of schools) in the research area. The unrest in the community and

subsequent delay of research made tracing of scholars from the clinical phase of the study difficult. Thus the sample sizes in the study and control groups are smaller than originally planned.

- Students (study n= 63, control n=62) over 12 years of age were randomly selected by study number, sex and age group from the control and study groups. If a student was not present at the time of interviewing, a replacement was identified from the appropriate age and sex category by use of random tables.
- Interviewing stations were set up in the high schools of the study and control areas. Food models and commonly used utensils were on display.
- The interviewer followed the interview as set out on the standardised questionnaire regarding dietary practices in the preceding 24-hour period.
- Students were encouraged to indicate, by using the models and utensils, the particular foods reported and utensils used during the preceding 24-hour period.
- All details were recorded on the 24-hour recall tool.
- Student information and food intake were coded, and the data were captured for computer analysis. Nutritional calculations were based on the NRIND (1986) tables (National Research Institute for Nutritional Diseases) and computed with the assistance of the MRC (Medical Research Council).

3.13 Statistical Methods

Statistical analysis was performed using the SAS and the SPSS statistical packages. Statistical tests used were the Student's t-test (for parametric data), Willcoxon test (when the sample was small and non-parametric), the Mann-Whitney and ANCOVA (analysis of covariance). LS means were used to run pairwise comparisons in the analysis of covariants. Bivariate relationships were tested using correlation (in some cases Spearman ranked correlation technique), regression analysis or Chi square tests. Growth was plotted using the NCHS

percentiles (National Centre for Health Statistics) for children aged 2 to 18 years and Z scores were calculated for each child.

Chapter IV

4.0 Results

4.1 Demographic Results

Sample size

The research sample was identified from schools in the relevant fluoride areas. A total of 614 scholars aged between 6 and 22 were selected in the study group (high fluoride area) and 613 scholars in the control group (lower fluoride area). The study and control groups were divided into 2 year age groups each consisting of approximately 40 scholars, except in the very young age group, 6-7 year olds, and the older ages, 20-22 year olds, where there were at least 20 in each group (Table 4.1). More females than males were willing to enter the study in the 20-22 (20, 21 and 22) year age group.

Table 4.1 Descriptive Data for the Survey Sample

Age Group	**Study Group**		**Control Group**	
	Males	Females	Males	Females
6-7	33	38	23	33
8-9	39	47	44	42
10-11	47	44	37	39
12-13	37	40	43	44
14-15	45	48	47	45
16-17	41	34	43	43
18-19	28	41	43	40
20-22	24	28	18	29
Sub-Group Total	**294**	**320**	**298**	**315**
Group Total	**614**		**613**	

4.2 Anthropometric Results

4.2.1 Height

4.2.1.1 Height-for-Age

Tables 4.2 and 4.3 present heights of males and females in the study and control groups respectively. National Centre for Health Statistics (NCHS) centile categories were used as the method of classification.

There were 214 scholars (≥ 18 years of age) who could not be classified by the NCHS centiles, as centiles are only available for children up to 18 years of age. These scholars were thus excluded from the analysis of anthropometric data.

To establish differences of males and females between the study and control groups, they were compared in each height-for-age centile category (≤ 5th; etc....) using contingency tables.

In both the study and control groups, the majority of the sample fell on or below the 50th centile. In the study group, 93% of males and females fell below the 50th centile compared with 94% of males and 90% of females in the control group (See Figs 4.3 and 4.4 - NCHS growth charts). The males of the study and control groups were more stunted than females, and the two groups were similar in their distribution across the centile groups (Chi-square 3.9; p=0.7). Females of the study and control groups were also similar in their distribution across the centile groups (Chi-square 5.5; p=0.5).

Comparing males and females within the study and control groups, 51% of males in the study group and 31% of the study females were below the 5th centile for height-for-age. In the control group, 46.3% of males were below the 5th centile for height-for-age, compared with 28% of females. Males and females within each

group showed a significant difference in their degree of stunting - study (p=0.0006) and control groups (p=0.00003) (Tables 4.2 and 4.3, Figs 4.3 and 4.4).

Table 4.2 Heights of Study Group Children According to NCHS Centiles (children < 18 years).

Percentile	**Females** (n)	**%**	**Males** (n)	**%**
>90 - ≤ 95th	2	0.8	1	0.4
>75 - ≤ 90th	3	1.2	3	1.6
>50 - ≤ 75th	13	5.1	11	4.5
>25 - ≤ 50th	68	26.7	31	13.2
>10 - ≤ 25th	56	22.0	44	15.6
> 5 - ≤ 10th	33	12.9	31	13.2
≤ 5th	80	31.4	120	51.0
Sex Total	**255**		**242**	
	Group Total		**497**	
	Chi-Square		(23.4 : DF 6: p=0.0006)	

Table 4.3 Heights of Control Group Children According to NCHS Centiles (children < 18 years).

Percentile	**Females** (n)	**%**	**Males** (n)	**%**
>90 - ≤ 95th	2	0.8	1	0.4
>75 - ≤ 90th	6	2.4	0	0
>50 - ≤ 75th	17	6.8	12	0.8
>25 - ≤ 50th	65	25.9	36	14.9
>10 - ≤ 25th	68	27.1	47	19.4
>5 - ≤ 10th	22	8.8	33	13.6
≤ 5th	71	28.3	112	46.3
Sex Total	**251**		**241**	
	Group Total		**492**	
	Chi-Square		(53: DF 6: p=0.0003)	

4.2.1.2 Z-Scores for Height

Standard deviation or z-scores were calculated for the age groups 6 to 17 years. The older groups were not included as data for the NCHS are not available as explained previously in this chapter.

The mean and median z-score for height increases in negative value as age increases in both study and control males (Tables 4.4 and 4.5 respectively). This reflects an increasing percentage of males falling further below the NCHS centiles as they grow older (Figs 4.1 and 4.2). Females follow a similar trend to males from 6-14 years of age, with a progressive deterioration of z-scores, however from 14 years of age females showed an improvement in z-scores with age. The patterns in the control and study groups were similar. These differences in height between males and females of the two groups were shown to be significant from the age of 14 years and greater when analysis of variance was carried out (14-15 year olds; and 16-17 year olds $p=0.0001$). There were no statistical differences in z-scores between females of the study and control groups by age group or between males of the two groups.

Table 4.4 Z-Scores for Height in the Study Group

Group	Males	Females	p value
6-7 years	n=33	n=38	
Mean	-1.09	-0.89	0.39
Median	-1. 1	-0.97	
Std Dev	1. 7	0.8 1	
Min : Max.	-3.83 : 1.37	-2.58 : 0.99	
8-9 years	n=39	n=47	
Mean	-0.94	-0.98	0.80
Median	-1.02	-0.81	
Std Dev.	1.04	0.82	
Min - Max.	-3.17 : 2.03	-3.58 : 1.5	
10-11 years	n=47	n=44	
Mean	-1.24	-1.34	0.64
Median	-1.13	-1.30	
Std Dev.	0.98	1.04	
Min - Max.	-3.13 : 0.56	-3.5 : 1.78	
12-13 years	n=36	n=40	
Mean	-1.70	-1.84	0.52
Median	-1.69	-1.75	
Std Dev.	0.77	1.14	
Min - Max.	-3.51 : -0.21	-4.83 : 1.37	
14-15 years	n=45	n=48	
Mean	-2.12	-1.11	0.0001
Median	-2.35	-1.02	
Std Dev.	0.95	0.82	
Min - Max.	-3.71: -0.53	-2.83 : 0.49	
16-17 years	n=41	n=34	
Mean	-2.37	-0.94	0.0001
Median	-2.12	-0.77	
Std Dev.	1.06	0.76	
Min - Max.	-3.84 : -0.79	-3.09 : -0.04	

Table 4.5 Z-Scores for Height in Control Group

Group	Males	Females	p value
6-7 years	n=23	n=33	
Mean	-0.85	-0.62	0.30
Median	-0.72	-0.52	
Std Dev.	0.89	0.78	
Min : Max.	-3.48 : 0.85	-2.27 : 0.93	
8-9 years	n=44	n=42	
Mean	-1.02	-1.04	0.92
Median	-0.93	-0.97	
Std Dev.	1.19	1.08	
Min - Max.	-4.36 : 1.73	-4.0 : 1.16	
10-11 years	n=37	n=39	
Mean	-1.31	-1. 2	0.58
Median	-1.32	-1.13	
Std Dev.	1.01	0.83	
Min - Max.	-4.43 : 0.54	-2.77 : 0.83	
12-13 years	n=43	n=44	
Mean	-1.62	-1.47	0.45
Median	-1.73	-1.43	
Std Dev.	0.86	0.96	
Min - Max.	-3.26 : 0.63	-2.76 : 0.7	
14-15 years	n=47	n=44	
Mean	-2.26	-1.01	0.0001
Median	-2.25	-1.65	
Std Dev.	0.99	0.85	
Min - Max.	-5.05 : 0.41	-3.38 : 0.59	
16-17 years	n=43	n=45	
Mean	-2.09	-0.85	0.0001
Median	-1.82	-0.93	
Std Dev.	1.22	0.92	
Min - Max.	-5.4 : 0.06	-3.98 : 0.64	

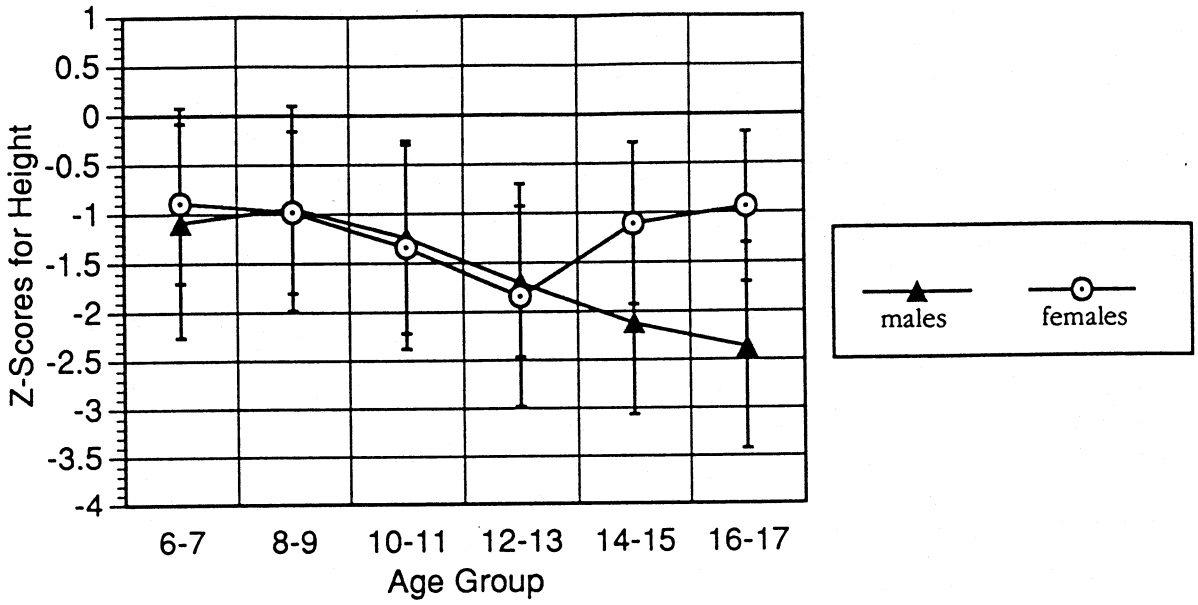


Fig 4.1 Height Represented by Z-Scores in the Study Group

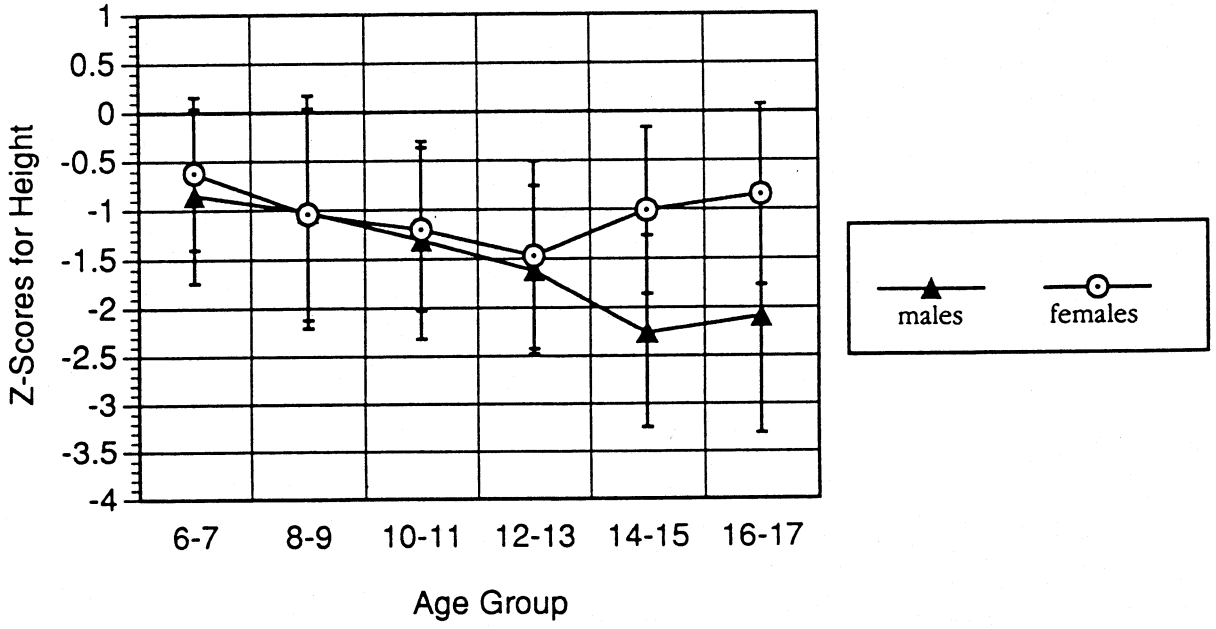


Fig 4.2 Height Represented by Z-Scores in the Control Group

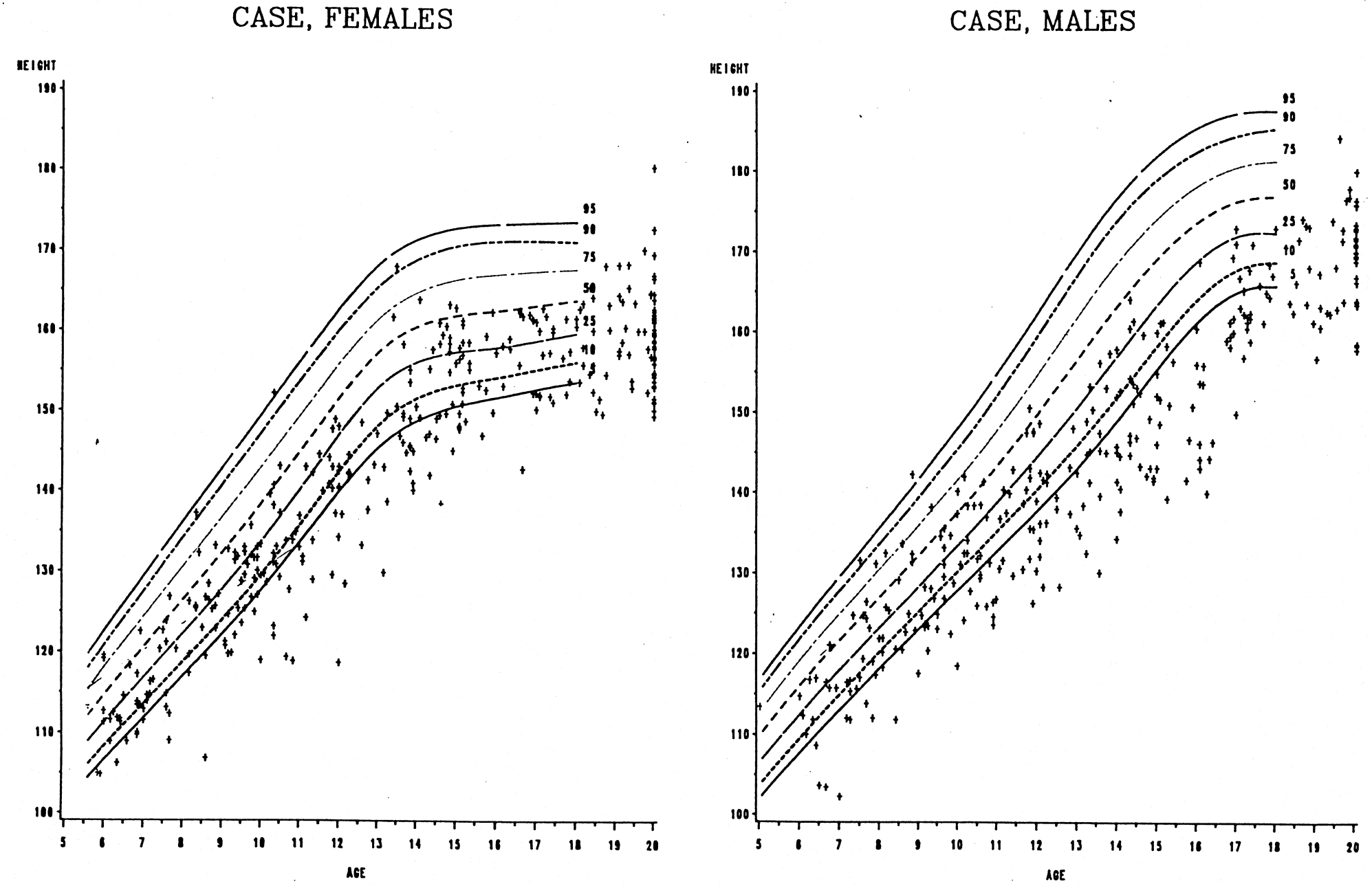


Fig.4.3 NCHS Centiles for Height in the Males and Females of the Study Group

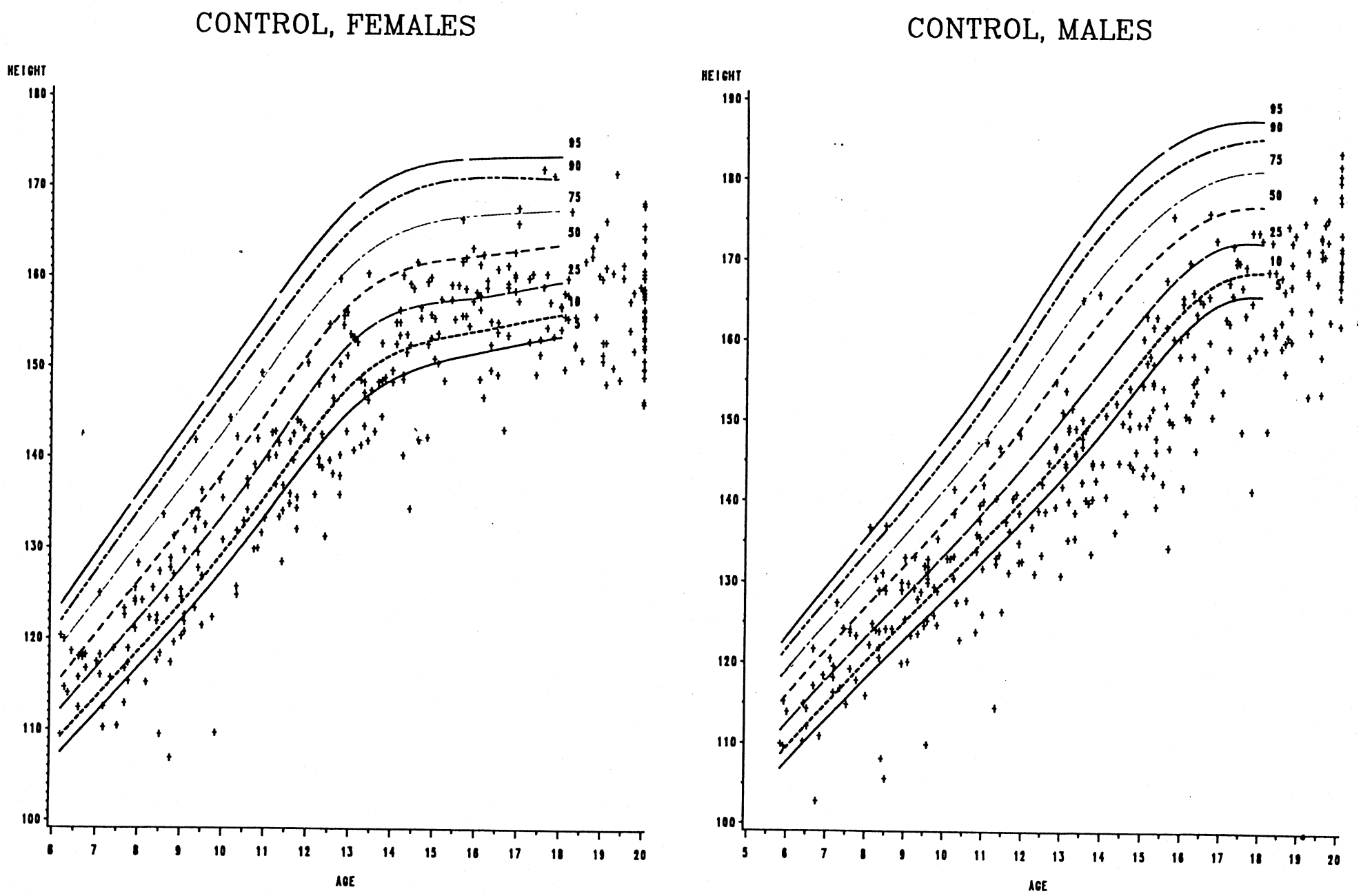


Fig.4.4 NCHS Centiles for Height in the Males and Females of the Control Group

4.2.2 Weight

4.2.2.1 Weight-for-Age:

Tables 4.6 and 4.7, and Figs 4.7 and 4.8 indicate weight-for-age measurements according to the NCHS centiles. In the female population of both groups, the majority of the population falls on or below the 50th centile (78% in the study group and 77% in the control group). This trend is even more pronounced in the male population, with 93% of the study group and 90% in the control group on or below the 50th centile. The Chi-square test found no significant difference between males of the study and control groups, nor between females of these two groups ($p=0.24$ and $p=0.25$ respectively).

To establish differences between males of the two groups they were compared in each weight-for-age centile category ($\leq$ 5th; etc...) using contingency tables. No significant differences were found between the two groups. The same was true for females of the two groups.

The males of both groups have approximately 55% of the population below the 5th centile for weight-for-age. This was considerably more than the females distributed below this centile: in the study group, 55.2% of males vs. 30.6% of females, and in the control group, 55% of males vs. 31.8% of females (see Fig 4.7 and 4.8 - NCHS growth charts). Significant differences were found between males and females of the same group using Chi-square ($p=0.0001$ for both the study and the control groups).

Table 4.6 Weight-for-Age NCHS percentiles for the Study Group

Percentile	**Females** (n)	**%**	**Males** (n)	**%**
>90 - ≤ 95th	2	0.8	1	0.4
>75 - ≤ 90th	5	1.9	2	0.8
>50 - ≤ 75th	26	10.1	7	2.9
>25 - ≤ 50th	63	24.4	20	8.3
>10 - ≤ 25th	54	20.9	37	15.4
>5 - ≤ 10th	29	11.2	41	17.0
≤ 5th	79	30.6	133	55.2
Sex Total	**258**		**241**	
	Group Total		**499**	
	Chi-Square		(53. 3: DF 6: p= 0.0001)	

Table 4.7 Weight-for-Age NCHS percentiles for the Control Group

Percentile	**Females** (n)	**%**	**Males** (n)	**%**
>90 - ≤ 95th	1	0.4	0	0
>75 - ≤ 90th	16	6.3	2	0.8
>50 - ≤ 75th	18	7.1	12	5.0
>25 - ≤ 50th	58	22.7	28	11.6
>10 - ≤ 25th	54	21 2	42	17.4
>5 - ≤ 10th	27	10.6	25	10.3
≤ 5th	81	31.8	133	55.0
Sex Total	**255**		**242**	
	Group Total		**497**	
	Chi-Square		(37. 4: DF 6: p=0.0001)	

4.2.2.2 Z-Scores for Weight

The median z-score for weight in study males decreased progressively with age until the 14-15 year age group and then the decline plateaued (Table 4.8 and Fig 4.5). The same pattern was seen in the males of the control group (Table 4.9 and Fig 4.6) although a slight improvement in z-score in the 16-17 year old age group was seen, but this was not significant.

Until the age of 14 years, the z-scores of females show a pattern similar to males, however there was a significant improvement in z-scores in the 14-15 and 16-17 year age groups in females. The age groups 14-15 and 16-17 show significant differences between the z-scores of males and females ($p=0.0001$) for both the study and control groups.

Table 4.8 Z-Scores for Weight in Study Group

Group	Males	Females	p value
6-7 years	n=33	n=38	
Mean	-0.81	-0.60	0.31
Median	-1.05	-0.61	
Std Dev.	0.91	0.78	
Min : Max.	-2.41 : 1.57	-2.23 : 1.34	
8-9 years	n=38	n=47	
Mean	-1.13	-1.17	0.80
Median	-1.3	-1.33	
Std Dev.	0.70	0.69	
Min - Max.	-2.26 : 1.46	-2.48 : 0.52	
10-11 years	n=47	n=44	
Mean	-1.48	-1.24	0.16
Median	-1.57	-1. 34	
Std Dev.	0.64	0.95	
Min - Max.	-2.68 : 0.26	-2.66 : 1.86	
12-13 years	n=37	n=40	
Mean	-1.80	-1.51	0.05
Median	-1.83	-1.64	
Std Dev.	0.48	0. 4	
Min - Max.	-2.83 : -0.55	-3.04 : 0.79	
14-15 years	n=45	n=48	
Mean	-2.23	-0.76	0.0001
Median	-2.35	-0.79	
Std Dev.	0.70	0.64	
Min - Max.	-3.71 : -0.53	-2.14 : 0.52	
16-17 years	n=41	n=34	
Mean	-2.22	-0.82	0.0001
Median	-2.12	-0.79	
Std Dev.	0.79	0.81	
Min - Max.	-3. 4 : -0.79	-2.48 : 0.59	

Table 4.9 Z-Scores for Weight in Control Group

Group	Males	Females	p value
6-7 years	n=23	n=33	
Mean	-1.21	-1.02	0.34
Median	-0.51	-0.93	
Std Dev.	0.76	0.74	
Min : Max.	-2.47 : 0.19	-2.15 : 0.47	
8-9 years	n=44	n=42	
Mean	-1.10	-1.25	0.33
Median	-1.18	-1.3	
Std Dev.	0.71	0.71	
Min - Max.	-2.35 : 0.52	-2. 3 : 0.27	
10-11 years	n=37	n=39	
Mean	-1.41	-1.18	0.22
Median	-1.55	-1.3	
Std Dev.	0.75	0.89	
Min - Max.	-2.92 : 0.38	-2.48 : 1.03	
12-13 years	n=43	n=44	
Mean	-1.58	-1.35	0.14
Median	-1.73	-1.43	
Std Dev.	0.71	0.77	
Min - Max.	-2.94 : 0.77	-2.76 : 0.7	
14-15 years	n=47	n=45	
Mean	-1.94	-0.81	0.0001
Median	-2.13	-0.76	
Std Dev.	0.96	0.71	
Min - Max.	-3.44 : 0.47	-2.28 : 0.91	
16-17 years	n=43	n=43	
Mean	-1.74	-0.42	0.0001
Median	-1.74	-0.44	
Std Dev.	0.94	0.99	
Min - Max.	-3.83 : 0.15	-2.5 : 1.28	

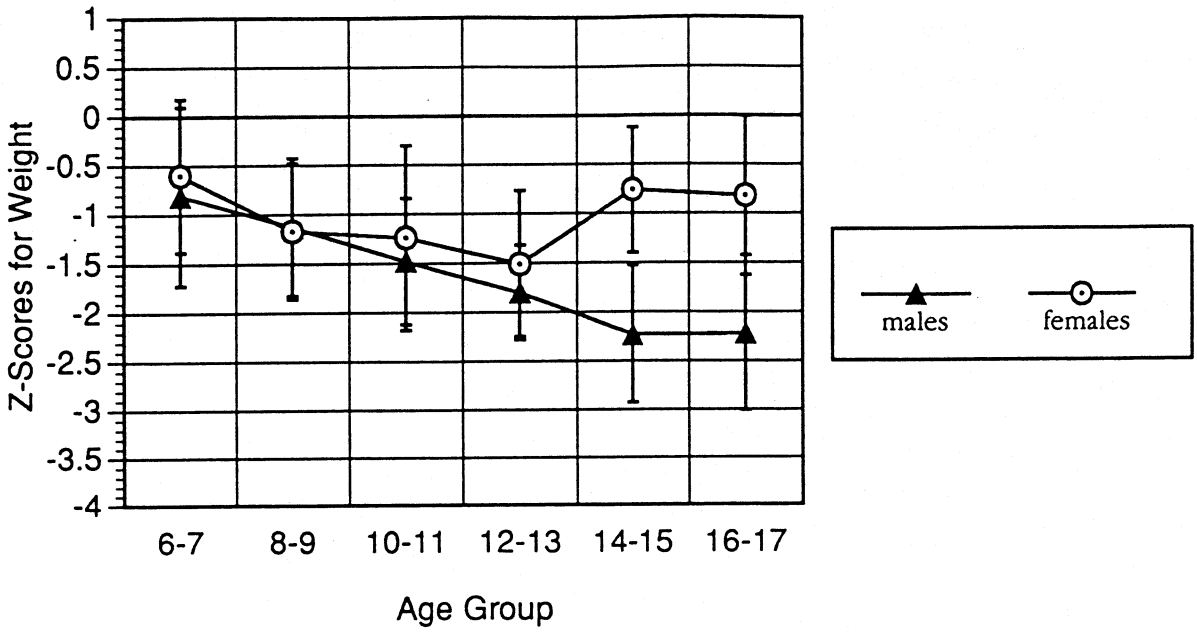


Fig. 4.5 Weight Represented by Z-Scores in the Study Group

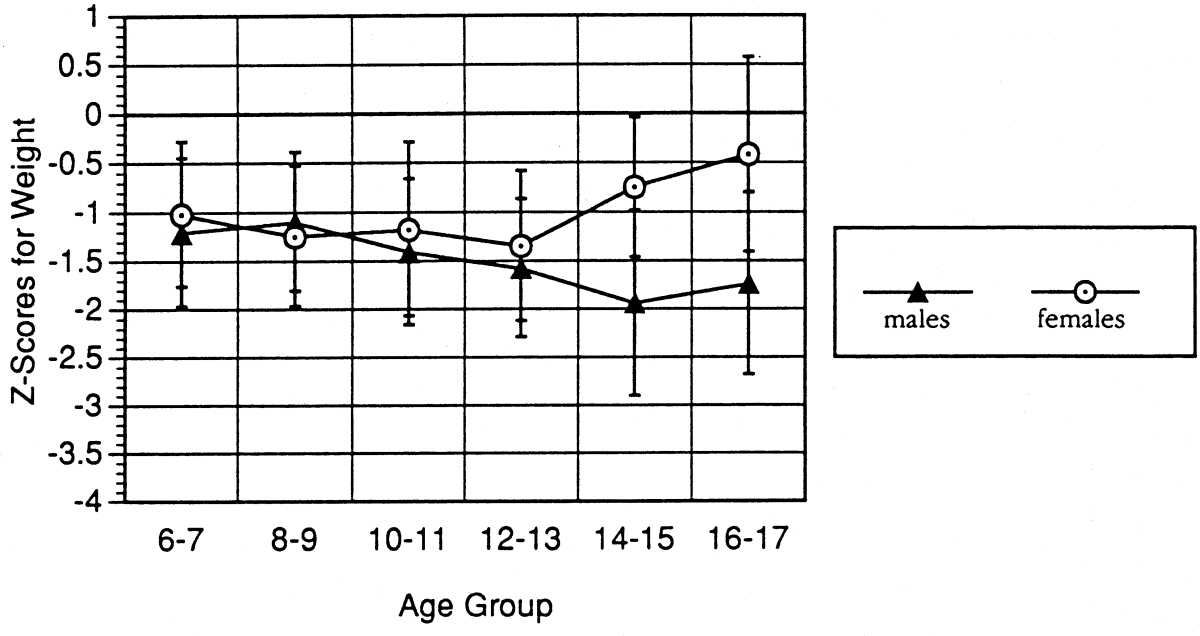


Fig. 4.6 Weight Represented by Z-Scores in the Control Group

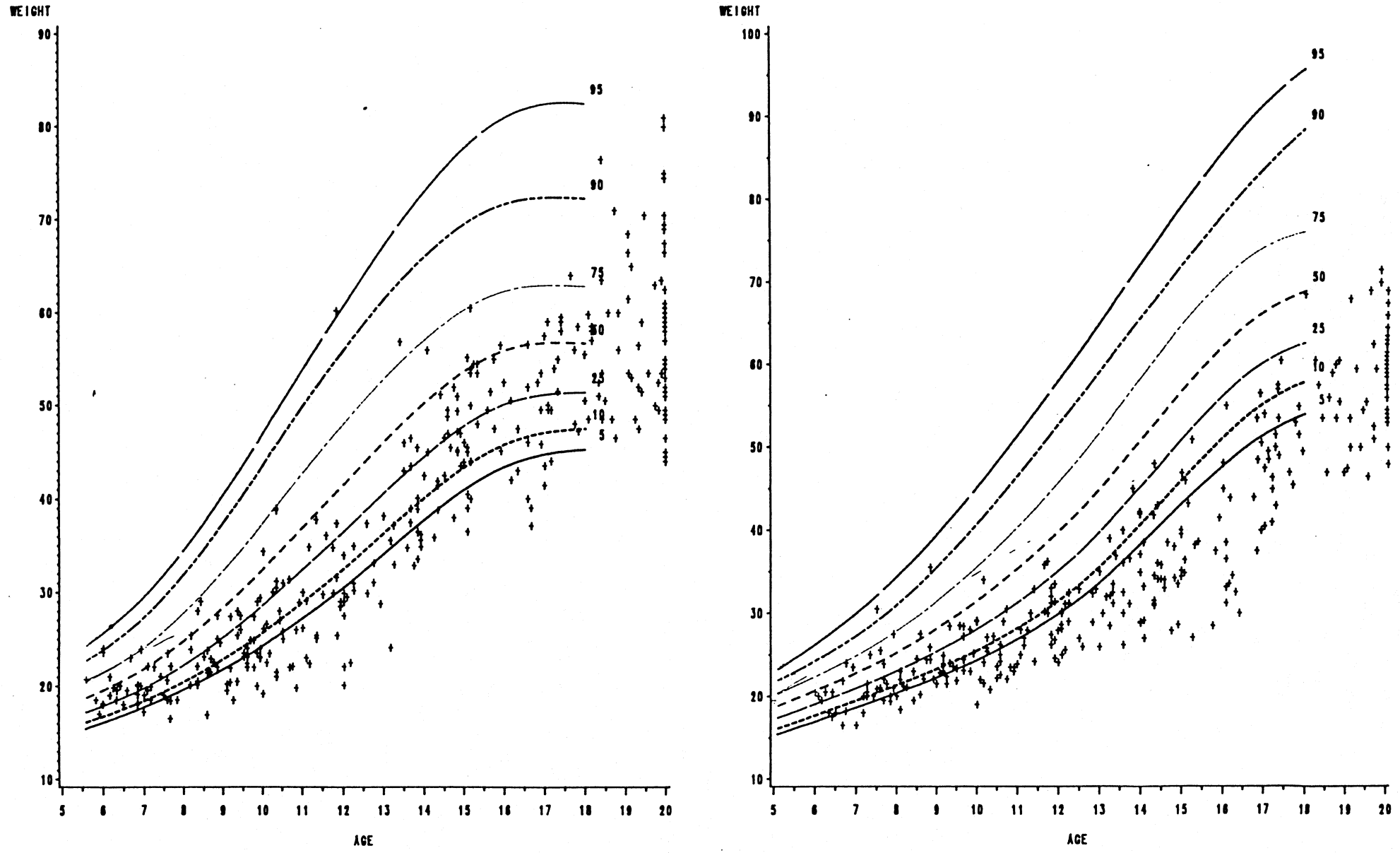


Fig.4.7 NCHS Centiles for Weight in the Males and Females of the Study Group

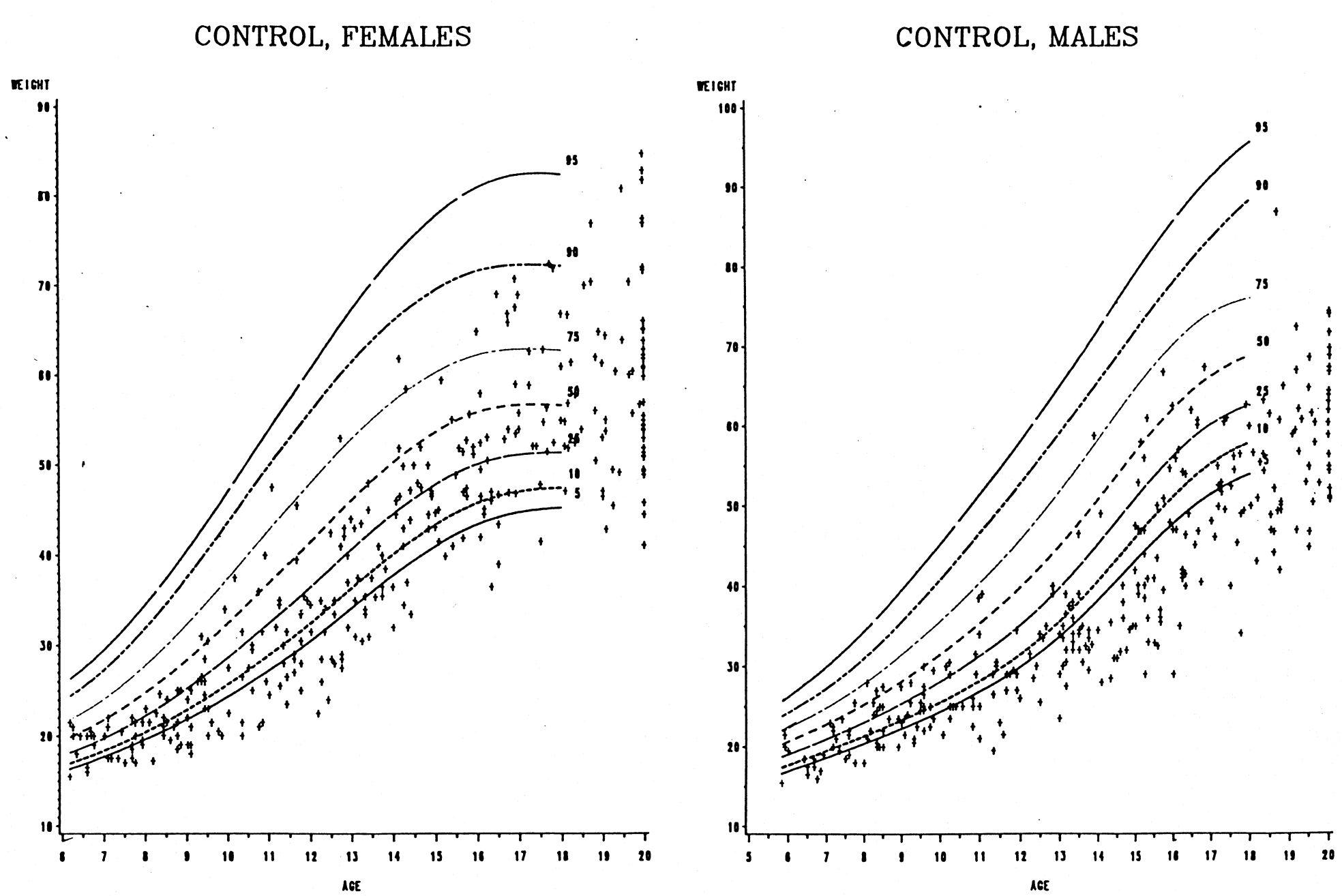


Fig.4.8 NCHS Centiles for Weight in the Males and Females of the Control Group

4.2.3 Body Mass Index

Body mass index (BMI) increased with age for both males and females of both groups (Tables 4.10 and 4.11 and Figs. 4.9 and 4.9a). The BMI of males in the study and control groups was similar in all the two year age groups, except the 6-7 and 12-15 year olds. A similar pattern was seen for the females with only the 6-7 year old and 16-17 year old age groups showing a significant difference between the study and control groups (Tables 4.12 and 4.13 and Figs. 4.9 and 4.9a).

Comparing the BMI of males and females in the same groups, the younger children had a similar BMI, but as the ages of the children increased, the BMI of females progressively diverged from that of the males, with the females having a greater BMI. In the study group, this divergence was first seen in the 10-11 year olds, while in the control group the difference only became significant in the 14-15 year age group.

BMI values were compared to the 50th centile data of the first National Health and Nutrition Survey (NHANES I) for Americans reported by Must et al. (1991). They report the 5th, 15th, 50th, 85th and 95th centiles for black Americans. BMI values for the males and females of both the study and control groups were followed roughly on or between 5th and the 50th centiles for black Americans (Tables 4.10 and 4.11). BMI for males of the study and control groups did not quite reach the 50th centile values in the corresponding age categories of the NHANES-I study (except for the 6-7 year old age group) (see Table 4.10). Females of the study and control groups had mean BMI values that were lower than the 50th centiles in the age groups 8-9, 10-11, 12-13, but in the 6-7, 14-15, 16-17, 18-19 and 20-22 year olds the BMI values were equal to the NHANES centile, and in some cases higher, however these are visual comparisons and statistical analysis between NHANES and this study were done (see Table 4.11 and Fig 4.10).

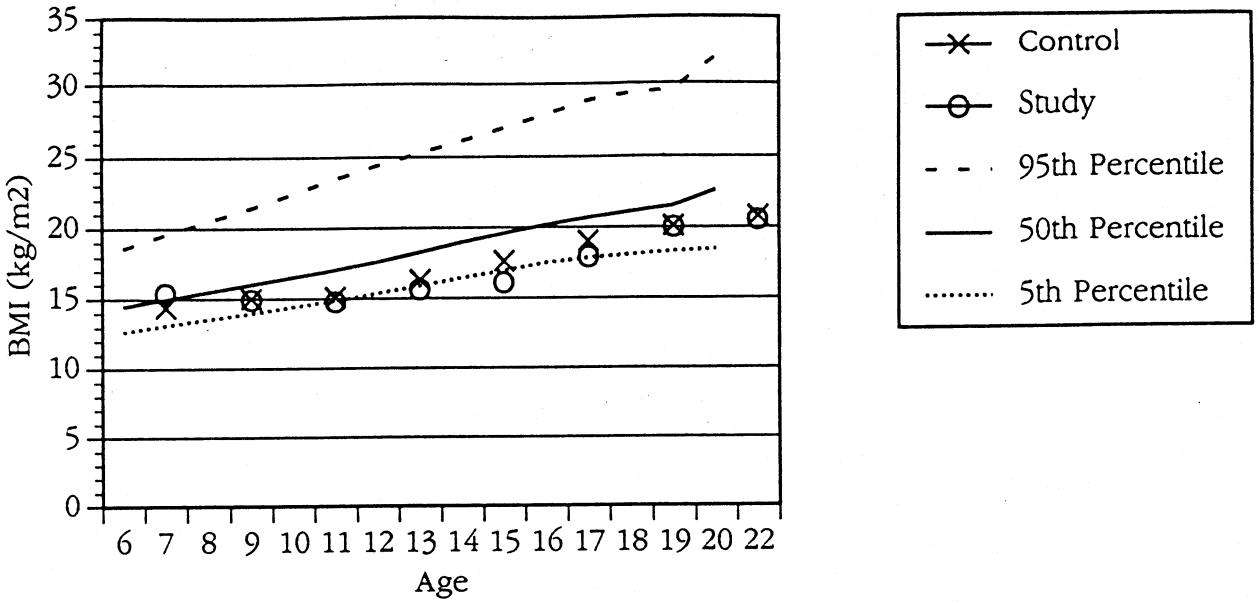


Fig 4.9 Body Mass Index Values for Study and Control Males Plotted Against Black Americans (Must et al.1991)

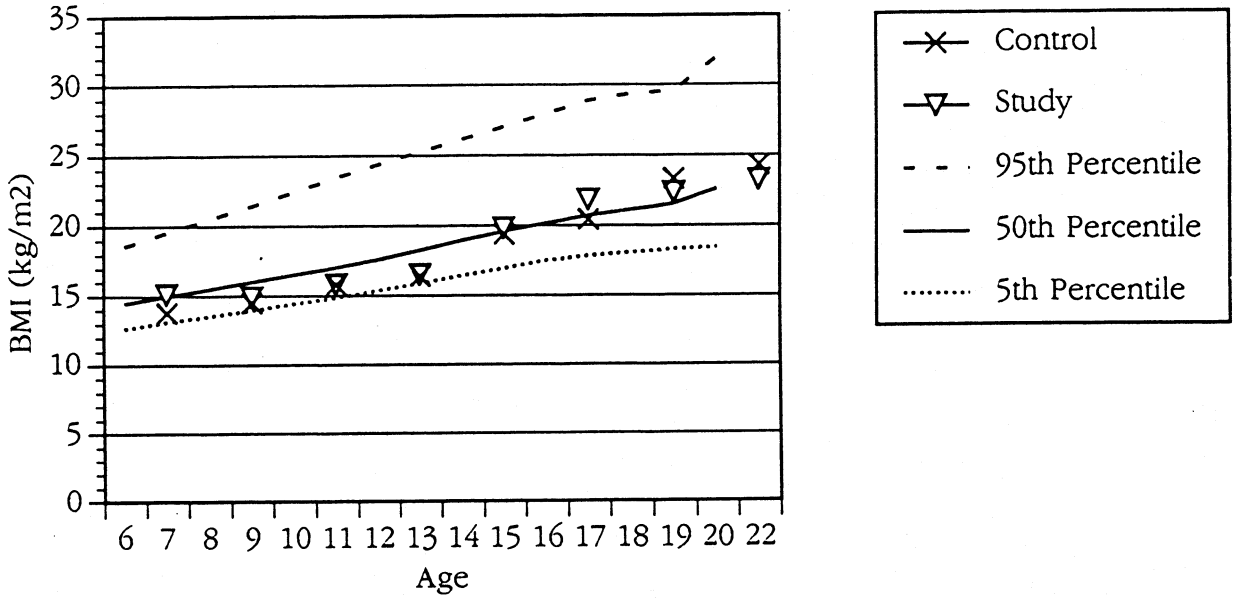


Fig 4.9a Body Mass Index Values for Study and Control Females Plotted Against Black Americans (Must et al.1991)

Table 4.10 Comparison of Body Mass Index between Males of the Study and Control Group

Group	N	Mean BMI kg/m^2	Std Dev.	p value	NHANES 50th centile
6-7					
Study	33	15.4	1.1	0.001 *	14.98
Control	23	14.4	1.3		
8-9					
Study	39	14.9	0.8	0.677	16.00
Control	44	15.0	1.4		
10-11					
Study	47	14.8	1.3	0.312	17.06
Control	37	15.1	1.8		
12-13					
Study	37	15.6	1.1	0.011*	18.28
Control	43	16.3	1.5		
14-15					
Study	45	16.1	1.5	0.0004*	19.56
Control	47	17.6	2.4		
16-17					
Study	41	17.9	2.0	0.127	20.70
Control	43	19.0	2.0		
18-19					
Study	28	20.0	1.9	0.904	21.51
Control	43	20.1	2.3		
20-22					
Study	24	20.5	1.2	0.637	22.59
Control	18	20.8	2.2		

* = significance

Table 4.11 Comparison of Body Mass Index between Females of the Study and Control Group

Group	N	Mean BMI kg/m^2	Std Dev..	p value	NAHNES 50th centile
6-7					
Study	38	15.1	1.1	0.0001 *	14.98
Control	33	13.8	1.0		
8-9					
Study	47	14.5	1.1	0.975	16.00
Control	42	14.5	1.5		
10-11					
Study	44	15.8	2.7	0.929	17.06
Control	39	15.8	2.4		
12-13					
Study	40	16.5	2.0	0.819	18.28
Control	44	16.4	1.8		
14-15					
Study	48	19.8	2.0	0.4.23	19.56
Control	45	19.4	2.4		
16-17					
Study	34	21.8	2.0	0.047*	20.70
Control	43	20.4	2.0		
18-19					
Study	41	22.3	2.8	0.114	21.51
Control	40	23.3	3.0		
20-22					
Study	28	23.2	3.6	0.218	22.59
Control	29	24.3	3.9		

* = significance

Table 4.12 Comparison of Body Mass Index by Sex in the Study Group

Group	N	Mean BMI kg/m^2	Std Dev.	p value
6-7				
Female	38	15.1	1.1	0.317
Male	33	15.4	1.1	
8-9				
Female	47	14.5	1.1	0.157
Male	39	14.9	0.8	
10-11				
Female	44	15. 8	2.7	0.032*
Male	47	14.8	1.3	
12-13				
Female	40	16 5	2.0	0.020*
Male	37	15.6	1.1	
14-15				
Female	48	19.8	2.0	0.0001*
Male	45	16.1	1.5	
16-17				
Female	34	20.4	2.6	0.0001*
Male	41	17.9	2.0	
18-19				
Female	41	22.3	28.1	0.0002*
Male	28	20.0	19.3	
20-22				
Female	28	23.2	3.6	0.0001*
Male	24	20.5	1.2	

* = significance

Table 4.13 Comparison of Body Mass Index by Sex in the Control Group

Group	N	Mean BMI	Std Dev. kg/m^2	p value
6-7				
Female	33	13.8	1.0	0.090
Male	23	14.4	1.3	
8-9				
Female	42	14.5	1.5	0.170
Male	44	15.0	1. 4	
10-11				
Female	39	15.8	2.4	0.180
Male	37	15.1	1.8	
12-13				
Female	44	16.4	1.8	0.942
Male	43	16.3	1.5	
14-15				
Female	45	19.4	2.4	0.0006*
Male	47	17.6	2.4	
16-17				
Female	43	21.8	3.4	0.0001*
Male	43	19.0	2.0	
18-19				
Female	40	23.3	3.0	0.0001*
Male	43	20.1	2.3	
20-22				
Female	29	24.3	3.9	0.0001*
Male	18	20.8	2.2	

* = significance

4.3 Measurements of the Leg Deformities

4.3.1 Inter-Patellar Distance

Children with inter-patellar distances of greater than 5 cm, which was considered clinically relevant, were found in each group. The study group had only 2 (0.3%) cases, and the control group 20 cases (3.2%). The Chi-square test shows the difference between the two groups to be significant, p=0.001.

Table 4.14 Inter-Patellar Measurements in the Study and Control Groups

	< 5 cm	> 5 cm
Study	612 (99.6 %)	2 (0.3%)
Control	593 (96.7%)	20 (3.2%)
Total	1 205 (98.2%)	22 (1.8%)

4.3.2 Inter-Malleolar Distance

The prevalence of knock-knees, defined by an inter-malleolar distance > 5 cm, was higher in the study group, in which 152 cases (24.7%) were found, compared with the control group, which had 85 cases (13.8%). The difference in the prevalence between the study and the control groups was significant (p=0.0001) using the Chi-Square test. When age was correlated with inter-malleolar distance, there was no systematic correlation for females or males.

Table 4.15. Inter-Malleolar Measurements in the Study and Control Groups

Group	< 5 cm	> 5 cm
Study	462 (75.2%)	152 (24.7%)
Control	528 (86.1%)	85 (13.8%)
Total	990 (80.7%)	237 (19.3%)

Gender played an important role in the prevalence of genu valgum. Females showed a higher prevalence than males, 23.1% versus 15.2 % respectively

(p=0.001. Chi-square test) (Table 4.16). Females in the study group had a 30% prevalence of genu valgum versus 16.2% in the control group (p=0.001), while study males had a prevalence of 19.0% versus 11.4% in control males which was not significant (p=0.09) (Table 4.17). Females of the study group also showed a trend to a higher frequency of genu valgum in the older age groups, from 12-13 years up, compared with the 10-11 year olds and younger (Table 4.17a). This tendency to a greater prevalence of genu valgum in higher ages of the study group was significantly different from the control group (p=0.001), in which the prevalence did not vary across the age groups.

Table 4.16 Inter-Malleolar Measurements by Sex in the Combined Samples

Group	≤ 5 cm	> 5 cm
Males	502 (84.8%)	90 (15.2%)
Females	488 (76.8%)	147 (23.1%)
Total	990 (80,68%)	237 (19.31%)

Table 4.17 Inter-Malleolar Measurements by Sex and Group

Group	Study		Control	
	≤ 5 cm	> 5 cm	≤ 5 cm	> 5 cm
Males	238 (80.95%)	56 (19.0%)	264 (88.6%)	34 (11.4%)
Females	224 (70,0%)	96 (30.0%)	264 (83.8%)	51 (16.2%)
Total	462 (75.2%)	152 (24.7%)	528 (86.1%)	85 (13.8%)

Table 4.17a Inter-Malleolar Distance > 5 cm : Frequencies per Age Group, Sex and Group

Age Group	Study Males	Study Females	Control Males	Control Females
6-7	5	6	4	4
8-9	1	6	2	6
10-11	9	6	5	11
12-13	6	13	9	9
14-15	10	21	8	6
16-17	13	13	3	9
18-19	8	20	2	5
20-22	4	11	1	1

4.3.3 Windswept Deformity

Out of the whole research population (n=1 227 scholars), only one case, a male from the study group, presented with windswept deformities of the lower limbs.

4.4 Biochemical Results

4.4.1 Serum Results

Mean serum values for calcium, magnesium, creatinine and albumin are included in Tables 4.18-4.21 and depicted graphically in Figs 4.10 - 4.25.

a. Serum Calcium

The mean serum calcium values in the study group fell within the normal reference range for males and females and were similar (2.40 ± 0.01 mmol/l and 2.41 ± 0.11 mmol/l respectively) (p=0.37) (Table 4.20). Only in the 14-15 year old age group did males and females show a significant difference in mean calcium values (males: 2.40 ± 0.08; females: 2.44 ± 0.09 mmol/l (p=0.04)). The males and females of the control group had similar mean serum values (2.43 ± 0.07 mmol/l and 2.44 ± 0.08 mmol/l respectively) (p=0.51) (Table 4.21 and Figs 4.10-4.13).

Serum calcium correlated significantly with age in the study group (r=0. 18 p= 0. 0001). Although a significant correlation was shown, age accounts for a small percentage of the variance found in the biochemical variables (only 3. 2% of the variance in serum calcium in the study group is due to age). In the control group serum calcium was not significantly correlated with age (r=0. 038 p=0. 30).

The mean serum calcium values in both males and females of the study group were significantly lower than those of the control group (males: 2.40 vs. 2.43 (p=0.0001): females; 2.41 vs. 2.44 mmol/l (p=0.0009) respectively), (Tables 4.18 and 4.19). Although mean serum calcium values in both groups fell within the normal reference range, 10% in the study group and 3% in the control group were hypocalcemic (≤ 2.25 mmol/l) (p = 0.0001). The number of hypocalcemic children was similar between the sexes in the study and control groups (23 males and 29 females) (Chi-square p= 0.65) and (3 males and 9 females) (Chi-square p=0.09) respectively.

b. Serum Magnesium

The mean serum magnesium values for study males and females fell within normal reference ranges and showed no statistical difference (0.84 ± 0.06 mmol/l and 0.85 ± 0.07 mmol/l respectively) (p=0.68) (Tables 4.20 and 4.21 and Figs 4.14-4.17). Although there was a significant difference between control group males and females (0.84 ± 0.07 mmol/l and 0.82 ± 0.07 mmol/l, respectively) (p=0.002), the difference was small.

Mean values in males of the control and study groups were not significantly different (0.84 ± 0.07 mmol/l and 0.84 ± 0.07 mmol/l, respectively) (p=0.17). However, females of the two groups showed differences that were significant (0.85 ± 0.07 mmol/l and 0.82 ± 0.07 mmol/l) (p=0.0001).

In the study group, serum magnesium correlated significantly with age (r =-0.21; p=0. 001), but this relationship was not seen in the control group (r= -0.6; p=0. 3).

c. Serum Creatinine

Mean serum creatinine values were similar between the sexes of each group with no significant differences. Mean serum creatinine values for males and females in the study group were 55 ± 9 µmol/l and 54 ± 8 µmol/l, respectively (p=0.08). Mean creatinine values for males and females of the control group were 55 ± 9 µmol/l and 55 ± 8 µmol/l, respectively (p=0.66) (Tables 4.20 and 4.21 and Figs 4.18 - 4.21).

Creatinine values showed no significant differences within children of the same sex in the two groups. Mean values in males of the study and control groups were 55 ± 9 µmol/l and 55 ± 9 µmol/l respectively, (p=0.7). In females of the study and

control groups, mean creatinine concentrations were 54 ± 8 μmol/l and 55 ± 8 μmol/l respectively (p=0.07). When age was correlated with serum creatinine values in the study group a significant positive correlation was found (r= 0.10 p=0. 01), however this was not seen in the control group (r=0.07 p= 0.08).

d. Serum Albumin

Mean serum albumin concentrations for study males and females were 42.3± 2. 3 g/l and 42.3 ± 2.2 g/l (p=0.70) respectively. Mean albumin concentrations for the control males and females were 43.7 ± 2.4 and 43 5 ± 2.2 g/l, respectively (p=0.69) (Tables 4.20 abd 4.21 and Figs 4.22-4.25).

The study group had lower mean albumin levels than the control group. In males serum albumin values were 42.3 ± 2.3 g/l in the study group compared to 43.7 ± 2.4 g/l in the control group (p=0.0001). Females of the study group also had a lower mean value than the control group (42.3 ± 2.2 g/l vs. 43.5 ± 2.2 g/l, (p=0.0001)). Although these differences are statistically significant, they are not clinically relevant. No children had values in the hypoalbuminaemic range (< 35 g/l). Age correlated significantly with serum albumin in both study group and control group (r=0.37 p=0.0001 and r=0.20 p= 0.0001 respectively).

The serum calcium levels in the study and control populations were corrected against serum albumin levels and did not influence the calcium levels. This was expected as the albumin levels fell within the clinically normal range.

Table 4.18 Mean Serum Values for Males in the Study and Control Groups

Group	n	Mean (Range)	Std Dev.	p value
Calcium mmol/l				
Study	263	2.40 (2.0-2.8)	0.101	0.0001
Control	292	2.43 (2.0-2.65)	0.077	
Magnesium mmol/l				
Study	263	0.85 (0.68-1.0)	0.071	0.176
Control	392	0.84 (0.7-1.05)	0.069	
Creatinine µmol/l				
Study	261	55 (40-72)	8.6	0.723
Control	293	55 (40-72)	8.5	
Albumin g/l				
Study	260	42.3 (38.5-47.6)	2.28	0.0001
Control	291	43.7 (38.4-47.8)	2.44	

Table 4.19 Mean Serum Values for Females in the Study and Control Groups.

Group	n	Mean (Range)	Std Dev.	p value
Calcium mmol/l				
Study	292	2.41 (2.0-2.7)	0.110	0.0009
Control	309	2.44 (2.15-2.65)	0.085	
Magnesium mmol/l				
Study	294	0.85 (0.7-1.05)	0.073	0.0001
Control	310	0.82 (0.6-1.1)	0.074	
Creatinine µmol/l				
Study	292	54 (41-72)	7.9	0.072
Control	312	55 (40-72)	7.9	
Albumin g/l				
Study	292	42.3 (38-47.6)	2.23	0.0001
Control	313	43.5 (38-47.5)	2.18	

Table 4.20. Mean Serum Values for Males vs. Females in the Study Group

Sex	n	Mean	Std Deviation	p value
Calcium mmol/l				
Male	261	2. 40	0. 10	0. 37
Female	292	2. 41	0. 11	
Magnesium mmol/l				
Male	261	0. 84	0. 064	0. 68
Female	294	0. 85	0. 073	
Creatinine µmol/l				
Male	261	55	8. 59	0. 08
Female	296	54	7. 86	
Albumin g/l				
Male	260	42. 3	2. 28	0. 70
Female	292	42. 3	2. 23	

Table 4.21. Serum Values for Males vs. Females in the Control Group

Sex	n	Mean	Std Deviation	p value
Calcium mmol/l				
Male	292	2. 43	0. 077	0. 51
Female	309	2. 44	0. 085	
Magnesium mmol/l				
Male	292	0. 84	0. 069	0. 002
Female	310	0. 82	0. 074	
Creatinine µmol/l				
Male	293	55	8. 54	0. 668
Female	312	55	8. 19	
Albumin g/l				
Male	291	43. 6	2. 44	0. 691
Female	313	43. 5	2. 18	

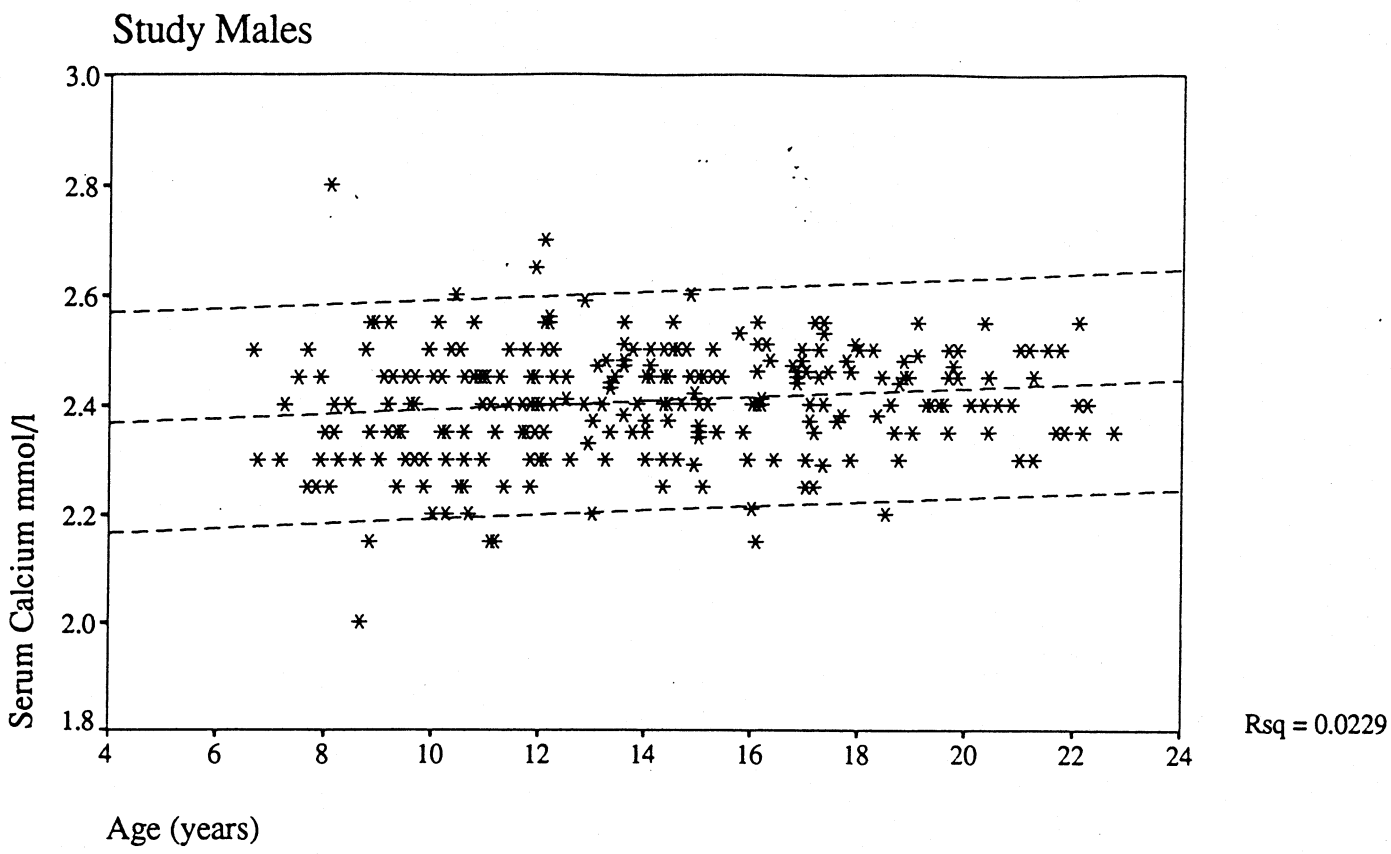


Fig 4.10 Serum Calcium Values for Study Males (Confidence Interval 95%)

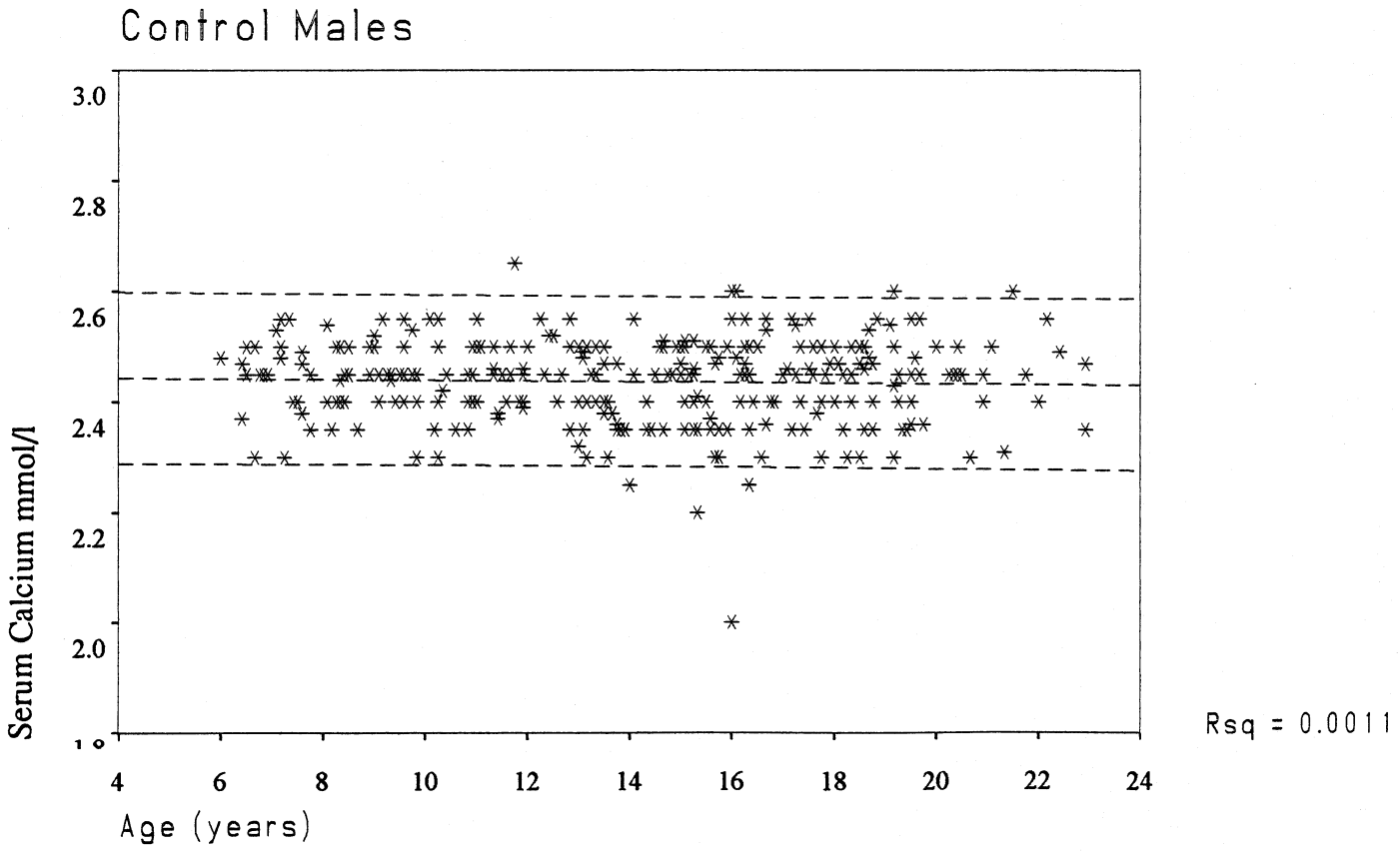


Fig 4.11 Serum Calcium Values for Control Males (Confidence Interval 95%)

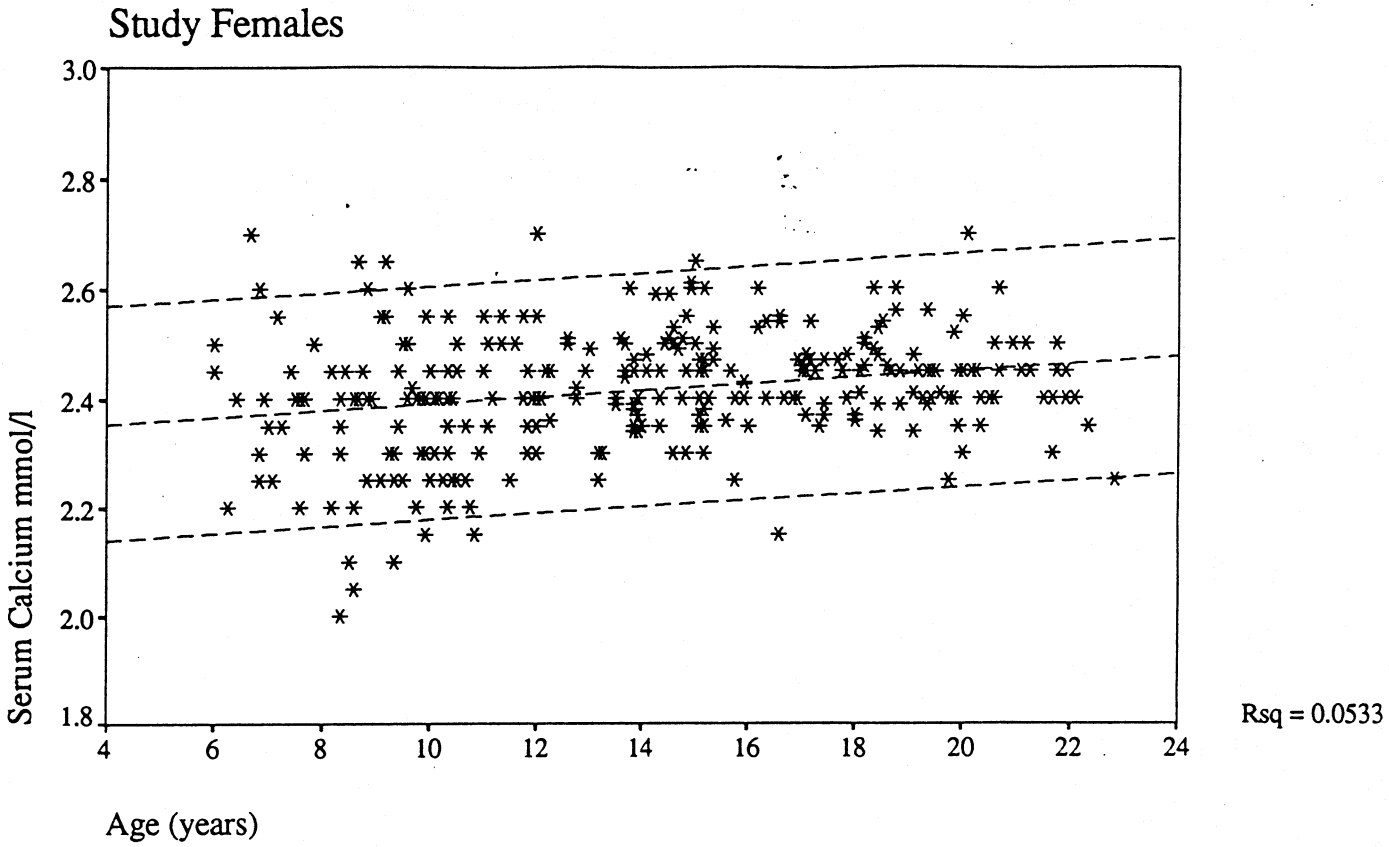


Fig 4.12 Serum Calcium Values for Study Females (Confidence Interval 95%)

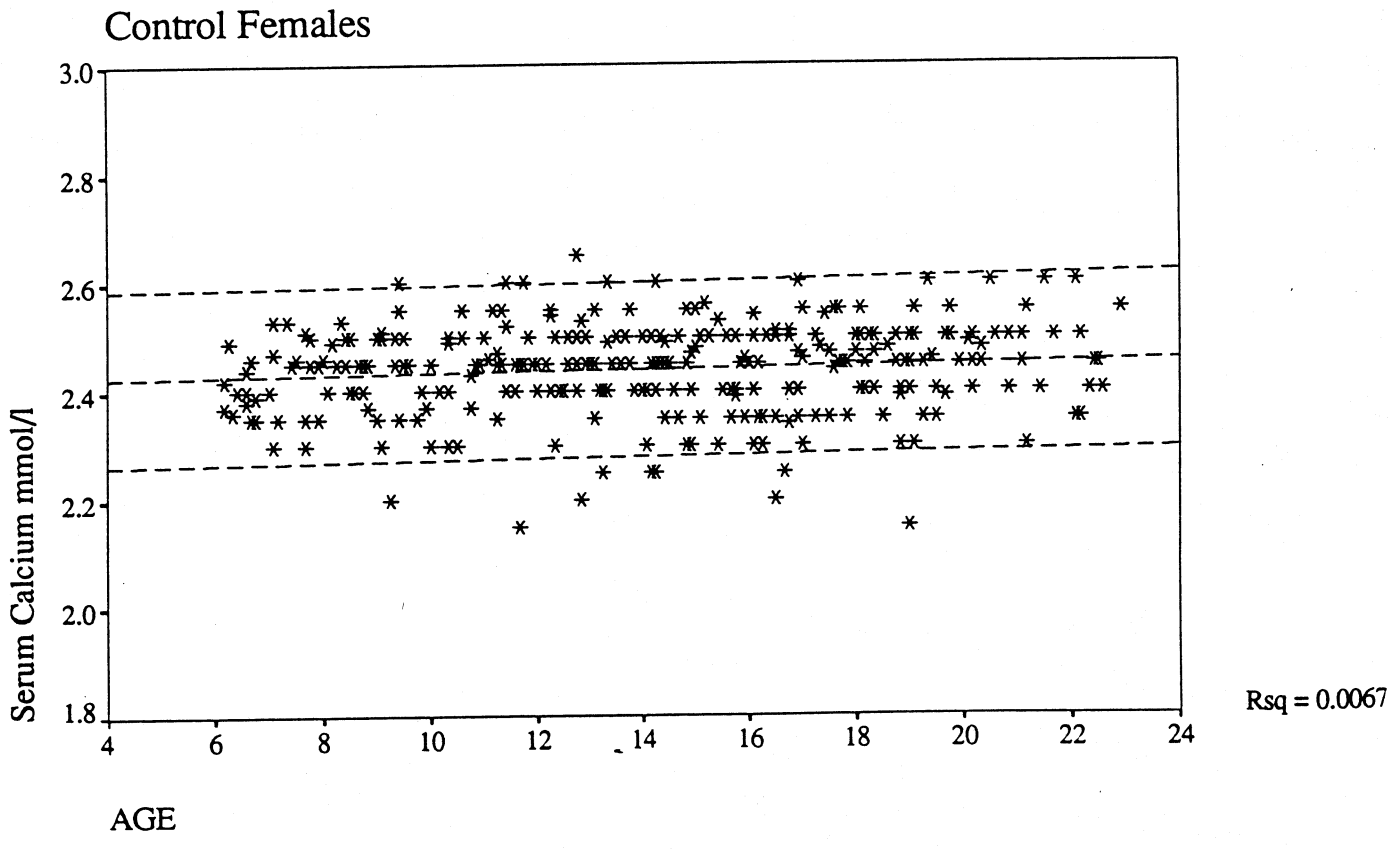


Fig 4.13 Serum Calcium Values for Control Females (Confidence Interval 95%)

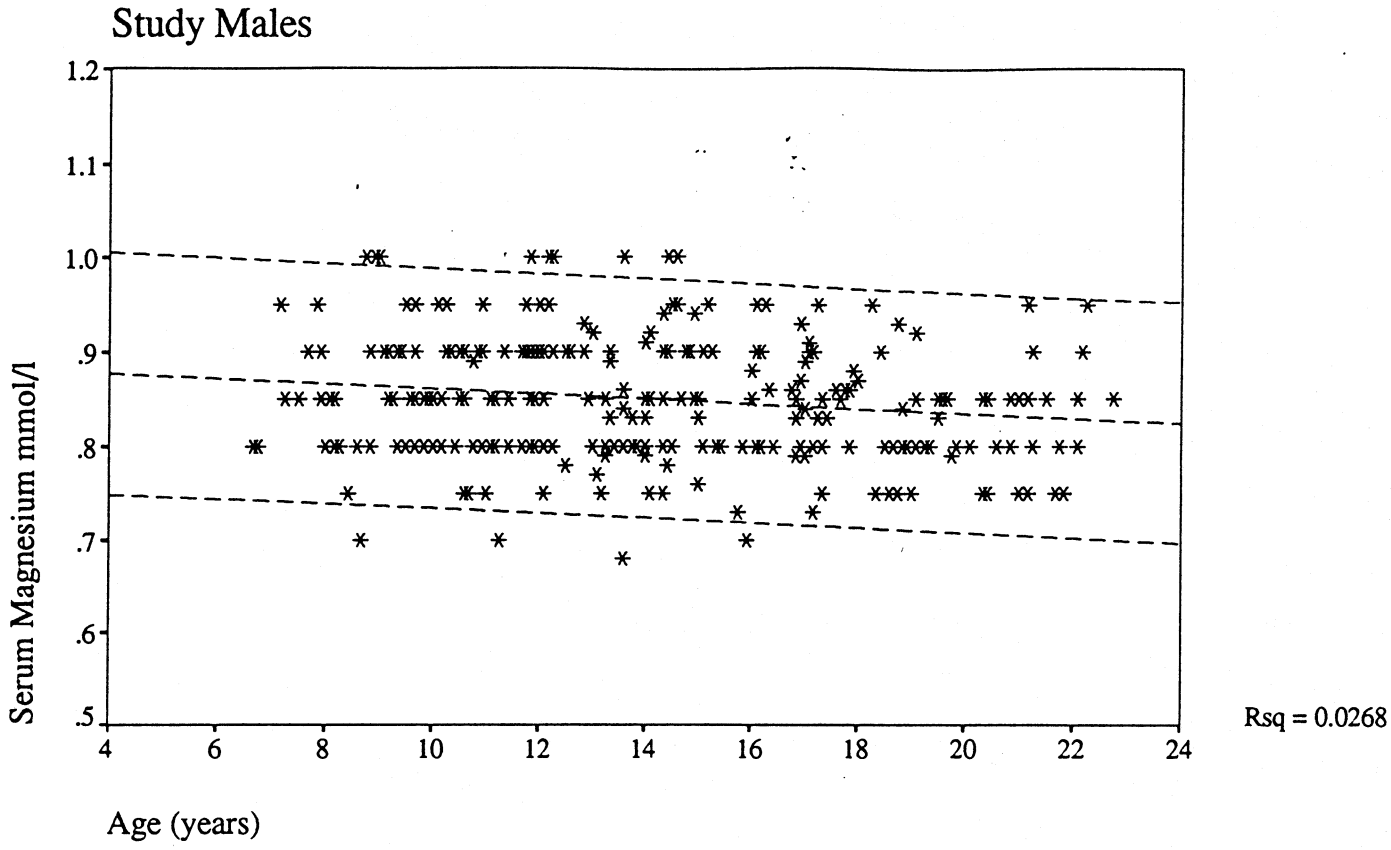


Fig 4.14 Serum Magnesium Values for Study Males (Confidence Interval 95%)

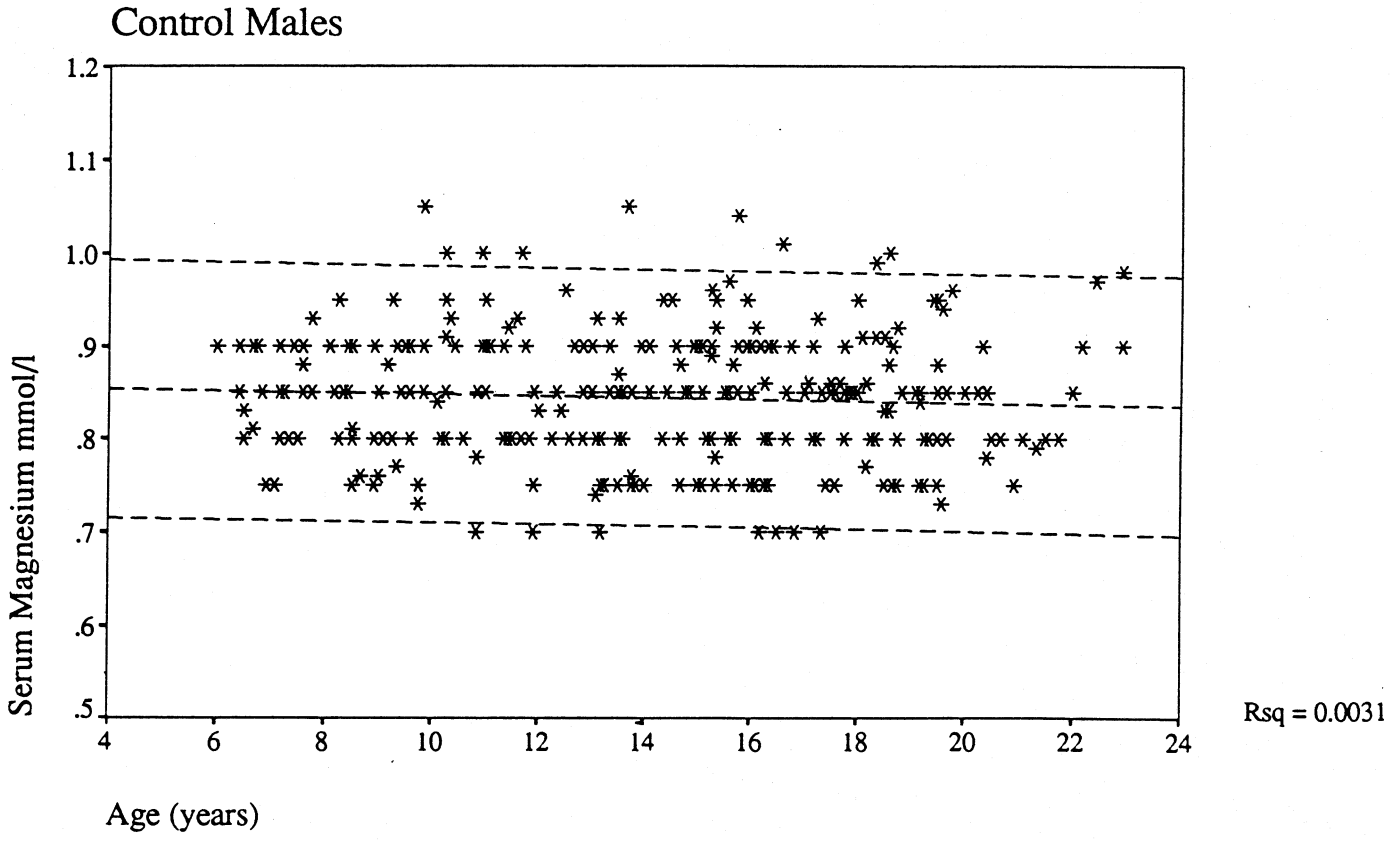


Fig 4.15 Serum Magnesium Values for Control Males (Confidence Interval 95%)

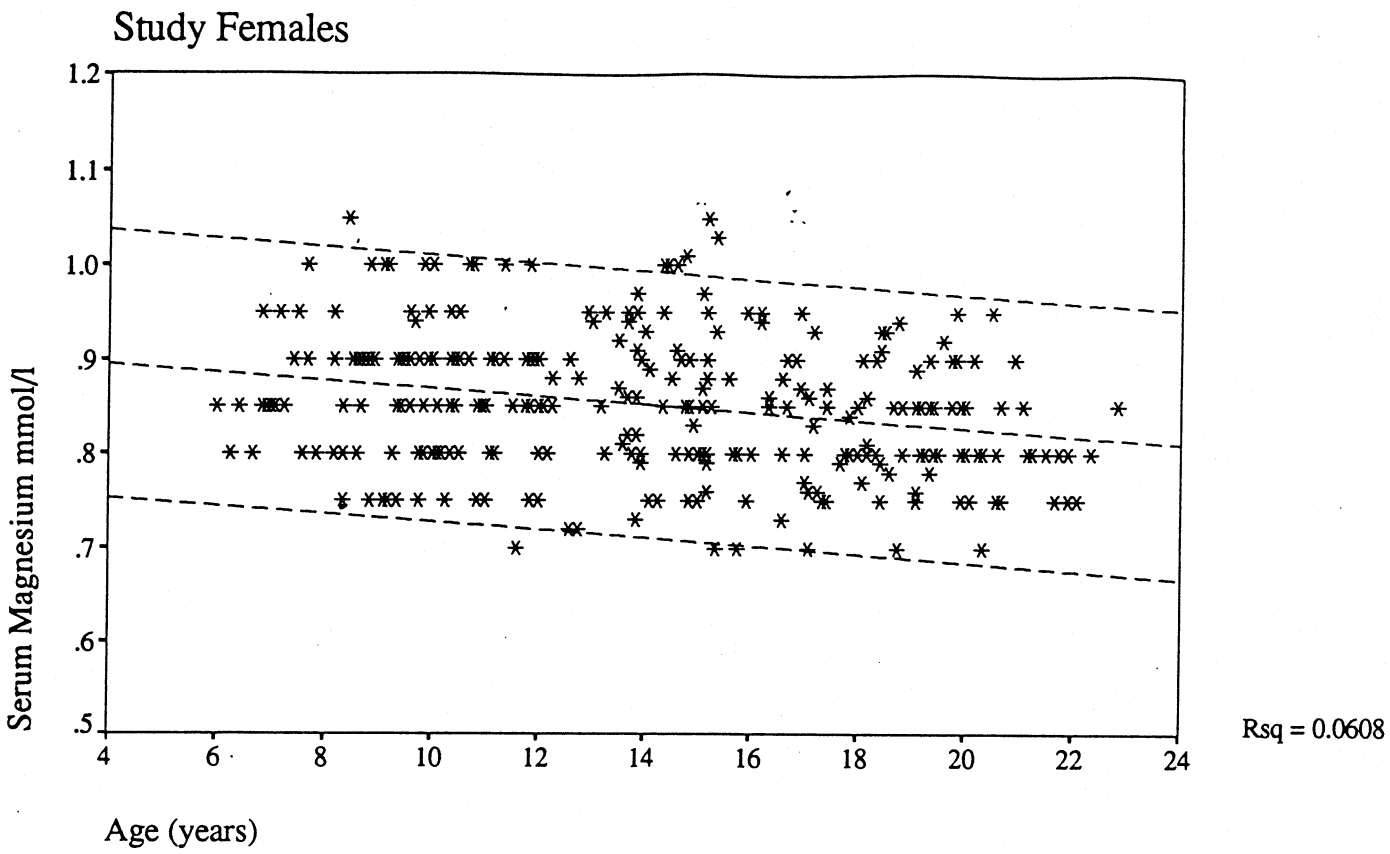


Fig 4.16 Serum Magnesium Values for Study Females (Confidence Interval 95%)

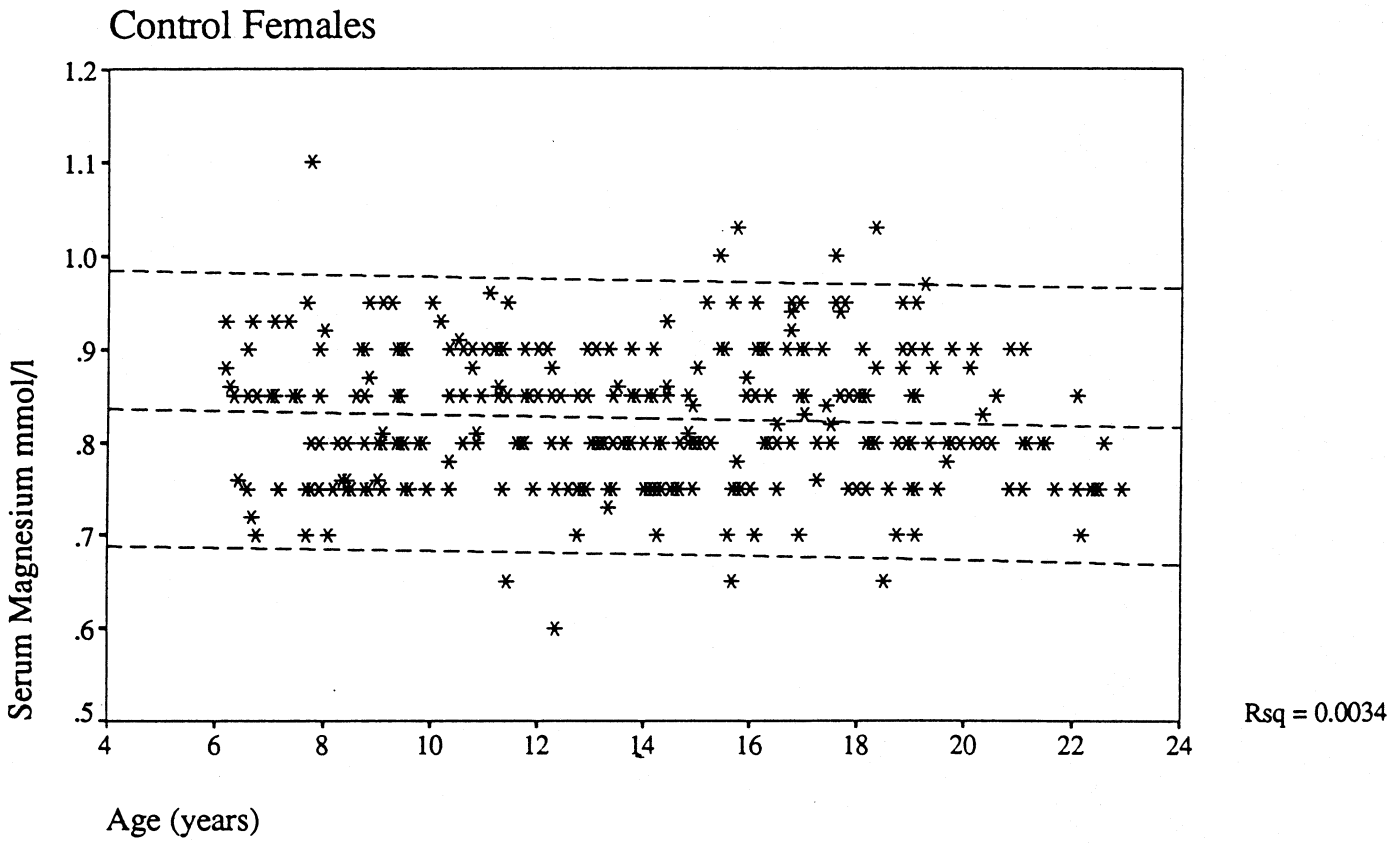


Fig 4.17 Serum Magnesium Values for Control Females (Confidence Interval 95%)

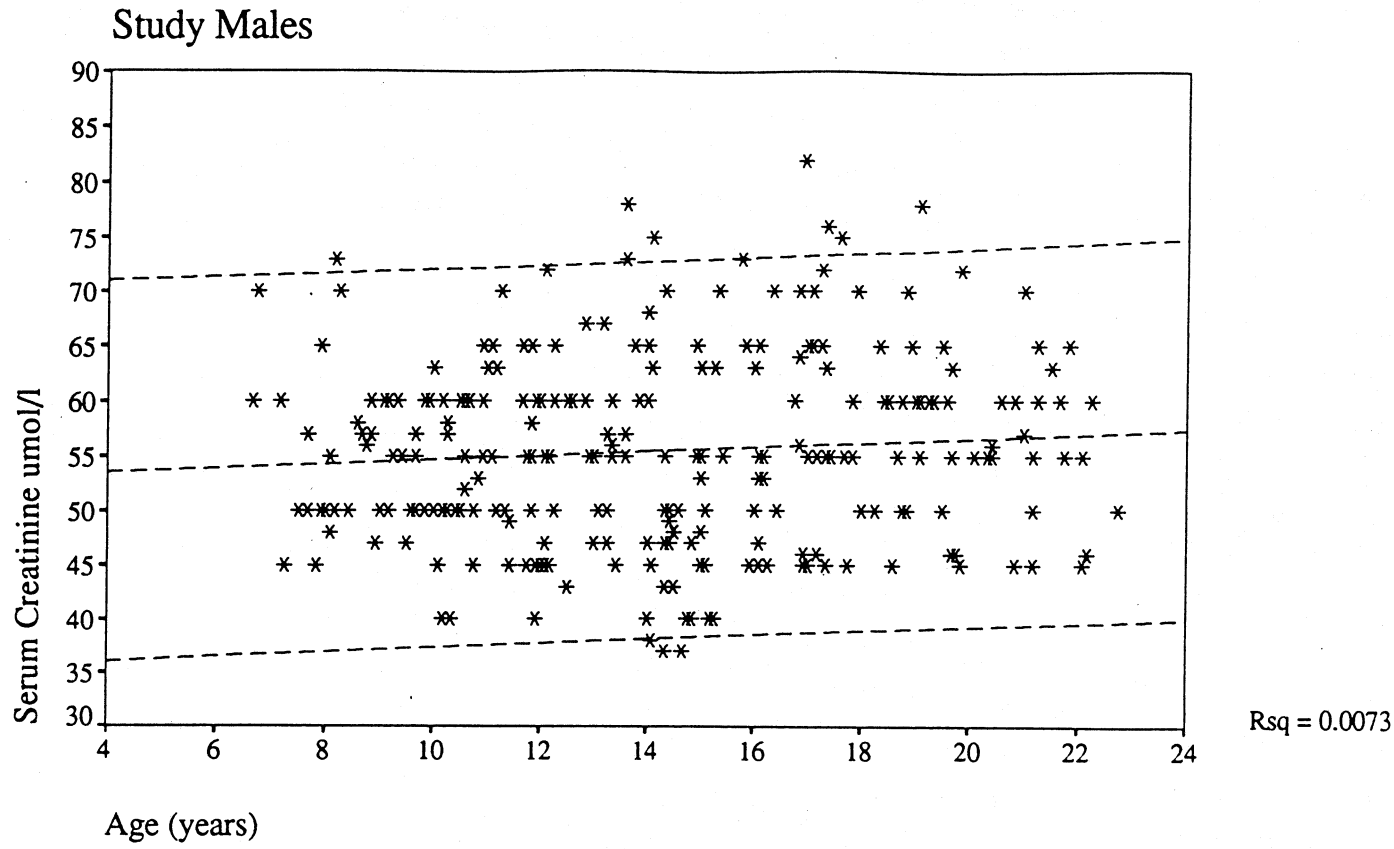


Fig 4.18 Serum Creatinine Values for Study Males (Confidence Interval 95%)

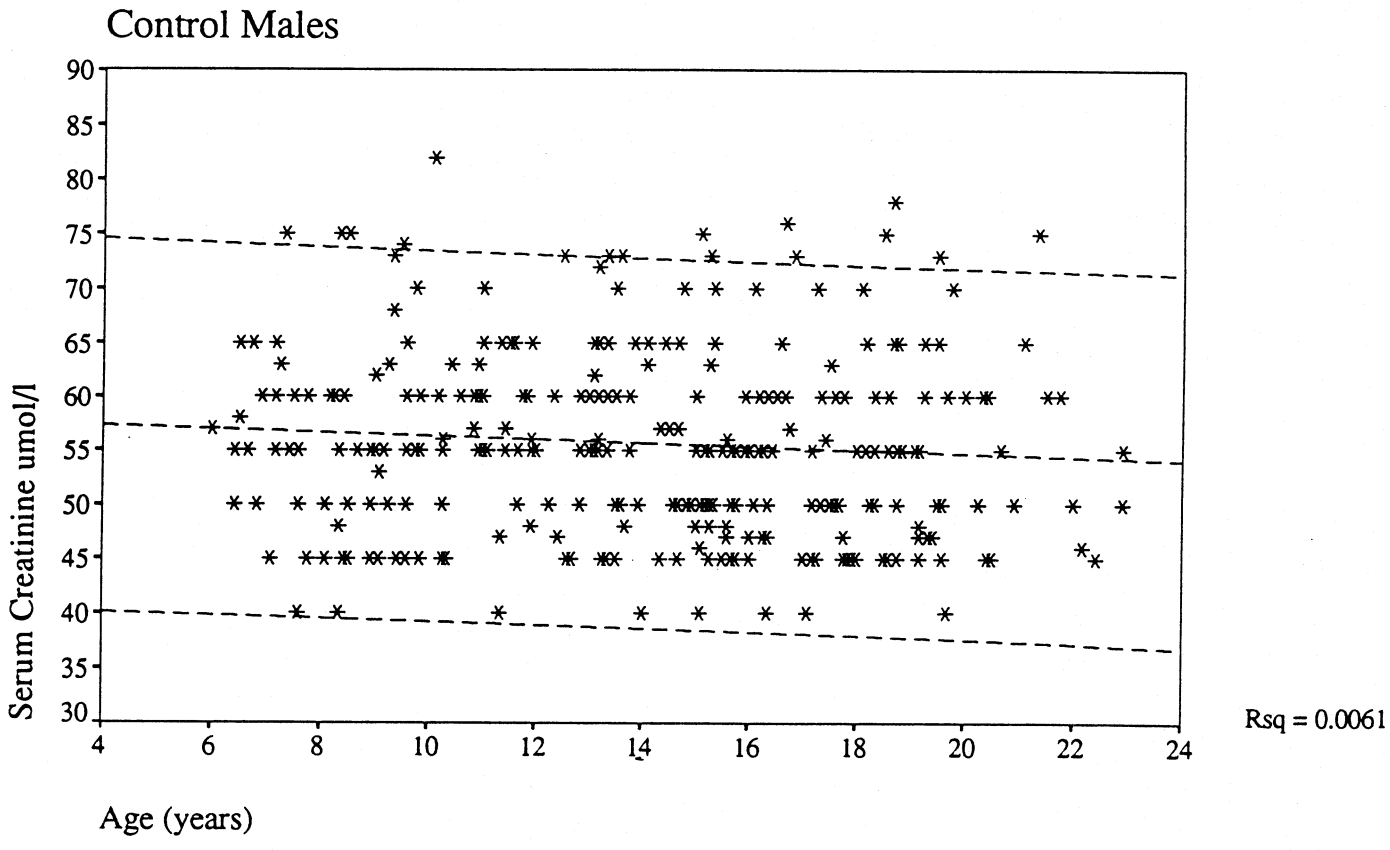


Fig 4.19 Serum Creatinine Values for Control Males (Confidence Interval 95%)

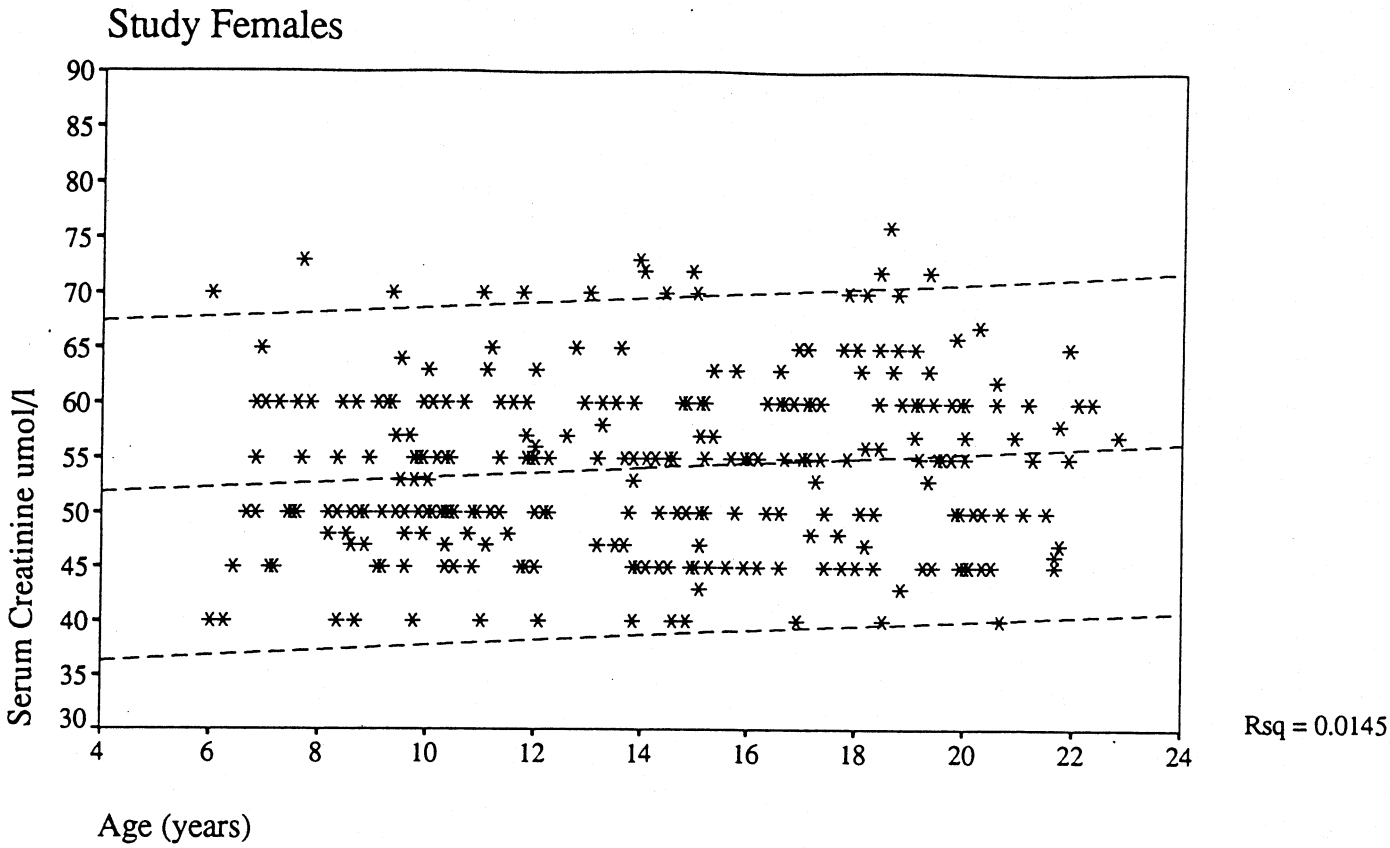


Fig 4.20 Serum Creatinine Values for Study Females (Confidence Interval 95%)

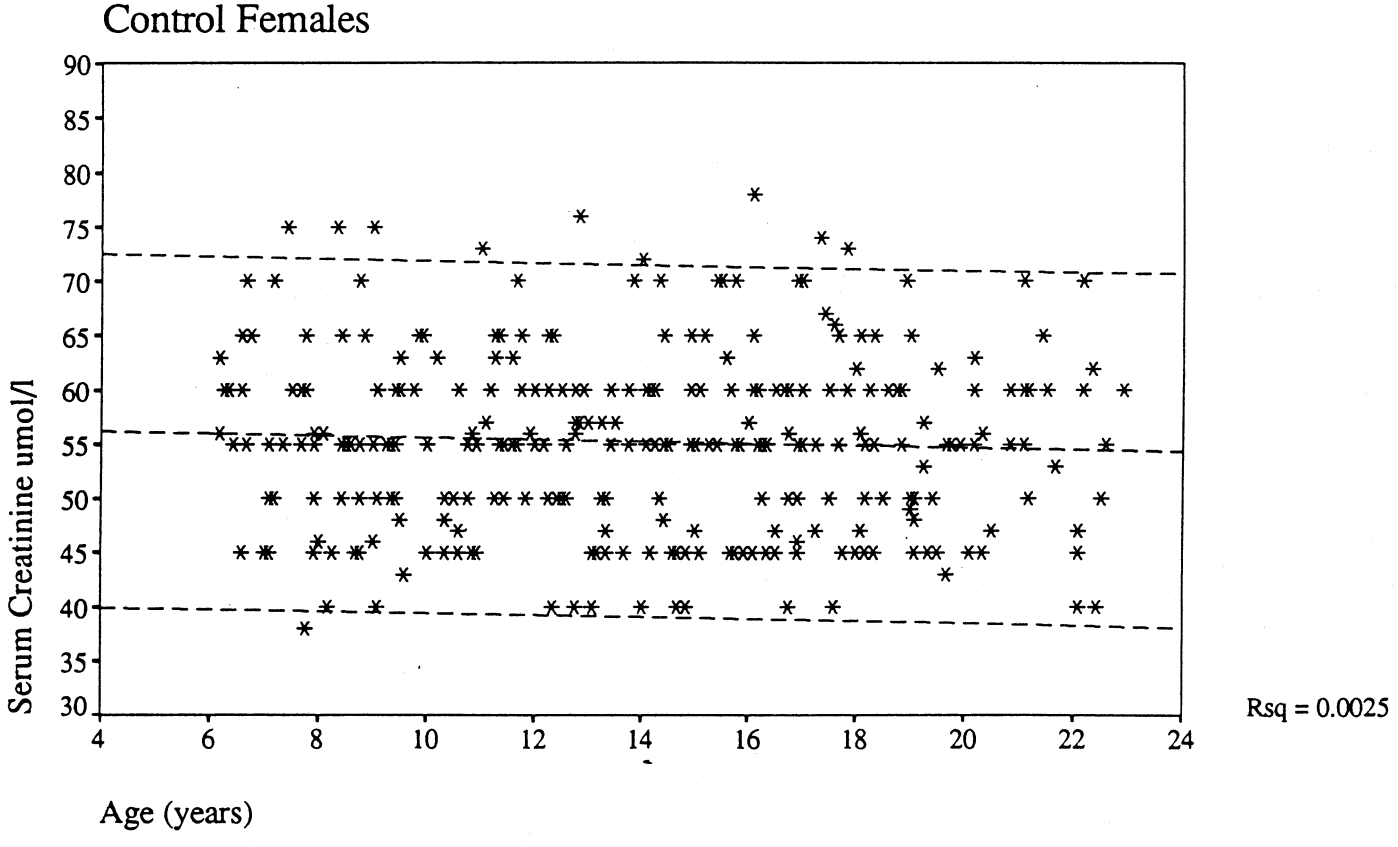


Fig 4.21 Serum Creatinine Values for Control Females (Confidence Interval 95%)

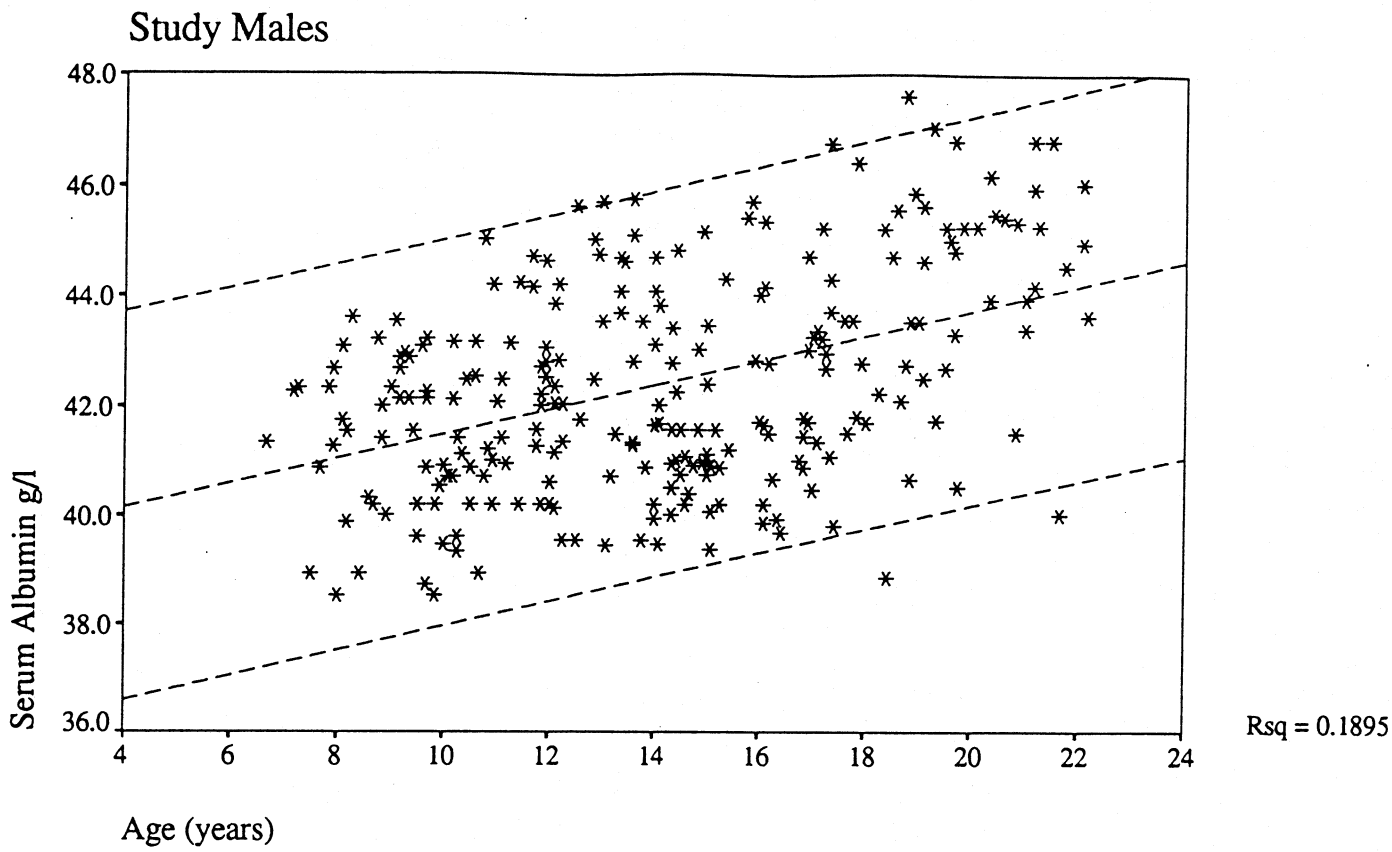


Fig 4.22 Serum Albumin Values for Study Males (Confidence Interval 95%)

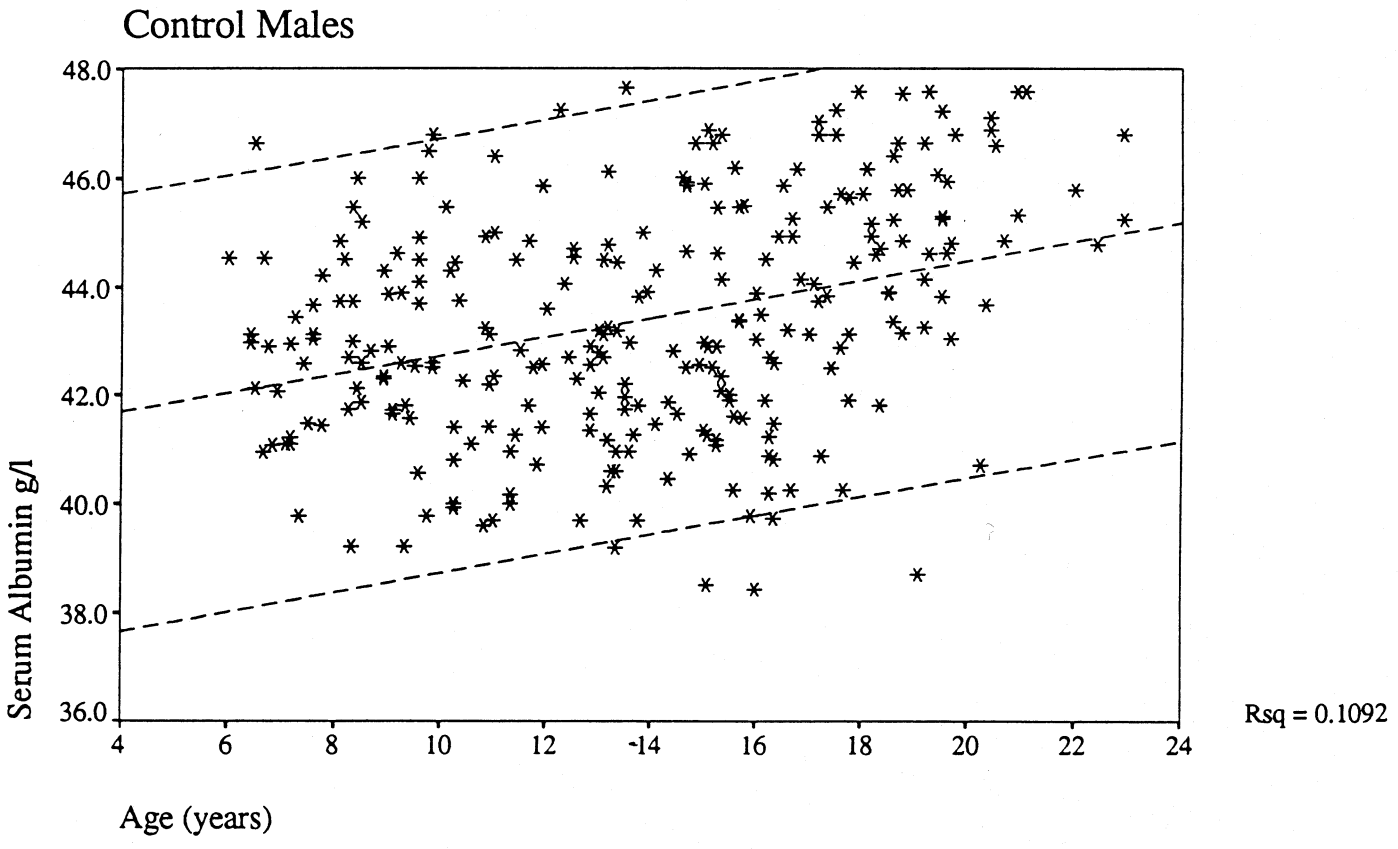


Fig 4.23 Serum Albumin Values for Control Males (Confidence Interval 95%)

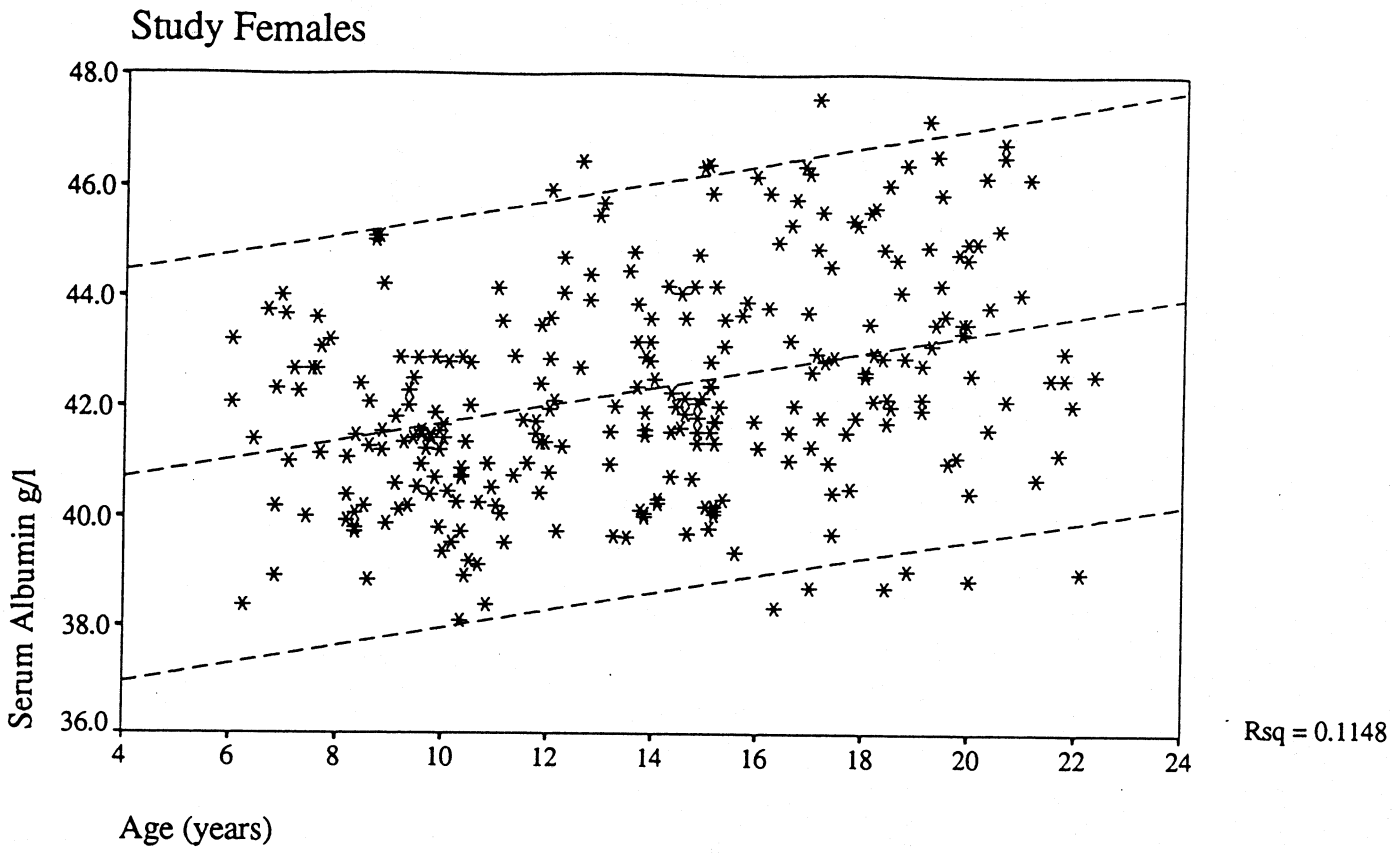


Fig 4.24 Serum Albumin Values for Study Females (Confidence Interval 95%)

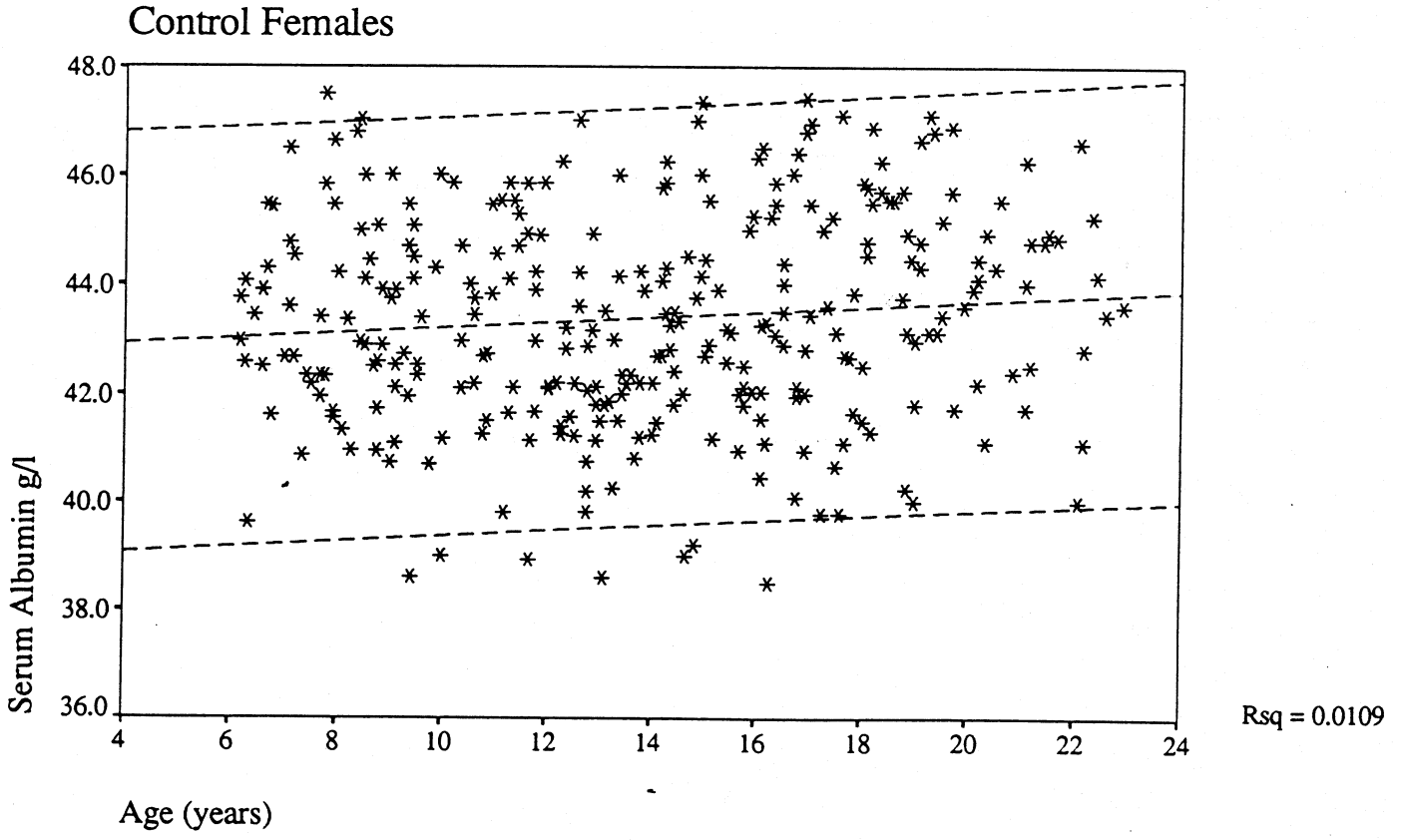


Fig 4.25 Serum Albumin Values for Control Females (Confidence Interval 95%)

e. Serum Phosphate and Alkaline Phosphatase

Serum phosphate and alkaline phosphatase are influenced by the onset of puberty (Whitby et al., 1993). Normal serum values decrease rapidly during infancy and more slowly during childhood. In puberty levels rise and then start to fall towards adult levels as growth slows down. When age groups of the study and control groups were included in the analysis of covariance (ANCOVA) there were significant differences. Serum alkaline phosphatase was significantly different between males of the study and control groups (p=0.0001) and females of these two groups (p=0.0001). This trend was also true for serum phosphate where a signficiant difference was seen between males of the two groups (p=0.0001) and females of the two groups (p=0.0001).

In order to determine the age at which the variables start to fall, values in one age group were compared with those of the next age group using the student t-test. More than one age group showed significant differences from the preceding group. The age at which the greatest p-value was achieved in combination with the graphic trends was used as the cut-off age for each variable. The student t-test was the appropriate test, even though the data are not completely normally distributed, because normality can be assumed due to the size of the samples tested ((Central Limit Theorem) Voelker and Orton, 1993).

Table 4.22 Inter-Age Group t-tests for Serum Phosphate and Alkaline Phosphatase: Study Females

Age Group	Alk Phos	p value	PO_4	p value
6-7 8-9	338 304	0.17	1.82 1.75	0.12
8-9 10-11	304 352	0.03	1.75 1.65	0.012
10-11 12-13	352 234	0.0001 ****	1.65 1.71	0.18
12-13 14-15	234 182	0.013	1.71 1.53	0.0006 ****
14-15 16-17	182 112	0.0002	1.53 1.52	0.91
16-17 18-19	112 88	0.03	1.52 1.38	0.03
18-19 20-22	88 98	0.206	1.38 1.16	0.0001

**** p value used to create cut-off

Table 4.22a Inter-Age Group t-tests for Serum Phosphate and Alkaline Phosphatase: Control Females

Age Group	Alk Phos	p value	PO_4	p value
6-7 8-9	222.8 197.9	0.04	1.75 1.55	0.0005
8-9 10-11	197.9 185.7	0.31	1.55 1.51	0.28
10-11 12-13	185.7 196.1	0.45	1.51 1.51	0.94
12-13 14-15	196.1 151.9	0.005***	1.51 1.44	0.05***
14-15 16-17	151.9 108.5	0.002	1.44 1.31	0.004
16-17 18-19	108.5 71.4	0.001	1.31 1.20	0.01
18-19 20-22	71.4 65.4	0.49	1.20 1.23	0.52

**** p value used to create cut-off

Table 4.23 Inter-Age Group t-tests for Serum Phosphate and Alkaline Phosphatase: Study Males

Age Group	Alk Phos	p value	PO_4	p value
6-7 8-9	317 311	0.82	1.76 1.67	0.22
8-9 10-11	311 307	0.90	1.67 1.65	0.66
10-11 12-13	307 230	0.003	1.65 1.68	0.586
12-13 14-15	230 297	0.008	1.68 1.62	0.18
14-15 16-17	297 286	0.70	1.62 1.56	0.327
16-17 18-19	286 153	0.001****	1.56 1.38	0.004***
18-19 20-22	153 130	0.27	1.38 1.15	0.0001

**** p value used to create cut-off

Table 4.23a Inter-Age Group t-tests for Serum Phosphate and Alkaline Phosphatase: Control Males

Age Group	Alk Phos	p value	PO_4	p value
6-7 8-9	195.8 181.2	0.24	1.75 1.57	0.0005
8-9 10-11	181.2 208.5	0.05	1.57 1.58	0.82
10-11 12-13	208.5 191.1	0.26	1.58 1.51	0.10
12-13 14-15	191.1 199.7	0.60	1.51 1.51	0.93
14-15 16-17	199.7 209.3	0.64	1.51 1.51	0.99
16-17 18-19	209.3 129.0	0.0001****	1.51 1.26	0.0001****
18-19 20-22	129.0 110.2	0.45	1.26 1.20	0.29

**** p value used to create cut-off

In combination with graphic trends (Figs 4.26-4.29) this testing revealed the age at which alkaline phosphatase started to decline significantly to be between 11 and 13 years in study females (Table 4.22) and 17 and 19 years in study males (Table 4.23). Using the same procedure for serum phosphate values, the cut-off points were 13 and 15 years in study females and 17 and 19 in study males (Tables 4.22 and 4.23, respectively). A similar pattern was seen in the control group males and females for both alkaline phosphatase and serum phosphates (Tables 4.22a and 4.23a).

e. i. Serum Phosphate

Mean serum phosphate levels were significantly higher in the study group than in the control group in both males and females under the ages of 18 and 14 respectively. A similar difference was found in the girls over 14 years of age, but there was no difference between the study and control groups in boys over 18 years of age.

Table 4.24 Serum Phosphate Values for Females in the Study and Control Groups.

Group & Age	n	Mean Phosphate mmol/l	Std Dev.	Range	p value
Study ≤ 14 y	143	1.72	0. 19	1.3-2.10	
Control ≤ 14 y	156	1.57	0.21	1.00-2.10	0.0001
Study >14 y	141	1.42	0.28	0.75-2.30	
Control >14 y	145	1.31	0.20	0.77-1.85	0.0003

Table 4.25 Serum Phosphate Values for Males in the Study and Control Groups.

Group & Age	n	Mean Phosphate mmol/l	Std Dev.	Range	p value
Study ≤ 18 y	213	1.64	0.21	1.00-2.10	0.0001
Control ≤ 18 y	235	1.56	0.22	0.98-2.08	
Study >18 y	50	1.25	0.22	0.85-1.90	0.77
Control >18 y	59	1.23	0.19	0.92-1.70	

Tables 4.26 and 4.27 compare the serum phosphate values in each age group between boys and girls. Mean serum phosphate levels were similar between males and females within the same age groups for the study (Table 4.26) and control groups (Table 4.27), except in the 14-15 year olds of the study group where the difference almost reached significance, and 16-17 year olds in the control group, where males and females were significantly different (Figs 4.26-4.27).

The normal range of phosphate concentration prior to puberty is 1.3-2.8 mmol/l (Walmsley and White, 1988). The control females ≤ 14 years of age had 12 cases with levels of phosphate levels below the 1.3 mmol/l concentration but there were no hypophosphatemic study females in this age

group. Males ≤ 18 years of age had 10 cases of hypophosphatemia in the study group and 26 cases in the control group, but this difference was not significant (p=0.75).

When analysis of variance was carried out controlling for the effect of age on serum phosphate concentrations for both males and females in both groups, the study group showed a significantly higher mean (Ls mean = 1.62 and Ls mean =1.63 respectively) compared with the control group (Ls mean =1.53 and Ls mean =1.46 respectively) (p=0.0001).

Table 4.26 Mean Serum Phosphate by Age Group and Sex: Study Group

Group	n	Mean PO_4	Std Dev.	p value
6-7				
Male	9	1.76	0.17	0.41
Female	18	1.82	0.13	
8-9				
Male	34	1.67	0.18	0.05
Female	46	1.75	0.16	
10-11				
Male	46	1.65	0.21	0.90
Female	41	1.65	0.20	
12-13				
Male	36	1.68	0.18	0.46
Female	37	1.71	0.20	
14-15				
Male	45	1.62	0.21	0.07
Female	47	1.53	0.26	
16-17				
Male	37	1.56	0.26	0.49
Female	30	1.52	0.26	
18-19				
Male	26	1.38	0.22	0.96
Female	40	1.38	0.27	
20-22				
Male	22	1.15	0.10	0.90
Female	25	1.16	0.12	

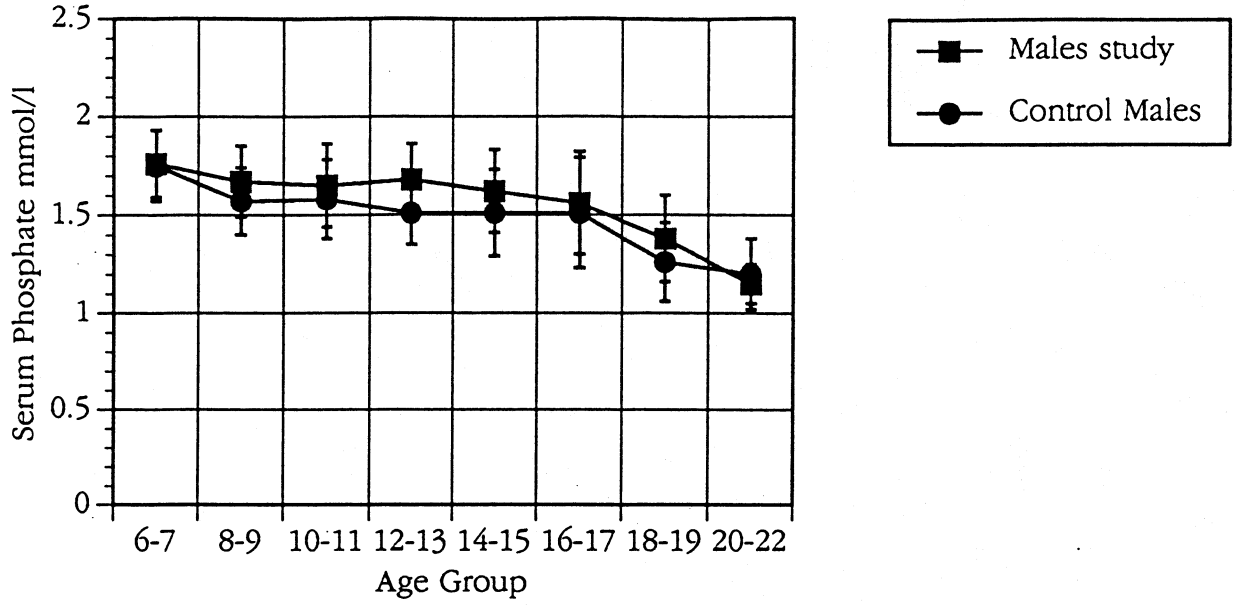


Fig 4.26 Serum Phosphate Values for Study and Control Males

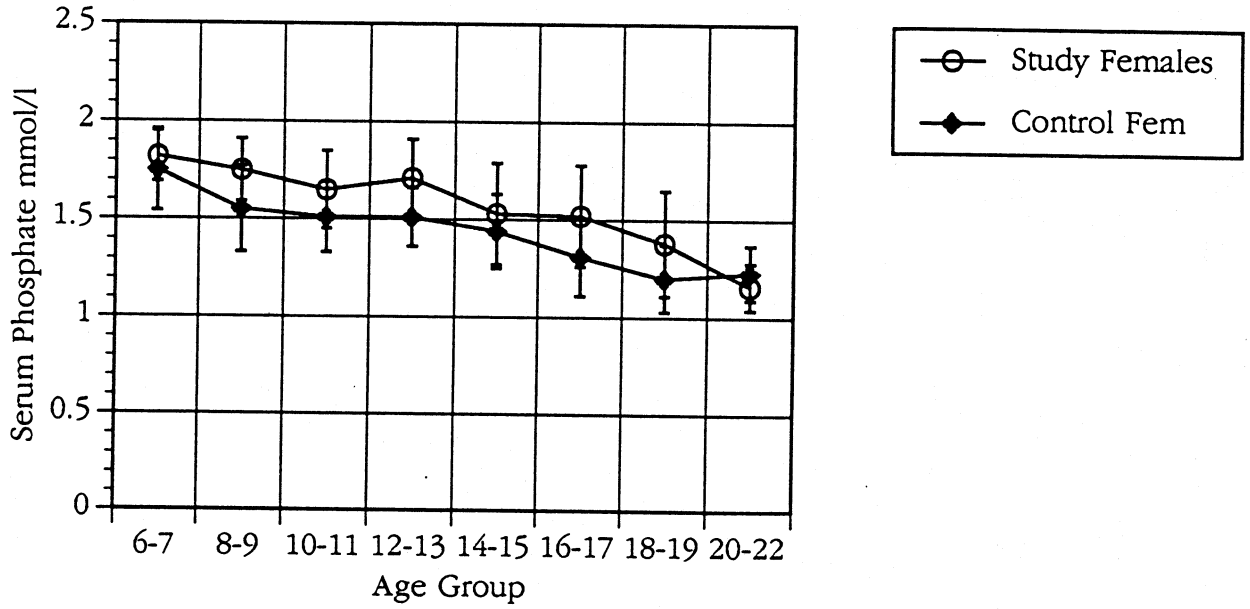


Fig 4.27 Serum Phosphate Values for Study and Control Females

Table 4.27 Mean Serum Phosphate by Age Group and Sex: Control Group

Group	n	Mean PO_4	Std Dev.	p value
6-7				
Male	23	1.75	0.18	0.86
Female	21	1.75	0.21	
8-9				
Male	41	1.57	0.17	0.67
Female	42	1.55	0.22	
10. 11				
Male	36	1.58	0.20	0.09
Female	38	1.51	0.18	
12-13				
Male	42	1.51	0.16	0.91
Female	43	1.51	0.15	
14-15				
Male	46	1.51	0.22	0.10
Female	45	1.44	0.19	
16-17				
Male	43	1.51	0.28	0.0004
Female	42	1.31	0.20	
18-19				
Male	43	1.26	0.20	0.22
Female	38	1.20	0.17	
20-22				
Male	18	1.20	0.18	0.55
Female	22	1.23	0.14	

e. ii. Alkaline Phosphatase

Mean serum alkaline phosphatase levels were significantly higher in the study group than in the control group. Significant differences were found between the study and control males (288 ± 124 IU/l vs. 197 ± 77 IU/l) (p=0.0001) and females of the two groups (328 ± 102 IU/l vs. 200 ± 53 IU/l) (p=0.0001) in the ≤ 18 and under ≤ 12 age categories respectively (Tables 4.29 and 4.28 and Figs 4.28 and 4.29).

Both groups had mean alkaline phosphatase values within normal limits (125-400 IU/l for pre-pubertal children (Whitby et al., 1993)). However, in the study group 70 (12.5%) children had serum alkaline phosphatase values over 400 IU/l compared to 4 cases (0.8%) in the control population (p=0.05, Kruskal-Wallis test Chi-square approx.).

In the study sample, 39 boys (<18 years) and 31 girls (<12 years) had elevated alkaline phosphatase values (>400 IU/l). In the control group, there were 4 males with elevated alkaline phosphatase and no females in the corresponding age groups.

Alkaline phosphatase levels were elevated above adult reference levels (> 125 IU/l) in the 28 male children (>18 years) in the study group and in 19 boys of the control group. This difference was not significant (p=0.23). Females of the two groups also had cases that were above adult reference ranges, 85 in the study group and 74 in the control group (p=0.74). However, interpretation of normal reference ranges for children during puberty is difficult as values rise and then fall, and the ages at which these changes occur are not clearly defined.

When analysis of covariance was carried out controlling for the effect of age on serum alkaline phosphatase concentrations for females in both groups, the study group showed a significantly higher mean (Ls mean =232 IU/l) compared with the control group (Ls mean =165 IU/l) (p=0.0001). When the same procedure was carried out on serum alkaline phosphatase concentrations for males in both groups, the study group also showed a significantly higher mean (Ls mean =274 IU/l) compared with the control group (Ls mean =187 IU/l) (p=0.0001).

Table 4.28 Mean Serum Alkaline Phosphatase Values for Females in the Study and Control Groups.

Group & Age	n	Mean Alk Phos IU/l	Std Dev.	Range	p value
Study ≤ 12 y	112	328	102	120-710	0.0001
Control ≤ 12 y	115	200	53	90-375	
Study >12 y	181	141	87	30-705	0.02
Control >12 y	198	123	76	30-365	

Table 4.29 Mean Serum Alkaline Phosphatase Values for Males in the Study and Control Group.

Group & Age	n	Mean Alk Phos IU/l	Std Dev.	Range	p value
Study ≤ 18 y	211	288	124	60-735	0.0001
Control ≤ 18 y	235	197	77	50-470	
Study >18 y	50	144	78	50-480	
Control >18 y	59	122	76	45-450	0.13

Mean alkaline phosphatase values were examined by age group and sex because of the strong pubertal influences on this variable. Mean alkaline phosphatase levels were significantly different between the sexes of the same group (study and control) at the onset of puberty (14-15 years old) with the exception of the control group where the 6-7 year olds showed a significant difference between sexes. From 14-15 year age group the differences between males and females carried on into the older age groups (Tables 4.30 and 4.31).

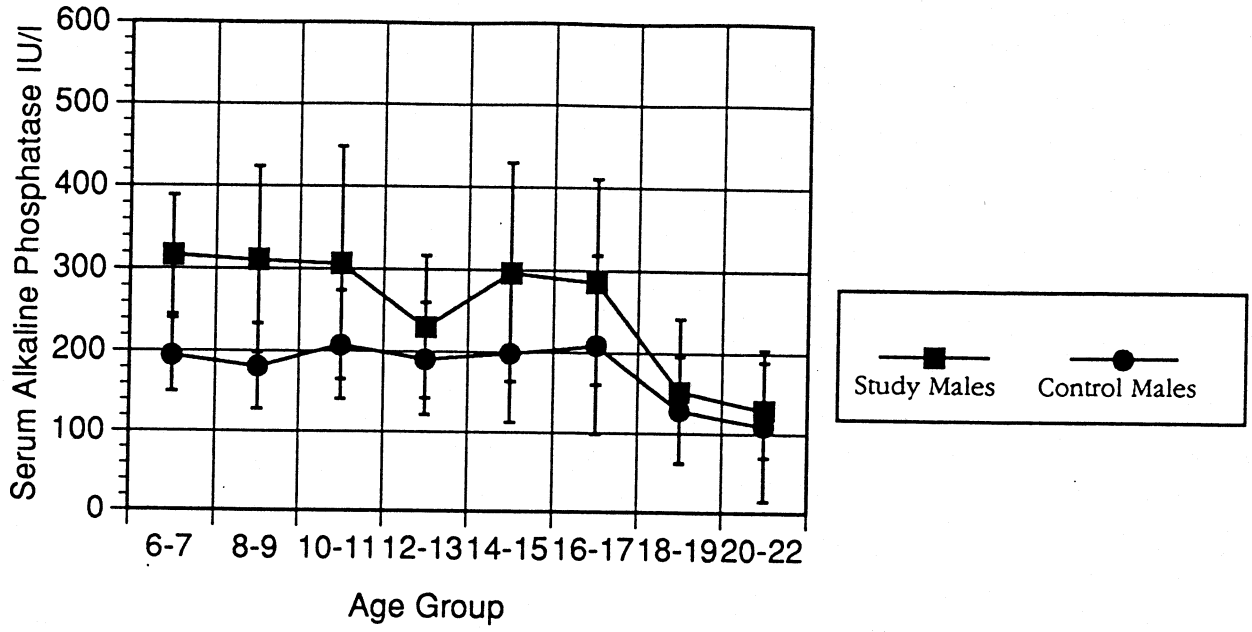


Fig 4.28 Mean Serum Alkaline Phosphatase in Study and Control Males

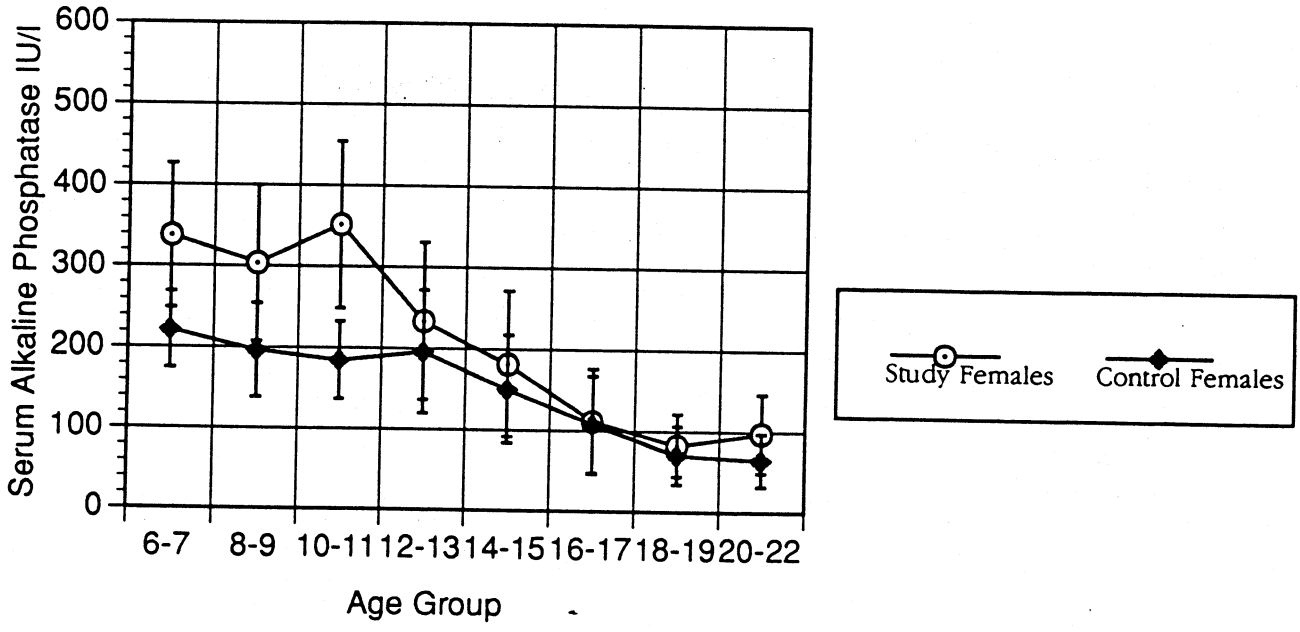


Fig 4.29 Mean Serum Alkaline Phosphatase in Study and Control Females

Table 4.30 Mean Alkaline Phosphatase Values for Males and Females of the Study Group

Group	n	Mean Alk Phos	Std Dev.	p value
6-7				
Male	9	317	72	0.49
Female	18	338	89	
8-9				
Male	32	311	113	0.80
Female	44	304	96	
10-11				
Male	46	307	141	0.09
Female	42	352	103	
12-13				
Male	37	230	87	0.85
Female	38	234	97	
14-15				
Male	45	297	133	0.0001
Female	48	182	90	
16-17				
Male	40	286	125	0.0001
Female	32	112	65	
18-19				
Male	27	153	90	0.0006
Female	41	83	39	
20-22				
Male	24	130	60	0.04
Female	28	98	49	

Table 4.31 Mean Alkaline Phosphatase Values for Males and Females of the Control Group

Group	n	Mean Alk Phos	Std Dev.	p value
6-7				
Male	23	195	45	0.03
Female	33	222	47	
8-9				
Male	42	181	53	0.17
Female	42	197	58	
10-11				
Male	36	208	67	0.10
Female	38	185	48	
12-13				
Male	42	191	69	0.75
Female	43	196	76	
14-15				
Male	47	199	86	0.004
Female	45	151	67	
16-17				
Male	43	209	110	0.0001
Female	43	108	60	
18-19				
Male	43	129	67	0.0001
Female	40	71	36	
20-22				
Male	18	110	95	0.07
Female	29	65	33	

4.4.2 Urine Results

Urinary values for calcium, fluoride, phosphorus and magnesium were expressed as creatinine ratios. The data were not normally distributed thus the Mann-Whitney test was used, differences between study and control groups (Tables 4.32 and 4.33) and between the sexes were tested (Tables 4.34 and 4.35).

a. Fluoride

As would be expected urinary fluoride:creatinine (mg/mmol) values were higher in the study group compared to controls for both the males and females (p=0. 0001). Males and females of the same group had similar fluoride:creatinine ratios for study and control groups (p=0.25 and p=0.76, respectively) (Tables 4.34 and 4.35 and Figs 4.30-4.33).

b. Calcium

Urinary calcium:creatinine values were lower in the study group than in the control group in both females (0.108 mmol/mmol in the study group vs. 0.139 mmol/mmol in the control group, p=0.004) and males (0.094 mmol/mmol in the study group vs. 0.113 mmol/mmol in the control group, p=0.05). Males and females of the study group had similar mean values (0.09 mmol/mmol and 0.108 mmol/mmol; p=0.16 respectively) while females of the control group had a higher mean calcium:creatinine ratio than males (0.139 mmol/mmol and 0.113 mmol/mmol; p=0.01 respectively) (Figs 4.34-4.37).

c. Phosphate

While urinary phosphate:creatinine levels were higher in children in the study group than in the control group, the difference was only significant in females (1.278 mmol/mmol vs. 1.116 mmol/mmol, respectively; p=0.015). Males and females of the study group had similar values while males of the control group had a higher phosphate:creatinine value than females (1.227 mmol/mmol and 0.116 mmol/mmol; p=0.03 respectively) (Figs 4.38-4.41).

d. Magnesium

Magnesium:creatinine levels were similar in the study and control groups. Values in males and females of each group were also similar with no statistical differences (Figs 4.42-4.45)

Table 4.32 Urine Values for Females in the Study and Control Groups

Group	n	Mean	Std Dev..	Mann -Whitney p value
F:Creat mg/mmol				
Study	300	1.356	0.978	0.0001*
Control	314	0.206	0.182	
Ca:Creat mmol/mmol				
Study	301	0.108	0.129	0.004*
Control	315	0.139	0.139	
PO4:Creat mmol/mmol				
Study	321	1.278	0.962	0.015*
Control	315	1.116	0.680	
Mg:Creat mmol/mmol				
Study	301	0.533	0.384	0.583
Control	315	0.550	0.369	

* = significant

Table 4.33 Urine Values for Males in the Study and Control Groups

Group	n	Mean	Std Dev.	Mann -Whitney p value
F:Creat mg/mmol				
Study	262	1.580	1.190	0.0001*
Control	301	0.212	0.192	
Ca:Creat mmol/mmol				
Study	263	0.094	0.111	0.050*
Control	299	0.113	0.121	
PO4: Creat mmol/mmol				
Study	291	1.273	0.975	0.503
Control	300	1.227	0.696	
Mg:Creat mmol/mmol				
Study	263	0.519	0.395	0.102
Control	299	0.572	0.363	

* = significant

Table 4.34 Urine Values for Males and Females in the Study Group

Group	n	Mean	Std Dev.	Mann -Whitney p value
F:Creat mg/mmol				
Males	262	1.58	1.190	0.25
Females	300	1.35	0.978	
Ca:Creat mmol/mmol				
Males	263	0.094	0. 111	0. 16
Females	301	0.108	0. 129	
PO4: Creat mmol/mmol				
Males	291	1.273	0.975	0.87
Females	321	1.278	0.962	
Mg:Creat mmol/mmol				
Males	263	0.519	0.395	0.64
Females	301	0.533	0.384	

Table 4.35 Urine Values for Males and Females in the Control Group

Group	n	Mean	Std Dev.	Mann -Whitney p value
F:Creat mg/mmol				
Males	301	0.212	1.192	0.76
Females	314	0.206	0. 182	
Ca:Creat mmol/mmol				
Males	299	0.113	0. 121	0. 01*
Females	315	0.139	0. 139	
PO4: Creat mmol/mmol				
Males	300	1.227	0.696	0.03*
Females	315	1.116	0.680	
Mg:Creat mmol/mmol				
Males	299	0.572	0.363	0.55
Females	315	0.550	0.369	

In the figures that follow (Figs 4.30 -4.45) the mean value for the group is represented by a dotted line (-------) drawn through the data.

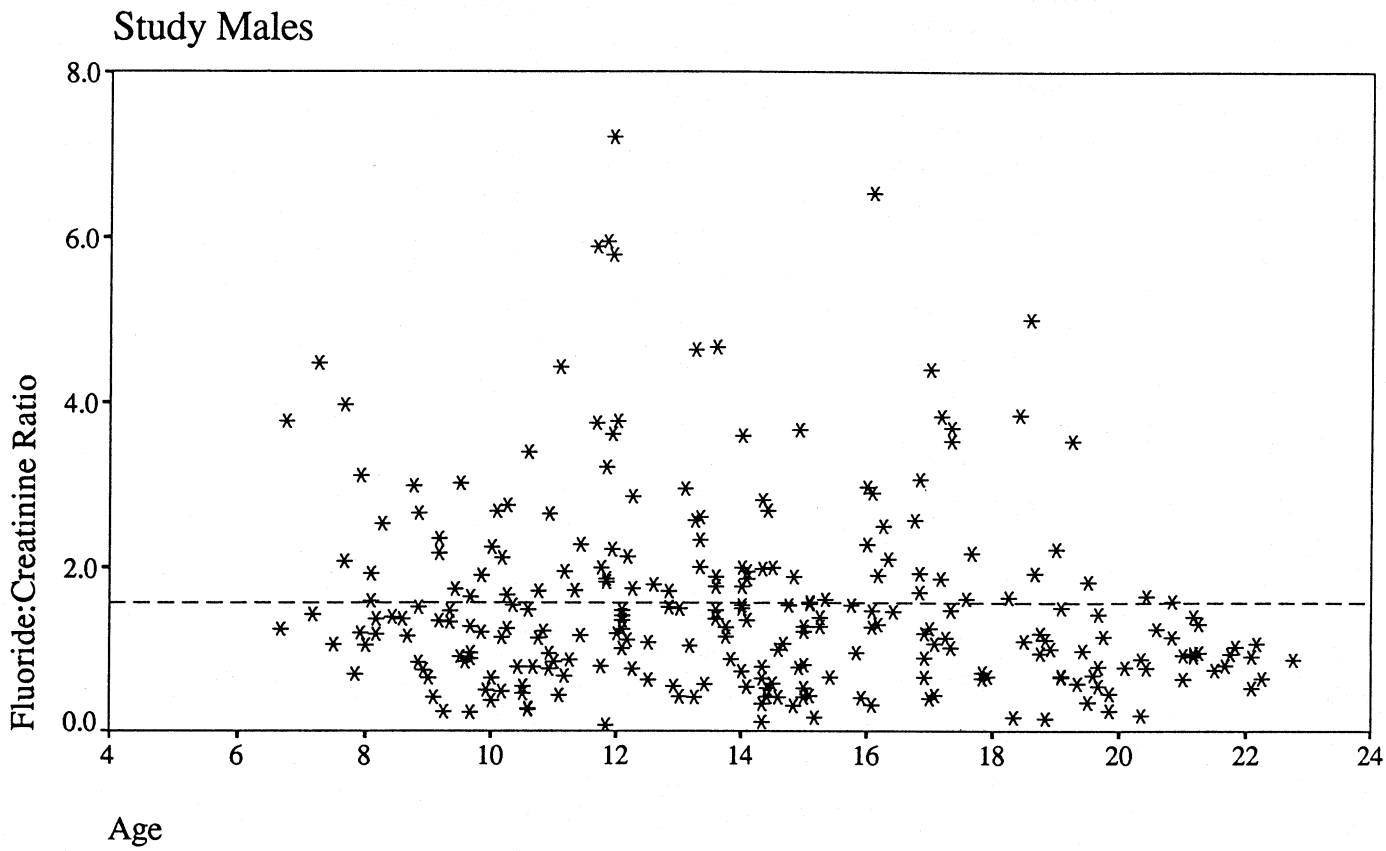


Fig 4.30 Urine Fluoride:Creatinine Values (mg/mmol) for Study Males

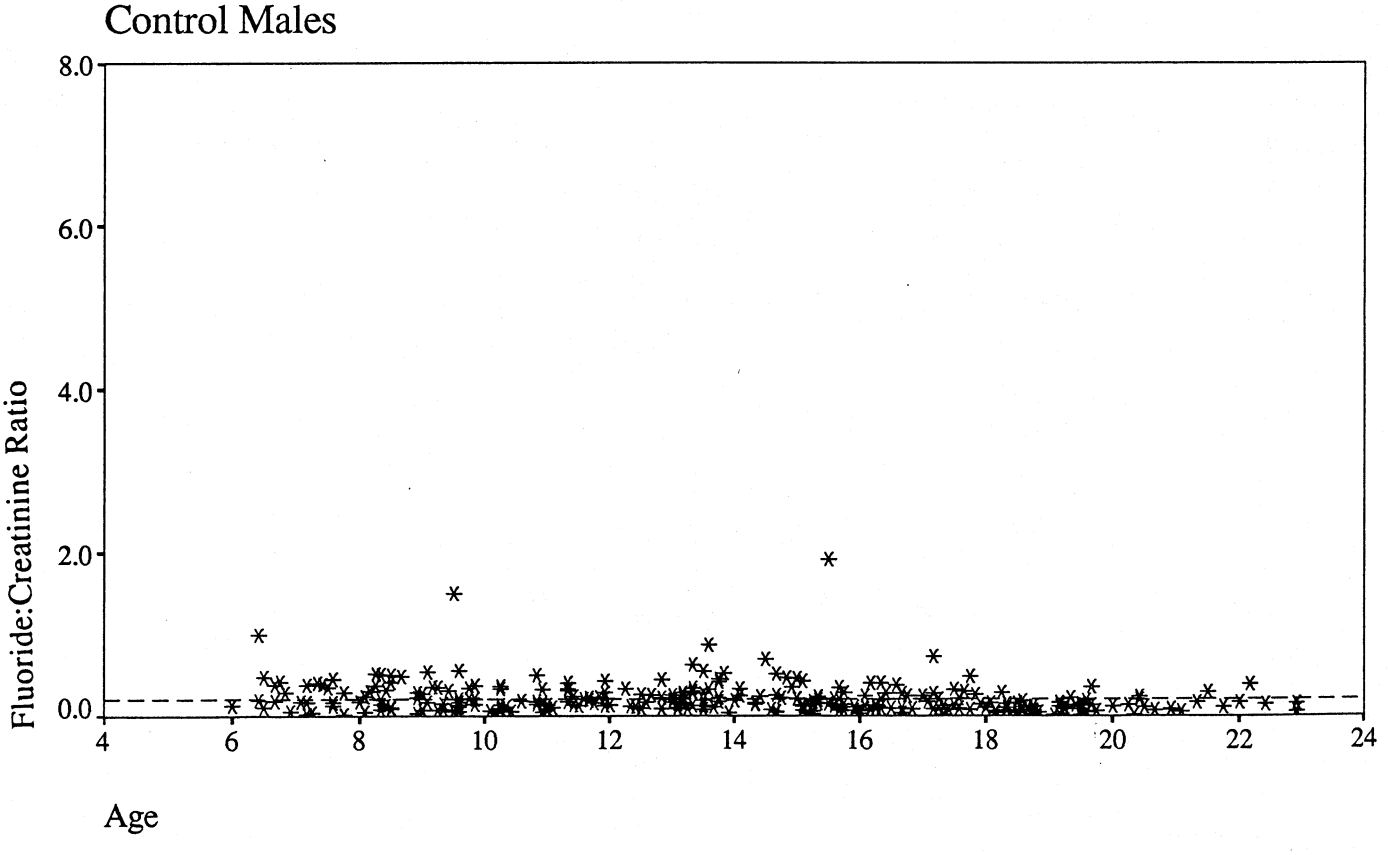


Fig 4.31 Urine Fluoride:Creatinine Values (mg/mmol) for Control Males

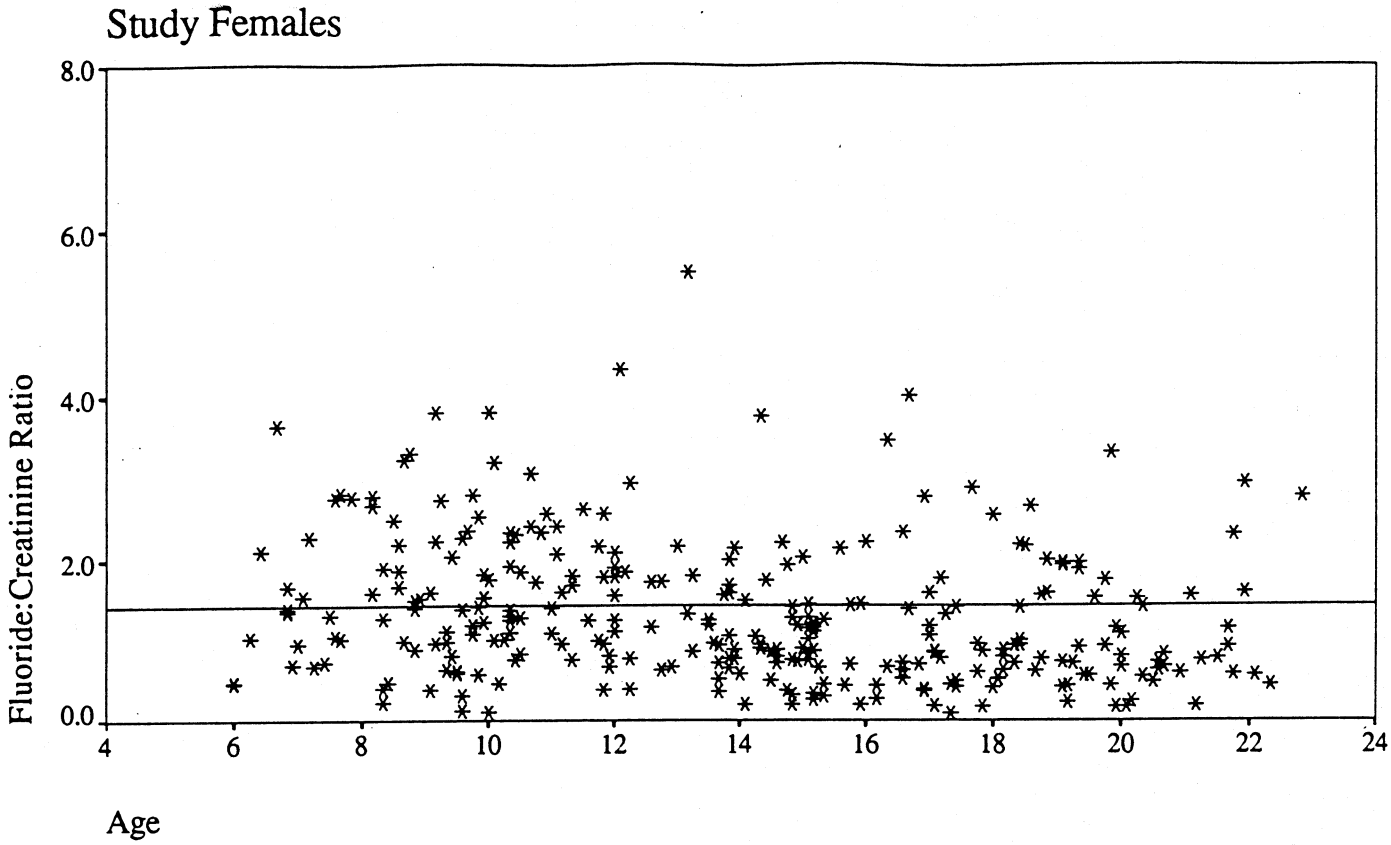


Fig 4.32 Urine Fluoride:Creatinine Values (mg/mmol) for Study Females

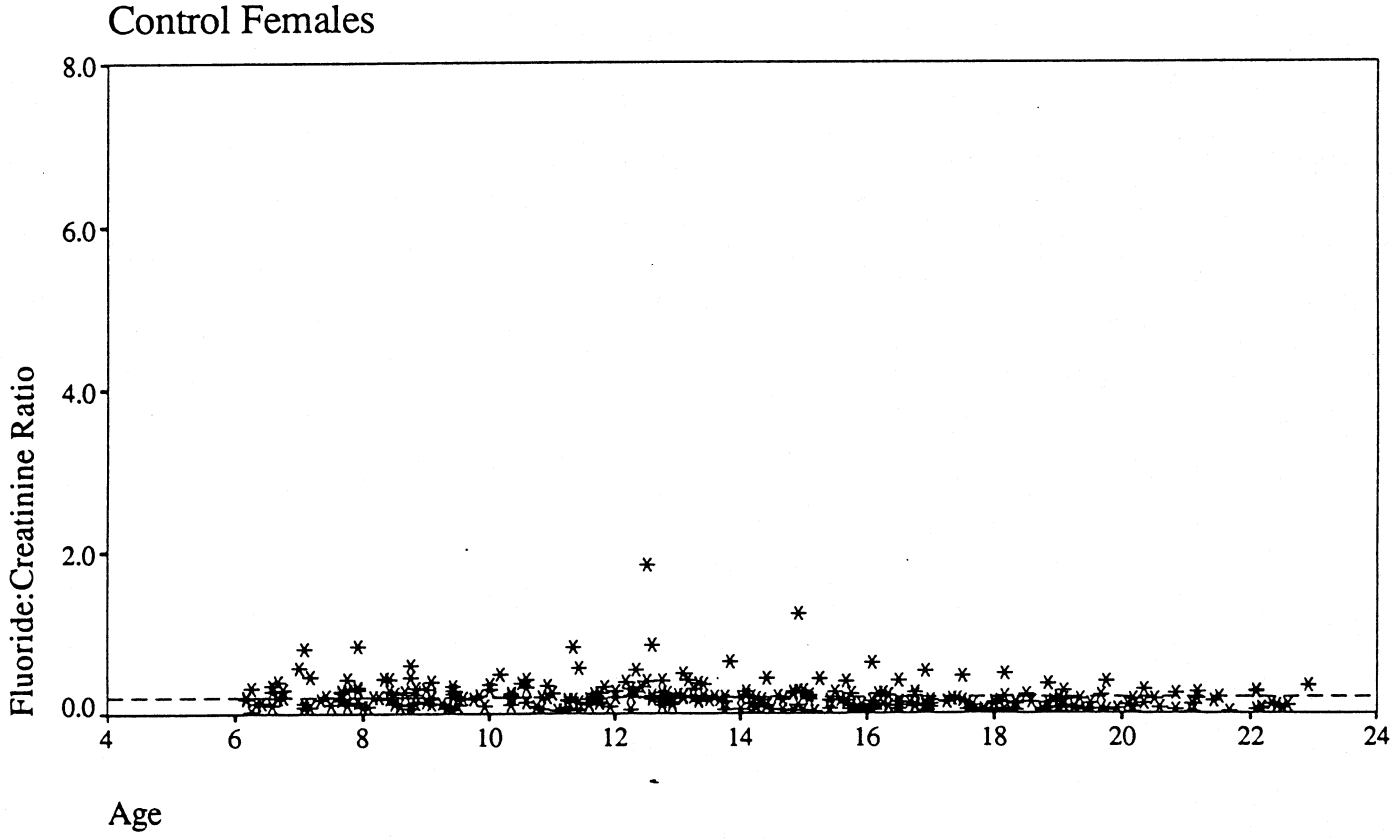


Fig 4.33 Urine Fluoride:Creatinine Values (mg/mmol) for Control Females

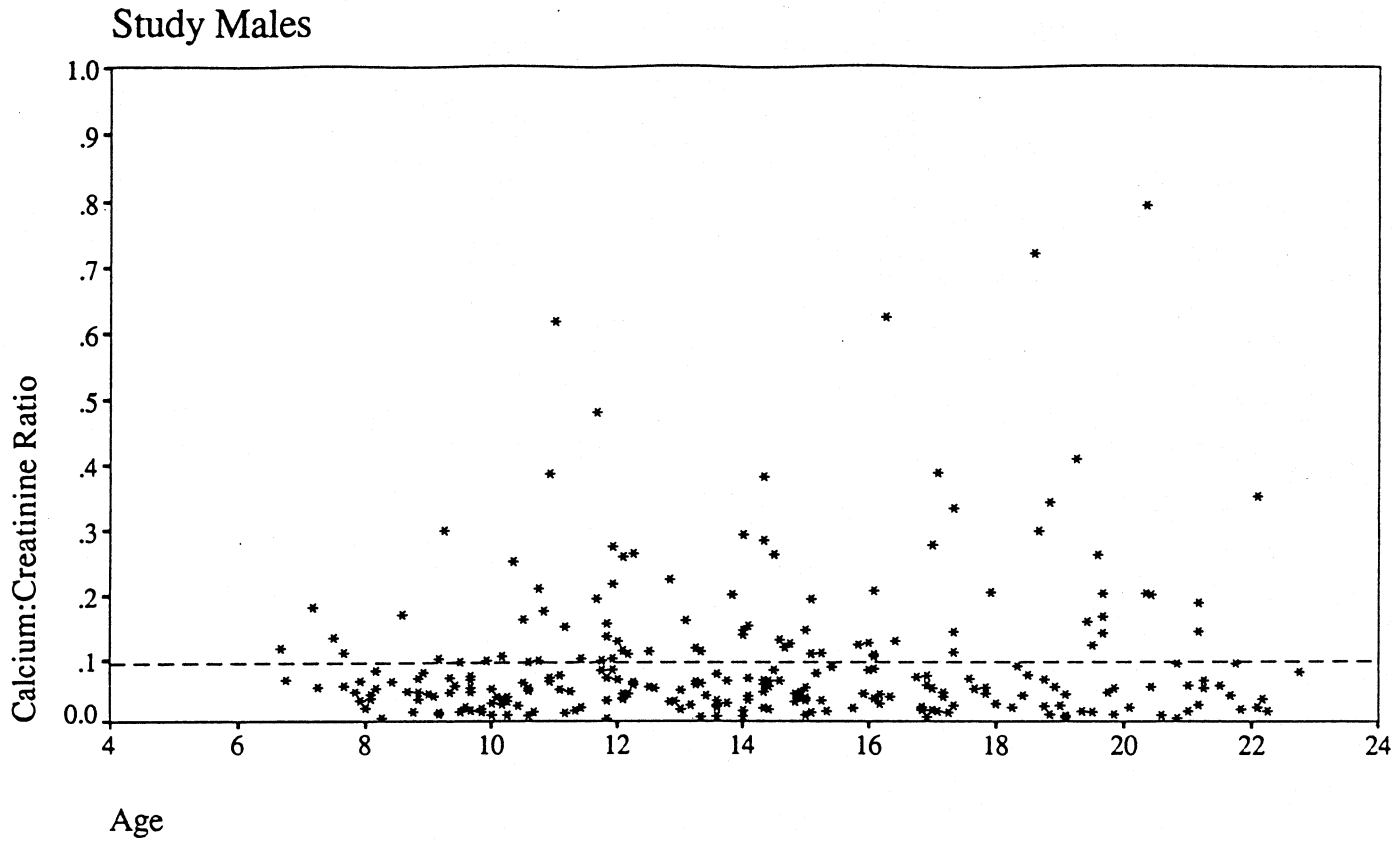


Fig 4.34 Urine Calcium:Creatinine Values (mmol/mmol) for Study Males

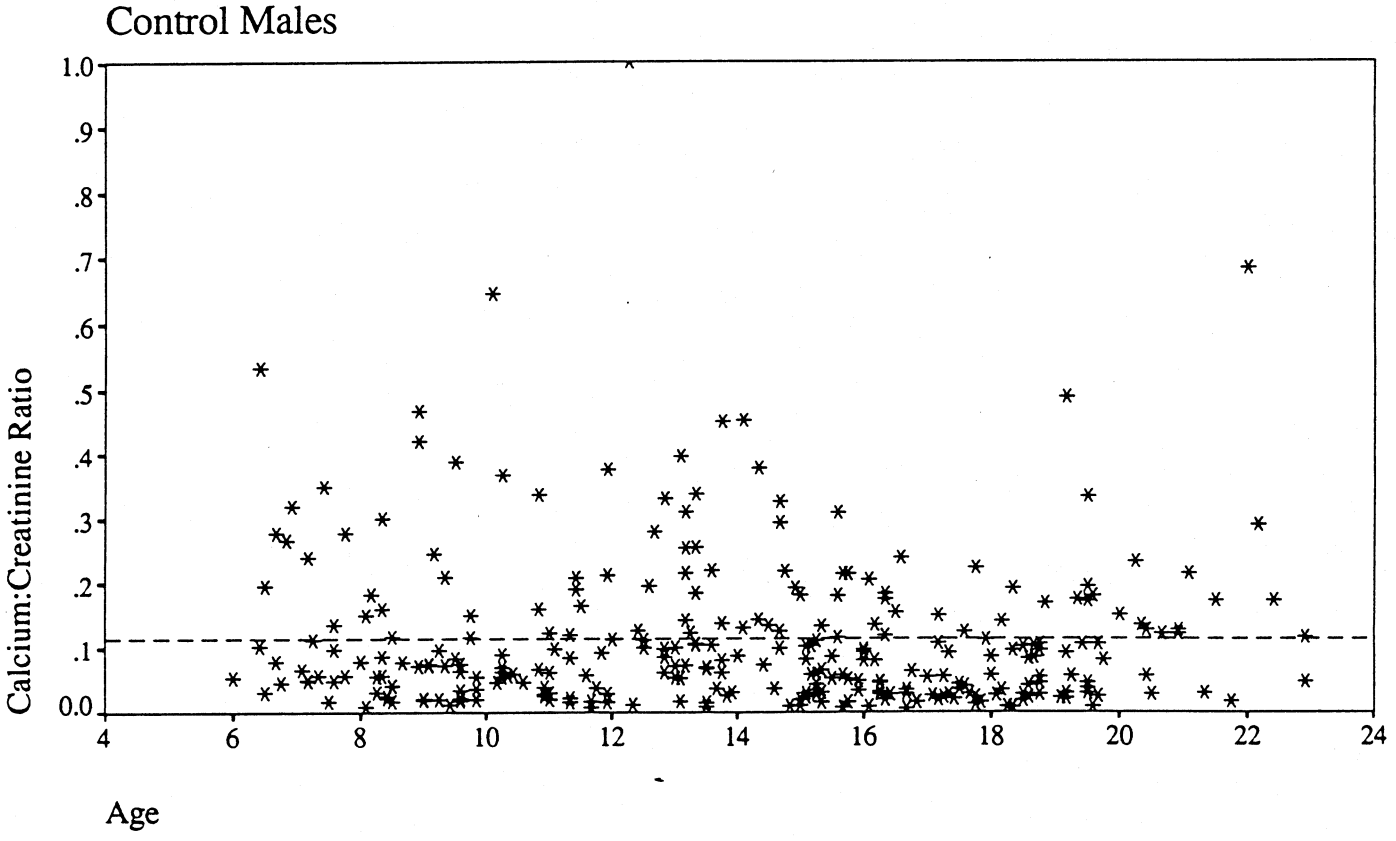


Fig 4.35 Urine Calcium:Creatinine Values (mmol/mmol) for Control Males

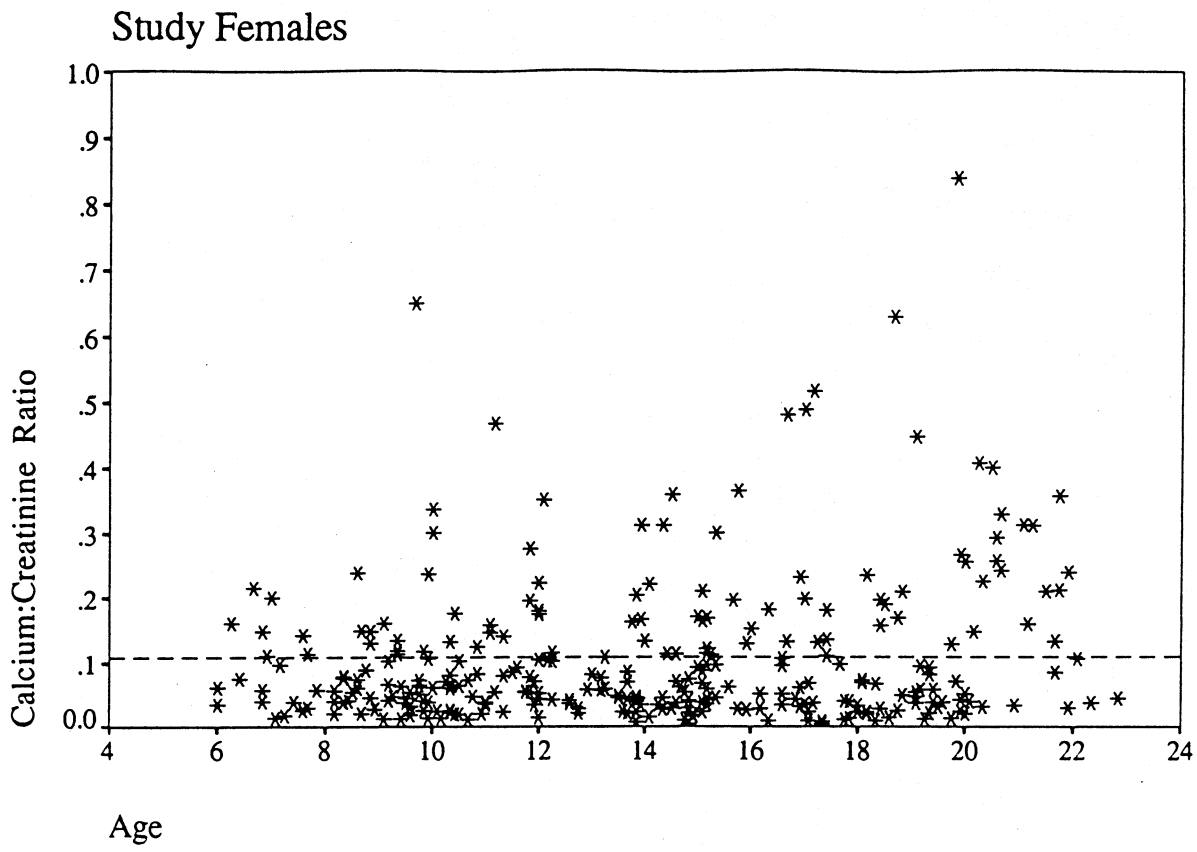


Fig 4.36 Urine Calcium:Creatinine Values (mmol/mmol) for Study Females

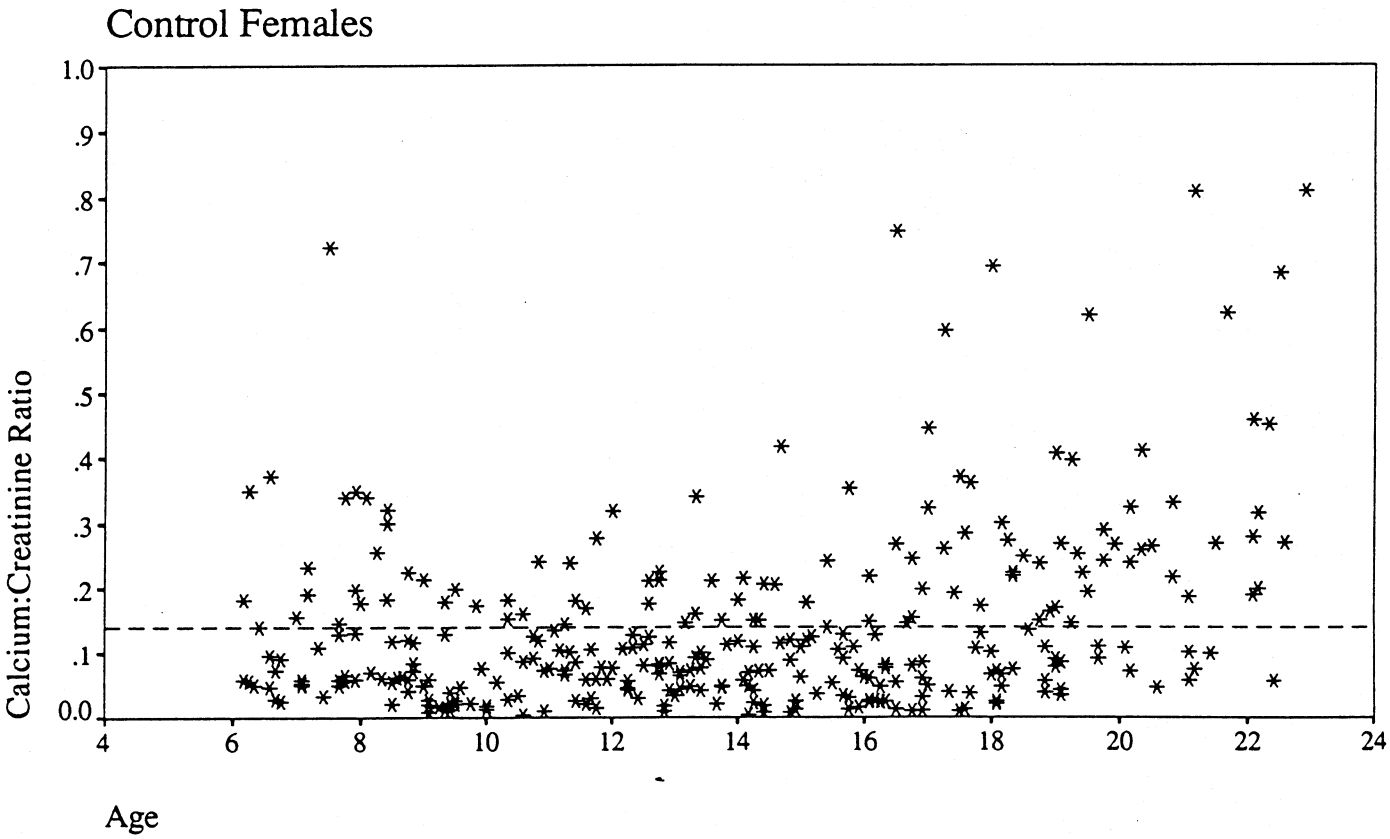


Fig 4.37 Urine Calcium:Creatinine Values (mmol/mmol) for Control Females

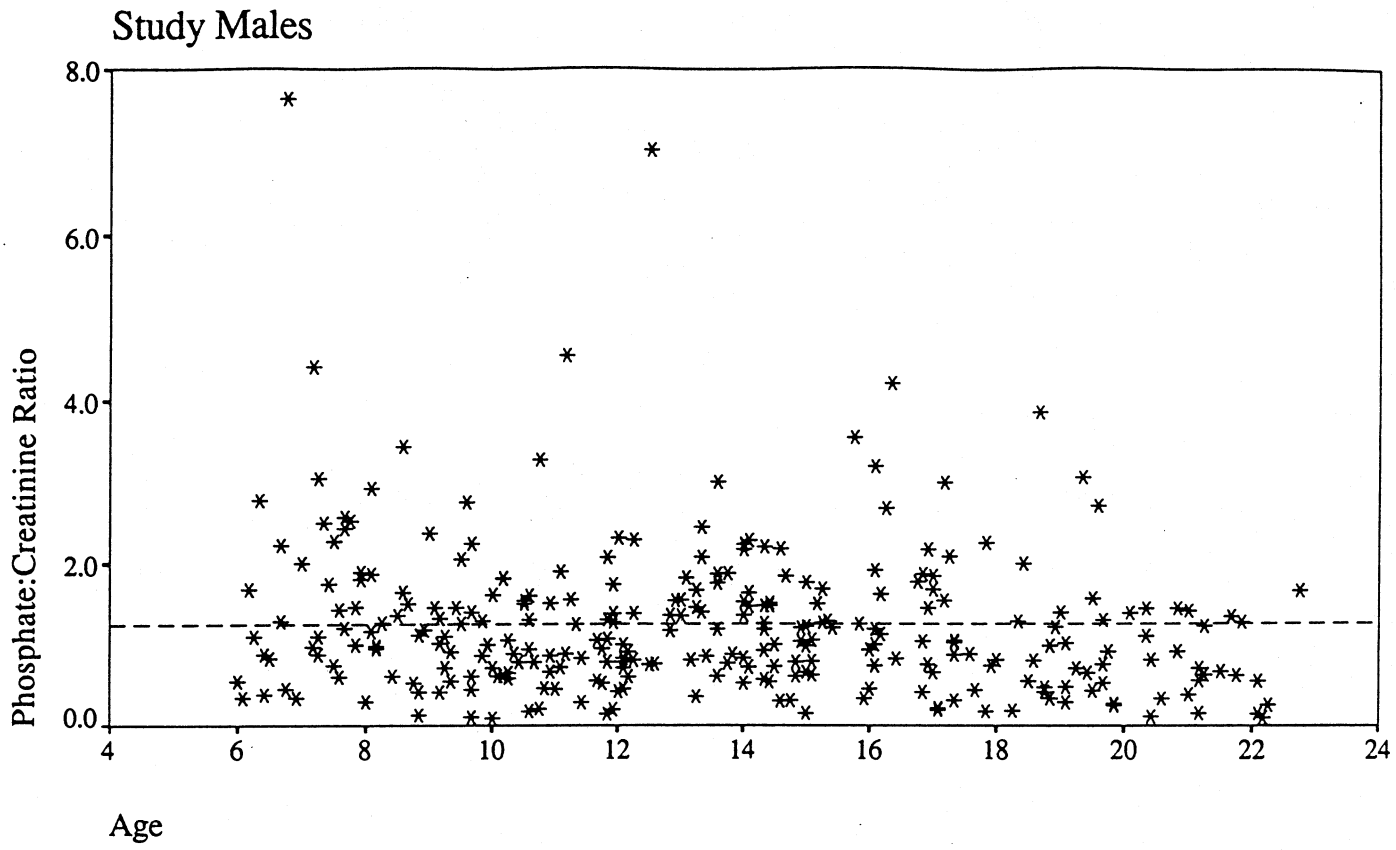


Fig 4.38 Urine Phosphate:Creatinine Values (mmol/mmol) for Study Males

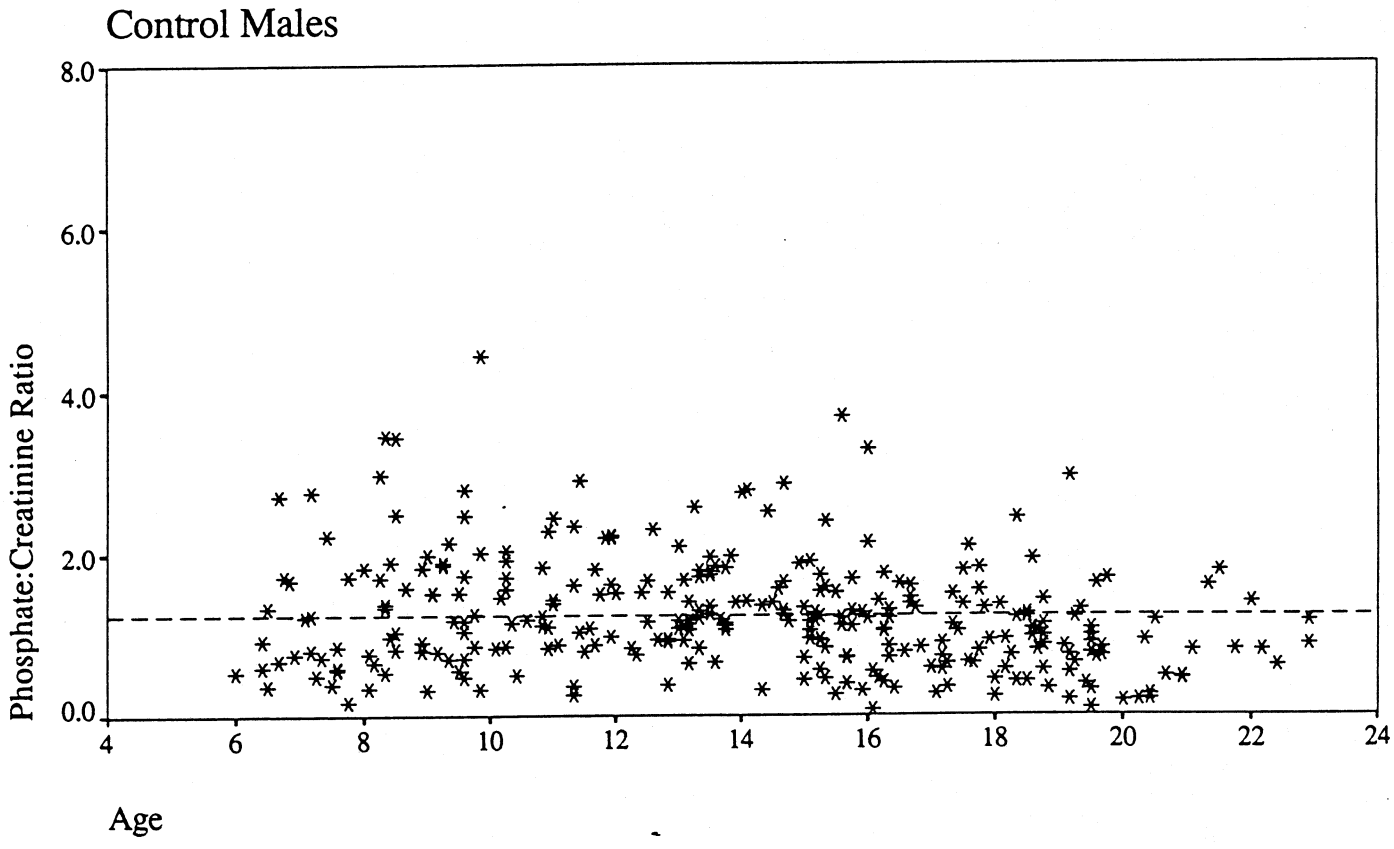


Fig 4.39 Urine Phosphate:Creatinine Values (mmol/mmol) for Control Males

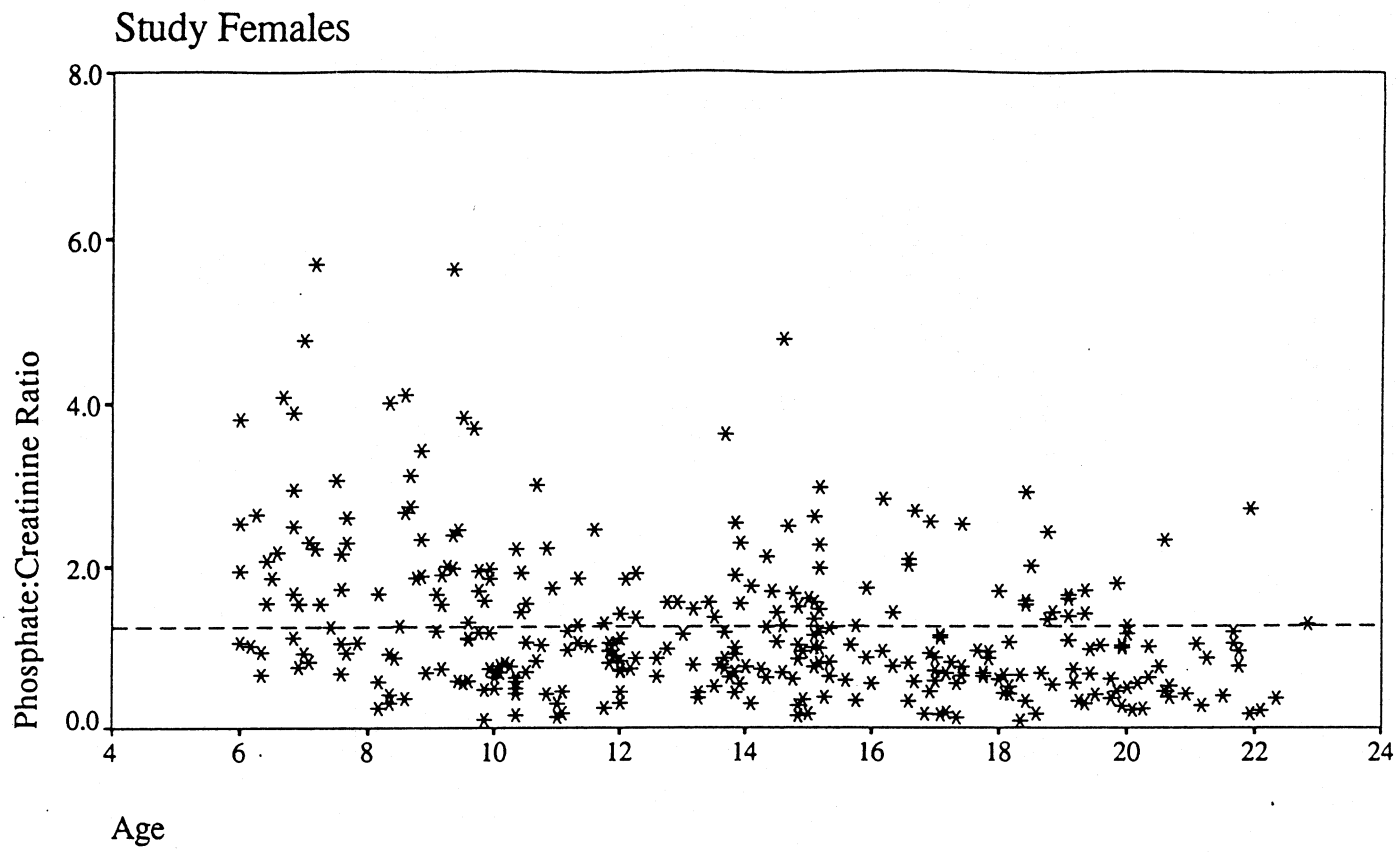


Fig 4.40 Urine Phosphate:Creatinine Values (mmol/mmol) for Study Females

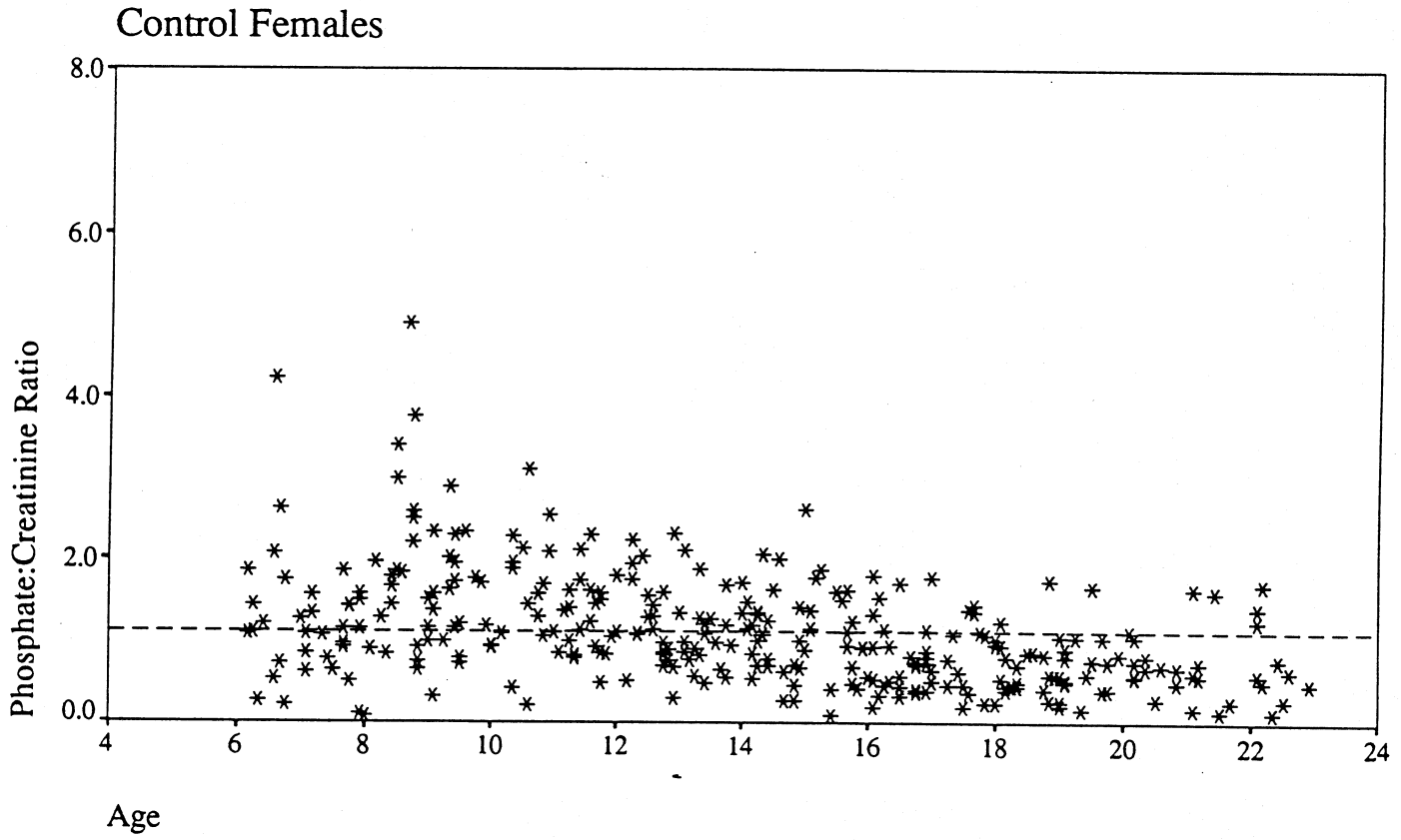


Fig 4.41 Urine Phosphate:Creatinine Values (mmol/mmol) for Control Females

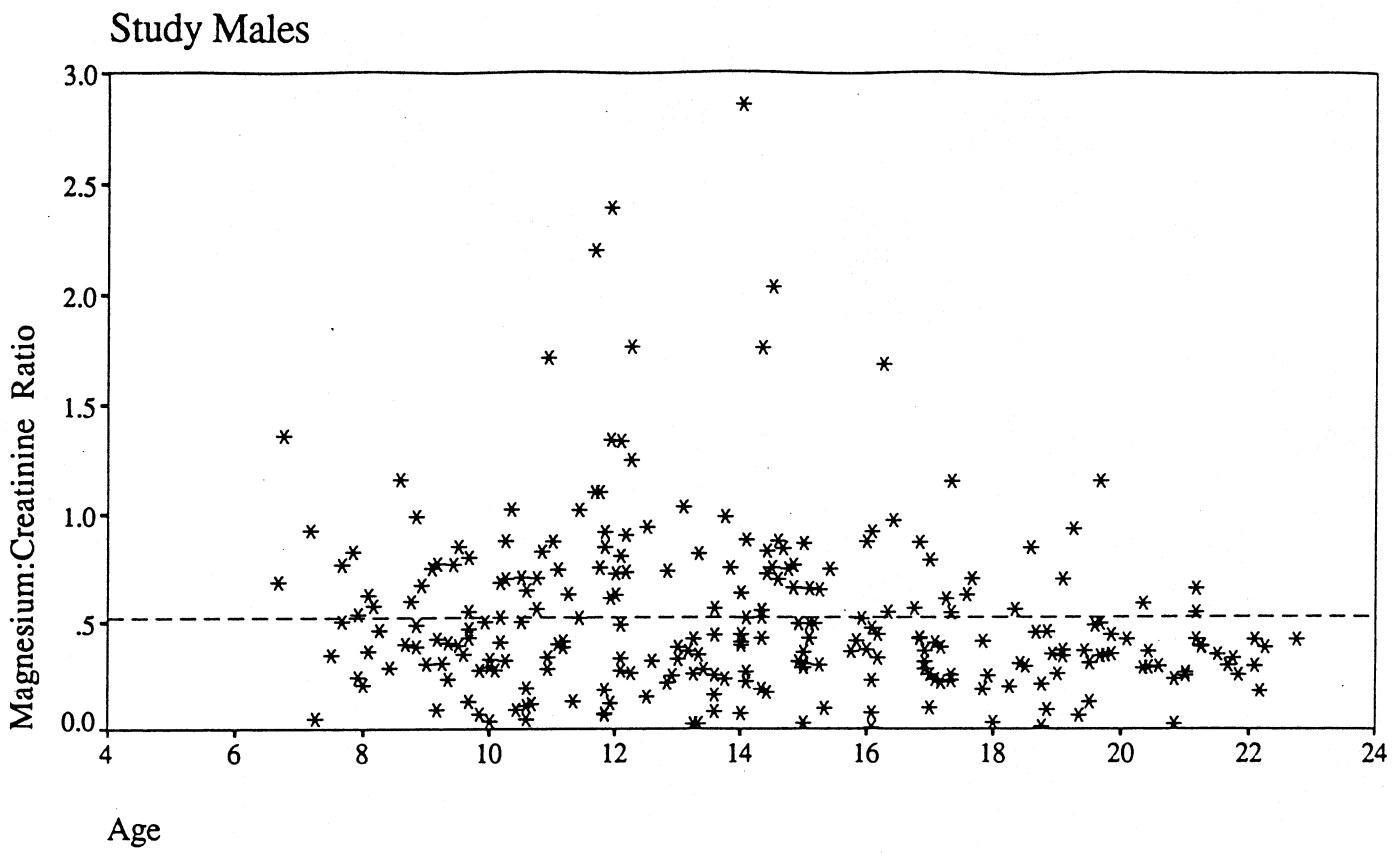


Fig 4.42 Urine Magnesium:Creatinine Values (mmol/mmol) for Study Males

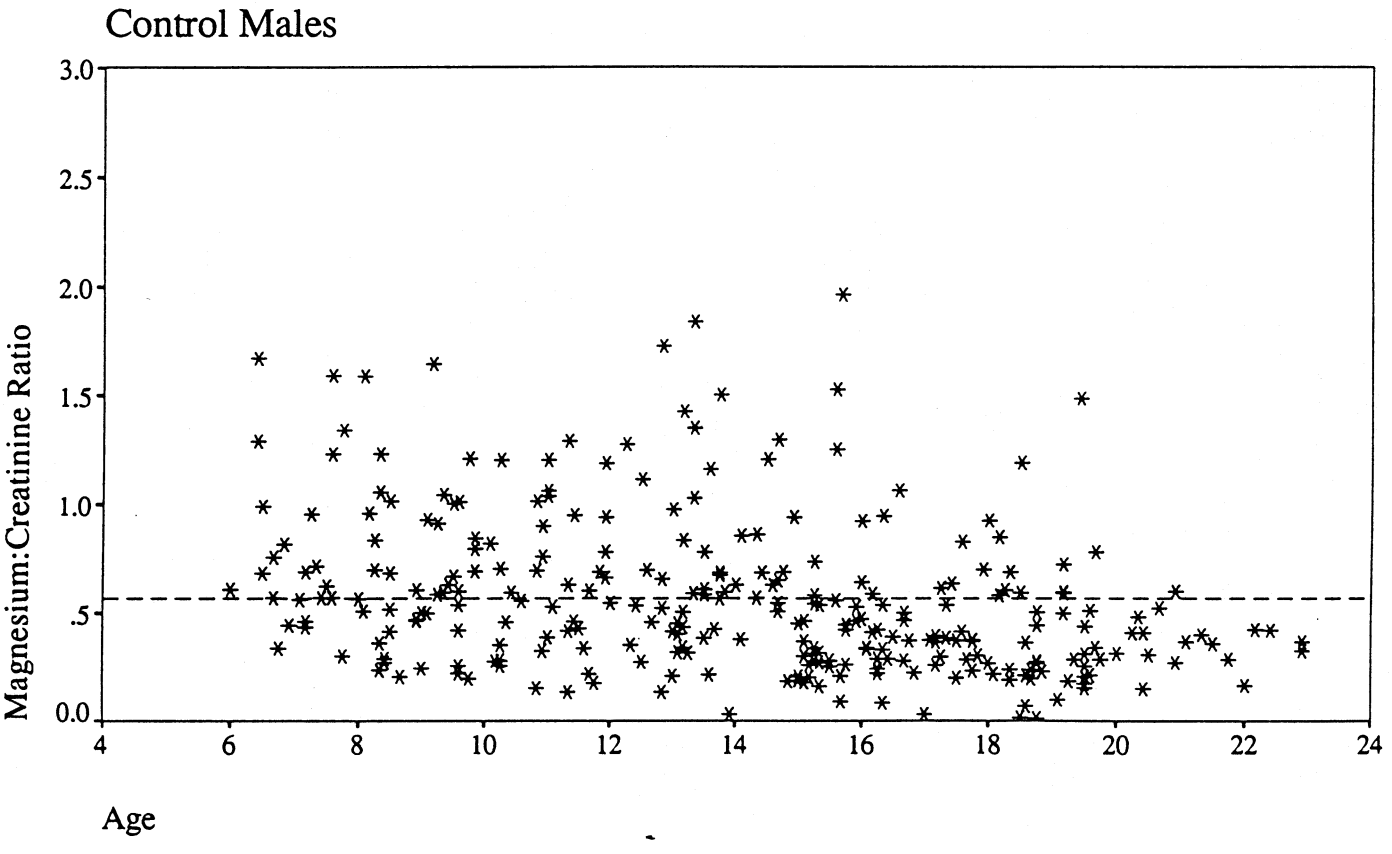


Fig 4.43 Urine Magnesium:Creatinine Values (mmol/mmol) for Control Males

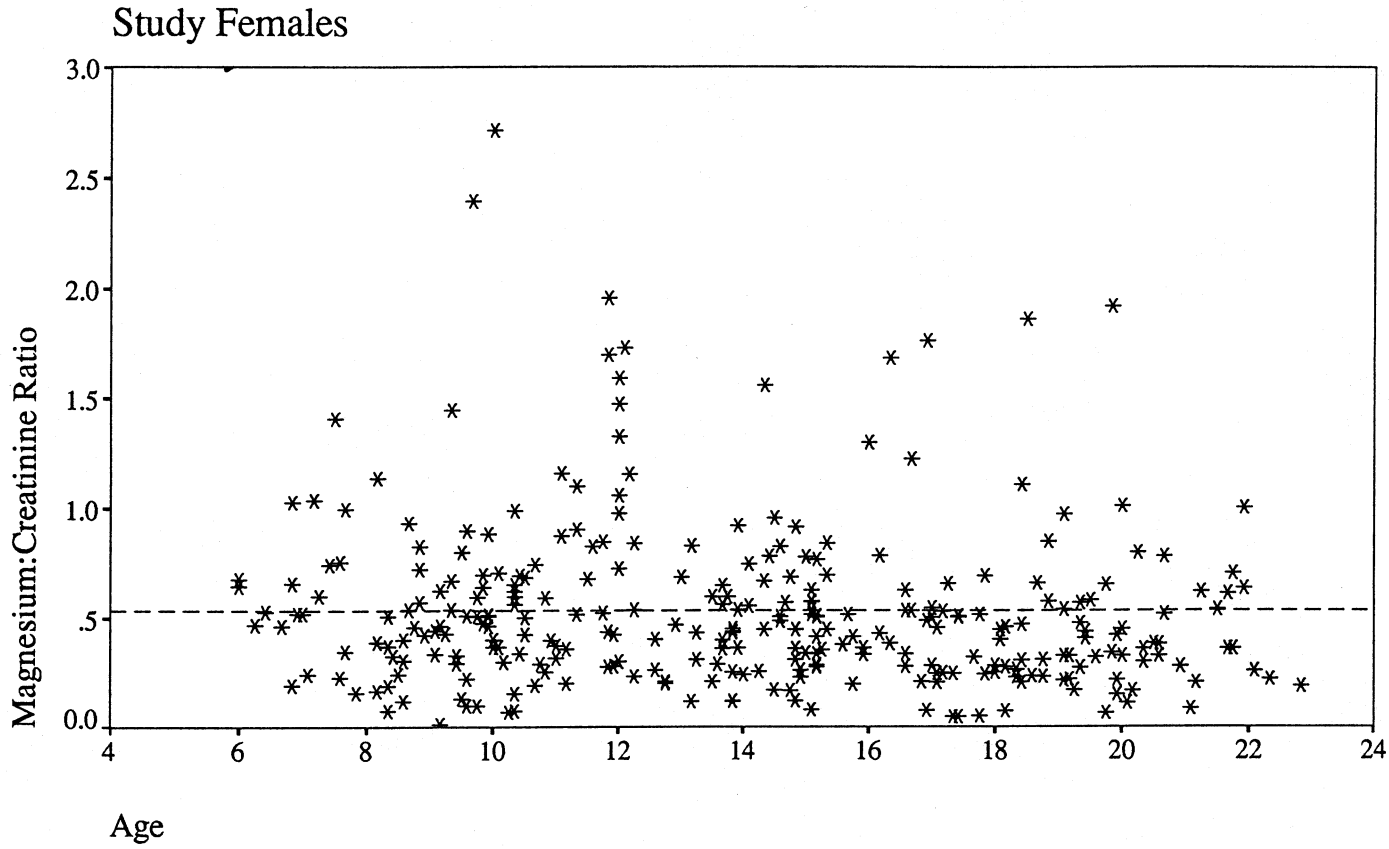


Fig 4.44 Urine Magnesium:Creatinine Values (mmol/mmol) for Study Females

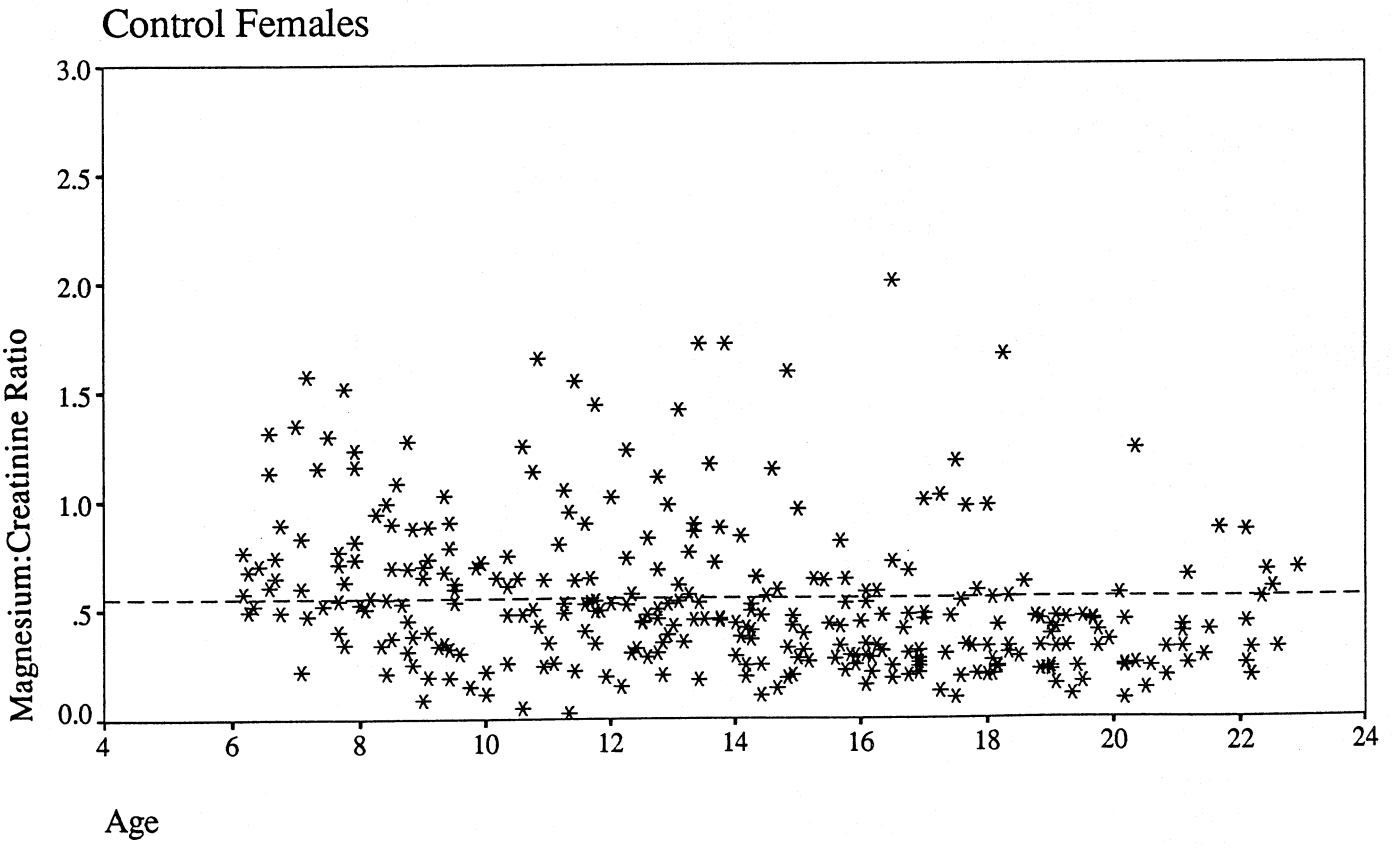


Fig 4.45 Urine Magnesium:Creatinine Values (mmol/mmol) for Control Females

4.5 Bone Densitometry Results

The bone variables bone width (BW), bone mineral content (BMC) and bone density (BD) of the distal and middle thirds of the radius were measured in the study and control groups. Study males and females had wider bones and less dense bones (p=0.0001 (Table 4.36) and p=0.001 (Table 4.37) respectively) than their counterparts in the control group. There was no difference in mean bone mineral content between the males or females of the two groups (p=0.88 and p=0.59 respectively).

Table 4.36 Mean Bone Width, Mineral Content and Density in Males by Group

Group	n	Mean	Std Dev.	p value
BW (cm)				
Study	293	1.23	0.25	0.0001
Control	298	1.13	0.23	
BMC (g/cm)				
Study	293	0.61	0.25	0.88
Control	298	0.60	0.22	
BD (g/cm^2)				
Study	293	0.48	0.12	0.0001
Control	298	0.52	0.10	

Table 4.37 Mean Bone Width, Mineral Content and Density in Females by Group

Group	n	Mean	Std Dev.	p value
BW (cm)				
Study	320	1.18	0.24	0. 0001
Control	315	1.05	0.21	
BMC (g/cm)				
Study	320	0.58	0.21	0.59
Control	315	0.58	0.20	
BD (g/cm^2)				
Study	320	0.48	0. 12	0. 001
Control	315	0.53	0. 11	

Comparison of mean bone width between males and females of the study group revealed that study males had significantly wider bones than females (p=0.004) (Table 4.38). There were no significant differences between study males and females for bone density or bone mineral content. The same pattern was found in the control group, where males had wider bones than females (p=0.0001) (Table 4.39) but bone density and bone mineral content were similar in the two sexes.

Table 4.38 Mean Bone Width, Mineral Content and Density in the Study Group by Sex

Group	n	Mean	Std Dev.	p value
BW (cm)				
Male	293	1.23	0.25	0.004
Female	320	1.18	0.24	
BMC (g/cm)				
Male	293	0.61	0.25	0.264
Female	320	0.58	0.20	
BD (g/cm^2)				
Male	293	0.48	0.12	0.404
Female	320	0.48	0.12	

Table 4.39 Mean Bone Width, Mineral Content and Density in the Control Group by Sex

Group	n	Mean	Std Dev.	p value
BW (cm)				
Male	298	1.13	0.23	0.0001
Female	315	1.05	0.21	
BMC (g/cm)				
Male	298	0.60	0.22	0.110
Female	315	0.58	0.20	
BD (g/cm^2)				
Male	298	0.52	0.10	0.151
Female	315	0.53	0.11	

4.5.1 Bone Parameters Relationships with Age and Group

Regression lines for bone parameters against age were plotted separately for males and females and are depicted in Fig 4.46 to Fig 4.48. All three bone mass variables were highly correlated with age. Bone width was greater for all ages in the study group, while bone density was lower compared with the control group.

Tables 4.40 and 4.41 tabulate the slopes and intercepts of the regression line for each bone variable. For bone width (BW) the slopes of the lines are the same but the intercepts are significantly different in both males and females ($p<0.0001$ and $p< 0.001$, respectively) thus bone width is greater in the study group compared with the control group. Bone density has different slopes and intercepts for the study and control group with the lower bone density values in the study group ($p < 0.0001$ for males and females).

A transformation procedure was carried out on the data as bone mineral content (BMC) does not appear to be different graphically, although regression analysis does show stastistical differences between the study and control groups. The males and females of the two groups show regression lines with similar slopes but different intercepts. This procedure transforms the data so both regression lines have intercepts of 0 and then tests only the difference between the slopes of the two lines. This procedure revealed that males of the two groups had significantly different slopes ($p=0.0001$) although females did not ($p=0.42$). This may be due to the large sample size. The difference between these regression lines does not reach clinical relevance and thus the statistical difference is questionable.

Table 4.40 Values for Regression Lines for Bone Variables in Males with Age

BW (cm)	Value	r value	Std Err	p value *
Study				
intercept	0.6156	0.79	0.157	< 0.0001
slope	0.0460			
Control				
intercept	0.5091	0.80	0.142	
slope	0.0445			
BMC (g/cm)				
Study				
intercept	-0.0841	0.88	0.122	0.0011
slope	0.0514			
Control				
intercept	-0.0243	0.85	0.119	
slope	0.0452			
BD (g/cm^2)				
Study				
intercept	0.1855	0.79	0.076	<0.0001
slope	0.0217			
Control				
intercept	0.2621			
slope	0.0188	0.76	0.069	

* p values represent the difference in slope and intercept of the regression lines between the study and control groups.

Table 4.41 Values for Regression Lines for Bone Variables in Females with Age

BW (cm)	Value	r value	Std Err	p value*
Study				
intercept	0.6869	0.70	0.178	<0.001
slope	0.0356			
Control				
intercept	0.5735	0.76	0.139	
slope	0.0345			
BMC (g/cm)				
Study				
intercept	0.0207	0.89	0.101	0.005
slope	0.0414			
Control				
intercept	0.0405			
slope	0.0385	0.89	0.096	
BD (g/cm^2)				
Study				
intercept	0.2068	0.81	0.0709	<0.0001
slope	0.2067			
Control				
intercept	0.2692			
slope	0.1911	0.80	0.067	

* p values represent the difference in slope and intercept of the regression lines between the study and control groups.

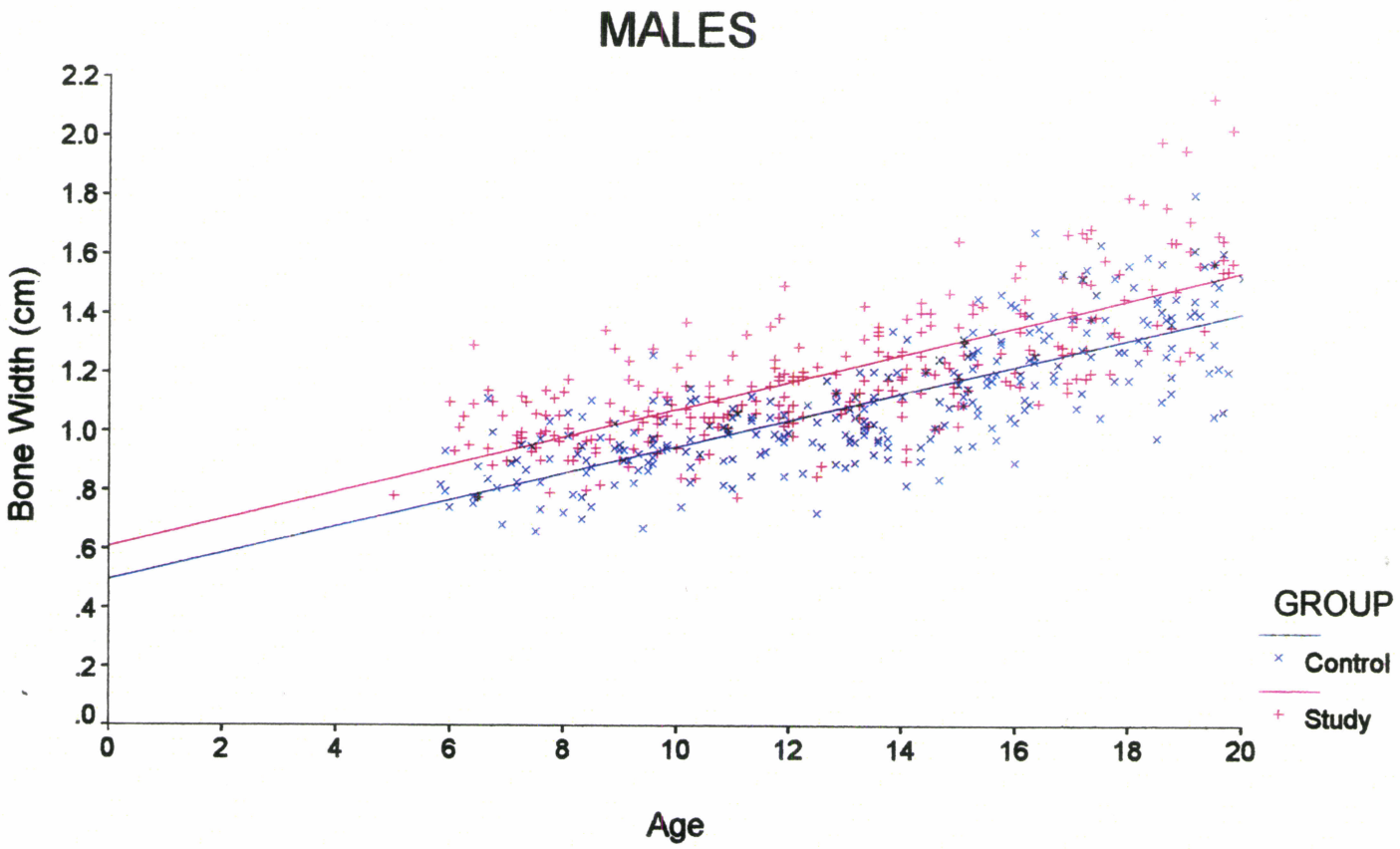


Fig 4.46a Bone Width for Males of the Study and Control Groups

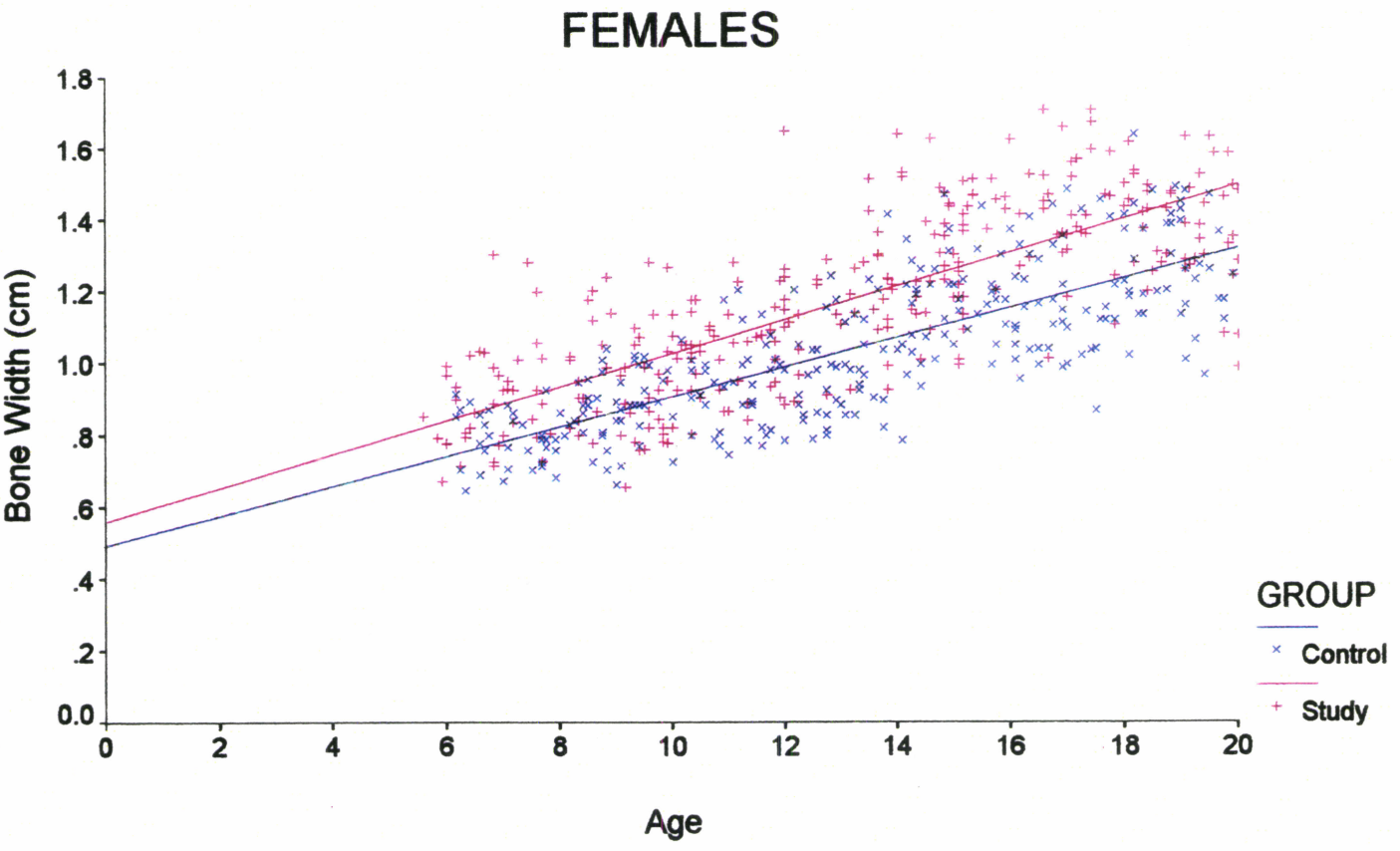


Fig 4.46b Bone Width for Females of the Study and Control Groups

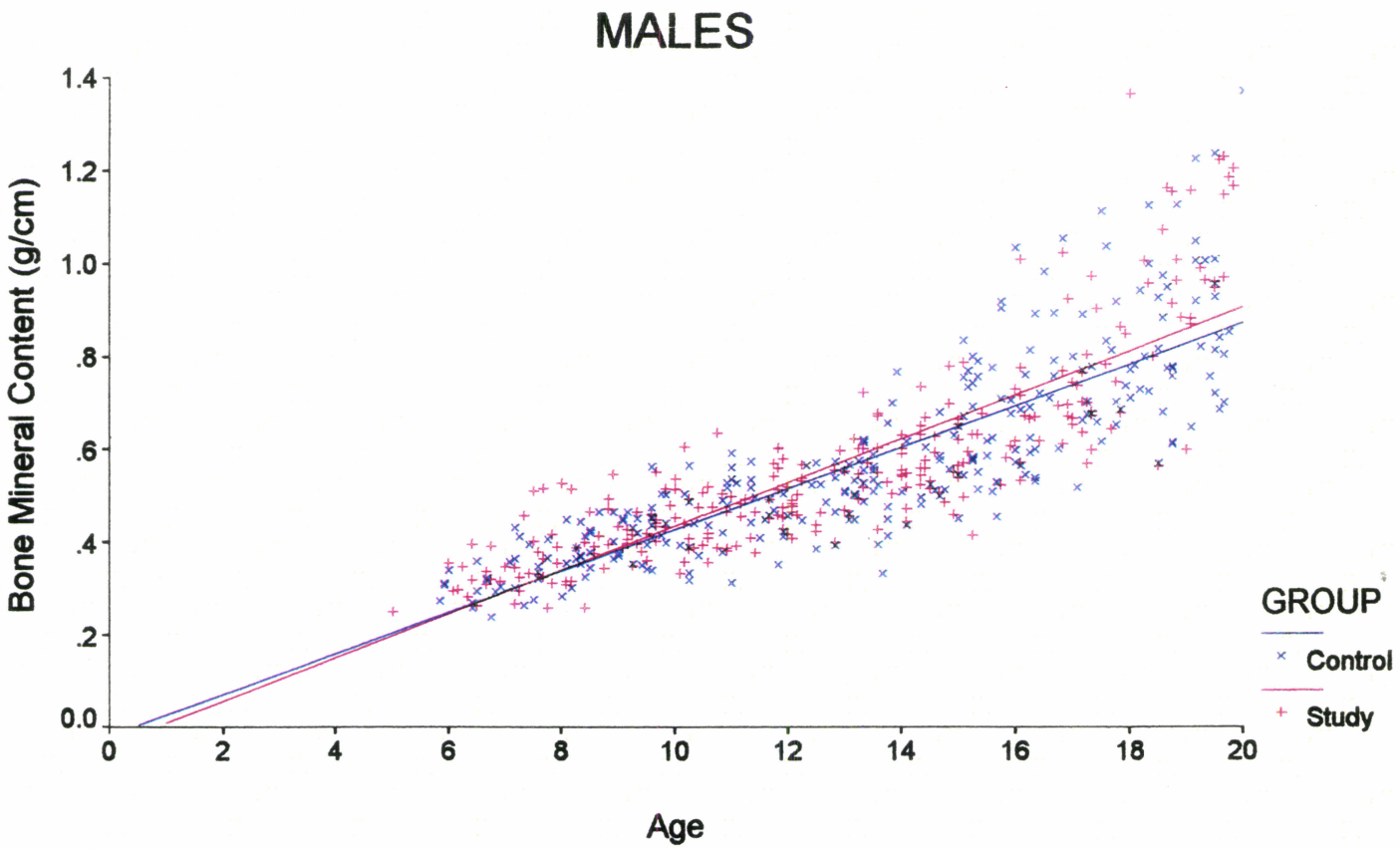


Fig 4.47a Bone Mineral Content for Males of the Study and Control Groups

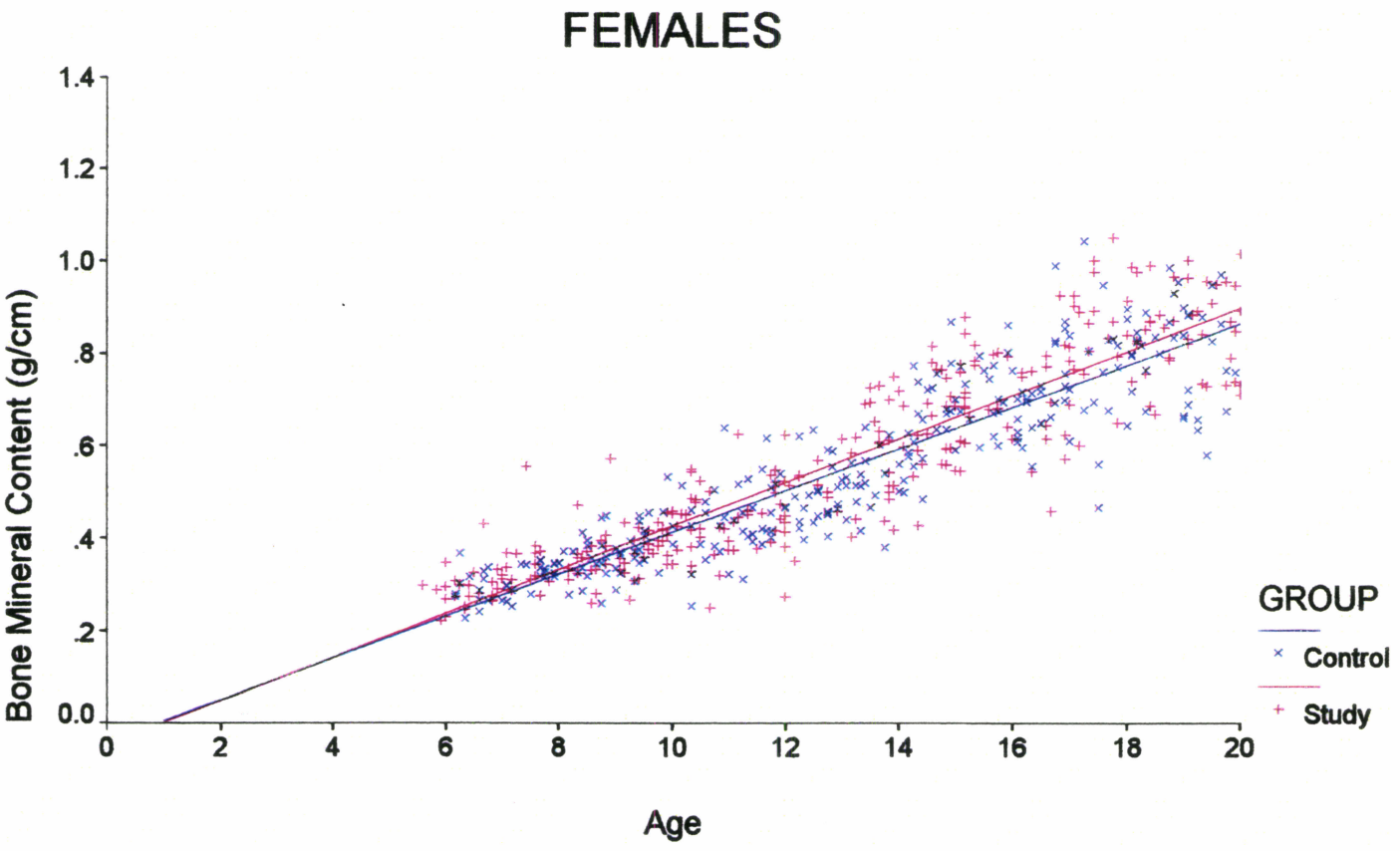


Fig 4.47b Bone Mineral Content for Females of the Study and Control Groups

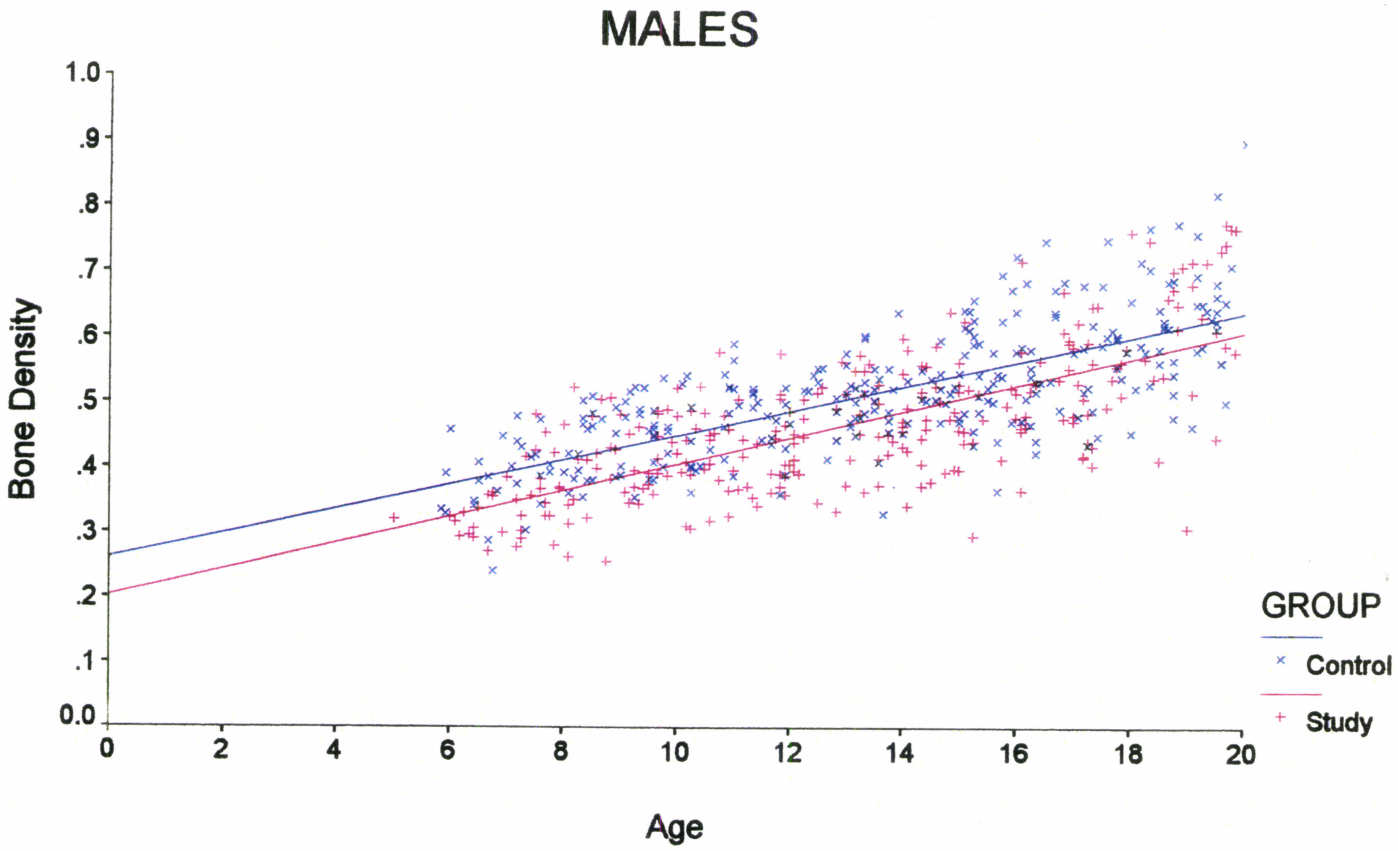


Fig 4.48a Bone Density for Males of the Study and Control Groups

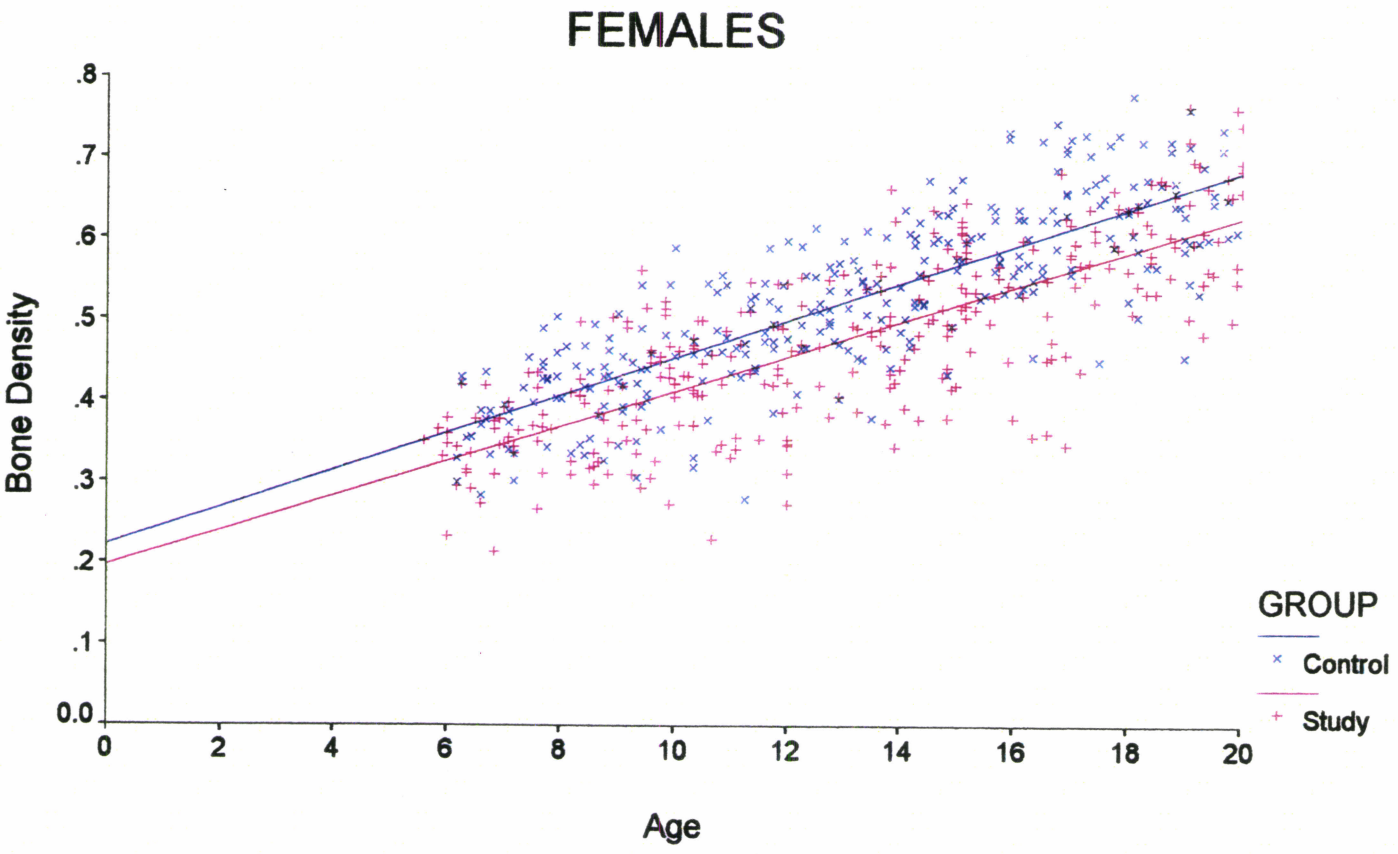


Fig 4.48b Bone Density for Females of the Study and Control Groups

a. Bone Width

As age increases, bone width increases for males and females of both groups (Tables 4.42 and 4.43). The males of the study group had consistently higher bone width values than those in the control group and were significantly different in all age groups. Study females showed significantly higher bone width values in all age groups except in the 20-22 year old group when compared with the control group.

Table 4.42 Bone Width Values by Age and Group: Males

Group	n	Mean BW (cm)	Std Dev.	p value
6-7				
Study	33	1.009	0.104	0.0001
Control	23	0.857	0.113	
8-9				
Study	39	1.035	0.125	0.0001
Control	44	0.924	0.116	
10-11				
Study	47	1.108	0.144	0.0001
Control	37	0.979	0.974	
12-13				
Study	37	1.153	0.125	0.0001
Control	43	1.030	0.113	
14-15				
Study	45	1.232	0.150	0.02
Control	47	1.160	1.155	
16-17				
Study	41	1.384	0.165	0.01
Control	43	1.291	0.168	
18-19				
Study	27	1.616	0.231	0.0001
Control	43	1.372	0.176	
20-22				
Study	24	1.600	0.138	0.02
Control	18	1.503	0.128	

Table 4.43 Bone Width Values by Age and Group: Females

Group	n	Mean BW (cm)	Std Dev.	p value
6-7				
Study	38	0.912	0.143	0.0001
Control	33	0.786	0.074	
8-9				
Study	47	0.945	0.150	0.01
Control	42	0.879	0.093	
10-11				
Study	44	1.026	0.114	0.0007
Control	39	0.934	0.124	
12-13				
Study	40	1.170	0.161	0.0001
Control	44	0.984	0.143	
14-15				
Study	48	1.321	0.164	0.0001
Control	45	1.157	0.135	
16-17				
Study	34	1.431	0.165	0.0001
Control	43	1.171	0.155	
18-19				
Study	41	1.405	0.127	0.0001
Control	40	1.278	0.153	
20-22				
Study	28	1.318	0.205	0.18
Control	29	1.253	0.152	

b. Bone Mineral Content

As with bone width, bone mineral content increased with age in both the study and control groups (Tables 4.44 and 4.45). Although males of the study group generally had similar bone mineral content to the control group, differences between the two groups were found in males of the 14-15 and 18-19 years old age groups (p=0.05 and p=0.0005 respectively). Females showed no significant differences in bone mineral content between the two groups except in the 18-19 year old age group (p=0.04).

Table 4.44 Bone Mineral Content Values by Age and Group: Males

Group	n	Mean BMC (g/cm)	Std Dev.	p value
6-7				
Study	33	0.347	0.062	0.24
Control	23	0.329	0.052	
8-9				
Study	39	0.416	0.068	0.53
Control	44	0.407	0.057	
10-11				
Study	47	0.466	0.074	0.72
Control	37	0.461	0.073	
12-13				
Study	37	0.524	0.078	0.78
Control	43	0.519	0.083	
14-15				
Study	45	0.583	0.081	0.05
Control	47	0.624	0.121	
16-17				
Study	41	0.727	0.116	0.63
Control	43	0.741	0.153	
18-19				
Study	27	1.015	0.185	0.0005
Control	43	0.853	0.160	
20-22				
Study	24	1.098	0.144	0.10
Control	18	1.015	0.175	

Table 4.45 Bone Mineral Content Values by Age and Group: Females

Group	n	Mean BMC (g/cm)	Std Dev.	p value
6-7				
Study	38	0.314	0.058	0.46
Control	33	0.305	0.035	
8-9				
Study	47	0.371	0.054	0.75
Control	42	0.368	0.055	
10-11				
Study	44	0.438	0.072	0.93
Control	39	0.439	0.079	
12-13				
Study	40	0.539	0.111	0.08
Control	44	0.503	0.066	
14-15				
Study	48	0.682	0.097	0.52
Control	45	0.669	0.093	
16-17				
Study	34	0.771	0.138	0.15
Control	43	0.728	0.115	
18-19				
Study	41	0.853	0.089	0.04
Control	40	0.808	0.105	
20-22				
Study	28	0.867	0.105	0.22
Control	29	0.830	0.119	

c. Bone Density

Bone density increases with age in males and females of both groups. Males and females of the control group had higher bone density values than the study group in most age groups. The differences were not significant for males of the 18-19, and 20-22 year age groups, and for females in the age groups 8-9, 18-19 and 20-22 year age groups (Tables 4.46 and 4.47).

Table 4.46 Bone Density Values by Age and Group: Males

Group	n	Mean BD (g/cm^2)	Std Dev.	p value
6-7				
Study	33	0.344	0.052	0.007
Control	23	0.387	0.059	
8-9				
Study	39	0.404	0.065	0.004
Control	44	0.443	0.051	
10-11				
Study	47	0.424	0.065	0.001
Control	37	0.471	0.059	
12-13				
Study	37	0.456	0.065	0.0009
Control	44	0.504	0.055	
14-15				
Study	45	0.478	0.075	0.0002
Control	47	0.538	0.069	
16-17				
Study	41	0.528	0.077	0.01
Control	43	0.573	0.084	
18-19				
Study	27	0.635	0.119	0.60
Control	43	0.622	0.084	
20-22				
Study	24	0.686	0.072	0.69
Control	18	0.675	0.101	

Table 4.47 Bone Density Values by Age and Group: Females

Group	n	Mean BD (g/cm^2)	Std Dev.	p value
6-7				
Study	38	0.347	0.051	0.0007
Control	33	0.391	0.054	
8-9				
Study	47	0.400	0.075	0.173
Control	42	0.420	0.056	
10-11				
Study	44	0.428	0.063	0.003
Control	39	0.472	0.068	
12-13				
Study	40	0.463	0.087	0.002
Control	44	0.515	0.057	
14-15				
Study	48	0.519	0.069	0.0001
Control	45	0.580	0.065	
16-17				
Study	34	0.541	0.090	0.0001
Control	43	0.623	0.072	
18-19				
Study	41	0.610	0.069	0.11
Control	40	0.635	0.070	
20-22				
Study	28	0.665	0.074	0.93
Control	29	0.663	0.067	

4.5.2 Bone Parameters in Relation to Age and Gender

a. Bone Width

The study group males had significantly higher bone width values than females, except in the age groups 12-13, 14-15, and 16-17 years old. In the 14-15 year old females actually had a significantly greater bone width than males (p=0.007) (Table 4.48 and Fig 4.49).

Table 4.48 Bone Width by Age and Sex : Study

Group	n	Mean BW (cm)	Std Dev.	p value
6-7				
Male	33	1.009	0.10	0.001
Female	38	0.91	0.14	
8-9				
Male	39	1.03	0.125	0.003
Female	47	0.94	0.150	
10-11				
Male	47	1.108	0.144	0.003
Female	44	1.026	0.114	
12-13				
Male	37	1.153	0.125	0.59
Female	40	1.170	0.161	
14-15				
Male	45	1.232	0.150	0.007
Female	48	1.321	0.164	
16-17				
Male	41	1.384	0.165	0.22
Female	34	1.431	0.165	
18-19				
Male	27	1.616	0.231	0.0001
Female	41	1.405	0.127	
20-22				
Male	24	1.600	0.138	0.0001
Female	28	1.318	0.205	

In the control group, males had significantly greater bone width than females except in the age groups 10-11,12-13 and 14-15 years old (Table 4.49 and Fig 4.50).

Table 4.49 Bone Width by Age and Sex : Control

Group	n	Mean BW (cm)	Std Dev.	p value
6-7				
Male	23	0.857	0.113	0.01
Female	33	0.786	0.074	
8-9				
Male	44	0.924	0.116	0.05
Female	42	0.879	0.093	
10-11				
Male	37	0.979	0.097	0.08
Female	39	0.934	0.124	
12-13				
Male	43	1.030	0.113	0.10
Female	44	0.984	0.143	
14-15				
Male	47	1.160	0.155	0.93
Female	45	1.157	0.135	
16-17				
Male	43	1.291	0.168	0.001
Female	43	1.171	0.155	
18-19				
Male	43	1.372	0.176	0.011
Female	40	1.278	0.153	
20-22				
Male	18	1.503	0.128	0.0001
Female	29	1.253	0.152	

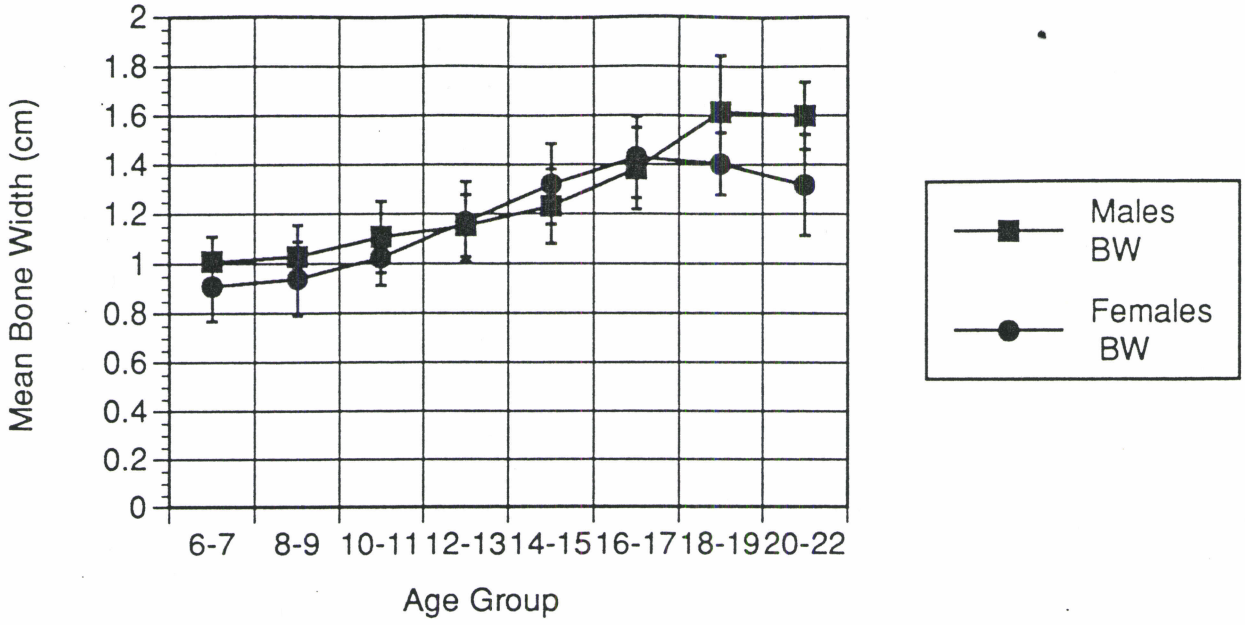


Fig 4.49 Mean Bone Width for Males and Females of the Study Group

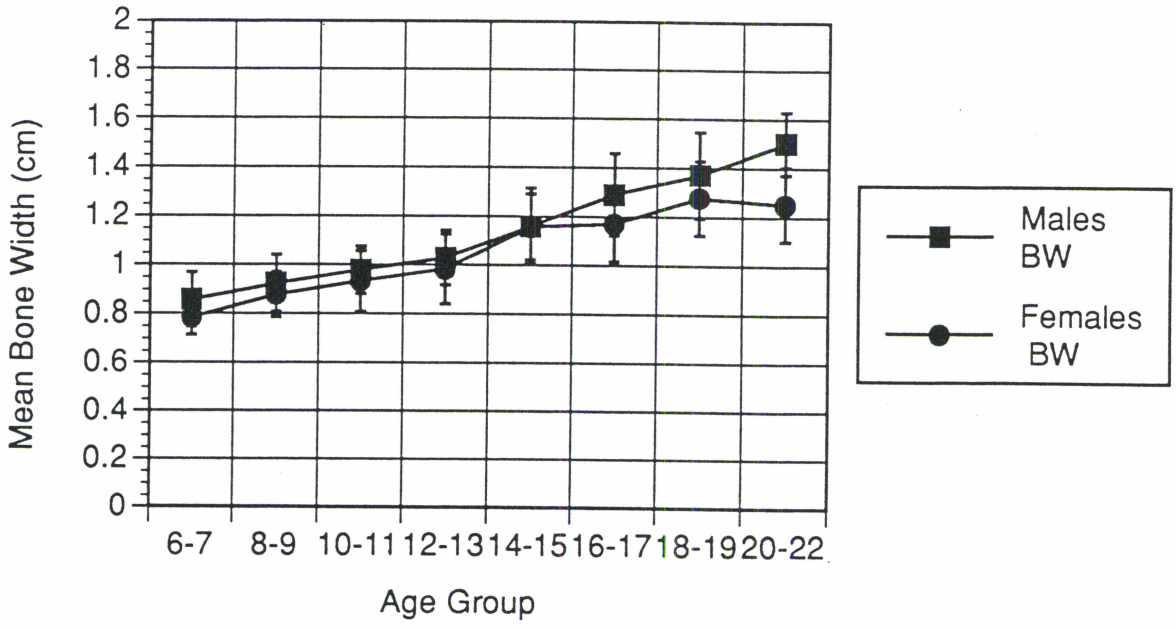


Fig 4.50 Mean Bone Width for Males and Females of the Control Group

b. Bone Mineral Content

Males generally had greater mean bone mineral content than females in the study group, and this reaches statistical significance in four age groups (6-7, 8-9, 18-19 and 20-22). A similar pattern was noted in the control group with males having significantly higher bone mineral content than females in three groups (6-7, 8-9 and 20-22 year olds). An interesting deviation from this trend is at the 14-15 year old age group where females of the study and control groups have significantly higher values than males (p=0.001 and p=0.04, respectively) (Tables 4.50 and 4.51, Figs 4.51 and 4.52).

Table 4.50 Bone Mineral Content Values by Age and Sex : Study

Group	n	Mean BD (g/cm)	Std Dev.	p value
6-7				
Male	33	0.347	0.010	0.02
Female	38	0.314	0.009	
8-9				
Male	39	0.416	0.068	0.001
Female	47	0.371	0.054	
10-11				
Male	47	0.466	0.074	0.06
Female	44	0.438	0.072	
12-13				
Male	37	0.524	0.078	0.49
Female	40	0.539	0.111	
14-15				
Male	45	0.583	0.081	0.001
Female	48	0.682	0.974	
16-17				
Male	41	0.727	0.116	0.14
Female	34	0.771	0.138	
18-19				
Male	27	1.015	0.185	0.0002
Female	41	0.853	0.089	
20-22				
Male	24	1.098	0.144	0.0001
Female	28	0.867	0.105	

Table 4.51 Bone Mineral Content Values by Age and Sex : Control

Group	n	Mean BMC (g/cm)	Std Dev.	p value
6-7				
Male	23	0.329	0.052	0.06
Female	33	0.305	0.035	
8-9				
Male	44	0.407	0.057	0.001
Female	42	0.368	0.055	
10-11				
Male	37	0.461	0.073	0.22
Female	39	0.439	0.079	
12-13				
Male	43	0.519	0.083	0.32
Female	44	0.503	0.066	
14-15				
Male	47	0.624	0.121	0.04
Female	45	0.669	0.093	
16-17				
Male	43	0.741	0.153	0.66
Female	43	0.728	0.115	
18-19				
Male	43	0.853	0.160	0.131
Female	40	0.808	0.105	
20-22				
Male	18	1.015	0.175	0.0005
Female	29	0.830	0.119	

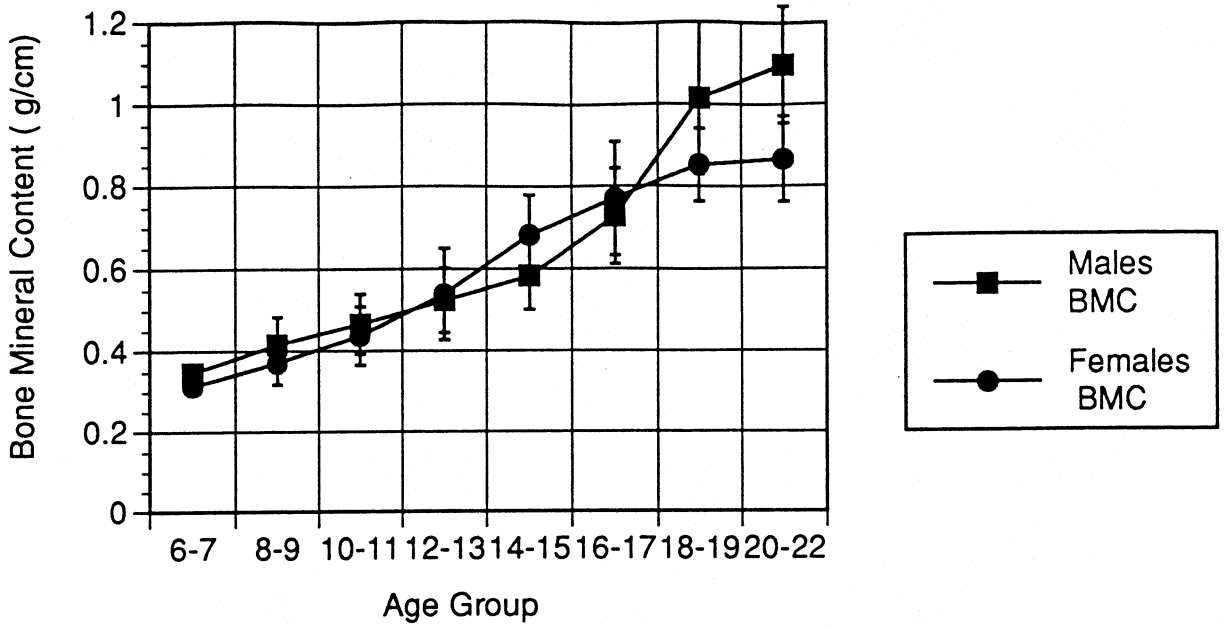


Fig 4.51 Mean Bone Mineral Content for Males and Females of the Study Group

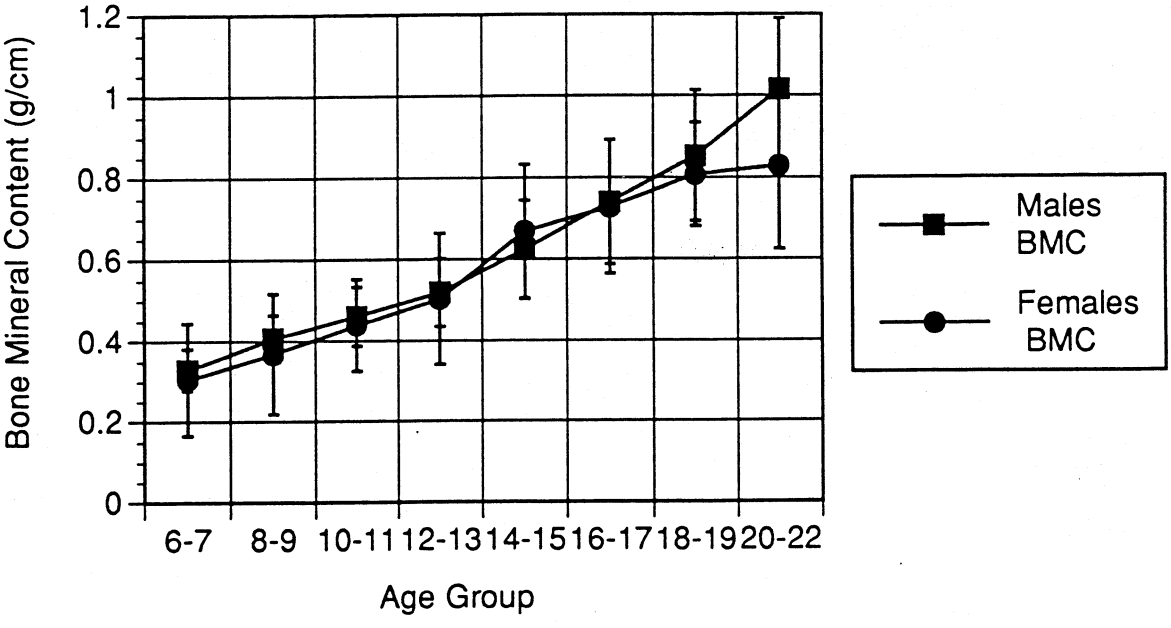


Fig 4.52 Mean Bone Mineral Content for Males and Females of the Control Group

c. Bone Density

In the study group, there were no significant differences between the sexes by age group, apart from the 14-15 year olds where females had significantly higher bone density values than males (p=0.007) (Table 4.52 and Fig 4.53). A similar pattern was seen in the control group, where females had greater bone density than males in the 14-15 and 16-17 year old age groups (p=0.003 and p=0.004 respectively) (Table 4.53 and Fig 4.54).

Table 4.52. Bone Density Values by Age and Sex: Study

Group	n	Mean BD (g/cm^2)	Std Dev.	p value
6-7				
Male	33	0.344	0.052	0.84
Female	38	0.347	0.051	
8-9				
Male	39	0.404	0.655	0.79
Female	47	0.400	0.750	
10-11				
Male	47	0.424	0.065	0.75
Female	44	0.428	0.063	
12-13				
Male	37	0.456	0.065	0.71
Female	40	0.463	0.087	
14-15				
Male	45	0.478	0.075	0.007
Female	48	0.519	0.069	
16-17				
Male	41	0.528	0.077	0.49
Female	34	0.541	0.090	
18-19				
Male	27	0.635	0.119	0.31
Female	41	0.610	0.069	
20-22				
Male	24	0.686	0.072	0.29
Female	28	0.665	0.074	

Table 4.53 Bone Density Values by Age and Sex : Control

Group	n	Mean BD (g/cm^2)	Std Dev.	p value
6-7				
Male	23	0.387	0.059	0.78
Female	33	0.391	0.054	
8-9				
Male	44	0.443	0.051	0.04
Female	42	0.420	0.056	
10-11				
Male	37	0.471	0.059	0.90
Female	39	0.472	0.068	
12-13				
Male	43	0.504	0.055	0.36
Female	44	0.515	0.057	
14-15				
Male	47	0.538	0.069	0.003
Female	45	0.580	0.065	
16-17				
Male	43	0.573	0.084	0.004
Female	43	0.623	0.072	
18-19				
Male	43	0.622	0.084	0.44
Female	40	0.635	0.070	
20-22				
Male	18	0.675	0.010	0.66
Female	29	0.663	0.067	

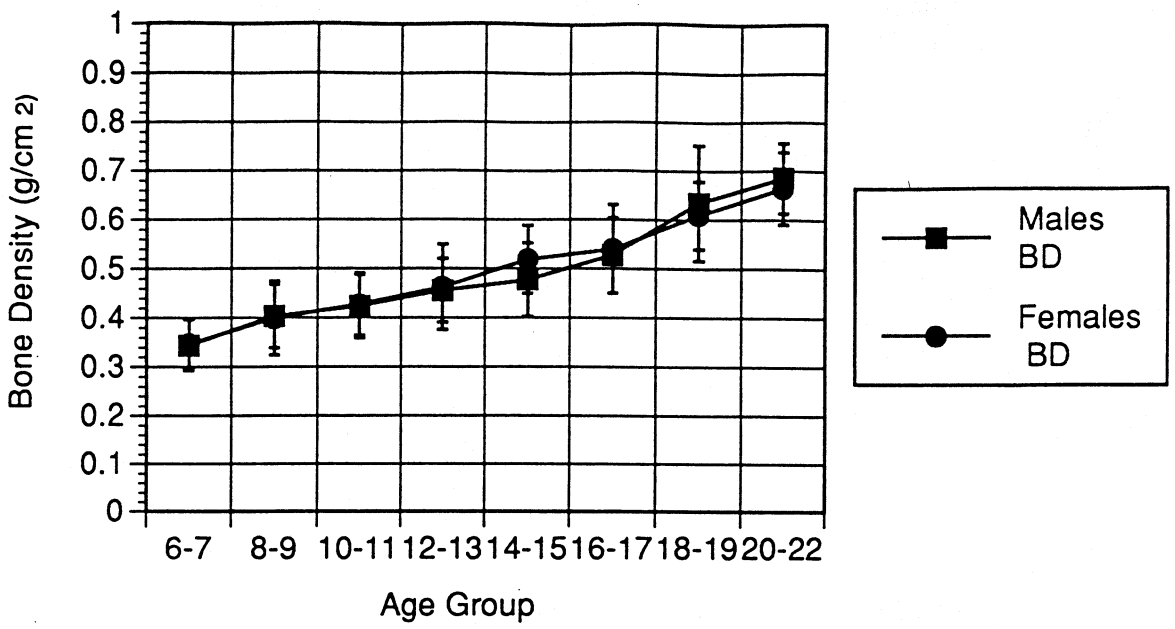


Fig 4.53 Mean Bone Density for Males and Females of the Study Group

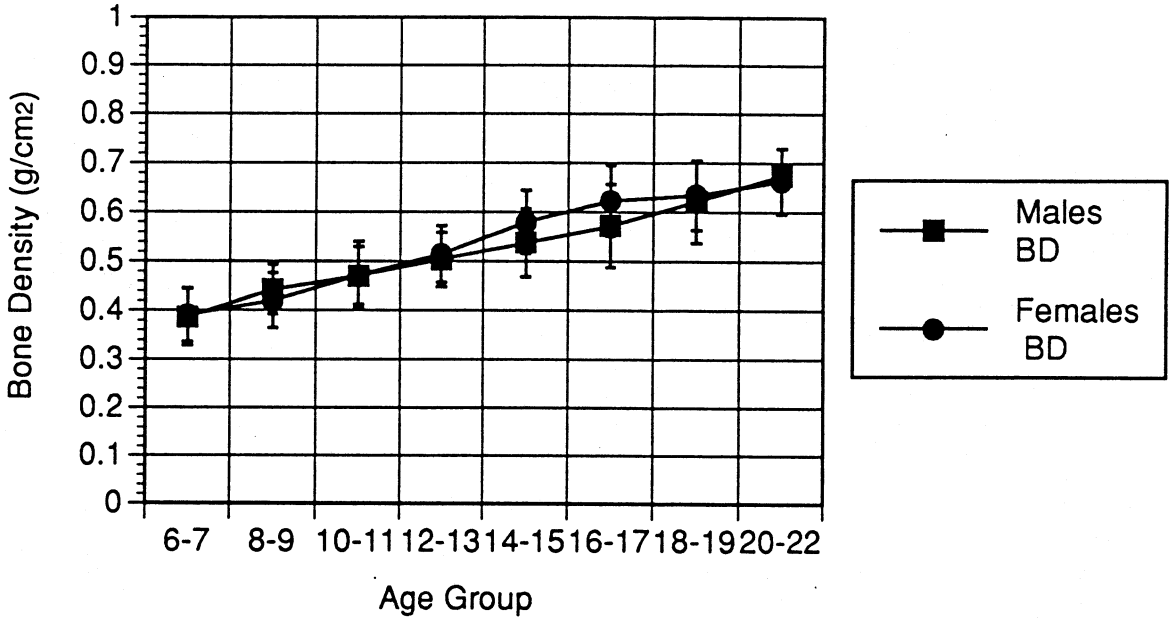


Fig 4.54 Mean Bone Density for Males and Females of the Control Group

4.5.3 Apparent Bone Density (ABD)

Females and males of the study group had mean ABD approximately 20% lower than those of the control group. These differences were significant for both groups (p=0.0001) (Table 4.54).

Table 4.54 Comparison of Apparent Bone Density between Groups by Sex

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
ABD (g/cm^3) **Females**				
Study	320	0.539	0.137	0.0001
Control	315	0.658	0.130	
ABD (g/cm^3) **Males**				
Study	293	0.501	0.113	0.0001
Control	298	0.602	0.115	

When males and females within each of the groups were compared, there were significant differences between the sexes; females had significantly higher mean ABD values than males (± 9 %) (Table 4.55).

Table 4.55 Comparison of Apparent Bone Density between Sexes by Group

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
ABD (g/cm^3) **Study**				
Male	293	0.501	0.113	0.0002
Female	320	0.539	0.137	
ABD (g/cm^3) **Control**				
Male	298	0.602	0.115	0.0001
Female	315	0.658	0.130	

a. Apparent Bone Density Values for Group by Age

Apparent bone density is significantly lower in males of the study than those of the control group in all but one group (20-22 year olds) (Table 4.56 and Fig 4.55). Females of the study and control groups display the same pattern in all but two age groups (8-9 and 20-22 year olds), in which the study females had lower but not statistically different mean ABD values than the controls (Table 4.57 Fig 4.56).

Table 4.56 Apparent Bone Density Values by Age and Group: Males

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
6-7				
Study	33	0.439	0.08	0.0001
Control	23	0.589	0.13	
8-9				
Study	39	0.507	0.11	0.0001
Control	44	0.623	0.12	
10-11				
Study	47	0.499	0.12	0.0001
Control	37	0.618	0.10	
12-13				
Study	37	0.513	0.11	0.001
Control	43	0.630	0.10	
14-15				
Study	45	0.506	0.12	0.003
Control	47	0.601	0.11	
16-17				
Study	41	0.495	0.10	0.001
Control	43	0.574		
18-19				
Study	27	0.515	0.13	0.02
Control	43	0.587	0.11	
20-22				
Study	24	0.550	0.07	0.36
Control	18	0.576	0.09	

Table 4.57 Apparent Bone Density Values by Age and Group: Females

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
6-7				
Study	38	0.500	0.11	0.0001
Control	33	0.644	0.12	
8-9				
Study	47	0.562	0.17	0.08
Control	42	0.617	0.12	
10-11				
Study	44	0.540	0.11	0.0001
Control	39	0.657	0.14	
12-13				
Study	40	0.515	0.13	0.0001
Control	44	0.684	0.13	
14-15				
Study	48	0.512	0.11	0.0001
Control	45	0.649	0.11	
16-17				
Study	34	0.490	0.108	0.0001
Control	43	0.691	0.132	
18-19				
Study	41	0.559	0.10	0.001
Control	40	0.644	0.12	
20-22				
Study	28	0.666	0.172	0.63
Control	29	0.685	0.121	

b. Apparent Bone Density by Age and Sex

Females of the study group tended to have higher mean ABD levels than the males of the same group, however this was only significant in two groups (6-7 and 20-22 year olds) (Table 4.58). Females of the control group also displayed a trend to greater mean ABD levels than males, which reached significance in all but three groups (6-7, 8-9 and 10-11 year olds) (Table 4.59).

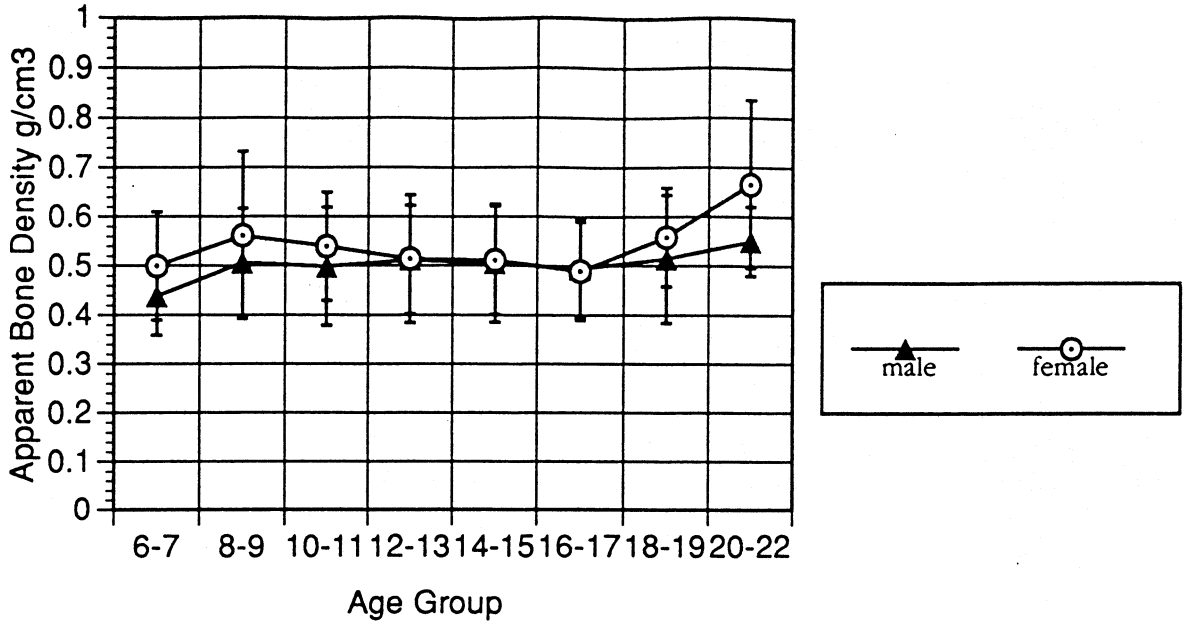


Fig 4.55 Mean Bone Apparent Bone Density for Males and Females of the Study Group

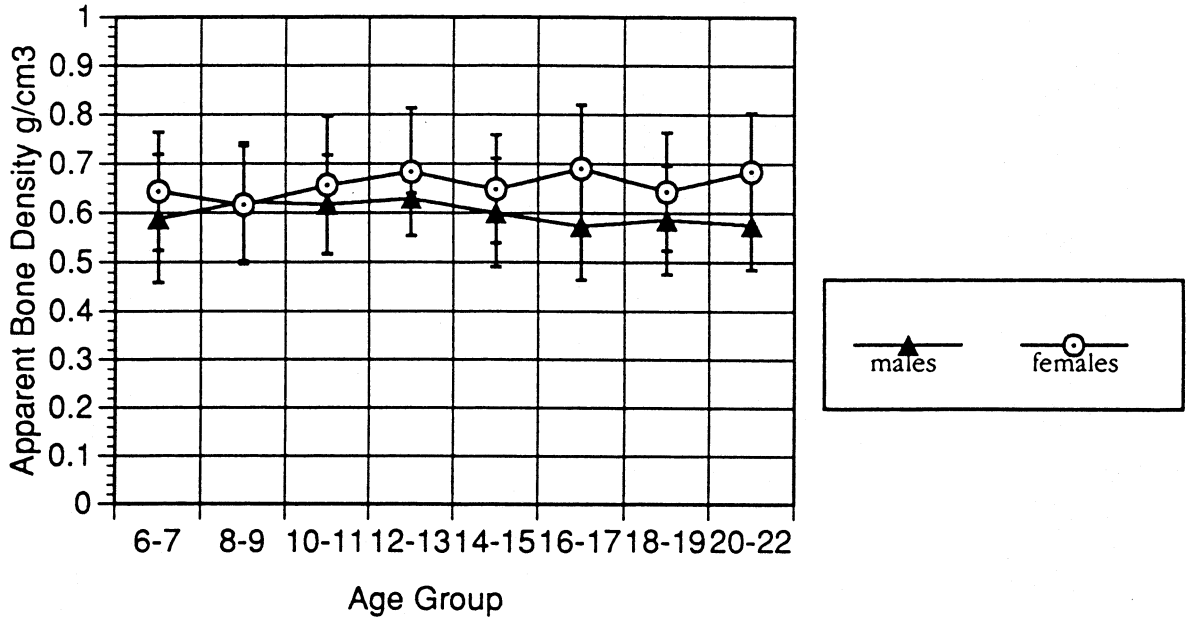


Fig 4.56 Mean Apparent Bone Density for Males and Females of the Control Group

Table 4.58 Apparent Bone Density Values by Age and Sex: Study

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
6-7				
Male	33	0.439	0.08	0.01
Female	38	0.500	0.11	
8-9				
Male	39	0.507	0.11	0.08
Female	47	0.562	0.17	
10-11				
Male	47	0.499	0.12	0.09
Female	44	0.540	0.11	
12-13				
Male	37	0.513	0.11	0.96
Female	40	0.515	0.13	
14-15				
Male	45	0.506	0.12	0.82
Female	48	0.512	0.11	
16-17				
Male	41	0.495	0.10	0.85
Female	34	0.490	0.10	
18-19				
Male	27	0.515	0.13	0.13
Female	41	0.559	0.10	
20-22				
Male	24	0.550	0.07	0.002
Female	28	0.666	0.17	

Table 4.59 Apparent Bone Density Values by Age and Sex : Control

Group	n	Mean ABD (g/cm^3)	Std Dev.	p value
6-7				
Male	23	0.589	0.13	0.14
Female	33	0.644	0.12	
8-9				
Male	44	0.623	0.12	0.82
Female	42	0.617	0.12	
10-11				
Male	37	0.618	0.10	0.17
Female	39	0.657	0.14	
12-13				
Male	43	0.630	0.01	0.04
Female	44	0.684	0.13	
14-15				
Male	47	0.601	0.11	0.05
Female	45	0.649	0.11	
16-17				
Male	43	0.574	0.11	0.0001
Female	43	0.691	0.13	
18-19				
Male	43	0.587	0.11	0.03
Female	40	0.644	0.12	
20-22				
Male	18	0.576	0.09	0.001
Female	29	0.685	0.12	

4.5.4 Factors affecting Bone Parameters

Correlations were made between bone parameters, and biochemical and anthropometric variables for males and females of each group. When interpreting correlations, it is essential to remember that a high correlation does not necessarily indicate a causal relationship exists between the two variables. Only those correlations with r values greater than 0.30 are reported, as lower r values represent less than 9% of the variance in the population.

a. Bone Width Correlations

Bone width was strongly correlated with age, weight and height with high r values describing approximately 59%-70 % of the variance in the population (Table 4.60). Biochemical variables had much weaker correlations. Bone width was positively correlated with serum albumin and negatively with alkaline phosphatase and phosphate in both groups. Urine variables did not correlate with bone width.

Table 4.60 Correlation Statistics for Bone Width

Correlation	Study Males r value *p value*	Study Females r value *p value*	Control Males r value *p value*	Control Females r value *p value*
BW with weight	0.80 *0.0001*	0.77 *0.0001*	0.84 *0.0001*	0.81 *0.0001*
BW with height	0.77 *0.0001*	0.76 *0.0001*	0.81 *0.0001*	0.75 *0.0001*
BW with age	0.78 *0.0001*	0.72 *0.0001*	0.80 *0.0001*	0.76 *0.0001*
BW with serum Alb	0.39 *0.0001*	0.33 *0.0001*	0.27 *0.0001*	0.17 *0.002*
BW with serum PO_4	-0.42 *0.0001*	-0.30 *0.0001*	-0.39 *0.0001*	-0.46 *0.0001*
BW with alkaline phosphatase	-0.39 *0.0001*	-0.59 *0.0001*	-0.21 *0.0002*	-0.53 *0.0001*

b. Bone Mineral Content Correlations

Bone mineral content increased with increasing age, height and weight for all groups with high r values (0.84 to 0.92 for different variables) (Table 4.61). Serum albumin was positively correlated in all but the control group females. Serum levels of alkaline phosphatase and phosphate were negatively correlated with bone mineral content in all groups. Urine phosphate:creatinine ratios were negatively correlated with bone mineral content in both study and control females as well as study males.

Table 4.61 Correlation Statistics for Bone Mineral Content

Correlation	Study Males r value *p value*	Study Females r value *p value*	Control Males r value *p value*	Control Females r value *p value*
BMC with weight	0.92 *0.0001*	0.92 *0.0001*	0.88 *0.0001*	0.91 *0.0001*
BMC with height	0.86 *0.0001*	0.87 *0.0001*	0.84 *0.0001*	0.85 *0.0001*
BMC with age	0.87 *0.0001*	0.90 *0.0001*	0.84 *0.0001*	0.89 *0.0001*
BMC with serum Alb	0.40 *0.0001*	0.32 *0.0001*	0.34 *0.0001*	NS
BMC w/ serum PO_4	-0.56 *0.0001*	-0.52 *0.0001*	-0.51 *0.0001*	-0.60 *0.0001*
BMC with alkaline phosphatase	-0.47 *0.0001*	-0.73 *0.0001*	-0.32 *0.0001*	-0.64 *0.0001*
BMC with PO_4:Creat	-0.18 *0.001*	-0.31 *0.0001*	0.15 *0.005*	-0.38 *0.0001*

NS=not significant

c. Bone Density Correlations

Bone density increases with weight, height and age in all groups (Table 4.62). Biochemical variables showed weaker correlations than the anthropometric variables, as was the case for bone width and bone mineral content. Alkaline phosphatase and phosphate fall as bone density rises in all groups.

Table 4.63 Correlation Statistics for Apparent Bone Density

Correlation	Study Males	Study Females	Control Males	Control Females
ABD with weight	0.16 0.005	NS	-0.15 0.006	NS
ABD with height	0.16 0.005	NS	-0. 14 0. 01	NS
ABD with age	r = 0. 15 p=0.007	r= 0. 17 p=0. 0001	NS	NS
ABD with serum Ca	NS	NS	NS	NS
ABD w/ serum PO_4	NS	r=-0.24 p=0. 0001	NS	r= -0. 16 p=0.003
ABD with F:Creat	r= -0.28 p=0. 0001	NS	NS	NS

NS = not significant

When ABD was correlated with serum calcium levels no significant correlations were found. In an attempt to determine whether serum calcium levels had any effect on the bone variable ABD, serum calcium levels were split into 3 groups: 1) $\leq$ 2.25 mmol/l calcium, 2) > 2.25 mmol/l and $\leq$ 2.45 mmol/l and 3) $\geq$ 2.46 mmol/l calcium. When these groups were correlated with ABD there were no significant correlations at the 5% level or better. Analysis of variance did not reveal any significant relationships between ABD and the above calcium groups.

e. Regression Analysis and Bone Mass

Stepwise regression analysis is helpful in exploring the relationships between the independent and dependent variables. Bone width, bone mineral content, bone density and apparent bone density were the dependent variables. All serum and urine variables as well as height, weight and age were included in the regression model as independent variables. The maximum R^2 improvement option was specified. This technique starts by building a one-variable model producing the highest R^2; another variable, the one producing the greatest increase in R^2, is added, etc. The technique determines if removing a variable and replacing it with another increases the R^2. After all possible switches are investigated, the "best" model which describes the variance is reported.

Tables 4.64 and 4.64a tabulate the dependent variables (bone variables) and two models: a) the most important variables in describing the major part of the variance and, b) the "best fit" model; and the coefficient of determination (R^2 x 100) for each model. For example, in study males, weight described 83.7% of the variance for bone mineral content. When age, height, serum phosphate and alkaline phosphatase were added to the model, it only increased the described variance by 2.6% (Table 4.64).

As a general approach to size adjustment, Prentice et al. (1994) recommend that BW, height and weight should be incorporated in all regression models of BMC. Thus BMC is reported with three models, the last being that of Prentice et al.

Table 4.64 Regression Equation for Bone Parameters in the Study Group

Study: Males	R^2 x 100
BMC = 0.0006 + 0.017 (wt)	83.7%
BMC = 0.457 + 0.014 (age) - 0.002 (ht) + 0.014 (wt) - 0.108 (PO_4) - 0.0001 (alkp)	86.3%
BMC = 0.13 + 0.16 (BW) + 0.013 (wt) - 0.12 (PO_4)	86.3%
BW = 0.708 + 0.014 (wt)	63.2%
BW = 0.695 + 0.014 (age) + 0.010 (wt) - 0.054 (PO_4) + 0.028 (F:Creat)	66.5%
BD = 0.233 + 0.007 (wt)	60.3%
BD = 0.230 + 0.007 (age) - 0.004 (wt) - 0.013(F:Creat)	63.2%
ABD = 0.545 - 0.025 (F:Creat)	7.5%
ABD = 0.712 + 0.002 (age) - 0.08 (Ca) - 0.025 (F:Creat)	8.7%
Females	
BMC = 0.089 + 0.012 (wt)	82.4%
BMC = 0.111 + 0.0184 (age) + 0.007 (wt) - 0.0002 (alkp)	86.4%
BMC = -0.109 + 0.0182 (BW) + 0.019 (age) +0.005 (wt)	87.6%
BW = 0.6947 + 0.012 (wt)	58.6%
BW = -0.611 + 0.003 (ht) + 0.009 (wt) + 0.115 (PO_4) - 0.016 (albumin)	62.6%
BD = 0.219 + 0.013 (age) + 0.002 (wt)	60.5%
BD = 0.338 + 0.011 (age) + 0.001 (wt) - 0.045 (PO_4) - 0.029 (Mg:Creat)	62.3%
ABD = 1.17 - 0.111 (PO_4) - 0.010 (albumin)	6.0%
ABD = 1.184 - 0.109 (PO_4) - 0.010 (albumin) - 0.02 (Mg: Creat)	6.7%
wt=weight, ht=height, PO_4 = phosphate, alkp = alkaline phosphatase	

Table 4.64a Regression Equation for Bone Parameters in the Control Group

Control : Males	R^2 x 100
BMC = 0.037 + 0.013 (age) + 0.096 (wt)	80.2%
BMC = 0.248 + 0.018 (age) - 0.002 (ht) + 0.011 (wt) + 0.164 (PO_4)	80.7%
BMC = -0.179 + 0.442 (BW) + 0.007 (wt)	85.4%
BW = 0.561 + 0.019 (age) +0.010 (wt)	70.9%
BW = 0.583 + 0.013 (age) 0.009 (wt) - 0.169 (Ca:Creat)	71.8%
BD = 0.283 + 0.009 (age) + 0.002 (wt)	58.8%
BD = 0.179 +0.008 (age) + 0.002 (wt) - 0.0001 (alkp) + 0.003 (albumin)	61.2%
ABD = 0.82 - 0.001 (wt) - 0.108 (PO_4)	5.8%
ABD = 0.79 - 0.001 (wt) - 0.093 (PO_4) + 0.122 (Ca:Creat)	7.6%
Females	
BMC = 0.018 + 0.019 (age) + 0.007 (wt)	87.3%
BMC = 0.079 + 0.017 (age) + 0.007 (wt) - 0.0001 (alkp)	88.0%
BMC = -0.122 + 0.361 (BW) + 0.007 (wt)	88.0%
BW = 0.570 + 0.014 (age) + 0.008 (wt)	67.0%
BW = 0.192 + 0.014 (age) + 0.007 (wt) + 0.008 (albumin) - 0.193 (Ca:Creat)	68.9%
BD = 0.245 + 0.042 (age) + 0.002 (wt)	70.5%
BD = 0.215 + 0.010 (age) + 0.001 (ht) + 0.001 (wt) - 0.051 (PO_4)	71.8%
ABD = 1.168 - 0.100 (PO_4) - 0.008 (albumin) + 0.037 (Mg:Creat)	5.6%
ABD = 1.164 - 0.087 (PO_4) - 0.008 (albumin) + 0.041 (Mg:Creat) - 0.02 (PO_4:Creat)	6.5%
wt=weight, ht=height, PO_4 = phosphate, alkp = alkaline phosphatase	

Weight, age and, to a lesser degree, height commonly describe the variance in the regression equations for bone parameters. Bone mineral content is most influenced by weight with or without age, describing over 80% variance in all groups. Using the technique of Prentice et al. (1994), BMC consistently had BW

and weight fitted to the model for all groups except the study females who had BW and age in the equation. All models explained over 85% of the variance for BMC. The variance in bone width and density were also affected by weight and age, explaining 60% -70% in all groups. After the influence of age, weight and height in explaining variance in bone parameters for the various groups, serum and/or urine biochemistry variables were fitted to the models and improved the R^2 value only slightly. No variable was consistently included in the regression equation, although in study males, urine fluoride:creatinine was included in the equation for BW and BD.

In the regression model for ABD, age or weight was only included in the equation for study and control males, and little of the variance of ABD could be explained by any of the measured variables included in the models (less than 9% in all groups).

f. Analysis of Covariance and Bone Mass

Analysis of covariance was performed using the GLM procedure in SAS controlling for age, weight and height simultaneously (Tables 4.65 and 4.66). It was found that without the influence of these variables both males and females of the study group had significantly greater values for BW and BMC, while BD values were significantly lower than the control group.

Table 4.65 ANCOVA Table for Mean Bone Mass Parameters in Males

Covariate	Group	Ls Mean	Covariants	p value
Bone Width	Study Control	1.25 1.11	Age Height Weight	0.0001
Bone Mineral Content	Study Control	0.59 0.63	Age Height Weight	0.0001
Bone Density	Study Control	0.49 0.51	Age Height Weight	0.0001

Table 4.66 ANCOVA Table for Mean Bone Mass Parameters in Females

Covariate	Group	Ls Mean	Covariants	p value
Bone Width	Study Control	1.19 1.05	Age Height Weight	0.0001
Bone Mineral Content	Study Control	0.60 0.57	Age Height Weight	0.0001
Bone Density	Study Control	0.49 0.54	Age Height Weight	0.0001

4.5.5 Comparison of Bone Parameters with those of Urban Children

The bone mass data of the study and control groups were compared with values obtained from urban black children in Johannesburg (Patel, 1990). The urban children's appendicular bone mass measurements were determined using the same equipment and techniques as those employed in the current study. Significant differences within the three groups are seen in Tables 4.67 and 4.68. Regression lines drawn through the data points for the various bone parameters in the urban, study and control groups are seen in Figs 4.57-4.62.

Comparisons in pairs indicated differences between the study, control and urban groups. The following pairs showed significant differences for the bone variables:

1) Bone width in males was significantly different between all groups (p=0.0001). Males in the study group had wider bone than both the control and urban groups, while the urban group had wider bone than the control group. In females bone width was also greatest in the study group compared with the control and urban groups (p=0.0001), but the control and urban groups showed no statistical difference for bone width (p=0.185).

Table 4.67 ANCOVA Table for Mean Bone Mass Parameters in Males

Covariate	Group	Ls Mean	Covariants	p value
Bone Width	Urban [2,3] Study [1,2] Control [1,3]	1.17 1.26 1.11	Age Height Weight	0.0001
Bone Mineral Content	Urban [2,3] Study [1,2] Control [1,3]	0.69 0.64 0.59	Age Height Weight	0.0001
Bone Density	Urban [2,3] Study [1,2] Control [1,3]	0.57 0.49 0.51	Age Height Weight	0.0001
Apparent Bone Density	Urban [1,2] Study [1,3] Control [2,3]	0.63 0.50 0.60	Age Height Weight	0.0001

[1,2,3] = groups with same superscripts are different from each other (p=0.0001)

Table 4.68 ANCOVA Table for Mean Bone Mass Parameters in Females

Covariate	Group	Ls mean	Covariants	p value
Bone Width	Urban [2] Study [1,2] Control [1]	1.07 1.19 1.05	Age Height Weight	0.0001
Bone Mineral Content	Urban [2,3] Study [1,2] Control [1,3]	0.65 0.60 0.57	Age Height Weight	0.0001
Bone Density	Urban [2,3] Study [1,2] Control [1,3]	0.58 0.49 0.53	Age Height Weight	0.0001
Apparent Bone Density	Urban [1,2] Study [1,3] Control [2,3]	0.69 0.55 0.66	Age Height Weight	0.0001

[1,2,3] = groups with same superscripts are different from each other (p=0.0001)

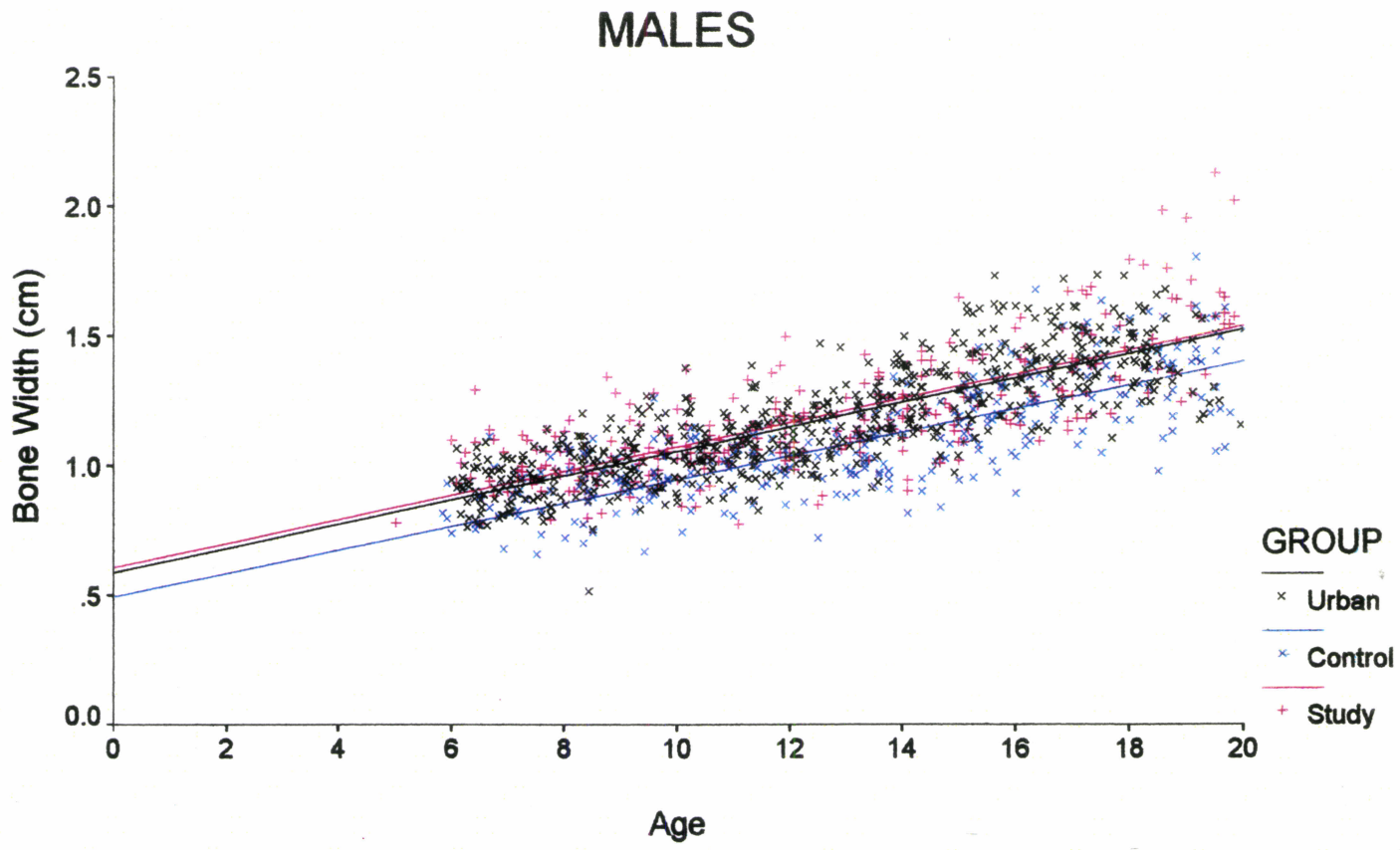


Fig 4.57 Bone Width for Males of the Study, Control and Urban Groups

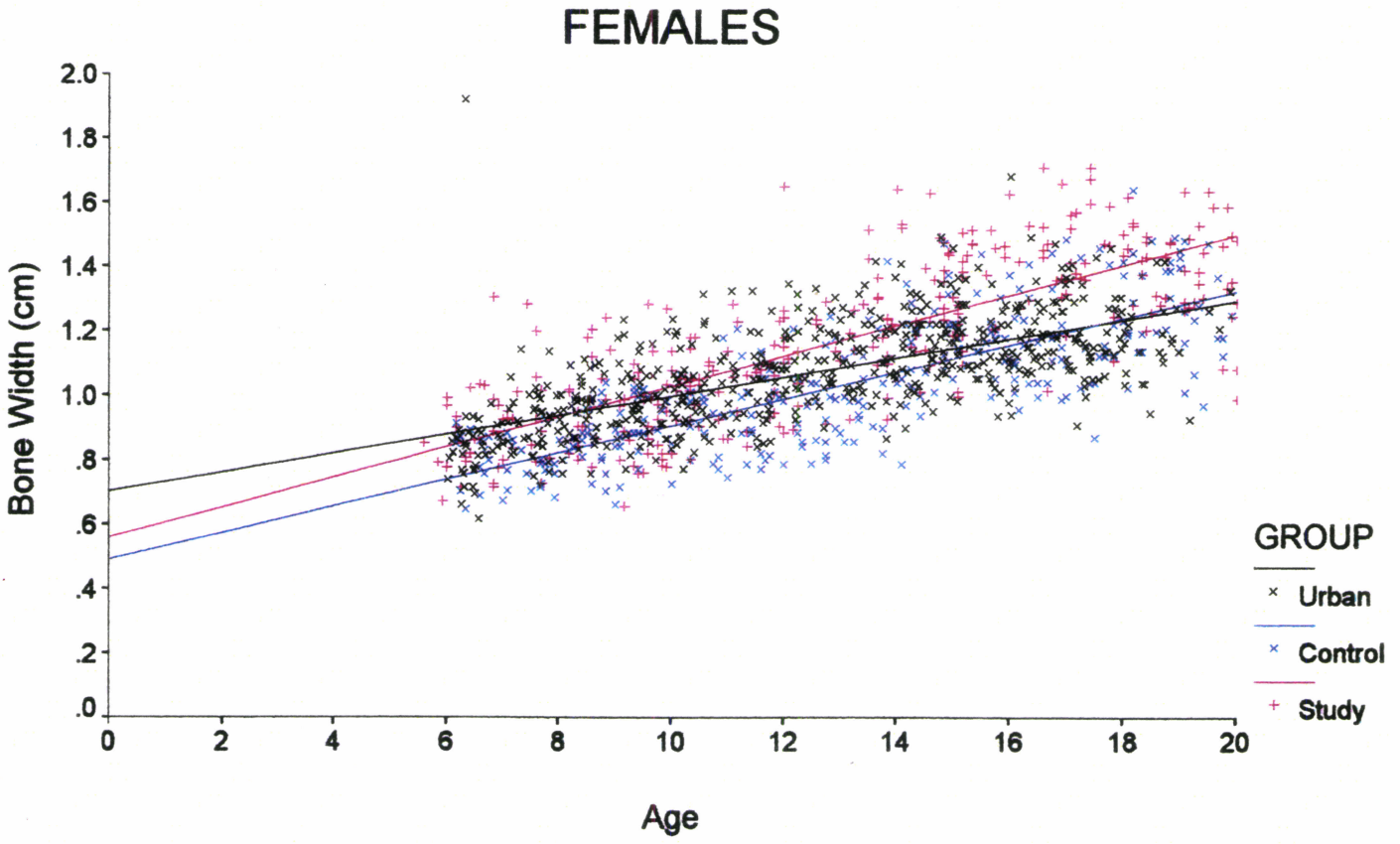


Fig 4.58 Bone Width for Females of the Study, Control and Urban Groups

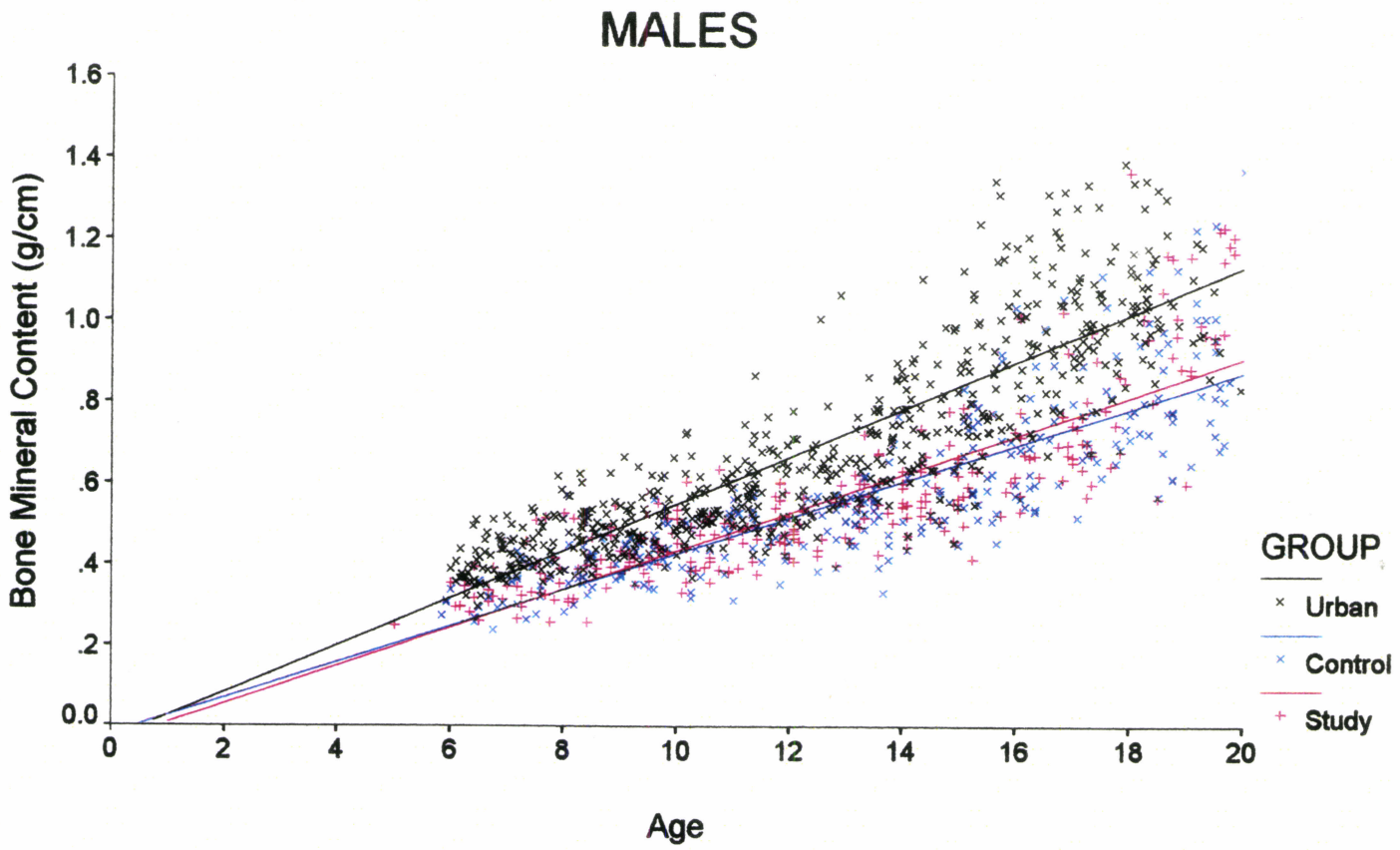


Fig 4.59 Bone Mineral Content for Males of the Study, Control and Urban Groups

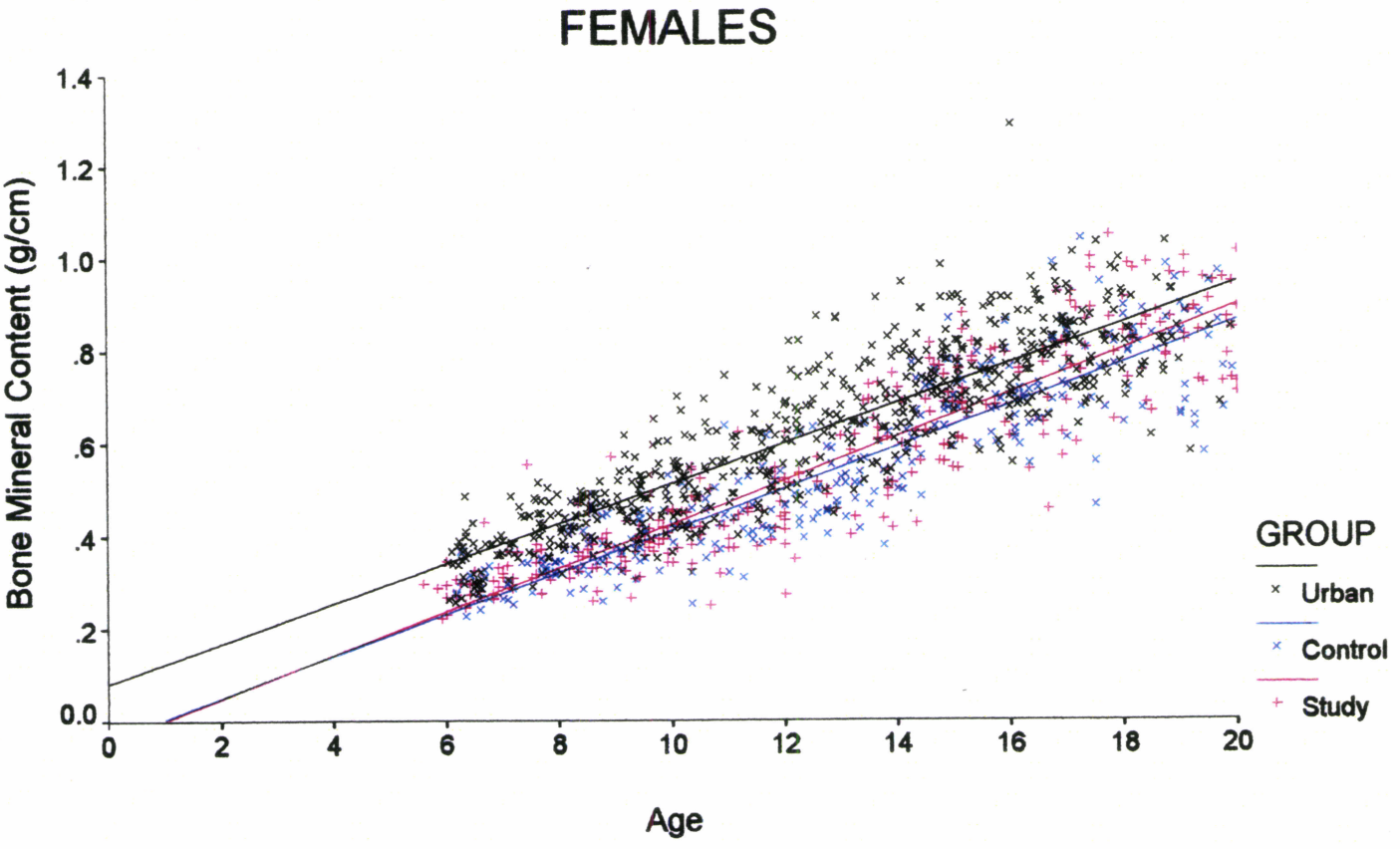


Fig 4.60 Bone Mineral Content for Females of the Study, Control and Urban Groups

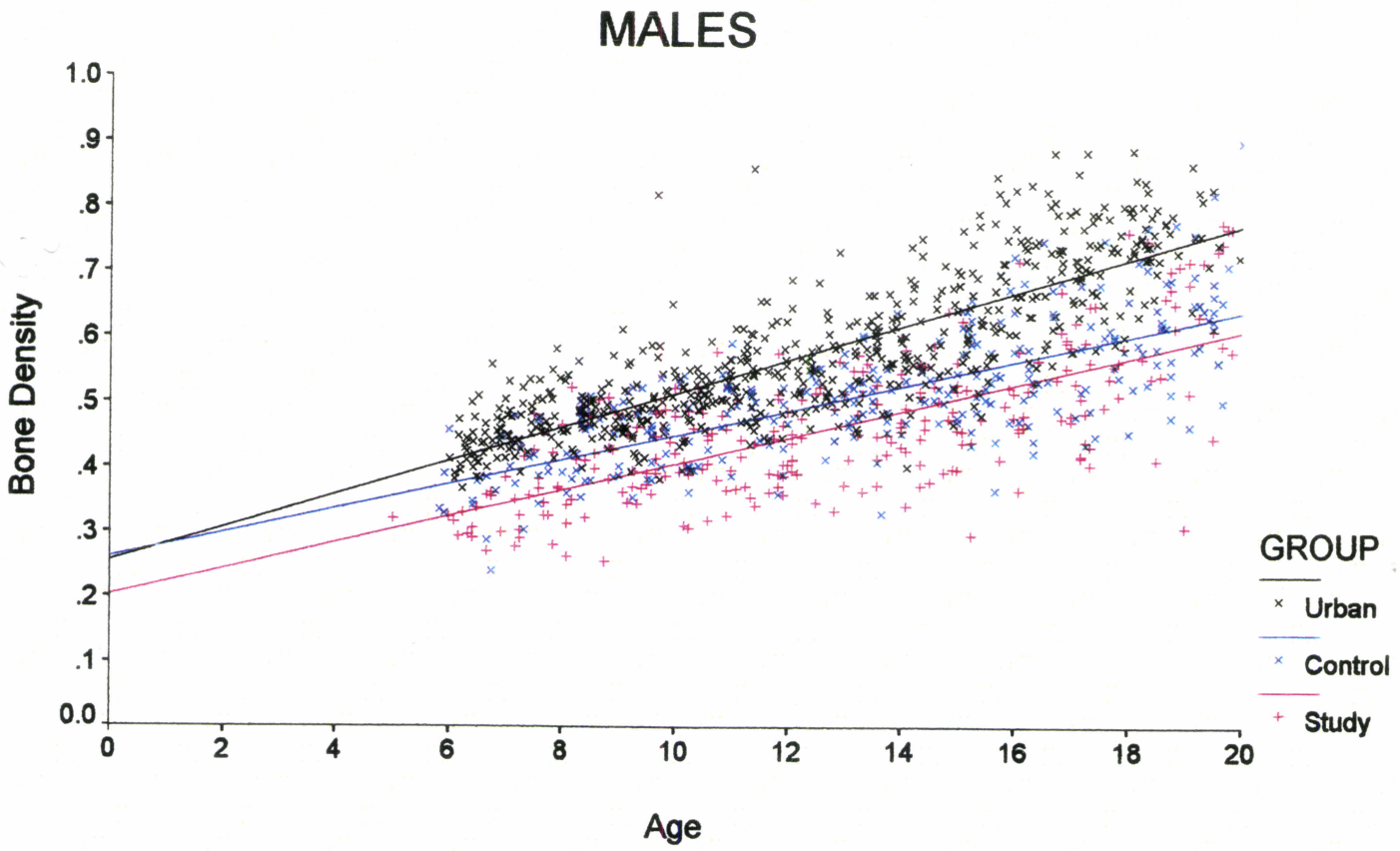


Fig 4.61 Bone Density for Males of the Study, Control and Urban Groups

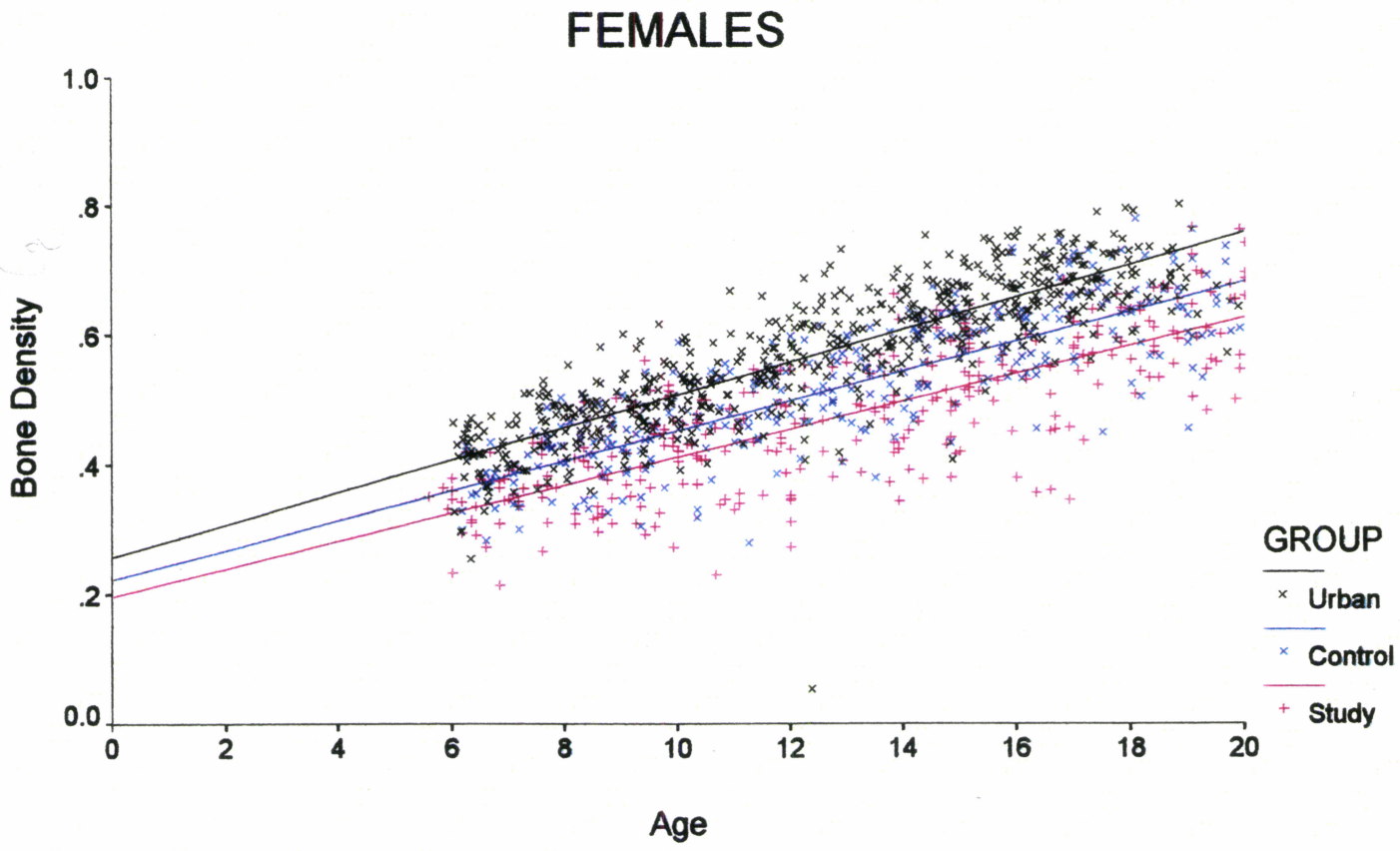


Fig 4.62 Bone Denstiy for Females of the Study, Control and Urban Groups

2) Bone mineral content in both males and females was significantly different between all three paired groups ($p=0.0001$). The children of the urban group had higher BMC than either the study or control groups while the study group had higher BMC than the control group .

3) Bone density in males and females shows significant differences between all three paired groups ($p=0.0001$); with the urban group being greater than the study or control group, and the control group, greater than the study group.

4) Apparent bone density in males shows significant differences between all three paired groups ($p=0.0001$). The urban groups have greater ABD than either the study or control groups, and the control groups have greater ABD than the study groups.

4.6.0 Dietary Results

4.6.1 Results of 7 Day Food Diaries

As was mentioned in the methods section, the food diaries were inadequately kept by the scholars. In an attempt to report the results, foods were grouped and the mean servings per weekday and weekend day are reported alphabetically for the study and control groups. The differences between the paired groups (weekday and weekend day) for each food category are reported as p values. However, conclusive dietary trends between weekends and weekdays or study and control groups should not be based on these data due to the above-mentioned problems with data collection.

Table 4.67 Food Frequency Table:
Mean Serving per Food Group and Standard Deviation Reported

Food Group	Study n=17	n=17		Control n=14	n=14	
	Mean weekday servings	Mean servings / weekend day	p value	Mean weekday servings	Mean servings / weekend day	p value
Bread {slice ± 1-2 cm thick}	1.93 (0.42)	1.32 (0.58)	0.001	1.65 (0.51)	1.54 (0.85)	0.68
Cereals {mealie meal, etc.}	1.42 (0.67)	1.88 (0.80)	0.009	1.54 (0.57)	2.08 (0.73)	0.003
Creamer - Non-dairy	0.64 (0.56)	0.56 (0.55)	0.37	0.60 (0.58)	0.69 (0.32)	0.62
Dairy Products (cheese, milk, sour milk)	0.93 (0.64)	1.35 (1.33)	0.15	0.95 (0.73)	1.35 (1.20)	0.17
Eggs	0.25 (0.20)	0.26 (0.24)	0.87	0.23 (0.11)	0.27 (0.10)	0.77
Fruit	0.84 (0.70)	1.29 (1.24)	0.06	1.20 (0.62)	1.73 (0.81)	0.03
Legumes and Nuts	0.29 (0.24)	0.24 (0.15)	0.58	0.35 (0.22)	0.19 (0.08)	0.02
Meat: red	0.55 (0.25)	0.85 (0.49)	0.04	0.55 (0.22)	0.88 (0.42)	0.02
Poultry and Fish	0.49 (0.35)	0.82 (0.64)	0.07	0.52 (0.40)	0.65 (0.43)	0.36
Sugar	1.02 (0.52)	1.26 (1.20)	0.52	1.31 (0.46)	1.04 (0.63)	0.23
Vegetables	1.46 (1.00)	1.88 (1.34)	0.32	1.77 (0.99)	2.19 (1.32)	0.40

Both the study and control populations consumed cereal, bread, sugar, fruit (apples, bananas and oranges) and vegetables (mainly cabbage, potatoes and tomatoes) more frequently than foods from other groups.

However, specific food groups did show significant variations between the frequency of consumption on weekdays and weekends. On weekdays, more bread was consumed in the study group ($p=0.001$), while cereals were eaten more frequently on the weekends in both groups (study: $p < 0.05$ and control: $p=0.003$). More fruit was eaten on weekends in the control group ($p=0.03$), and items from the legumes/nuts group were more frequently eaten during weekdays in the control group ($p=0.02$).

The study and control groups were similar in the food items consumed during weekdays and weekend days. Using the Wilcoxon Ranked Pairs test to compare total servings on weekend days and weekdays, it was found that they did not differ significantly in the study group ($p=0.107$) or in the control group ($p=0.143$).

4.6.2 Dietary Calcium, Daily Intake

Mean calcium intakes calculated from the 24-hour recall data were very low in all groups (Table 4.68). The minimum and maximum values show large variations in calcium intake within all subgroups. Males and females of the study population had higher mean calcium intakes than did their counterparts in the control population. These differences were significant in males ($p=0.03$) but not in females ($p=0.08$). When females and males were compared within the same group, there were no statistical differences (study males vs. females, $p= 0.15$; control males vs. females, $p= 0.64$).

Calcium is found in water as well as food sources. In the study group the calcium levels 18 mg/l and in the control group from 24 mg/l. Assuming an average intake of 1.5 liters of water during the day the study group would have an additional 27 mg/day and the control group 36 mg/day. However, water as a source of was not included in the calculation of dietary caclium intakes.

Table 4.68 Calcium Intake (mg/day): Group Means

Group	**Females** Study	Control	**Males** Study	Control
cases	n=32	n=31	n=31	n=31
Min-Max.	46-638	16-492	73-1078	20-640
Mean	244	190	319	205
Std Dev.	130	109	254	140
Median	146	176	227	154
		p=0.08		p=0.03

In an analysis by age group, it is shown that all age groups have mean intakes below 67% of the RDA and most groups fall near or below 33% of the RDA for their particular age categories (RDA, 1989) (Table 4.69). The control males aged 12-13 and 14-15 years had the lowest mean calcium intakes (157 mg/day for both age groups) and the highest mean calcium intake was 384 mg/day in study males in the 14-15 year category.

Group means were analysed using the Kruskal-Wallis (non-parametric) test to detect if there were significant differences in mean calcium intakes between the age groups. This was carried out for each sex in the study and control groups (Table 4.70). No significant differences in calcium intake were found between the age groups of the study or the control group.

Table 4.69 Calcium Intake per Age Group (mg/day)

	Females Study	**Control**	**Males Study**	**Control**
12-13 years	n=6	n=6	n=6	n=6
Min-Max.	46-317	84-350	94-399	98-213
Mean	*162*	*161*	*197*	*157*
Std Dev.	98	98.1	109	47
Median	142	121	174	157
RDA 1 200 mg				
14-15 years	n=6	n=6	n=7	n=7
Min-Max	101-440	16-492	73-1078	64-242
Mean	*234*	*245*	*384*	*157*
Std Dev.	147	192	350	69
Median	169	225	249	142
RDA 1 200 mg				
16-17 years	n=7	n=5	n=6	n=6
Min-Max	123-466	134-343	76-784	20-640
Mean	*259*	*214*	*365*	*215*
Std Dev.	116	82	280	220
Median	244	193	290	138
RDA 1 200 mg				
18-19 years	n=6	n=9	n=6	n=7
Min-Max	164-310	66-296	80-728	146-621
Mean	*264*	*168*	*263*	*245*
Std Dev.	53	81	242	172
Median	282	176	197	154
RDA 1 200 mg				
20-22 years	n=5	n=7	n=6	n=5
Min-Max	70-638	108-238	139-740	116-420
Mean	*290*	*178*	*374*	*262*
Std Dev.	189	56	239	131
Median	252	177	303.5	296
RDA 1 200 mg				

Table 4.70 Summary statistics for Calcium Intake by age group: Kruskal-Wallis test

Group	ChiSq	Degree Freedom	p value
Study			
Males	1.49	4	0.91
Females	6.49	4	0.16
Control			
Males	2.79	4	0.59
Females	1.96	4	0.74

When dietary calcium for group and sex was correlated with apparent bone density, serum calcium , inorganic phosphate and alkaline phosphatase levels, no significant correlations were found. Dietary calcium intake correlated significantly with inter-malleolar distance in study females (r=0.44; p=0.01).

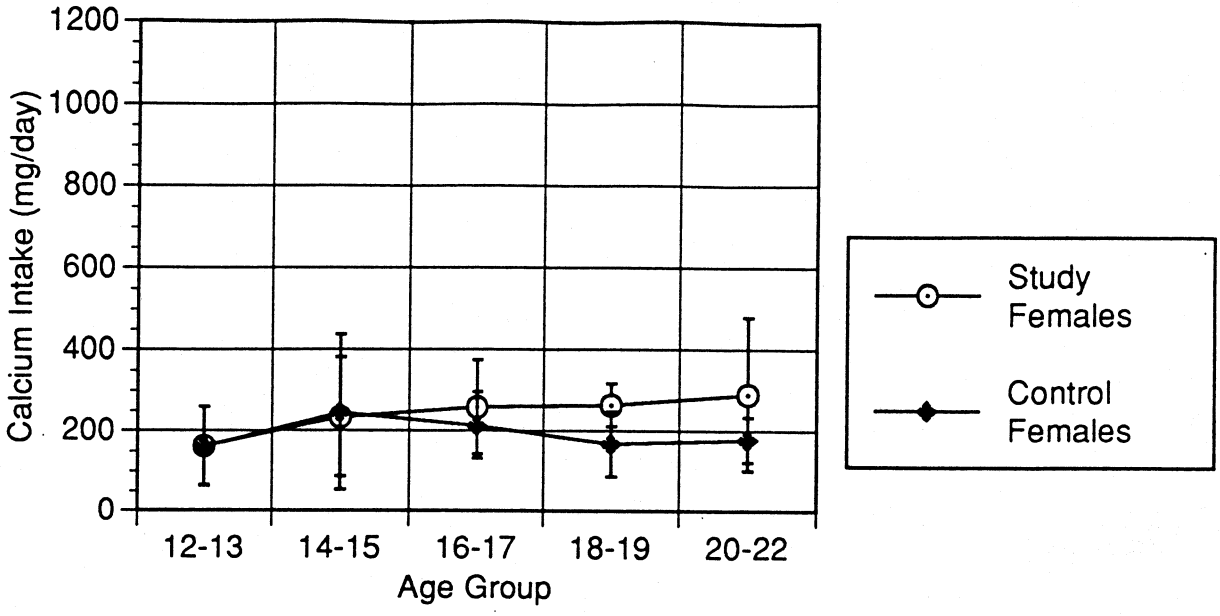


Fig 4.63 Mean Dietary Calcium Intake for Females of the Study and Control Groups

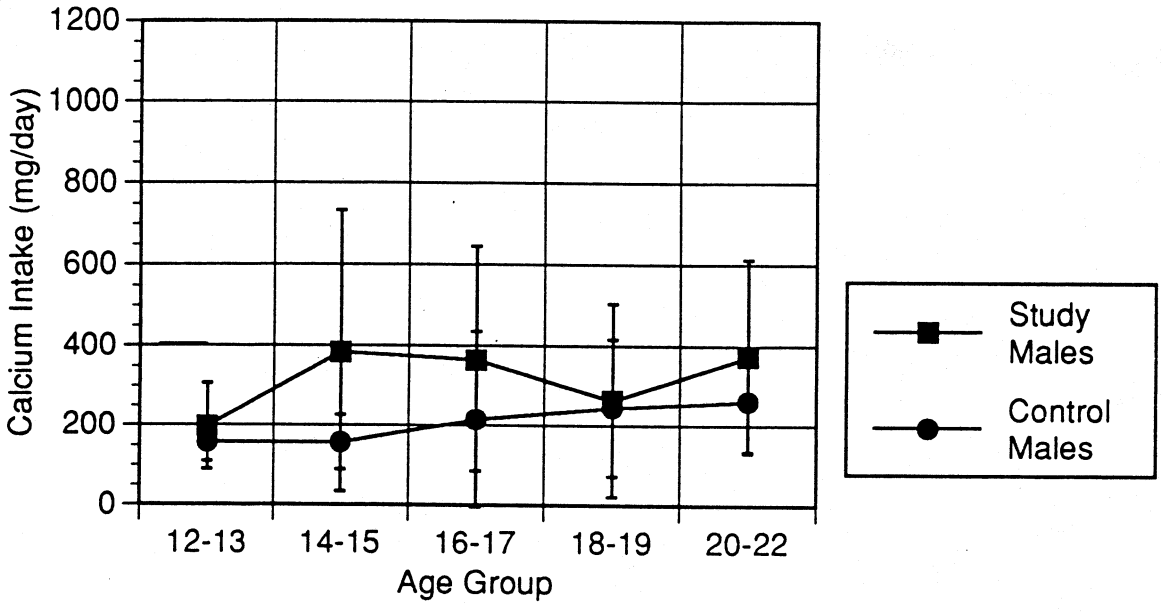


Fig 4.64 Mean Dietary Calcium Intake for Males of the Study and Control Groups

4.6.3 Dietary Protein, Daily Intake

The RDA for protein for females 11-14 and 19-24 years of age is 46 g/day, and for 15-18 years it is 44 g/day (RDA, 1989). The RDA for males 11-14 years of age is 45 g/day, for 15-18 years it is 59 g/day, and for 19-24 years it is 58 g/day (RDA, 1989).

The majority of the female age groups have median protein intakes approaching or above 67% of the relevant RDA, except in the youngest age group (12-13 years of age) (Table 4.72). A similar trend is seen in the males of both control and study groups.

When both males and females of the study and control groups were compared, no differences were found in mean protein intake ($p=0.71$ and $p=0.66$ respectively). Mean protein intakes within the same group were not different between females and males (study $p=0.32$, control $p=0.78$).

The World Health Organisation's (WHO) recommended protein intakes are roughly 15 to 20 g lower than the RDA's across age groups (WHO, 1974). When the WHO dietary recommendations are used as the reference, the study and control groups generally exceed the recommended protein intakes. Study females, (12-13 and 14-15 year age groups) and control males (12-13 year olds) had slightly lower intakes than the recommended amount for their age groups.

Table 4.71 Protein Intake (g/day): Group Means

Statistic	Study Females	Control Females	Study Males	Control Males
n	32	31	31	31
Mean	39.9	42.4	45.7	43.7
Std Dev.	24.5	20.4	21.9	18.8
Min-Max	8.2-124.5	15.4-92.4	14.9-100.4	82.9-15.8
Median	35.9	38.3	43.7	39.7

Table 4.72 Protein Intake by Age Group (g/day)

Statistic	Study Females	Control Females	Study Males	Control Males
12-13 years	n=6	n=6	n=6	n=6
Min-Max	19.8-37.7	20.2-46.5	14.9-44.2	15.8-39.7
Mean	*27.1*	*30*	*30.2*	*25.8*
Std Dev.	6.7	8.8	12.1	7.7
Median	25.5	28.2	32.7	24.4
	RDA 46g		RDA 45g	
14-15 years	n=6	n=6	n=7	n=7
Min-Max	21.4-44.0	30.4-64.1	24-88.9	19.4-81
Mean	*35.3*	*42.6*	*55.1*	*43.8*
Std Dev.	7.6	12.9	21.4	22.8
Median	36.3	39.1	51.7	35.5
	RDA 46g		RDA 45g	
16-17 years	n=7	n=5	n=6	n=6
Min-Max	20.5- 89.6	30.3- 92.4	20- 76.8	32.4- 61.2
Mean	*44.3*	*59*	*42.6*	*49*
Std Dev.	25.7	29.3	20.8	10.4
Median	37.2	45.2	37.3	49.1
	RDA 44g		RDA 59g	
18-19 years	n=6	n=9	n=6	n=7
Min-Max	8.2-124.5	15.4-91.3	19.5-52.2	24.3 -62.4
Mean	*58.8*	*38.6*	*37.7*	*41.0*
Std Dev.	40.2	23.7	13.1	11.7
Median	54.8	38.3	38.5	37.6
	RDA 44g		RDA 59g	
20-22 years	n=5	n=7	n=6	n=5
Min-Max	11.7-69.3	30.1-66.9	22.1-100.4	30.2-82.9
Mean	*34.2*	*47.0*	*61.2*	*62.9*
Std Dev.	20.0	14.1	28.3	21.8
Median	32.5	44.2	61.4	64.1
	RDA 46g		RDA 58g	

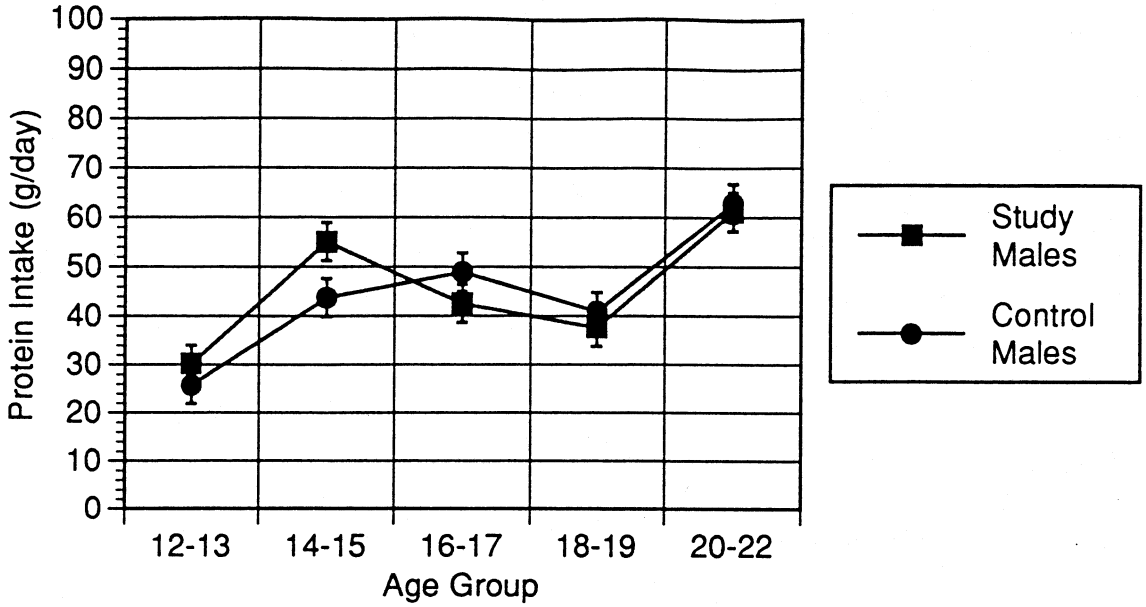


Fig 4.65 Mean Dietary Protein Intake for Males of the Study and Control Group

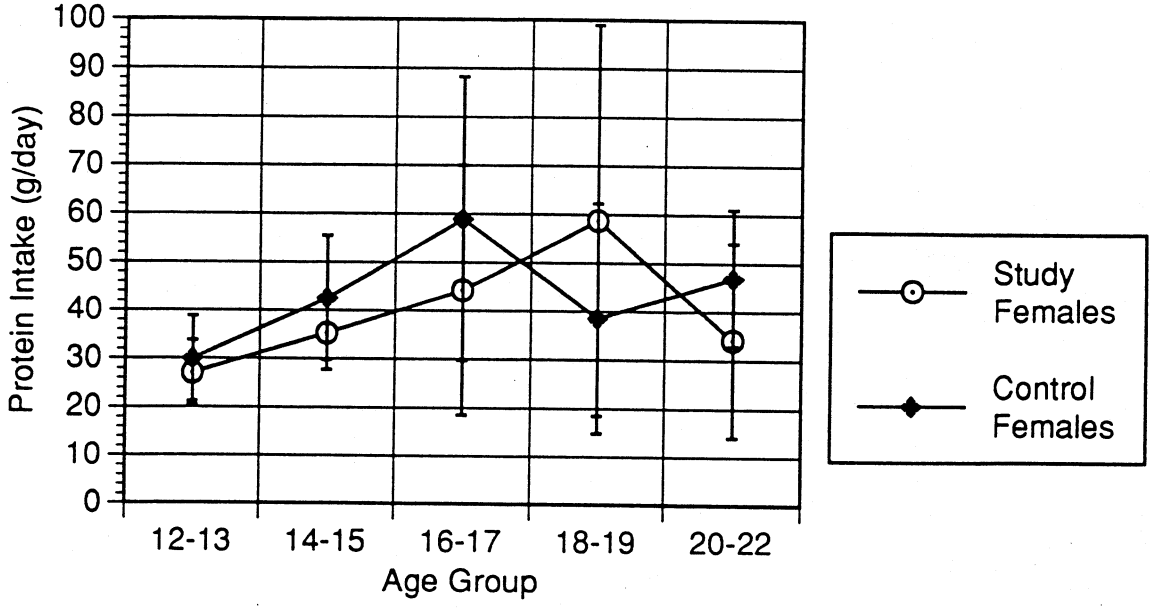


Fig 4.66 Mean Dietary Protein Intakes of Females of the Study and Control Groups

4.6.4 Dietary Fluoride, Daily Intake

Fluoride intake was estimated from water intake in tea, coffee and other beverages using local water, as reported in the 24-hour recall interview. These values do not reflect water intakes from local taps drunk from cupped hands (a common way of drinking water during school breaks and during times away from home facilities). Neither do they reflect fluoride intake from cooking processes, or from foods grown in local soils using local irrigation. Thus it can be safely assumed that these are minimum daily intakes.

The study population consistently had higher levels of fluoride intake than the control group for all age groups and both sexes ($p=0.001$). The lowest reported intake in the study group was 3.67 mg/day (females 20-22 years), which was still considerably higher than the highest reported intake in the control group (1.24 mg/day, control males of 16-17 years).

Table 4.73 Fluoride Intake from Drinking Water (mg/day) in Males

Age Group	Study	Control
12-13 years Mean Std Dev.	 4.51 1.22	 0.84 0.36
14-15 years Mean Std Dev.	 4.90 2.62	 1.10 0.35
16-17 years Mean Std Dev.	 4.70 1.75	 1.24 0.68
18-19 years Mean Std Dev.	 5.35 3.07	 0.74 0.21
20-22 years Mean Std Dev.	 5.79 2.66	 1.18 0.57

Table 4.74 Fluoride Intake from Drinking Water (mg/day) in Females

Age Group	Study	Control
12-13 years Mean Std Dev.	 6.11 2.77	 0.93 0.28
14-15 years Mean Std Dev.	 6.18 3.03	 0.86 0.21
16-17 years Mean Std Dev.	 4.09 1.45	 0.92 0.67
18-19 years Mean Std Dev.	 5.82 3.21	 1.04 0.37
20-22 years Mean Std Dev.	 3.67 1.34	 1.2 0.59

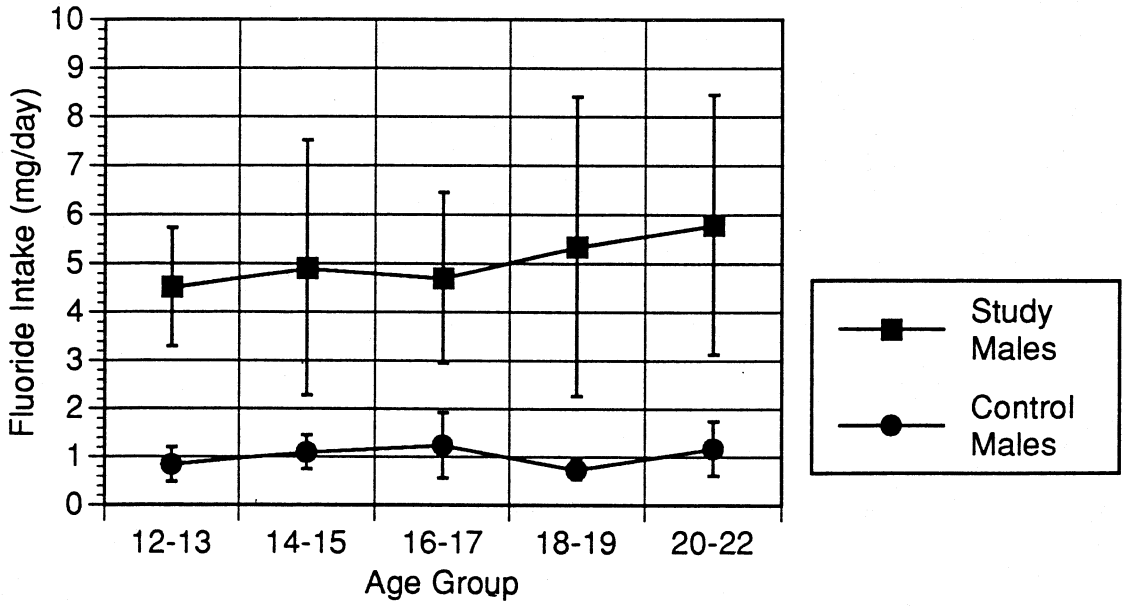


Fig 4.67 Dietary Intake of Fluoride in Males of the Study and Control Groups

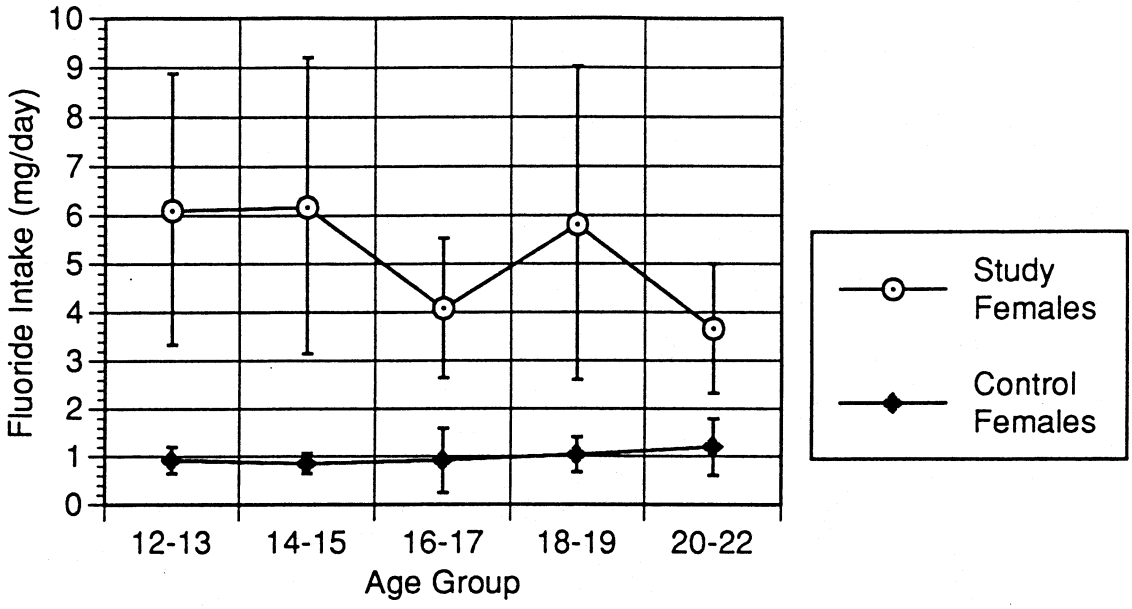


Fig 4.68 Dietary Intake of Fluoride in Females of the Study and Control Groups

Chapter V

5.0 Discussion

5.1 Overview of Fluorosis

In this study, a school-going population in Klipplatdrift, KwaNdebele, was investigated to determine the effects of high water fluoride levels (8.8 -9.4 ppm) on growth, serum and urine biochemistry and bone parameters. A demographically similar population 15 km away in Pieterskraal with lower water fluoride levels (1.6 - 2.0 ppm) was used as a control group.

The two groups had similar climatic, topographical and socio-economic profiles and consisted predominantly of Ndebele-speaking residents. The major difference between these two communities was the source of water. Klipplatdrift had water pumped from boreholes into a local reservoir while Pieterskraal had a natural lake nearby from where water was pumped into a reservoir. Both communities had water piped from reservoirs to street taps where residents collected their daily supply in buckets.

The classification of fluorosis in this study population would be considered primarily asymptomatic with some cases falling into the early symptomatic phase (those presenting with genu valgum). The picture of endemic fluorosis found in the study population was depicted by increased bone width and decreased bone density compared with the control population. Bone mineral content did not show as marked differences between the study and control groups until age, weight and height were controlled for, and then the study group showed higher mean bone mineral content than the control group. Mean apparent bone density was significantly lower in the study group compared with the control population. Elevated serum phosphate and serum alkaline phosphatase concentrations, and decreased urinary calcium excretion characterised the biochemical picture of

fluorosis in this endemic population. Hypocalcemia was found in 10% of the study population, but in only 3% of the control group.

Fluoride is a cumulative factor which can alter accretion and resorption of bone tissue, and it thus affects the homeostasis of bone tissue. The total dose of ingested fluoride is the single most important factor which determines the clinical course of the disease. In endemic fluorosis, one would expect to find clinical, skeletal and biochemical changes in the resident population. Krishnamachari attempted to classify the stages of skeletal fluorosis and proposed the following classification: 1) asymptomatic phase, 2) early symptomatic phase, 3) established skeletal phase, 4) phase of complications, 5) crippling fluorosis and 6) phase of incapacitation (Krishnamachari, 1986). The asymptomatic phase is described by Jolly et al. (1971) where there was radiological evidence of fluorosis, but patients were asymptomatic. The early symptomatic phase is generally recognisable by musculo-skeletal involvement. More advanced cases of severe arthralgia, arthritis associated with stiffness and limitation of movements of the spine would be recognised. In phase three, the established skeletal phase, marked limitation of movement will be evident in the knees, ankles and pelvis. The phase of complications presents with a variety of neurological defects including paraplegia, radiculopathy, cervical spondylosis and peripheral neuropathy. Muscle wasting is commonly observed at this stage. In phase five, crippling fluorosis, restriction of spinal movements eventually lead to the phase of incapacitation. Endemic genu valgum in its severe form is also included in this phase (Krishnamachari, 1986). This spectrum of change has been well documented, particularly by researchers in India and Africa (Teotia et al., 1971, 1979; Krishnamachari and Krishnaswamy, 1974; Christie, 1980; Pettifor et al., 1989).

This study on endemic fluorosis in children is unique in a number of aspects: 1) it uses single photon absorptiometry in the assessment of bone changes; previous studies have looked at radiological changes (Jackson, 1962; Steyn et al., 1962; Krishnamachari and Kamla, 1973; Krishnamachari and Sivakumar, 1976; Sivakumar and Krishnamachari et al., 1976; Teotia et al., 1971, 1976; Teotia and Teotia, 1973; Christie, 1980), light microscopy (Chongwan et al., 1986), histomorphometric measurements (Pettifor et al., 1989) and scintigraphic findings (Gupta et al., 1993); 2) it is the largest prevalence study reported with age-matched controls; and 3) it is one of the few studies that investigates dietary calcium intakes.

The current study includes anthropometric, dietary, biochemical, skeletal and bone densitometry data which will be discussed independently and then in conjunction with each other.

5.2 Anthropometry

Anthropometric indicators have long been used to separate groups of children into those who are "normally nourished" and those who are "malnourished." Significant growth retardation does not occur during a short-term episode of nutritional deficiency, but frequent episodes of acute food shortage, infection, or a sustained period of inadequate food intake may result in undernutrition manifesting as underweight for age, stunting or wasting. The nutritional profile of both the study and control communities was one of undernutrition and growth retardation in males and females, however males displayed greater degrees of growth retardation.

The indicators of nutritional status measured in this study are the anthropometric measurements of height-for-age and weight-for-age. The trend to fall below the 5th centile for height and weight was seen most markedly in males (height: study and control, 51% vs. 46.3% ; weight: 55.2% vs. 55%, respectively), although females

also had considerable numbers falling below the 5th centile for height and weight (height: study and control, 31.4% vs. 28.3% ; weight: 30.6% vs. 31.8%, respectively).

This pattern of growth retardation has been reported by a number of authors working in similar rural black communities (Richardson, 1973; Kotze et al., 1982; Shuenyane et al., 1977; Wagstaff et al., 1987; Pettifor et al., 1989). In an anthropometric survey of different cultural groups in South Africa, Kotze et al. (1982) found that there was a considerable difference in growth-rate between 6-9 year olds in the four ethnic groups investigated (Caucasian, mixed ethnic origin, Asian and black) with black children having the lowest growth-rates in weight and height. Kotze et al. also showed increasing percentages of children falling under 80% of NCHS values of weight for age with increasing age; 14% in black males aged 6 rising to 34% in 9 year olds and 19% in black females rising to 42.5% (Kotze et al., 1982). In a study which investigated the nutritional status of boys 10 years old (Voster et al., 1994) from a multicultural private school in Johannesburg, Voster et al. showed that a larger percentage of black boys fell below the 5th centile for height and weight than white, "coloured" and Indian boys (7.7% and 15.4% respectively). Pettifor et al. (1989), working in an area within 20 km of the current study and control areas, found that children aged 6 to 15 had increased percentages falling below the 5th centile for weight-for-age and height-for-age with increasing age. In males, no cases fell below the 5th centile for weight-for-age in the 6-7 year age group but there were 75% in the 14-15 year age group, while females had 4% below the 5th centile in the 6-7 year group and 43% in the 14-15 year age group. The same trend was seen for height, with 9% of males below the 5th centile in the 6-7 year group, increasing to 62% in 14 -15 year olds, and 17% in females aged 6-7 increasing to 86% in the 14-15 year age group (Pettifor et al., 1989).

Although growth retardation is commonly reported in rural black communities, Cameron et al. (1993) showed a catch-up growth phase. Cameron et al. (1993) investigated the growth of South African rural black children and found that by the start of adolescence approximately 50% of the children were below the 10th centile for the American norms. They found that the onset of adolescence in all groups was delayed but there appeared to be a catch-up growth phase in the late teenage years (Cameron et al. 1993).

The question arises of using Western standards, namely the NCHS growth charts, to measure a Third World population. Cameron (1986) suggests that Western standards for height and weight are not appropriate and that local standards should be compiled. However, Patel (1990) measured black urban children from private schools using the NCHS growth charts and found this population to follow the Western standards favourably (only 9.5% of the population falling below the 5th percentile) (Patel, 1990). The WHO recommendation is to continue the use of NCHS percentiles as international standards, as nutrition and infection are the main determinants of weight and height and not genetic factors (WHO, 1986).

Growth percentiles do not present the clearest picture for expressing data from developing countries since substantially large numbers of children have anthropometric values below the 5th or 3rd percentile. Percentages of median provide a distribution of low values but do not relate to the overall distribution of these values in the reference population (Graitcer et al., 1981). Standard deviation scores (z-scores) describe the approximate probability distribution values and are the preferred method for presenting anthropometric values from nutrition surveys in developing countries. Z-scores of greater than -2.0 for weight-for-age and height-for-age (about 95% of the reference population) are classified as underweight or stunted respectively (WHO, 1986).

The median z-score for weight in the study group decreased with age to the lowest level in 14-15 year old males (-2.35). The same trend was seen in the control group, with the lowest z-scores in males of the 14-15 year old category (-2.13). Males of these groups would be classified as underweight. Females followed a similar trend of decreased z-scores for weight with increased age until reaching the lowest median z-scores at 12-13 years of age (-1.64 and -1.43 for the study and control group respectively). However after this low point females of both groups improved the mean z-scores in the older age groups, revealing significant differences between the females and males of the 14-15 and 16-17 year age groups ($p=0.0001$ for both age groups of the study and control groups). This improvement in z-score in females corresponds with the earlier onset of puberty in girls than in boys and the resultant increase in weight. Calculations of body mass index confirmed that females aged 14-22 years were significantly fatter than males in both the study and control groups.

Z-scores for height follow the same growth trends as for weight. Males and females have mean z-scores that are relatively similar until the 12-13 year age group, after which female z-scores improved compared with those of males. Males of the study and control group dropped to the lowest values in the 16-17 year olds and 14-15 year olds (-2.35 and -2.25 respectively). Females dropped to the lowest z-scores of -1.75 in the 12-13 year olds of the study group and -1.65 in the 12-13 year olds of the control group.

This trend indicates a tendency to growth retardation until the growth spurt associated with puberty occurs and then there seems to be a "catch-up" period, however this cannot be clearly quantified by z-scores as the tables used in this study only go to 17 years and 11 months. Cameron et al. (1993) noted a similar delay in the growth spurt associated with puberty and reported a catch-up growth period.

The difference in growth trends between males and females may be explained by the delayed onset of puberty in males and thus a delayed pubescent growth spurt. There is little data on the onset of puberty in rural black boys, but Patel (1990) showed the mean onset of pubic hair development in urban black males to be 12.86 ± 1.22 years. Cameron et al. notes in a 1993 study that rural black children are consistently delayed in the age at which they entered the events of puberty and took longer to pass through each of the stages of puberty (Cameron et al., 1993). This implies that the onset of puberty in rural males would be later than 12.86 years as was the case in Patel's study. This is confirmed in the study by Cameron et al. (1993) where the onset of pubic hair development in rural males begins at the mean age of 13.8 ± 1.72 years (Cameron et al., 1993). The onset of puberty in rural black females starts earlier. Cameron and Wright calculate that most rural black girls are expected to have started puberty (onset of breast development) by the age of 11.7 years. Onset of menarche for these girls is at a mean age of 14.6 ± 1.2 years (Cameron and Wright, 1990). This trend to differing ages in the onset of puberty in females and males, and a delayed growth spurt in males, may explain the differences between males and females of the current research groups between the ages of 12-13 and more markedly in the 14-15 and 16-17 year age groups for height and weight.

Comparing the body mass index (BMI) of males and females in the same groups showed that the younger children had similar BMI, but as the ages increased the females' BMI progressively diverged from that of the males. In the study group, this divergence was first seen in the 10-11 year olds, while in the control group the difference only became significant in the 14-15 year age group.

BMI is a measurement which has elicited considerable debate as the current reference data is based on adolescents in the United States who have elevated and skewed age-specific distributions of BMI levels in the upper percentiles (> 85th centile). However, for the sake of uniform reporting and in the absence of other data specifying optimum cut-off values for BMI in adolescence, it is recommended that the BMI-for-age data for US children published by Must et al. (1991) should be used on a provisional basis until better reference data for adolescent growth are available (de Onis and Habicht, 1996).

BMI values were compared to the 50th centile data of the first National Health and Nutrition Survey (NHANES-I) for black Americans reported by Must et al. (1991). They reported BMI values for the 5th, 15th, 50th, 85th and 95th percentiles. BMI values for the males and females of both the study and control groups were closer to the 50th centile than to any other reported centile for BMI. BMI for males of the study and control groups were below the 50th centile values in the corresponding age categories of the NHANES-I study (See Table 4.10). Females of the study and control groups had mean BMI values that were lower than the 50th centiles in the age groups 8-9, 10-11 and 12-13 but in the 6-7, 14-15, 16-17, 18-19 and 20-22 year olds the BMI values were equal to the NHANES-I centile and in some cases higher (See Table 4.11). However, females in the study and control population with higher BMI values than the 50th centile did not reach the next reported centile (85th centile) of the NHANES-I data. The data suggest that although the majority of rural school children are stunted they are not wasted, as the BMI approximates the 50th centile for BMI in black children in the United States.

Although there are interesting differences in growth trends between males and females, these observations are seen between sexes of both the study and control groups. The anthropometric data do not highlight any differences between the

study and control populations. Both communities are growth retarded. It can be concluded that fluoride does not have any discernible effect on the anthropometric outcome in this study.

5.3 Dietary Intake

Nutritional requirements of young people are influenced primarily by the spurt of growth which occurs at puberty. A lack of adequate nutrition during the periods of rapid growth tends to unmask underlying problems in adolescent children. In this study the adolescent population fell into the category of "undernourished". How these nutritional factors influenced the effect of fluoride are not easily determined. The major nutrients of interest in this study are 1) calcium intake and its relationship to bone parameters and 2) the estimated fluoride dose ingested.

The word "monotonous" would be an appropriate description of the local diet in the study and control areas. There are limited food choices available in the local shops. The economics and cultural norms of the area dictate that basic foods like bread and mealie meal form the staple constituents of the diet. Vegetables are grown in a few gardens in the community and these are usually cabbage and potatoes. Most residents buy their food from the local shops. Tomatoes and onions are frequently bought along with cabbage and potatoes. Little, if any, fruit is grown in the area and the fruits reflected on the food frequency questionnaire are those available in the local shops. The food frequency lists revealed small differences between weekend and weekday food choices. Based on the economic profile of the area, it is suspected that greater variations in diet may be seen at the end of the month when wage-earning family members return home. The typical daily pattern of food choices is repetitive with little variation from day to day.

As was stated previously in the results chapter, it was intended that the food frequency and the 24-hour recall be correlated to examine the reliability of the

food intake information collected. This approach was also used by Nieves et al. (1995) when investigating calcium intake in relation to bone mineral density. They found that there was a high correlation (Spearman's ranked correlation) between 24-hour recall and food frequency information (Nieves et al., 1995). Unfortunately it was not possible to correlate the 24-hour recall data with the 7 day food diary data in the current study due to inadequate recording procedures carried out by the scholars selected. The data from the 7 day food diary is reported as food frequency data and used as general descriptive data. Specific measures of nutrients are reported from analysis of 24-hour recall data.

5.3.1 Dietary Calcium

The RDA is 1 200 mg per day for children 11-18 years of age and 800 mg per day for 6-10 and 19-22 year olds. The World Health Organisation recommends that calcium intakes for children aged 6-9 years should be 400-500 mg/day, for children aged 10-15 years the intake should be 600-700 mg/day and for those of 16-19 years intake 500-600 mg per day. The mean intakes of the study and control populations fall far below these conservative recommendations (WHO, 1974).

In the same way that water is a source of fluoride, water can be a significant source of calcium depending on the area and geological structures. Water samples in the area tested in 1984 and 1985 found borehole water to have calcium levels ranging between 3 and 18 mg/l (CSIR Water Research Unit, personal communication). These levels are not high enough to make a significant difference to the total dietary calcium intakes. Assuming a child would drink a maximum of approximately 1.2 - 2 litres of local water per day, this would contribute at most between 6 and 32 mg to the daily calcium intake.

Maize is the staple food of the rural diet and of this area and is particularly low in calcium (13 mg/100g). Dairy products are the major source of calcium in the diet, and the 24-hour recalls indicate that these items are not freely available, or are too

expensive to be consumed in large amounts. The seven day food diary data indicate that dairy products are a preferred food choice when available, with an average of 1.32 servings on weekend days and 0.9 servings on weekdays. Non-dairy creamers are used extensively, which may indicate a storage problem of fresh dairy products. Use of non-dairy creamers may also indicate a lack of awareness that they are not milk powders or that milk powders are more expensive than non-dairy creamers. This is an unfortunate trend as non-dairy creamer is not as rich a calcium source as milk, 22 mg Ca/100g non-dairy creamer vs. 119 mg Ca/100 g whole milk (NRIND tables, 1986). A study conducted in the Cape province of South Africa with 983 respondents from a black residential area aged 15-64 years of age looked at recent trends in dietary intakes (Bourne et al., 1994). Milk intake is reported to be low, with less than half of the two recommended portions per day being consumed. Cheese intake was negligible. This tendency to low dietary calcium intakes seems to be common in black communities. It is not clear if this trend is based in food preferences and cultural norms or poor economic factors limiting availability of calcium-rich foods. In many rural communities, sour milk forms a traditional food, but in those communities which do not have land to keep cattle, the cost of dairy products may be prohibitive and they are therefore not utilised.

Males and females in the study population had higher calcium intakes than did their counterparts in the control population. These differences were significant at $p=0.05$ in females and $p=0.03$ in males. When females and males were compared within the same group, there were no statistical differences. The mean calcium intakes of the various age groups ranged from 157 mg to 384 mg/day.

All calcium intakes in the studies mentioned are far below WHO recommend-ations. Dietary calcium intakes in this study were extremely low (the mean

calcium intake for the study population was 281 mg/day and for the control group 198 mg/day, both less than 33% of the RDA).

Krishnamachari and Krishnaswamy (1974) conducted an epidemiological study in an endemic area and included a dietary survey of 50 households. The authors do not state the methods used in collection of dietary data. The survey included 22 children (11 male and 11 female). They reported low median calcium intakes in children, (males: 153 mg/day (range 22-391); and females: 137 mg/day (range 68-213)).

Comparing mean calcium intakes of the study and control groups with those of South African dietary studies of similar age and race group, namely Steyn (1986), Lubbe (1973) and Eyeberg et al.(1986), revealed that the current study had low intakes of calcium similar to those of comparative studies (see Table 5.1).

Table 5.1 Comparative Studies of Calcium Intake

Area/Author & Age		Girls	Boys	Boys & Girls
KwaNdebele	12-22 study 12-22 control	244 mg 190 mg	319 mg 205 mg	
Steyn	11	285 mg	307 mg	
Lubbe	10-11	261 mg		
Eyeberg	8-10			199 mg study* 338 mg control**
	13-16			229 mg study* 378 mg control**

* study = serum Ca ≤ 2.25 mmol/l and serum alkaline phosphatase ≥ 300IU/l
** control = normal serum biochemistry

The study reported by Eyeberg et al. (1986) had a study group consisting of children in three age categories with serum calcium levels of ≤ 2.25 mmol/l and raised serum alkaline phosphatase ≥ 300 IU/l. The control group was from the same community. Children were age and sex matched and had normal serum

biochemistry. The study population reported by Eyeberg et al. (1986) has similar mean calcium intakes, although it is noted that the children, (8-10) years were younger than in the current research group. The 13-16 year olds' calcium intakes compared closely with those of similar ages in the current study.

Low dietary calcium in childhood can give rise to the development of rickets in childhood and osteomalacia in adult life.

Nutritional rickets was described by Pettifor et al. (1981) in a population of children in South Africa who consumed little milk or dairy products and whose daily calcium intake was estimated to be only 125 mg, while phosphate intake was adequate. It was previously believed that low or excessive dietary calcium intakes could be sustained without apparent signs of deficiency or excess, however Pettifor in his work has shown this not to be true. Dietary calcium deficiency has been the major agent in the development of rickets, osteomalacia and bone deformities in children from rural areas of South Africa and has subsequently been described in other parts of the world as well (Pettifor, 1991). Low dietary calcium intakes resulting in nutritional rickets in the Argentinian province of Tucuman were reported by Oliveri et al. (1991), where children had calcium intakes less than 180 mg/day. Low serum calcium, low urinary calcium excretion and elevated alkaline phosphatase levels were also seen in this population.

Poor socio-economic and cultural factors appear to be fundamental issues in low dietary calcium intakes and are common to both the study and the control group. The study group, although they had marginally higher dietary intakes of calcium, had more cases with hypocalcemia (10%) than the control group (2.1%). This increase in hypocalcemia in the study group is suggestive of a factor, possibly fluoride, resulting in the hypocalcemia. This is supported by Pettifor et al. (1979)

who suggest that endemic fluorosis increases the dietary calcium requirements and exacerbates the prevelence of hypocalemia.

5.3.2 Protein Intake

Food proteins provide amino acids for the synthesis of body protein and other important nitrogen-containing compounds, such as peptide hormones and neurotransmitters. Proteins and other nitrogenous compounds are being degraded and resynthesized continuously. Several times more protein is turned over daily within the body than is ordinarily consumed, thus reutilisation of amino acids is a major feature of the economy of protein metabolism (RDA, 1989).

There are two well established reference groups for which protein requirements can be regarded as reasonably reliable. These are young children and young male adults. For other age groups much less direct information is available, and protein needs must be assessed largely by interpolation or by extrapolation based on reasonable biological principles (Pellet, 1990). There is a wide range between sufficient protein intake to compensate for losses or permit growth in an individual and intakes that may be associated with harmful effects. For a group of similar persons, safe intake is considered to be an amount that will meet or exceed the requirements of practically all members of the group, allowing for individual variations (Pellet, 1990). Recommended daily allowances of protein vary with age. Females have RDAs for protein of 46 g/day for 11-14 and 19-24 years of age, and 44 g/day for 15-18 year olds (RDA, 1989). The RDA for males 11-14 years is 45 g/day, and 59 g/day for 15-18 year olds, decreasing to 58 g/day in the 19-24 year old group (RDA, 1989).

Protein intake, along with low calcium intakes, has been shown to be a significant factor in the development of osteopenia in endemic fluorosis (Mithal et. al., 1993).

That study showed that subjects with the lowest calcium and protein intakes had the highest mean alkaline phosphatase levels and showed a propensity to osteopenic bone. There were 65 randomly selected subjects (51 male and 14 female; age range 4-64 years). All had high values of serum and urinary fluoride compared with normal subjects. Dietary evaluations were carried out using the 24-hour recall method on three occasions with at least two weeks between assessments. The 65 subjects were grouped radiologically, with group 1 having a normal radiological pattern (30%), group 2 showing predominant osteosclerosis (30%), group 3 exhibiting coarse trabecular pattern (28%) and group 4 showing predominant osteopenia (12%). Group 1 had lower fluoride in the drinking water (4.02 mg/l) than group 2 (8.01 mg/l), group 3 (10.33 mg/l) or group 4 (7.32 mg/l). The protein and calcium intake was lower in group 4 (45 ± 8 g/day) than in groups 2 (57 ± 24 g/day) and 3 (51 ± 30 g/day) ($p=0.04$ and $p=0.02$ respectively). It is shown that subjects that had osteopenia of long bones had significantly lower calcium and protein intakes than other groups (Mithal et al., 1993).

Table 5.2 Dietary Calcium, Protein and Serum Alkaline Phosphatase Concentrations related to Bone Conditions (Mithal et al., 1993).

Group	Calcium (mg/day)	Protein (g/day)	Alkaline Phosphatase
Normal radiology	706 ± 325	61 ± 23	122. ± 43.7
Predominant osteosclerosis	638 ± 413	57 ± 24	164 ± 38.7
Coarse trabecular pattern	713 ± 421	51 ± 30	243 ± 93
Predominant osteo-penia	323 ± 180	45 ± 8	320 ± 337

In their epidemiological study of Krishnamachari and Krishnaswamy (1974), they describe subjects with genu valgum and evidence of skeletal changes such as sclerosis of the spine. In the dietary survey, they report male children to have a median protein intake of 44.7 g/day (range 6.1-108) vs. 39.1 g/day (range 20.5 -58.8)

for female children. These levels are similar to those of Mithal et al. (1993) and to the present study.

In the present population, the protein intakes in both the study (males 45.7 g/day, females 39.9 g/day) and control (males 43.7 g/day, females 42.4 g/day) groups were approximately 67% of the RDA in most groups. Daily protein intakes are comparable to group 4's intake in the study cited above, in both the study and control groups for males and females. Dietary calcium intakes in the study and control groups were also similar to those in group 4 of the study by Mithal et al.. In the light of the similarities of intakes of calcium and protein between the current research and that of group 4 of Mithal et al., which showed predominantly osteopenic bone, it would be interesting to compare alkaline phosphatase levels. However, this is difficult to do as the age group reported (4-64 years) includes the period of normally elevated alkaline phosphatase concentration associated with puberty, and Mithal et al. do not clarify the specific ages included in group 4. Thus it is not clear whether the increased alkaline phosphatase concentrations are due to normal physiological function or due to low calcium and protein intakes in association with high fluoride intake.

Dietary calcium and protein intakes were similar in the study and control populations. The protein intake of the two populations was low (67% of the RDA), however it was not as low as the calcium intake (< 33% of the RDA). The literature supports the important role that low dietary calcium plays in the development of endemic fluorosis as do the results reported by Mithal et al. (1993). It would appear that low dietary calcium intake is more influential in the development of altered bone homeostasis than protein intake. Fluoride, in the presence of low dietary calcium and protein intakes, plays a major role in the obvserved increase in bone width and decrease in bone density in the study

population. These nutrients did however not correlate significantly with bone or serum parameters.

5.3.3 Fluoride Dose

Fluoride dose is calculated from estimated water intake in beverages and from the drinking of water from local taps. Because scholars often drink from their hands when drinking from taps, accurate quantification is difficult. The fluoride intake ranged from 4.51-5.79 mg/day in study males and 3.67-6.18 mg/day in study females. The control group had considerably lower fluoride intakes, control males ranged from 0.74-1.18 mg/day, and in females ranged from 0.86-1.2 mg/day. It must be noted that some of the control population has elevated intakes, optimal fluoride intake being 1 mg/day.

There are other issues to be considered when looking at the consumed fluoride dose, namely climate, boiling of water and tea consumption. In a dental study done in Sri Lanka, the authors question the WHO appropriate upper limit for fluoride (1.5 mg/l) in hot, dry climates based on findings of fluorosis. Work done in Kenya and Senegal is cited and the authors recommend that the appropriate upper level should be 0.8 mg/l fluoride for hot, dry climates (Warnakulasuriya et al., 1992). Similar findings are reported by Brouwer et al. who also question the upper limit of the fluoride recommendation and report a more acceptable level to be 0.6 mg/l (Brouwer et al., 1988). The above authors maintain that increased climatic temperatures increase the amount of fluid intake, and in endemic areas this increases the fluoride dose. These conclusions are supported by the findings in the present study - even though the fluoride levels were within the recommended WHO range in the control community of the current study, dental fluorosis was found in 80% of the dental cohort. (Lewis et al. 1992).

Manji et al. (1986) looked at the incidence of dental fluorosis at sea level, 1 500 and 2 400 metres above sea level. They showed a significant increase in dental fluorosis with increased altitude. Manji et al. claim that their study for the first time establishes that populations living at high altitudes may be more susceptible to dental fluorosis than those living at low altitudes for a given concentration of fluoride in drinking water and that altitude must be taken into account when fluoride dose is investigated (Manji et al., 1986).

The boiling of water causes evaporation by steam and thus increases the fluoride concentration in the remaining boiled water. Grimaldo et al. examined risk factors in human exposure to fluoride and showed that boiling water and preparing food and beverages with boiled water increased the fluoride risk to humans (Grimaldo et al., 1995). The subjects living in the current area of research used boiled water extensively. The method of choice for cooking vegetables is boiling. Water is brought to a rapid boil before adding maize meal to make the staple food, maize meal porridge. Hot tea is the beverage of choice in the early morning and evening. These practices would most likely increase the fluoride dose.

When determining fluoride dose, the other issue for consideration is the local population's tea intake. Tea is naturally high in fluoride, and this is highlighted by children from Jordan who consumed 1.2 mg fluoride daily in tea (Fraysse et al., 1989). Sergio Gomez et al. (1989) investigated three brands of tea and measured the fluoride content after 5 minutes of infusion, the mean concentration being 2.36 ppm. They found that 68% of the children in the population drank tea (Sergio- Gomez et al., 1989). The children in KwaNdebele have high tea intakes - although specific quantities were not recorded, tea was reported as the beverage of choice in the morning and often again in the evening. Tea drinking, and the boiling of the water in tea preparation, may contribute to a higher fluoride dose than has been reflected in the current figures.

Studies where subjects showed evidence of symptomatic fluorosis cite water fluoride concentrations, but the actual dose ingested is not reported. The current study found water fluoride levels of 8.8-9.4 mg/l. Fluoride concentrations reported in the literature varied and ranged from 1.6 to 21 mg/l. Bagga et al. (1979) reported some of the lowest fluoride levels, 1.6-3.9 mg/l and Teotia et al. (1976) reported some of the highest levels, 24-26 mg/l (Teotia et al., 1976), along with Christie (1980), 21mg/l. Most of the studies ranged between approximately 4 and 12 mg/l (Teotia and Teotia, 1973; Mithal et al., 1993; Gupta et al., 1993; Krishnamachari and Krishnaswamy, 1973; Krishnamachari and Sivakumar, 1976; Siddiqui, 1955).

5.4 Skeletal Deformities

Deformities of the lower limbs in endemic fluorosis areas have been reported in India, Kenya, South Africa and other countries. Genu valgum (knock-knees) in particular seems to be more common in fluorotic areas (Krishnamachari et al., 1976; Christie, 1980; Steyn et al., 1962; Pettifor et al., 1989). Our results support these findings, with the prevalence in the study population of 24.7% vs. 13.8% in the control population ($p=0.0001$). Krishnamachari et al. reported a greater proportion of males affected by genu valgum than females, with a ratio of 15:1 (Krishnamachari and Kamla, 1973) and a 10:1 ratio in their 1976 study (Krishnamachari and sivakumar, 1976). This finding is supported by Christie (1980) and Pettifor et al. (1989), although the reason for more males than females presenting with genu valgum is unknown.

Contrary to all other studies reported, females in the present study were affected in higher numbers than males. Females in the study group had a prevalence of 30% vs. 19% in the males ($p=0.001$). This trend was also seen in the control group, with 16.2% in females vs. 11.4 % of the males but was not significant. The study

females showed a significant difference compared to the control group in the prevalence of genu valgum (30% vs. 16%) (p=0.001), however males of the two groups did not show significant differences. The reason for this predominantly female manifestation of genu valgum is unknown. A possible explanation for this may be that the female population were not as growth retarded as males. Females were more obese (they had greater BMI values, and fewer fell below the 5th percentile for weight-for-age compared with males). This may have resulted in a higher incidence of genu valgum in females because of increased body mass stressing fluorotic bone (possibly osteomalacia). Males, on the contrary, were "slower" growing and lighter in weight, and this may have protected them from the expected fluoride effect.

A similar female preponderance has been reported by Richardson (1974) in school children in South Africa. Black girls tended to be heavier than boys and there was an increased prevalence of genu valgum in older girls. In her data, Richardson saw a clear relationship between the severity of knock-knees and body mass index. In contrast, Indian girls with the lowest body mass index, showed little increase in genu valgum with age. A case is made that the heavier girls were the more likely to develop genu valgum (Richardson, 1974).

Prevalence studies in endemic areas may reveal different trends in the progression of fluorosis compared with studies which select subjects who are already symptomatic. The selection bias of symptomatic patients may mask trends seen in pre-symptomatic stages. Previous studies in which the prevalence of genu valgum was assessed were not random samples of a community, thus it is possible that males self-selected themselves because of more severe bone disease. This could have introduced bias into the results. The current study is the first reported in which the subjects were selected randomly from those attending school. This may have also introduced bias, as the most severely affected children could

possibly not have been attending school. However, the communities were well aware of the studies being undertaken and no cases of severe crippling fluorosis were brought to our attention.

Genu valgum and genu varum may be related to dietary calcium deficiency more than to effects of high fluoride intakes. Schnitzler et al. (1994) histomorphometically investigated South African black children (adolescents) with genu valgum and varum without radiologic rickets. Osteopenia was present in 46% with normal or low bone turnover, 19% had mildly increased bone turnover, 15% had histologic hyperparathyroidism, 8% had preosteomalacia and 12% had osteomalacia with features of hyperparathyroidism. The authors suggest that calcium deficiency may have caused the increase in bone turnover and the mineralisation defects (Schnitzler et al., 1994).

Genu varum measured by inter-patellar distance was not as commonly found as genu valgum. The total prevalence in the combined group of 1.8% (22 cases out of 1 205) falls below that found in a normal rural black population according to Richardson, who found genu varum (> 2.5 cm) ranging between 4-21% in rural black children (Richardson, 1974). It should be noted that the definition used for genu varum in the current study was >5 cm and not > 2.5 cm as used by Richardson. It is interesting to note that when study and control children were looked at separately, there was a tenfold increase in prevalence in the control population (n=20) when compared with the study group (n=2).

Possible reasons for the development of genu valgum as opposed to genu varum may include factors such as the age of onset of the deformities, the mechanical stress around the knee and the lines of force during weight bearing, or the natural differences between boys and girls as far as points of stress during weight bearing - resulting in males being more knock-kneed than females.

5.5 Serum Biochemistry

The biochemical variables measured were chosen to provide more information on altered mineral homeostasis (calcium, magnesium, phosphorus and alkaline phosphatase) and nutritional status (albumin and creatinine). In general, serum magnesium, creatinine and albumin concentrations were similar in the two groups, however statistical differences in serum calcium, phosphorus and alkaline phosphatase were detected.

5.5.1 Serum Calcium

Calcium is the principal mineral constituent of bone tissue. It is present in plasma in three forms: ionised (45-50%), bound to protein, mostly albumin (40-45%) and complexed (5-10%). The ionised calcium is the physiologically active fraction that directly affects neuromuscular excitability and release of PTH from the parathyroid glands (Walmsley and White, 1988). The total calcium concentration is most commonly measured for clinical purposes and was the index used in this study.

Rural areas of South Africa are regions where residents typically have poorer diets than their urban peers. The anthropometric data reveal that children in urban areas (Patel, 1990) are better nourished than those living in rural areas. Pettifor et al. (1979) also report that children (7-12 years old) in an urban setting are heavier and taller than their rural counterparts. The latter study included children from three areas, a rural community (Driefontein), a black residential suburb of a small town (Piet Retief) and an urban area (Soweto). Serum calcium concentrations in the urban group were significantly higher than in their rural peers, with those from Piet Retief falling between the rural and urban groups. The semi-urban area (Piet Retief) reported 6.5% hypocalcemia and the rural area 13.2% hypocalcemia (Pettifor et al., 1979).

Mean serum calcium levels in both study and control groups were within normal limits, however both groups had cases with hypocalcemia (≤ 2.25 mmol/l). The study group had 10% hypocalcemia (n=55) compared with 2.1% in the control group (n=13). Hypocalcemia may be a reflection of protein-energy malnutrition and associated hypoalbuminemia. Correction of total serum calcium for albumin concentrations did however not lower the prevalence of hypocalcemia in the study group.

Hypocalcemia has been reported by Pettifor et al. (1989) in a population neighbouring the current study area with a prevalence of 23% hypocalcemia. This population also had high hydric fluoride intakes (8-12 ppm). Dietary calcium intakes were low and some children presented with rickets. Pettifor et al. drew a comparative picture with another research area, a rural black community, low in fluoride, where hypocalcemia prevalence in children was 13.2%. These children also had low dietary calcium intakes (mean daily intake of 212 mg), (Pettifor et al., 1979). They point out that the prevalence of 13.2% hypocalcemia in this community is less than the 23% hypocalcemia found in the endemic fluoride area and suggest that endemic fluorosis increases calcium requirements and exacerbates the prevalence of hypocalcemia. This is supported by the data from the current study with 10% hypocalcemia in the study population and only 2.5% in the control although the study group had the higher dietary calcium intakes of the two groups. Hypocalcemia has not been reported in previous endemic fluorosis investigations, except for Opinya et al. (1991) who mentioned that two cases had calcium levels in the lower range of normal, and Zhang et al. (1994) who described a marked decrease in serum calcium in pigs from an endemic area as compared with pigs in a control group from a non-endemic area.

The question arises of dietary calcium intake in relation to fluoride dose. Most previous studies in India (Krishnamachari and Kamla, 1973: Krishnamachari and Sivakumar, 1976; Sing et al., 1963; Teotia et al., 1971, 1976, 1978; Teotia and Teotia, 1973) did not report calcium intakes. However, Teotia et al. cites research where the incidence and severity of bone disease and of bony leg deformities were greater in residents of endemic areas where there was calcium deficiency (intakes were not reported). Serum calcium levels were not reported (Teotia et al., 1983). The only study to carry out a dietary survey was that of Krishnamachari and Krishnaswamy (1974) where they report mean intakes ranging from the lowest, 137 mg/day in female children, to the highest, 297 mg/day in adult men. The sample of four groups they interviewed, male and female adults and male and female children, totalled 40 cases. Although low dietary calcium is reported, serum calcium levels were not investigated (Krishnamachari and Krishnaswamy 1974).

The relationship between high fluoride dose and low dietary calcium intake in relation to raised prevalence of hypocalcemia is unclear. In experimental fluorosis in rats, low dietary calcium intakes increase fluoride absorption and the toxicity of a given fluoride dose (Harrison et al., 1984). Radioisotope turnover studies in endemic fluorosis revealed increased calcium accretion and turnover rates (Narasinga Roa et al., 1979). Reddy and Narasinga Roa found increased retention of ^{45}Ca in monkeys receiving fluoride in combination with low dietary calcium, along with higher accretion rates and higher concentrations of alkaline phosphatase (Reddy and Narasinga Rao, 1971). Srikantia and Siddiqui found significantly higher retention of calcium in patients suffering from fluorosis (Srikantia and Siddiqui, 1965). One of the actions of fluoride on bone is a stimulatory effect on the osteoblast which leads to increased osteoid formation and thus increased calcium demands. Children in the present study area, where hypocalcemia is reported, and children in the study by Pettifor et al. (1989) had habitually low dietary calcium intakes. These low intakes were probably unable to meet the requirements of the

growing skeleton resulting in hypocalcemia, secondary hyperparathyroidism and increased bone turnover, these changes resulting in increased bone width and a decrease in bone density.

Although hypocalcemia has not been reported frequently, hyperparathyroidism has been described in a number of studies (in children: Teotia and Teotia, 1973; Pettifor et al., 1989: and adults Teotia et al., 1983; Krishnamachari and Kamla, 1973, Krishnamachari and Sivukumar, 1976; Krishnamachari, 1986; Opinya et al., 1991). It is likely that the stimulus for hyper-parathyroidism is a fall in ionised calcium, however there is evidence that fluoride itself may directly stimulate PTH secretion (Farley et al. 1990). Thus hypocalcemia or a low ionised calcium concentration is likely to be more common than the reported prevalence.

Small sample sizes may be a part of the reason that other clinical studies which assessed serum calcium levels conducted in children did not show hypocalcemia as in the current study and that of Pettifor et al. (1989). However in the study of Teotia et al. (1979) 200, patients were investigated and serum calcium concentrations were reported to be normal.

Table 5.3 Sample Sizes in Endemic Fluorosis Studies

Author	*Sample Size*
Krishnamachari and Kamla. (1973)	n = 24
Teotia et al. (1971)	n = 22 children
Teotia and Teotia (1973)	n = 20 adults with skeletal fluorosis
Pettifor et al. (1989)	n = 260 prevalence study
Greeff (current)	n = 614 prevalence study - study group
	n = 613 control group

5.5.2 Serum Inorganic Phosphate

Serum phosphate levels are very dependent on pubertal development. Levels fall during puberty and decrease to adult levels in late adolescence (Portale, 1990). For this reason, serum phosphate levels were investigated in an age-related manner; above and below the age of 13 in females and 18 in males. In both the study and control groups, the mean serum levels dropped with age. The study group had significantly higher mean serum phosphate levels than the control group, although the mean values for each group were within normal reference ranges. Despite mean values within the range of normal, the range revealed cases of hyperphosphatemia. Although, characteristically, endemic fluorosis presents with normal serum phosphorus (Teotia et al. 1979) findings by Pettifor et al. showed hyperphosphatemia in individual patients who presented with clinical bone changes such as genu varum and genu valgum (Pettifor et al. 1989).

The mechanism for the higher phosphate levels in the study than control groups is unclear. Although hypocalcemia would have been expected to result in an increase in PTH secretion, which would have increased renal phosphate excretion and thus lower serum phosphate levels, this was not found. Hyperphosphatemia has also been found in children admitted with dietary calcium deficiency and rickets (Pettifor et al., 1981). He suggests that the increased levels of 1,25-$(OH)_2D$, as a result of the high PTH levels, increase phosphate absorption from the gut, and together with a down regulation of renal PTH receptors, result in an increase in serum phosphate levels. Support for the down regulation of renal PTH receptors was provided in a study of children with rickets due to endemic fluorosis (Pettifor et al., 1989).

5.5.3 Alkaline Phosphatase

The major sources of alkaline phosphatase in the plasma are bone and liver, and the total concentration represents the sum of the activity from both. Measurement of the tissue-specific isoenzyme provides the ability to distinguish between these two sources, and Leung et al. found high levels of the bone-specific alkaline phosphatase in a normal population of adolescents, whereas levels of the liver-specific enzyme did not vary from adult levels (Leung et al. 1993). Kattwinkel et al. also showed that the major proportion of alkaline phosphatase found during childhood is derived from bone (Kattwinkel et al. , 1973).

Skeletal alkaline phosphatase is an enzyme localised on the membrane of the osteoblasts and released into the circulation by a mechanism which is unclear. It is the most commonly used marker of bone formation (Delmas, 1993). Alkaline phosphatase levels, like inorganic phosphate, are very dependent on the age of the child. Values are fairly constant in prepubertal age groups, rise with puberty and fall sharply to adult values in late adolescence (Bennett et al., 1976).

Leung et al. (1993) investigated bone-specific alkaline phosphatase in different bone and liver disease states. The control group, cosisted of 92 males and 78 females aged 10 to 80 years and over, showed peak levels at age 10 (males 240 IU/l; females 110 IU/l) and thereafter dropping steadily to adult levels (Leung et al., 1993). Kattwinkel et al. (1973) state that total alkaline phosphatase is highly age-dependent, and values as high as four times those of adult levels may be found at the end of puberty (18 years of age). Females showed a slightly earlier decrease to adult values than males did - a fact which corresponds to the earlier onset and completion of puberty in females. The ages at which onset of elevated alkaline phosphatase levels occur is approximately 10 to 14 years, dropping to adult levels between 17 and 18 years (Kattwinkel et al. 1973). From the trends described in

these studies, the females in the current research have similar ages (10-12 years) at which the alkaline phosphatase levels reach their highest concentations, corresponding with the onset of puberty. However, males in the present research are slightly slower in reaching the ages at which alkaline phosphatase levels reach maximum concentration, approximately 17-19 years.

This age-related trend in alkaline phosphatase levels was seen in both the study and control groups with mean levels fairly constant until puberty and then falling progressively to adult levels in the older age groups. The decline in alkaline phosphatase values in females started to occur in the 12-13 year old age group, while in males this puberty-associated fall occurred some five years later in the 18-19 year old age group. There was no difference in the age of onset of the adolescent decline between the study and control groups.

Both males and females in the study group had higher mean alkaline phosphatase concentrations than their peers in the control group. These differences were highly significant in the prepubertal children (288 vs. 197 IU/l in males; 328 vs. 200 IU/l in females: $p=0.0001$) but were less so in the adolescents. The possible reason for not showing a statistical difference is that the values were declining in both groups and had not reached the adult values, and secondly, the cut-off point used in males was 17 years, thus there were fewer adolescents for comparison.

It is difficult to compare the age of onset of elevated alkaline phosphatase levels (or puberty) with other studies of endemic fluorosis as age in relation to this measurement is not clearly reported. Teotia and Teotia (1973) investigated children with endemic fluorosis and showed elevated serum alkaline phosphatase levels in 4 of 22 children (15 males and 7 females). All 4 children were male aged 11 or 12 years and had alkaline phosphatase concentrations between 18 and 98 KA units (normal below 16 KA units). In the 1979 study by Teotia et al., they report

elevated alkaline phosphatase (mean 47.72 ± 13.48 KA) in all children investigated (n=200) aged 4 to 15 years old, however they do not report mean alkaline phosphatase related to age, nor do they clarify trends associated with puberty, but report the variable as a group mean (Teotia et al., 1979). Alkaline phosphatase levels in children in the study of Pettifor et al. (1989) (n=260) showed approximately a quarter of the children with elevated levels (> 300 IU/l). However, in those children who were admitted with advanced fluorosis and had bony symptoms (8 males and 1 female) only the female, aged 8 years, had normal alkaline phosphatase levels. The males ranged in age from 8 to 19 years. All had levels over > 300 IU/l (range 345 - 1 830 IU/l).

Raised alkaline phosphatase values have been found in children living in rural areas (Pettifor et al., 1979). In their study of urban, semi-urban and rural groups, described previously, rural children were noted to have the highest levels of alkaline phosphatase concentrations and the urban group to have the lowest concentrations. The frequency of elevated alkaline phosphatase concentrations was 41.5, 29.3 and 28.3% for the areas Driefontein, Piet Retief and Soweto, respectively. Pettifor et al. report that these results might be influenced by dietary calcium intakes as there is a significant correlation between urinary calcium excretion and both serum calcium and alkaline phosphatase (Pettifor et al., 1979).

Characteristically, endemic fluorosis patients have elevated alkaline phosphatase levels in children (Teotia and Teotia, 1973; Pettifor et al., 1989) and adults (Teotia et al., 1984; Krishnamachari and Kamla, 1973; Krishnamachari and Sivukumar, 1976; Krishnamachari, 1986; ; Opinya et al., 1993) and this study confirmed that finding. Mean alkaline phosphatase levels in the study females ≤ 12 years old were 328 ± 102 U/l, vs. 200 ± 53 IU/l in the control group. In males ≤ 18 years old, values were 288 ± 124 IU/l, vs. 197 ± 77 IU/l in the controls.

In the study group, 70 (12.5%) cases had serum alkaline phosphatase levels over 400 IU/l compared with 4 cases (0.8%) in the control population. These levels over 400 IU/l are clearly elevated, Pettifor et al. (1979) used 350 IU/l as the upper normal limit. When the mean serum calcium values for these cases were assessed they fell within normal range, 2.3 mmol/l in the study, 2.4 mmol/l in the control group. These elevated alkaline phosphatase levels in the study population probably indicate increased osteoblastic activity, which is a well documented fluoride effect (Farley et al., 1984, 1990).

In skeletal fluorosis, metabolism of bone-related minerals is altered to varying extents. Table 5.4 provides a summary of serum results published by a number of researchers in endemic fluorosis. Unfortunately alkaline phosphatase is reported in KA units not in international units as in the present study. Alkaline phosphatase is a sensitive indicator of altered bone metabolism in fluorosis.

Table 5.4 Biochemical Findings in Endemic Fluorosis Research

Author	Year	Age	Study Group	n	Ca mmol/l	PO4 mmol/l	Alk. Phos KA units
Srikantia & Siddiqui	1965	A*	fluorosis *reference*	31 -	2.53 *2.47*	1.16 *1.22*	16.9 *3.9*
Teotia et al.	1973	A*	fluorosis	5	2.55	1.22	53 (26-80)
Jolly	1971	A*	fluorosis *reference*	25 -	2.55 *2.47*	1.06 *1.09*	13.3 *8.7*
Teotia et al.	1979	C*	fluorosis *reference*	200	2.43 *2.25-2.62*	1.22 *1.45-2.5*	47.72 *6.16*
Pettifor et al.	1989	C*	fluorosis *reference*	9 -	2.04 *2.25-2.75*	1.77 *1.3-1.8*	867 IU/l *<300*

C* = children, A* = adults

It is characteristic of patients with skeletal fluorosis to have elevated alkaline phosphatase, normal serum calcium and phosphate values (Teotia et al., 1978). Patients in Teotia and Teotia's research showed high alkaline phosphatase levels and increased phosphate clearance (Teotia and Teotia, 1973). Increased urinary

excretion of phosphate is also reported (Teotia et al., 1971, 1979) however it is difficult to interpret the reported phosphaturia in the presence of normal serum phosphate levels as the authors do not state whether fasting urine specimens were used). Teotia also reported increased urinary excretion of fluoride and low urinary calcium levels (Teotia and Teotia, 1973). Opinya and Imalingat (1991) investigated skeletal fluorosis in two studies from an endemic area in Kenya with 10 ppm fluoride in Kenya, which is a similar fluoride level to the current study population. Biochemical tests confirmed the above findings and revealed normal serum calcium and phosphate and raised serum alkaline phosphatase (Opinya and Imalingat, 1991).

Serum 25 (OH)D levels have generally found to be normal in fluorosis (Mithal et al., 1995; Srivastava et al., 1989; Teotia et al., 1978). Sera from five patients with skeletal fluorosis were investigated for total calcium, ionised calcium, phosphate, alkaline phophatase, 25 (OH)D, 1,25 $(OH)_2D$, parathyroid hormone and osteocalcin concentrations (Srivastava et al., 1989). Patients with low calcium concentrations also had low 25 (OH)D, 1,25 $(OH)_2D$, and elevated PTH concentrations. Osteocalcin was markedly elevated in all patients as was alkaline phosphatase activity indicating increased osteoblastic activity. A different trend was seen in the study of Pettifor et al. (1989) where 9 children with skeletal symptoms of fluorosis were investigated. They reported elevated alkaline phosphatase concentrations in all but one patient, normal 25 (OH) D concentrations in two patients and elevated 1,25 $(OH)_2$ D concentrations in 7 patients, in keeping with the hypocalcemia which was found in 6 of the nine patients (Pettifor et al., 1989).

There is not agreement on the state of parathyroid function in fluorosis as various studies report completely different outcomes. Some workers have reported cases of hyperparathyroidism, others have found normal parathyroid function in most cases (Pettifor et al., 1989; Srivastava et al., 1989; Teotia and Teotia, 1973). There is

consensus on the existence of increased bone turnover in fluorosis, regardless of the state of parathyroid function. Bone scans show evidence of increased tracer uptake and turnover in virtually all patients with overt skeletal fluorosis (Gupta et al., 1993). Markers of resorption, like urinary hydroxyproline, are also increased (Srivastava et al., 1989; Mithal et al., 1995).

5.6 Urine Biochemistry

Urinary fluoride levels reflect the physiological balance related to recent and previous fluoride intake, degree of fluoride deposition and rate of fluoride mobilisation from bone. Fluoride is eliminated almost exclusively via the kidneys. As would be expected in an endemic fluorosis area, there were elevated urinary fluoride:creatinine ratios in the study population (males, 1.58 mg/mmol ± 1.19; females, 1.35 mg/mmol ± 0.98) compared with the control (males, 0.21 mg/mmol ± 0.19; females, 0.20 mg/mmol ± 0.18).

Hodge and Smith (1970) considered urinary fluoride to be the best indicator of fluoride intake level. Measurement of urinary fluoride over 24 hours shows concentrations that vary between 1.2 and 10.0 mg/24 hours. Narasinga Rao et al. (1979) correlated 24-hour urinary fluoride to the clinical stage of the disease in 50 patients who had radiological evidence of the disease. In their series they had values ranging from 1.2 to 20.0 mg/day. In crippling stages of the disease, a urinary fluoride value 6.6 mg/day was observed. Patients who developed radiculopathy as a complication of disease invariably excreted more than 3.0 mg/day. Dental mottling was present even when the urinary fluoride was as low as 1.2 mg/day (Narasinga Rao et al., 1979). Elevated urinary fluoride was also found by other researchers working in the area of endemic fluorosis (Krishnamachari and Kamla, 1973, Krishnamachari and Sivukumar, 1976; Sing et al., 1963; Teotia et al., 1971, 1976, 1978; Teotia and Teotia, 1973; Pettifor et al., 1989). The present study

population had a six- to sevenfold greater urine fluoride:creatinine ratio than the control population.

In addition to increased urinary fluoride levels, the study population had decreased urinary calcium levels. This is most likely due to increased uptake of calcium into bone and/or to increased PTH secretion which would lead to renal tubular reabsoption of calcium. The calcium:creatinine ratios for study and control males were 0.094 mmol/mmol ± 0.111 vs. 0.113 mmol/mmol ± 0.121, respectively, and for study and control females 0.108 ± 0.129 mmol/mmol vs. 0.139 mmol/mmol ± 0.139, respectively. This trend is supported by Teotia and Teotia (1973) Teotia et al. (1978, 1979) and Pettifor et al. (1989), where similar reduced urinary calcium findings are reported. Low urinary calcium levels along with high levels of serum alkaline phosphatase suggest elevated parathyroid hormone (PTH). Although PTH levels were not measured in this study, increased PTH levels in endemic fluorosis were reported by Teotia et al. (1978) and Sivakumar and Krishnamachari (1976). Pettifor et al. (1979) report low urinary calcium levels in children living in rural areas compared to urban children living in Soweto. They suggest that low dietary calcium intakes contribute to this by stimulating PTH secretion which increases renal uptake of calcium. Reduced urinary calcium in the current study is most probably due to low dietary calcium intakes in addition to effects of high levels of fluoride.

Urinary magnesium levels were similar between the study and control groups for both males and females.

Fasting urine specimens are needed to establish phosphaturia, and the current study used random specimens which reflect dietary phosphate intake.
The phosphate:creatinine ratios were similar in the study and control groups as would be expected because the dietary intakes were similar. The

phosphate:creatinine ratio was slightly increased in the study females when compared with the control females (p=0.015), but this trend was not seen in the males of the two groups.

5.7 Bone Densitometry Parameters

The human skeleton is formed and modelled during childhood and youth through the constant influence of daily mechanical loading. In young adulthood (± 20-25 years) the skeleton achieves its maximum mass and strength (Mosekilde, 1993) although Sabatier et al. (1996) report that the age at which maximal bone mass is reached is still debated.

It is well known that bone mass in adulthood is greater in men than in women, there being a significant sex difference in size and cortical thickness of most axial and appendicular bones. This gender difference develops during puberty - no consistent differences are observed in bone mass between male and female prepubertal children (Carrie-Fassler and Bonjour, 1995). Bone mass accrual accelerates at 11 years of age and 13 years of age in Western girls and boys respectively, and bone mineral density increases at a high rate for three consecutive years in girls, as compared with four years in boys. Carrie-Fassler and Bonjour report that studies suggest the greater bone mass in young adult males may be due to a longer pubertal maturation rather than to a greater rate of bone mass gain, and possibly to a greater duration or magnitude of the "consolidation period" in boys than in girls. The two years additional prepubertal growth in boys may also be an important determinant of the sex difference in bone mass (Carrie-Fassler and Bonjour, 1995).

Bone mass is generally estimated from measurement of bone mineral content (BMC) or bone mineral density (BMD) in the entire skeleton or at given sites. A number of non-invasive methods, with varying accuracy and precision depending

on the skeletal region assessed, equipment being used, are currently available. The most commonly used approach is dual x-ray absorptiometry (DXA). Single- and dual-energy photon absorptiometry, typically of the appendicular skeleton, which measures bone width (cm), bone mineral content (g/cm) or bone density (g/cm^2) is useful in paediatric populations (Carrie-Fassler and Bonjour, 1995). The junction of the middle and distal thirds of the radius was measured for bone mineral content, bone width and bone density using the Norland single energy (^{125}I) photon absorptiometer (Model 278A). At this site the bone is mainly cortical bone, and bone mass measured at the mid-radial site which is also cortical correlates well with the weight of other long bones, as well as with total skeletal weight and total body calcium (Johnston, 1981).

Most studies using absorptiometry have concentrated on bone mass in adults, especially postmenopausal females. There are few studies concerning bone mass acquisition during youth using absorptiometry (SPA) or quantitative computed tomography (QCT) (Sabatier et al., 1996). There is even less research on ethnic differences during adolescence using bone densitometry. Patel (1990) was the first to look at bone mass indices in adolescents of urban racial groups in South Africa.

Skeletal fluorosis has been investigated by radiographs and bone biopsies, but absorptiometry data have not been reported from an endemic fluoride area before.

It is difficult to determine the fluoride effect on bone when it is changing in width, mineral content and density as a result of normal growth. The control population provides a much needed reference in highlighting the fluoride effect in the research population.

5.7.1 Bone Width

Bone width increases with age, and this was evident in both the study and control populations. In both groups, males had consistently wider bone than females ($p=0.004$ and $p=0.0001$ respectively). This sex-related difference was also noted by Patel (1990) who found males to have greater bone width and bone mineral content than females of the same age groups in an urban paediatric population.

An unusual gender-related difference is seen in the 14-15 year age group where females of the study group have greater mean bone width values than their male counterparts ($p=0.007$). This trend is also seen in the 12-13 and 16-17 year age groups, however the differences are not significant ($p=0.59$ and $p=0.22$, respectively). The control group does not exhibit this difference in bone width, although a similar trend was present. Bone width increases as longitudinal growth occurs, and it is likely that the wider bone seen in females 14-15 years old is due to the earlier pubertal growth spurt of girls, with males lagging behind until their growth spurt.

Study males in this age group had significantly higher alkaline phosphatase levels than their female counterparts (297 IU/l vs. 182 IU/l respectively $p=0.0001$). Concentrations in females had started dropping to adult levels with the earlier onset of puberty.

Mean bone width was greater in the study population (1.18 gm/cm^2) than in the control population (1.05 gm/cm^2) ($p=0.0001$). Although other absorptiometric findings are not available in endemic fluorosis studies, our findings are consistent with radiographic trends. Radiological findings in endemic fluorosis show increased bone width in children (Teotia et al. 1976; Jackson, 1962; Christie, 1980; Krishnamachari and Sivakumar, 1976; Pettifor 1989). In a study of 127 (13-62 years)

clinically proven endemic fluorosis cases, radiographs showed a clear and consistent trend to diaphyseal widening of the long bones (Wang et al., 1993).

Maximum bone width is reached in the study group by the 16-17 year age group in females (1.43 cm) and by the 18-19 year age group in males (1.61cm). The control group shows maximum bone width values in the 18-19 year age group for females (1.27 cm), and the highest value in males is seen in the 20-22 year age group (1.50 cm). It cannot be determined if this is the highest value as densitometry data were not collected beyond this age. These data highlight that an earlier maximum bone width is achieved in the study group compared with the control group. Secondary hyperparathyroidism may be the mechanism causing this earlier maximum bone width in the study group, due to increased calcium requirements of fluoride stimulated bone resulting in increased PTH levels and thus higher periosteal new bone formation and greater bone width.

Although correlations are helpful in explaining relationships between variables, caution must be exercised when generalising the relationship to the whole population. Regression analysis is more useful in describing a model. As would be expected anthropometric variables explained more of the variance in the population than biochemical variables. Regression analysis of bone width showed weight to be the most influential in describing the variance in the males and females of the study population (63.2% and 58.6% respectively), compared with weight and age in the control population males and females (70.9% and 67% respectively).

Bone parameters from a well-nourished black urban population of children 6-18 years old (Patel, 1990) were compared with those from the study and control groups and used as a reference group. In the urban group the fluoride levels are low at 0.21-0.33 ppm (Grobler and Dreyer, 1988). The urban group, had greater

physical stature and better nutrition (the majority of this group was between the 25th-75th centile for height and weight compared with the study and control groups which had approximately half the population below the 5th centile for height and weight). Thus one would have expected the urban group to have the greatest bone width. However, the study population had greater bone width values than both the urban and control groups. The urban group had greater bone width than the control group as one would expect (See Figs 4.57-4.62). The three groups were significantly different from each other (p=0.001). The study and control populations were similar in growth and nutritional profiles which indicates an additional factor causing abnormally wide bone. High fluoride intake in the study group is most probably the major contributing factor in increased bone width.

5.7.2 Bone Mineral Content

Bone mineral content (g/cm), like bone width, is a direct measurement carried out by absorptiometry. Bone mineral content increases with age, but the age at which peak bone mineral content is achieved is not clear. The maximum amount of bone an individual acquires during the first two decades of life is an important determinant of bone mass throughout the rest of life (Sabatier et al., 1996).

Males of the study group had greater BMC than females, and this reached significance in four age groups (6-7, 8-9, 18-19, and 20-22). However, deviation from this trend is seen at the 14-15 year age group where females have greater BMC values than males (p=0.001), a pattern similar to that seen in bone width. Females continue with greater BMC values in the 16-17 year age group, but this is not significant (p=0.14), and in the 18-19 year age group males again have greater BMC values. Females in the control group also exhibit greater BMC values than males in the 14-15 year age group (p=0.04). As this reversal trend is seen in both groups, the influence of growth on BMC in females may play a role. The more

marked trend in the study females may be a fluoride effect in addition to growth trends. The changing pattern between males and females during the early adolescent period is probably a reflection of the earlier onset of puberty in females causing a greater increase in bone mass (growth).

On comparing the mean bone mineral content between the study and control groups no significant difference was found. Using covariant analysis and controlling for height, weight and age, bone mineral content was significantly higher in the study group than in the control group. The two groups were significantly different from each other; for males ($p=0.0001$) and females ($p=0.0001$) (see Tables 4.65 and 4.66). These differences may be explained by the direct effectof fluoride on osteoblastic bone formation. A case may be made that increased calcium demands stimulate increased PTH levels, which lead to increased bone width via increased preiosteal new bone formation, while at the same time the fluoride effect seems to increase osteoid via osteoblastic stimulation.

Unlike linear bone growth, bone mass seems to continue increasing into early adulthood (± 30 years) (Carrie-Fassler and Bonjour, 1995). Findings in the study and control groups would indicate that peak BMC may be reached after the age of 22 years, as mean values increased progressively to the 20-22 year age group in males and females of both groups.

Ogle et al. used DEXA in a cross-sectional study looking at body composition of 265 normal subjects aged 4-26 years. They demonstrated an increase in BMC and lean tissue mass (LTM) with age and found that males were leaner than females. They found that BMC lags LTM by 1-2 years; therefore, peak values are obtained approximately one year after linear growth ends. A significant correlation between BMC and LTM was seen, and they report that this strong relationship in

normal healthy subjects suggests that peak BMC is determined by the LTM, which in turn is a function of body size (height and weight) and body phenotype (Ogle et al., 1995). This is consistent with findings in the current study, where 83.7% of the variance in BMC in study males and 82.4% in study females is explained by weight. In control males and females, 80.2% and 87.3% of variance in BMC is explained by age and weight respectively.

In a study conducted by Chaturvedi et al. (1993), single photon absorptiometry was carried out on the distal radius. Bone mineral content was measured in 70 children aged 2 to 14 years, with one group comprising 34 normal healthy children and the other 36 malnourished children. There was a significant reduction in bone mineral content in the malnourished group compared with the healthy children (Chaturvedi et al., 1993). When the well-nourished urban group of Patel (1990) was included in comparisons with the current study and control populations a similar trend was observed, with lower bone mineral content being found in the undernourished groups. Results of covariant analysis when height, weight and age were controlled for, showed that bone mineral content was highest in the urban group, next highest in the study group and lowest in the control group. The three groups were significantly different from each other for males ($p=0.0001$) and females ($p=0.0001$).

5.7.3 Bone Density

Using dual photon absorptiometry, bone mineral content and bone density of the total body and lumbar spine were assessed in 97 healthy Caucasian children aged 3-14 years. High correlations were found between bone mineral content and bone density on the one hand and age, height and weight on the other (Proesmans et al., 1994). This was also seen in the current study: bone density increased with age in children and this was seen in both the study and control groups. The study group had lower mean bone density values for males and

females compared with the control group (males p=0.0001, females p=0.0001). One would expect these findings, as bone density is derived from bone mineral content divided by bone width.

Sex-related trends revealed that males and females within the same group were not significantly different from each other for bone density, except in the 14-15 year age group where the study females had greater bone density values than the study males (p=0.007). Control females also showed significantly more dense bone than males in the 14-15 year age group (p=0.003), and the trend continued in the next two age groups, 16-17 (p=0.004) and 18-19, however the difference in the 18-19 year age group was not significant. This consistent trend to greater bone width, bone mineral content and bone density in study females aged 14-15 compared with study males must be related to the increased velocity of pubertal growth in females at this age and compounded by the effect of fluoride on growing bone, while males exhibit a delayed pubertal growth spurt, as discussed earlier.

Bone density of the radius and lumbar spine measured by DEXA in 121 normal growing Spanish children aged 3 - 18 years showed a steady increase with age and a steeper increase during puberty for lumbar and radial bone density (Moreira-Andres et al., 1995). There was a good correlation between spinal and radial bone density, and both were highly correlated with growth parameters. In regression analysis, the best predictors for radial bone density were chronological age and weight (Moreira-Andres et al., 1995). In the current study, regression predictors were similar; the regression model for radial bone density in study females revealed that age and weight explained 60.5% of the variance, while in study males weight alone explained 60.3% of the variance. Control males and females both included age and weight in their regression models, explaining 58.8% and 70.5% of variance, respectively.

These findings of lower bone density in the study population are in keeping with radiological findings of demineralised bone and rarefaction of the long bones in studies of endemic fluorosis (Christie, 1980; Jackson, 1962; Krishnamachari and Sivakumar, 1976; Pettifor et al., 1989; Teotia et al., 1976). This osteopenia of the long bones is reported to be due to a secondary hyperparathyroidism, where children living in endemic areas present with elevated alkaline phosphatase levels, increased bone width via increased periosteal new bone formation, increased renal uptake of calcium and low dietary calcium intakes.

When the urban group's bone density results were compared with those of the study and control population there were significant differences between all three groups ($p=0.0001$), with the urban group being higher in bone density than the other two groups. This is probably reflective of the improved nutrition and growth in the urban group.

5.7.4 Apparent Bone Density

Apparent bone density (ABD) is a parameter calculated to account for changes in the cubic volume of bone. Absorptiometric data are expressed as bone mineral densities and are calculated as described above. However, these calculations assume that BMC and BW are directly proportional to one another, while this is not usually true. Bone density is not a true measure of density because absorptiometry provides no information about the depth of bone in the scan path nor does it distinguish between osseous and non-osseous areas within the bone envelope (Prentice et al., 1994). By calculating apparent bone density, one assumes the radius is cylindrical in shape at the point of measurement (ABD = BMC/ (22/7 x (BW/2 x BW/2)) and attempts to reduce the effects of increased bone size on actual bone density (Katzman et al., 1991). ABD is a

relatively geometry free value used for the purpose of measuring individuals of different size.

There were basically no age-related changes of significance for ABD in the study or control group. Regression analysis confirmed this where age was not fitted to the model for ABD for males and females of the study group and control females. In the control group males, weight (which is age-related) was included in the model for ABD, but in combination with another variable and this only described 5.8% of the variance in the population. Thus the calculation of ABD essentially removed the age-related effect of increase in bone size on bone density. ABD is therefore a linear reflection of actual bone density.

The study group showed significantly lower mean ABD values (± 20%) for both males and females compared with the control group (study females 0.54 g/cm^3 vs. 0.65 g/cm^3 in control females, and study males 0.50 g/cm^3 vs. 0.60 g/cm^3 control males). Study males had consistently lower ABD values than control males in all the age groups, and the difference was significant in all but the 18-19 and 20-22 year olds. Study females also show lower values for ABD than their control counterparts in each age group. These differences were significant in all but the 8-9 and 20-22 years age groups.

Gender-specific trends show that the study females had higher ABD values than the study males. This difference reached significance in the 6-7 and 20-22 year age groups. The 14-15 year age group, which has consistently shown a propensity for study females to have higher values than study males for the various bone parameters, does not show any gender-related growth effects at all for the variable ABD. This lack of any difference during the pubertal growth spurt of females suggests that the increase in bone density (BD) recorded in girls compared with boys in the age group is related to an increase in bone size rather than a true

increase in bone density. Higher ABD values are also seen in the control females compared to the control males, the differences are significant in the 12-13, 14-15 and 20-22 year age groups. When mean BD values were compared between males and females of the same group, no differences were found. However with the calculation of ABD, females show greater bone mineral density than males, and this is most significant in the oldest age group (20-22 years), in both study and control groups. This may be reflective of peak ABD in females, males possibly lagging behind and only achieving peak ABD later. This is supported by Ogle et al. (1995) who reported that peak bone mineral content is reached approximately two years later in males than in females.

Females have higher ABD values than males. This is an unexpected trend but may be explained by the cylindrical nature of the radius when measured. If BMC remains constant and BW increases (i.e. the area of the bone increases) then ABD would be lower. Males of both groups have greater BW than females, therefore it is likely that males would have lower ABD than the females who have less wide bone.

Age, weight and height are removed from the regression models as predictors of variance for ABD. Models for the study and control groups describe very little variance of ABD in the two populations, less than 10%. In study males the urine fluoride:creatinine ratio describes 7.5% of the variance, and in study females serum phosphate and albumin describe 6.0% of the variance. The control males have the only model to include a growth variable (weight) which, along with serum phosphate, describes 5.8% of the variance. In control females the serum phosphate, albumin and urine magnesium:creatinine ratio described 6.5% of the variance.

Mean apparent bone density is greatest in the urban group (0.66 g/cm^3), followed by the control group (0.63 g/cm^3), and then the study group with the lowest mean value (0.52 g/cm^3). These three groups are significantly different from each other (p=0.0001).

BD was shown to be lower in the study group compared with the control group when analysis of covariance (controlling for age and growth) was performed. However bone mineral content was shown to be greater in the study group compared with the control group when tested under the same conditions. An explanation for this may be the way in which BD is calculated. As detailed previously, BD is calculated by the formula BD = BMC/BW. Although the BMC is greater in the study group, they also had significantly wider bone than the control group, resulting in an overall lower bone density. This is supported by the ABD measurements. The significantly lower apparent bone density of the study group compared with the other groups (p=0.0001), after the effects of age, weight and height are removed, implies that fluoride causes thinner but wider bone i.e. lower ABD.

5.8 Fluoride Effect on Bone

Fluoride stimulates the differentiation of preosteogenic mesenchymal cells into osteoblasts and subsequent deposition of bone matrix (Hall, 1987). Fluoride has been shown to cause increased osteoblast proliferation and an increase in alkaline phosphatase activity of bone cells in vitro (Farley et al., 1983). It is a highly reactive element, with an affinity for deposition in regions undergoing active ossification. It becomes incorporated into the bone mineral either by fixing at the surface of the hydroxyapatite crystal or by exchanging with the hydroxyl ion at the surface or inside the crystal (Gryndpas, 1990). The resultant mineral is resistant to osteoclastic resorption. Overall, fluoride excess results in increased bone remodelling with unbalanced coupling in favour of bone formation.

Both the axial and appendicular skeleton are affected by fluoride. Involvement of the axial skeleton is characteristic, and changes are most marked in the spine, pelvis and ribs (Fisher et al.; 1989; Sing et al., 1962; Stevenson and Watson, 1957). Osteopenia is one of the spectra of bone changes occurring in fluorosis and has been reported frequently (Christie, 1980; Teotia and Teotia, 1973; Lian and Wu, 1986; Wang et al., 1994). Lian and Wu (1986) reported that osteopenia of the long bones, such as the distal part of the radius, appeared before any other changes in the development of skeletal fluorosis. They also noted that osteosclerosis in the spine and pelvis was always combined with osteopenia of the long bones. They considered this an indication that the axial skeleton undergoes a different pathologic process from the appendicular skeleton over many years. Krishnamachari reported severe osteoporosis of the distal part of the femur and the proximal part of the tibia, along with rarefaction of the metacarpal bones as the most striking radiological features of fluorosis (Krishnamachari, 1986).

Although we do not have the descriptive radiological data in this study, the more precise densitometry measurements reveal that the fluorotic population has wider, less dense bone than its control, indicating a fluoride effect either directly or indirectly. Osteoblastic activity is presumably increased due to stimulation by fluoride and PTH (suggested by elevated levels of alkaline phosphatase). The demand for calcium is increased in the growing, fluorotic skeleton, and low serum calcium results in less dense bone because of the increase in bone width. The ABD measurements clearly show that the study group has the less dense bone of the two groups.

The findings of this study may be strongly influenced by low dietary calcium intakes and growth. It is characteristic of endemic fluorosis to present with the simultaneous presence of osteosclerosis, osteoporosis and osteomalacia in the

same individual (Krishnamachari and Kamla, 1973). Mithal et al. (1993) found this spectrum in subjects living in the same village and sharing the same water source. They found the osteopenic radiological picture more common in the poorer and more undernourished (mean calcium intake 323 mg day, mean fluoride intake 7.32 mg/l)) population of the village. None of the patients with the recommended daily intake of calcium (800 mg/day) showed a predominantly osteopenic picture. Other workers have shown that adequate calcium and vitamin C in the diet can relieve the toxic effects of fluoride in monkeys (Reddy and Narasinga Rao, 1971).

In this study population with high fluoride intake (indicated by high water levels of fluoride and increased renal fluoride excretion) and low dietary calcium, appendicular bone is affected. The tendency to hypocalcemia found in the study group could have been induced by the effect of fluoride on the osteoblast, causing increased osteoid formation and therefore increased calcium demand. Fluoride has been shown to stimulate the osteoblast directly, which in turn initiates new bone formation and mineralisation. In the presence of low dietary calcium intakes, it is thought that there is not adequate calcium to mineralise the new osteoid as was reported in the study by Pettifor et al. (1989). Calcium bound to fluoride as fluorohydroxyapatite has been shown to be resistant to osteoclastic resorption and thus compounds the low calcium availability. This, in addition to the low dietary intakes may stimulate increased PTH secretion, which in turn stimulates osteoblastic activity. Although one of the most obvious cellular functions of PTH is to release calcium and phosphate from bone by increased osteoclast number and activity, osteoclasts paradoxically contain no PTH receptors. Instead PTH, receptors are found on osteoblasts, which secrete factors which in turn stimulate osteoclasts to resorb bone (Kronenberg, 1990). This action of PTH compounds the effect of fluoride on bone formation. Reduced urine calcium levels seen in the study group are in keeping with this picture of elevated PTH. Although PTH was not measured in this study, other authors

researching endemic fluorosis have shown these levels to be elevated (Teotia et al., 1978, Krishnamachari et al., Sivakumar et al. 1976). Secondary hyperparathyroidism is characterised by increased surface and volume, but not the thickness of the osteoid. There is accelerated loss of mainly cortical bone.

An unusual finding of hyperphosphatemia occurred in the study group. This is not consistent with the diagnosis of secondary hyperparathyroidism. Classically serum calcium and phosphate levels are *normal*, but serum alkaline phosphatase levels may be elevated in 80% of patients (Rao, 1993). However, in the current study and that of Pettifor et al. (1989), there is evidence of hypocalcemia and hyperphosphatemia. Although the mechanism resulting in hyperphosphatemia is not known, it is suggested that the increased levels of 1,25-$(OH)_2D$, resultant of the high PTH levels, increase phosphate absorption from the gut and in association with a down regulation of renal PTH receptors, result in an increase in serum phosphate levels (Pettifor et al., 1989).

Anthropometric data do not reveal a direct effect of fluoride on growth as both the study and control communities had similar degrees of undernutrition. However, body mass seemed to play a role in the prevalence of genu valgum, with a propensity towards females. Females, being more obese than their male peers, showed a higher degree of genu valgum. The delayed pubertal growth spurt in males seemed to play a protective role in the prevalence of genu valgum. This delay in growth spurt in males was independent of fluoride and has been shown in other rural communities.

The above historical, biochemical and densitometry data present a picture consistent with a modified secondary hyperparathyroidism, although the important serum markers, vitamin D and parathyroid hormone were not measured.

Exposure to fluoride increases the rate of bone turnover, with unbalanced coupling in favour of bone formation, through its action on the osteoblast. Calcium deficiency induced bone turnover, on the other hand, has a resorptive bias. Hence when high fluoride intake is combined with calcium deficiency, osteopenia accompanies or replaces osteosclerosis. The high turnover state induced by calcium deficiency also increases the skeletal uptake of fluoride. Fluoride exposure can, by itself induce a state of relative calcium deficiency by increasing the calcium demand resulting from fluoride stimulated bone turnover and formation. In addition to the above, calcium deficiency aggravates fluoride induced mineralisation defects. Undermineralised soft bones maybe responsible for the greater incidence of long bone deformities seen in calcium deficient fluorotic children.

5.9 Summary

The aims of this study, which were to investigate the effects of high intakes of water fluoride on children living in an endemic area and comparing these children with a control group living in an area with lower water fluoride concentrations, were achieved. Anthropometric measurements revealed that both the study group and the control group showed growth retardation, and this was more marked in males. Genu valgum was a significant clinical finding in the study group with a propensity to affect females. Dietary data highlighted low intakes of calcium with adequate to low protein intakes in both groups. Alterations in mineral homeostasis were seen, with evidence of hypocalcemia, elevated alkaline phosphatase and low urinary calcium excretion. Alterations in appendicular bone mass in the endemic group revealed wider, less dense bone compared with the control population.

This randomised, age-matched study has contributed to the understanding of endemic fluorosis by the use of bone densitometry in the investigation of endemic fluorosis. It is suggested that fluoride increases the calcium requirements of growing bone and exacerbates the prevalence of hypocalcemia. This, along with elevated alkaline phosphatase levels (suggestive of elevated PTH), results in wider bone and lower bone density - a picture consistent with secondary hyperparathyroidism. Thus, children living in an endemically high fluoride area (the study group) show evidence of altered bone mineral homeostasis and reduced dietary calcium intake.

Radiological examination has, in the past, been regarded as the best method for diagnosing fluorosis, especially when the disease is in the asymptomatic phase (Krishnamachari, 1986; Teotia and Teotia. 1973). In this study, single photon absorptiometry clearly showed altered bone mineral homeostasis due to fluorosis consistent with a picture of secondary hyperparathyroidism during the asymptomatic phase.

Absorptiometry would not be the ideal measurement in the diagnosis of individuals in a clinical setting as the need for age-matched controls or other reference data would be needed for interpretation of bone densitometry findings. However, absorptiometry is a useful research tool as it provides measurement of specific bone parameters and the equipment is more portable than radiographic equipment allowing investigations of populations not easily accessible from locations where medical facilities are available.

Skeletal fluorosis existed in the study population due to continual intake of hydric fluoride and is identified by dental, biochemical and densitometry data. The water sources in this community have been changed from boreholes to piped surface water, and this will markedly improve the skeletal health of future populations.

The role of calcium intake is a confounding factor in the skeletal outcome of endemic fluorosis. Dietary calcium intakes of populations living in endemic areas are often not investigated, and, if reported, little methodology is given, which makes it difficult to assess the validity of the data. Most studies ascertain water fluoride levels (ppm) in endemic areas, however the fluoride dose ingested is generally not calculated. The literature supports the fact that the fluoride/calcium interaction is an important one and that the disease presents differently with different doses of fluoride intake. This research has highlighted the need for careful attention to and further investigation into establishing calcium intake, fluoride dose and the relationship between the two in altered bone mineral homeostasis in endemic fluorosis.

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DATA FORM Addendum A

Name: _____ Study No: ☐☐☐☐

School:_____ ☐

Classs:_____ ☐☐

Sex: M / F ☐

Date of Birth: Year: ☐☐☐☐ Months ☐☐

Height ☐☐☐☐

Weight ☐☐☐☐

Intermalleolar distance (cm) ☐☐

Interpatellar distance (cm) ☐☐

Windswept deformity ☐☐

Blood Collected ☐

Urine Collected ☐

Bone Densitometry: BMC ☐☐☐☐☐

BW ☐☐☐☐☐

BD ☐☐☐☐☐☐

24 Hour Recall Questionnaire : English\Ndebele

Name_____ **Study no.** _____
School_____ **Sex** _____ **Age**_____

1. When you woke up yesterday did you eat or drink anything? ☐ **Yes** ☐ **No**

Uma uvuka ekuseni izolo kukhona yini okudlile nokuphuzile na?

If yes: RECORD ON FOOD QUANTITIES SHEET and proceed to question 2.

If no: proceed to next question

When did you first eat or drink yesterday?_____ ☐

Be kungasikhathi sisi uma uqala ukudla nokuphuza izolo?

Was this the <u>first time</u> you had anything to eat or drink yesterday? ☐ Yes ☐ No

Be kuwokokuqala nqha na?

If yes: What did you have to eat or drink ? RECORD ON FOOD QUANTITIES SHEET ☐

Uye wadla ini? Waphuza ini?

If no: proceed to the next question

2. When did you next eat or drink something? _____ ☐

Wabuya wadla nini futhi na? Wabuya waphuza nini futhi na?

What did you have to eat or drink? RECORD ON FOOD QUANTITIES SHEET ☐

Wadla ini? Waphuza ini?

Did you have anything to eat or drink before this time that you have not mentioned?

Yes ☐ No ☐

Kukhona yini okunye okudlile noma okuphuzile ongakushongo na phambi kwalesikhathi?

If yes: What did you have to eat or drink? RECORD ON FOOD QUANTITIES SHEET ☐

If no : proceed with questions

3. When did you eat or drink again? _____ ☐

Ubuye wadla nini futhi? Waphuza nini futhi?

What did you have to eat or drink? RECORD ON FOOD QUANTITIES SHEET ☐

Udle ini? Wphuza ini?

Did you have anything to eat or drink before this time that you have not mentioned?

Yes ☐ No ☐

Kukhona okunye okudlile nokuphuzile phambi kwalesikhathi ongakushongo na?

If yes: What did you have to eat or drink? RECORD ON FOOD QUANTITIES SHEET ☐

If no : proceed with questions

Did you have any sweets or colddrinks that you might have forgotten? Yes ☐ No ☐

Amaswidi no colddrink? Awukhohlwanga ukuzisho na?

If Yes : when did you eat or drink them? _____ ☐ RECORD ON SHEET

Udle ini? waphuza ini?

If no : proceed with questions

4. Did you eat or drink again? Yes ☐ No ☐

Ubuye wadla noma waphuza futhi?

If yes : When _____ ☐ RECORD ON FOOD QUANTITIES SHEET

Nini _____ Wadla ini?

If no: Proceed with questions

5. *Wabuya wadla noma waphuza futhi izolo na?* *Yes* ☐ *No* ☐

If yes: *udle nini ? waphuza nini?*

udle ini ? waphuza ini?

6. Have you told me everything you had to eat or drink yesterday? Yes ☐ No ☐

Ungithshene konke okudlile nokuphuzile izolo na?

If no : When did you eat or drink_____ ☐ RECORD FOODS ON SHEET

udle nini ? waphuza nini?

udle ini ? waphuza ini?

If yes: Proceed with questions

7. Did you have any sweets or colddrinks you may have forgotten Yes ☐ No ☐

Akukho amaswidi nesiphuza okhohiwe ukuzisho na?

If yes : When _____ ☐ RECORD ON FOOD QUANTITIES SHEET

If no: Proceed with questions

8. Did you have anything to eat or drink before you went to bed ? Yes ☐ No ☐

Kukhona okudlile noma okuphuzile phambi kokuba uyolala na?

If yes : What _____ ☐ RECORD ON FOOD QUANTITIES SHEET

Yini okunye?

If no: Proceed with questions

9. Are you sure you have remembered all the foods you ate or drank yesterday?

Yes☐ No☐

Uneqiniso lokuthi ukhumbule konke okudlile nokuphuzile izolo na?

If no : What esle did you eat ? ☐ RECORD ON FOOD QUANTITIES SHEET

Yini okunye?

If yes: Proceed with questions

10. Is there anything else you may have forgotten? Yes ☐ No ☐

Kukhona okunye ongabe ukhohiwe ukukusho na?

If yes : What _____ ☐ RECORD ON FOOD QUANTITIES SHEET

udle nini ? waphuza nini?

udle ini ? *waphuza ini?*

If no: Proceed with questions

11. Is this and accurate recall of what you ate yesterday? Yes ☐ No☐

Kiliqiniso ngempela yini lokhu okuchilo ngezidla nezipbuza zakho zayizolo na?

If yes: Proceed to No. 12

If no : What is not accurate? _____

Yikupi okunye?

If addtional food are listed - RECORD ON FOOD QUATITIES SHEET

12. Thank you for helping us with these questions.

Siyabonga kakhulu ngosizo lwakho kulemibuzo!

Addendum C

KwaNdebele Dietary Interview
24-Hour Recall Survey
March 1988

Study No☐☐☐☐

Subject Name: _____
School:_____
Class:_____
Teacher:_____
Area Lived in:_____

Child = 1; Repeat = 2; Mother = 3; ☐

Questions:

Was yesterday a routine day? Yes_____ No_____

Where did you eat yesterday? School_____ Home_____ Other_____

How many cups of water did you drink yesterday? _____

Where do you get your water from? _____

FOOD ITEMS	QUANTITY	Break	snack	lunch	snack	supp	snack	Total grms	CODE		
Breakfast foods:											
tea	200ml 300ml 400ml										
coffee	200ml 300ml 400ml										
sugar in drink	1t = 6g								9012		
condensed milk	1t = 10g								"0002		
non-dairy creamer	1t = 3g								"0039		
Milk blend	20 ml - tea cup								"0068		
skim milk powder + water	35 ml - tea mug								"0007		
whole milk powder + water	40 ml -coffee cup								"0009		
Whole milk(fresh + long life)	75 ml -coffee mug								"0006		
Sourmilk (commercial)	small = 175 ml large = 500ml								"0001		
Water: Size mug/cup used	200ml 300ml 500ml								water		
Vetkoek	sm=40g med=60g lg=80g								4148		
bread brown	10 mm = 30 g								4002		
bread white	20mm=60g, 30=100g 1/2 L=375g								4001		
rolls brown	round 10cm = 45g								4001		
rolls white	long 14 cm = 45g								4002		
Oats	1/2 cup =125 ml								4032		
Maltabella	" " "								4034		
Soft porridge	1c = 40g								4031		
Butter	spread :- thin medium thick								6502		
Margarine hard	5g 7g 10g								6508		
Jam, Honey, Syrup	5g 7g 10g								9008		
Peanut Butter	5g 10 g 20g								6509		
Eggs -boiled	1 egg = 50g								1001		
-fried in marg	1 egg = 52g								1012		
-scrambled WM + marg	1T = 35g, 1SP =80g								1025		
	1/2c = 115g (2 eggs)										
Lunch /Dinner Foods:											
Cheese - cheddar	slice 85x25x2mm = 8 g								"0010		
Mealie meal stiff porridge	1c = 100g								4031		
	T SP 1/2 c										
Maize rice	25g 45g 65g								4043		
Samp	55 95 115								4043		
Rice white	25 60 65								4040		
Spaghetti/ Macaroni	35 70 90								4062		
Spaghetti in tomato sauce	45 80 130 small tin 225g								4058		
Baked beans	50 105 135 small tin 225g								3504		
Cooked beans	50 85 135								3515		
FOOD ITEMS	QUANTITY	Break	snack	lunch	snack	supp	snack	Total grms	CODE		
Peas Tinned	15 35 85								8026		
Mixed veg canned/cooked	25 40 75								8035		

Tinned Meats /Fish											
meat balls in gravy	50mm diam = med = 60g								1563		
spaghetti + meat balls	lg sp=75, 1/2c =125g, med can=420g								4060		
corned meat	68mmx54mmx10mm = 40g								1535		
-savory + potato + onion	T = 45g;1/2c=120g								1606		
sardines, canned, oil	s/s = 10g, l/s = 20g								2502		
sardines, canned, tomato sauce	10g 20g								2539		
Fresh meat or chicken											
Beef											
mince	T = 40g, SP = 90g, 1/2 c = 115g								1505		
savory mince + tomato+ onion	T = 40g, SP = 90g, 1/2 c = 115g								1585		
beef stew: carrot,peas, pot.	1SP=105, 1/2c=125								1557		
roast beef	slice: sm= 35g med = 70g lg = 90g								1539		
stewing beef									1603		
Chicken:											
Boiled + skin	breast=110g								1521		
	thigh = 65 g										
Roast Chicken	drumstick = 45g								1520		
	wing =30g								1609		
Chicken feet	1 foot = 30 g										
Stew- carrot, peas,potato	1 SP = 105g								1618		
- cabbage, potato	1/2 c = 125g								1619		
Mutton:											
Chop, fried	chop = 40g								1522		
Roast	thick slice = 90g								1542		
Stew - plain + veg	SP=55g,1/2 c=130								1511		
Liver: fried, lamb	patty = 60g								1550		
fried, beef	patty = 60g								1515		
Offal	SP=105.1/2 c=125								1616		
Oxtail stew	SP=105.1/2 c=125								1573		
Polony	5mm= 8g, 1cm = 16g								1514		
Sausage: beef	sm = 30, med = 45, lg = 90								1526		
pork	sm = 30, med = 45, lg = 90								1527		
Viennas or canned sausages	9cm = 25g,10 = 35g								1531		
Toppers	SP = 85g, 1/2 c = 120g								3527		
Vegetables	T SP 1/2 c										
Green Beans Fresh HM	40 80								8098		
Green Beans -Pot - Onion HM	60 95 120								8102		
Beetroot + sugar	85								8005		
FOOD ITEMS	QUANTITY	BREAK	SNACK	LUNCH	SNACK	SUPPER	SNACK	TOTAL GRAMS	CODE		
Cabbage	40 50 80								8066		
Cabbage +pot + onion HM	60 120								8123		
Carrots	20 50 80								8067		
Carrots + pot+ onion	35 70 105								8132		
Cauliflower	40 80								8135		
Mealies (corn)	1 ear = 95								8033		

Mixed veg (tin)	40 105 140								8145		
Onions	5 0								8154		
Peas (tin)	30 65 85								8166		
Potato mashed, HM + milk	50 115 125								8046		
Potato - peeled , boiled	60 90 140								8187		
Pumpkin - Butternut	60 100 120								8202		
Spinach - Pot - onions	50 105 110								8212		
Sweet Potato	50 110 145								8214		
Turnips	25 45 90								8222		
Fruit											
Apple	1 med = 150 g								7001		
Banana	sm = 80g,m =100								7009		
Orange	med = 150g								7031		
Pawpaw	slice 180x50mm = 80g								7034		
Peaches	med = 150 g								7036		
Watermelon									7047		
	T SP 1/2 c										
Pudding : Instant	45 95 145								4066		
Jelly	60 120								9004		
Custard	15 35								'0004		
Cookies - plain	average = 10 g								4007		
Cookies - with filling	average = 15 g								4008		
Cake - home made plain HM	cup cake = 35 g								4010		
Drinks\sweets\candybars											
Cold drinks - carbonated	175ml, 300ml, 500ml, can=340 1L,								9001		
Chocolate bar	see addendum								9024		
Sweets Hard / and soft jelly									9009		
Sweets soft - toffee, fudge	eclair = 10g , wilson = 10g								9014		
Peanuts	tbs = 10g, 1/2c = 80g								6007		
Peppermints	1mint = 2g								9031		
Popcorn	handful = 6g								4163		
Potato crisps	sm pkt = 30g, lg pkt = 150g								8049		
Fritos - Nik Naks	sm pkt = 30 g / Nik Nacks= sm pkt = 24g								4067		
Chappi / Beechie	1 = 2 g								9019		
FOOD ITEMS	QUANTITY	Break	snack	lunch	snack	supp	snack	Total grms	CODE		
Chutney	1t = 6g								9524		
Curry powder	5ml 10ml 1tbs								9522		
Tomato Sauce Commercial	1t = 6g 1T = 30g								9505		
School Soup : veg, curry, flour	sm mug = 200g, 1/2 c = 125g								4222		