The Effect of a High Calcium Dairy Based Supplement on Parameters of Bone Health in Pre-Pubertal New Zealand Children

A thesis presented in partial fulfilment of the requirements for the degree of Masters of Science in Nutritional Science

At Massey University, Albany, New Zealand.

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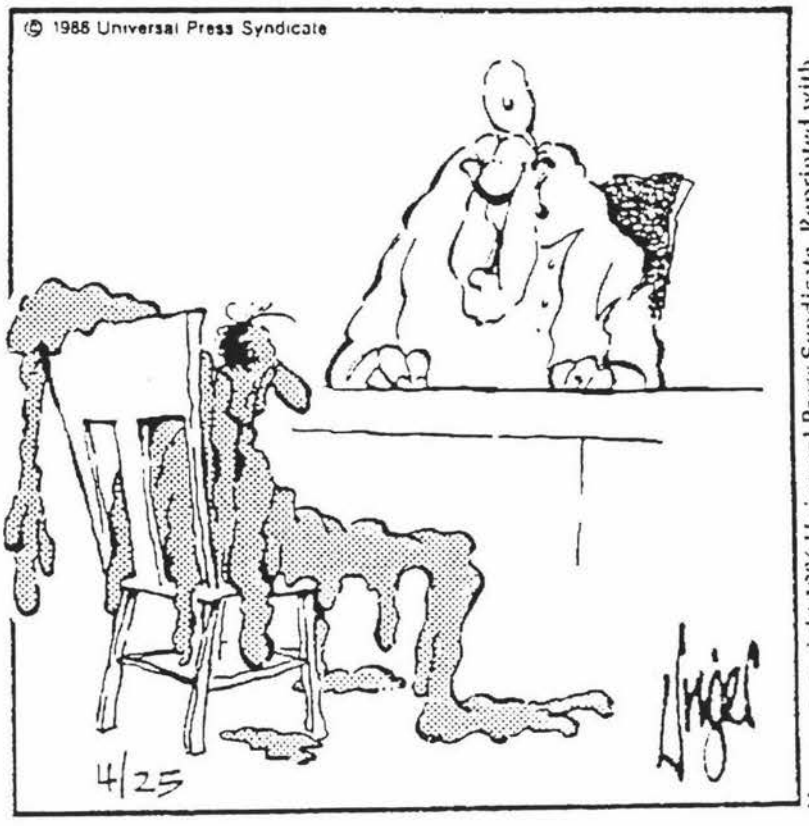

"You're not getting enough calcium."

Abstract

With an ageing population and an increased awareness of the rising health costs of fractures caused by osteoporosis (1); the focus of osteoporosis research is changing from treatment to prevention. More recent studies have looked at the effect of calcium supplementation during childhood and adolescence as a method of increasing the peak bone mass (2-10). It is postulated this will lead to a decrease in fractures in later life.

This study investigated the effect of a calcium enriched milk drink on bone density, bone mineral content and bone size in both male and female 8-10 year old New Zealand (NZ) pre-pubertal children.

One hundred and fifty four NZ pre-pubertal boys and girls were randomized to receive a high calcium dairy (Ca^{2+} =1200mg) drink or a control (Ca^{2+} =400mg) drink for 18 months. They were assessed at baseline and then every 6 months for the first 18 months, during the supplementation period; they were then followed up 12 months later. Bone mineral density, and bone mineral content was assessed at the total body, hip and spine. Indicators of bone size were measured at the spine. Anthropometric data was collected and Tanner stages of pubertal development, dietary calcium intake, compliance and medical questionnaires were administered. The calcium food frequency questionnaire was validated against a 3 day weighed food record at baseline.

There was no significant difference between the 2 groups for bone mineral density or bone mineral content observed either before or after the intervention. Trends were seen in bone mineral density in the total hip ($p=0.081$) and the trochanter ($p=0.088$). There was no difference in vertebral height or width at any stage of the study, indicating no additional influence on bone size. There were no significant differences between height, weight, lean mass or fat mass. Both groups had high habitual calcium intakes at baseline and this continued throughout the study, resulting in calcium intakes above the estimated calcium threshold for both groups.

In this 2½ year study (18 months supplementation, 1 year follow-up) there was no difference in bone mineral density in children aged 8-12 years. This is most likely due to a high habitual dietary calcium intake, that even with minimal addition of calcium to the diet a threshold level was reached where no further benefit was seen.

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List of Abbreviations Used

Abbreviation	*Full word(s)*	*Abbreviation*	*Full word(s)*
BMD	*Bone mineral density*	*PICP*	*Procollagen 1 carboxy-terminal propeptide*
DEXA	*Dual energy x-ray absorptiometry*	*HPro*	*Hydroxyproline*
PBM	*Peak bone mass*	*Pyr*	*Pyridinoline*
OA	*Oligo-amenorrhoeric*	*DPyr*	*Deoxypyridinoline*
EA	*Eumenorrhoeric*	*TRAP*	*Plasma tartrate-resistant acid phosphatase*
OCA	*Oral contraceptive users*	*Hyl*	*Hydroxylysine and glycosides*
PTH	*Parathyroid hormone*	*kJ*	*kilojoules*
BMC	*Bone mineral content*	*kcal*	*calories*
QCT	*Quantitative computed tomography*	*g*	*grams*
mg/d	*milligrams per day*	*µg*	*microgram*
mg	*milligrams*	*IU*	*International units*
%	*percent*	*mA*	*milliAmps*
RDI	*Recommended daily intake*	*kg*	*kilograms*
AI	*Adequate intake*	*mm*	*millimetres*
USA	*United States of America*	*n*	*number*
NZ	*New Zealand*	*SPSS*	*Statistical package for social sciences*
UL	*Tolerable upper intake level*	*SEM*	*Standard error of mean*
IGF-1	*Insulin-like growth factor-1*	*PRN*	*As often as required*
hr	*hour*	*NCHS*	*National children's health survey*
DASH	*Dietary approaches to stopping hypertension study*	*L1-L4*	*Lumber 1 to lumber 4*
$1,25(OH)_2D$	*1,25-dihydroxyvitain D*	*FMV*	*First morning volume*
FFQ	*Food frequency questionnaire*	*BAP*	*Bone alkaline phosphatase*
OC	*Osteocalcin*		

1. Introduction

With an ageing population and an increased awareness of the rising health costs of fractures caused by osteoporosis (1); the focus of osteoporosis research is changing from treatment to prevention. More recent studies have looked at the effect of calcium supplementation during childhood and adolescence as a method of increasing the peak bone mass (2-10). It is postulated this will lead to a decrease in fractures in later life.

Puberty is a period of marked change during the lifespan from childhood to adulthood. The influence of hormones and accelerated growth means it is a period of rapid skeletal development (11). It is therefore important to consider whether calcium intervention is more important prior to puberty or during puberty. Studies to date have looked across the age-span from 7 years to 18 years and all have found positive results during the period of intervention (2-10). The calcium supplied must be adequate to meet the requirements for both skeletal and physiological development.

A food based supplement may be better than a calcium salt due to the influence of the other nutrients on the absorption of the calcium, and the effect of the other nutrients on bone health (12). However, it is important to consider the acceptability of a food-based product and how the acceptability will affect compliance over the intervention period.

The methods of assessment for both dietary intake and bone parameters need to be sensitive enough to see small indications of change. Dietary assessment is known to have many inherent errors (13-14) and it is important the conclusions drawn reflect this. In comparison dual energy x-ray absorptiometry has a very low coefficient of variance and is a precise measure of change in bone parameters.

2. Literature Review

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone, leading to enhanced bone fragility and a consequent increase in fracture risk (15). In New Zealand osteoporosis is a major public health problem involving postmenopausal women and ageing individuals. In New Zealand it is estimated that, 56% of women and 29% of men aged over 60 will experience a fracture as a result of osteoporosis (1).

At present, the accepted method for assessing the fracture risk of an individual is the measurement of bone mass or bone mineral density (BMD). However recent studies (16) have looked at the ability of bone markers to predict fracture risk and these may be a better predictor than BMD. Bone mineral density increases during childhood until the peak bone mass is achieved, around the age of 18-20 years (11,17-19). Thereafter, bone mass stabilizes and then decreases progressively in both sexes after 35-40 years of age with a steeper decline in women after the menopause.

Studies in post-menopausal women showed for each standard deviation decrease in BMD there is a 2-3 fold increase in fracture risk (20-21). The peak bone mass acquired during adolescence and the subsequent rate of bone loss determines bone mass later in life. Low peak bone mass results in a higher risk of osteoporosis. A high peak bone mass provides a larger reserve in later life (11).

It is an important public health requirement that the recommendations for calcium intake in children correspond with the optimal calcium intake required to maximize their peak bone mass and meet their physiological requirements, thus serving as a preventative measure against osteoporosis in later life, while still meeting their requirements during growth.

The purpose of this literature review is therefore to:

- Review the role of calcium in the bone health of pre-pubertal children, and identify the calcium intake level at which calcium has the most favourable effect on bone.
- Comment on the role of other nutrients on bone health.
- Determine the best methods of assessing the effect of additional calcium on the bone status of children.

A computer-based literature search using Medline, was conducted using the key words: calcium, bone, children, osteoporosis, puberty, growth and peak bone mass. These words were all entered separately then combined to limit the search. Other searches on Medline were conducted to examine the methods to be used using each key word: DEXA or dual x-ray absorptiometry, dietary assessment, hedonic scales and intervention studies. A manual search was conducted of the references cited in the relevant literature papers, and of available reference textbooks. References included were those dated no earlier than 1990, unless of historical importance, and in English. Articles in other languages were not used due to resource constraints in using translators.

2.1 Bone and Peak Bone Mass

2.1.1 Bone

Bone consists of an organic matrix, primarily collagen fibres, around which salts of calcium and phosphate are deposited in combination with hydroxyl ions in crystals of hydroxyapatite (22). The tensile capacity of collagen and the compressional ability of calcium salts combine to give bone its strength.

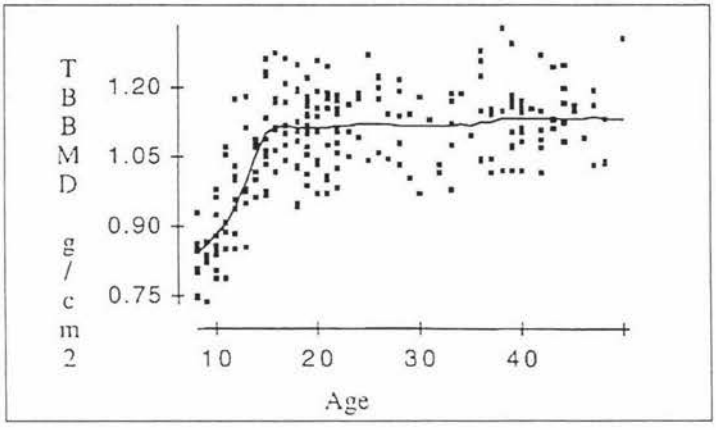
The largest part of the skeleton (~80%) is made up of compact cortical bone. The remainder is the trabecular bone that occurs in the knobbly ends of the long bones, the iliac crest of the pelvis, the carpel bones of the wrist, scapulae and vertebrae. Trabecular bone is spongy and less dense than cortical bone, and these are the areas that most osteoporotic fractures occur in later life (23).

2.1.2 Normal Peak Bone Mass

Peak bone mass (PBM) can be defined as the amount of bony tissue present at the end of skeletal maturation (11), and it is important to look at how this can be optimised. This biological variable is an important determinant of osteoporotic fracture risk in later life, since the mass of bony tissue present at any one time during adult life is the difference between the amount achieved at skeletal maturity and that lost with ageing (18).

One of the most critical periods in skeletal development is during the time of the most rapid bone modeling and turnover of the adolescence. The process of bone modeling that takes place from birth until the cessation of longitudinal bone growth is characterized by changes in the volume and the shape of the bones. Thereafter, bone tissue within the existing skeletal structure is continuously being resorbed and formed with minimal change in bone size through the remodeling process (refer figure 2.1).

Figure 2.1: Scatter-plot with Trewess smoothing of bone mineral density of the whole body (TBBMD g/cm^2) in premenopausal women (24).



There are a number of factors that can determine or effect an individual's bone mass. These include genetics, race, hormonal and nutritional factors, and physical activity.

Hereditary and racial factors are probably the most important determinants of peak bone mass. It is well documented African American children have higher bone densities than Caucasian children (25-28). Pocock et al (29) showed that bone density was significantly better correlated in monozygotic twins than in dizygotic

twins. The BMD at the lumbar spine was better correlated than the BMD at the proximal femur or the distal forearm, this suggests there is a large genetic effect. It is difficult to alter the load at the lumber spine, compared with the hip and forearm where many daily activities apply a mechanical force. In this study twins were used to control for environmental factors, however the mean age of the monozygotic twins was 47 years and the dizygotic twins was 40 years, and so they would most likely have been living in different environments within the twin pairs. This is different from studies in twin children where they are living in the same environmental condition. Other studies have shown a correlation between the bone densities of teenage girls and their mothers and fathers (27,30-31).

Secondly, hormonal factors also appear to play a major role in the attainment of peak bone mass. Oestrogen deficiency has serious detrimental effects on bone mass. Proinflammatory cytokines stimulate the proliferation and differentiation of osteoclasts leading to an increase in bone resorption and subsequent bone loss (23). Oestrogen suppresses this response, so with inadequate oestrogen there is increased bone loss. Oophorectomy[1] results in a rapid loss of bone, and women with various causes of secondary amenorrhoea have low bone density (32). In athletes with amenorrhoea the mechanism of bone loss is often questioned. Gremion et al (33) looked at oligo-amenorrhoeic (OA) distance runners and the incidence of bone loss. When they compared OA, eumenorrhoeic (EA) and oral contraceptive users (OCA) they found the sex hormone levels in the OA group were all significantly lower, with the exception of follicle stimulating hormone. This

[1] Oophorectomy is the removal of the ovaries in a female prior to menopause. This results in a cessation in the release of sex hormones, which causes premature bone loss.

resulted in decreased bone mineral density in the trabecular bone of the lumbar spine in the OA group. Another finding of this study was parathyroid hormone (PTH) levels were lower in the OA and OCA groups. This was probably due to the higher calcium intake in these two groups (EA = 1147mg/d compared with OA = 1620mg/d and OCA = 1526mg/d). This demonstrated that the lower oestrogen levels were not acting on the PTH feedback loop, but acted directly on the bone. It is therefore of importance to consider hormonal status when looking at studies of bone gain.

Thirdly, activity and exercise also affect bone mass. Bone responds to compressive forces, (specifically the longitudinal forces exerted by gravity), by increasing bone mass. The converse is also true; bedrest leads to rapid bone loss as has been observed in patients with spinal trauma (34-35). Bone demineralization and atrophy of antigravity muscles occur under weightless conditions because of the absence of adequate mechanical loading of the body. Under free-fall conditions, the body is not subjected to the forces of gravity. This has been researched to try and minimize the bone demineralization that occurs in astronauts during space flights (36-38).

Longitudinal and prospective studies (39-41) have indicated weight-bearing physical activity in childhood and adolescence is an important predictor of BMD, while non-weight-bearing activity is not. The size of the effect of physical activity is typically between 5 and 15% (42). It is probable that activities that involve high stress on the bone, developed rapidly and distributed unevenly, may be particularly osteogenic. Further work needs to be carried out to establish the optimal period within childhood to perform activities to promote bone growth. Adult bone appears to be relatively unresponsive to all but the most vigorous of exercise regimes. However, there is

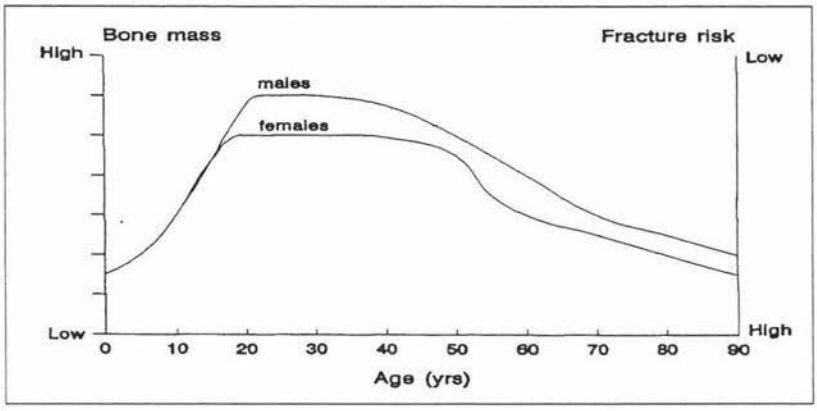
some evidence physical activity during the intermediate pre-pubescent and pubescent years may be crucial for maximizing bone mass (43-44). It is therefore important to consider the effect of physical activity as a possible bias when you are looking for changes in bone mass through intervention.

Finally, nutrition also has an effect on bone mass. There are many nutrients that have an effect, namely calcium, vitamin D, protein, energy, phosphorus, sodium, vitamin K, fibre and caffeine. Most studies have focused on calcium through different lifestages (2-10,45-47). This will be looked at in greater depth in section 2.6.

2.1.3 Puberty and gender

Puberty is the period during which the gender difference in bone mass observed in adult subjects first becomes expressed. Before puberty there is no consistent gender difference in bone mass of either the axial or the appendicular skeleton (11,48-52). There is no evidence of gender difference in bone mass at birth, volumetric bone mineral density appears to be similar between female and male newborns (11). This absence of a gender difference in bone mass is maintained until the onset of pubertal maturation (see figure 2.2).

Figure 2.2: Schematic lifetime presentation of bone mass and fracture risk (53).



The sizes of most bones are on average greater in men than in women. Morphometric studies have shown there is a gender difference in the cortical thickness of most appendicular and axial bones (11,18,54). It is important to highlight that gender differences in mean areal[2] BMD/bone mineral content (BMC) observed at several sites of the skeleton after pubertal maturation do not appear to be due to a difference in volumetric BMD. By using 3 methods (11), histomorphometry, gravimetry and quantitative computed tomography (QCT), it has been shown there is no difference in volumetric trabecular density between sexes at the end of the period of maturation.

The significantly greater mean lumbar, mid-femoral and mid-radial BMDs observed in young healthy adult males as compared to females appears essentially to be due to a more prolonged period of pubertal maturation rather than a greater maximal rate of bone accretion (18).

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[2] The areal measurement of bone is a 2 dimensional measure, while bone is a 3 dimensional structure.

2.1.4 Childhood Growth

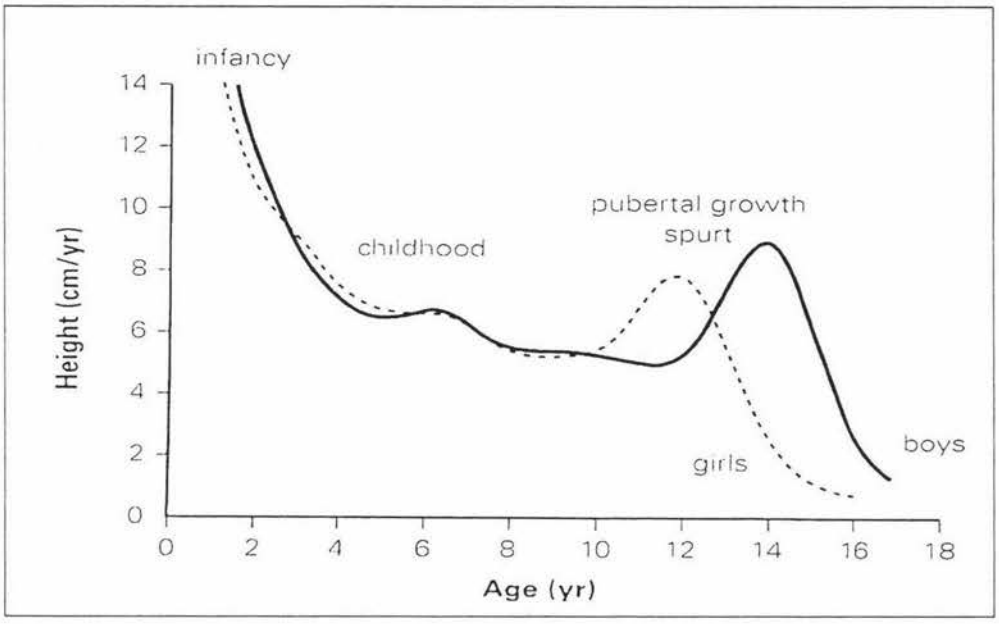
It is important to know the overall growth rates of children as this will have an effect on the growth of the skeletal tissue.

In children prior to puberty and older than 1-2 years, there is minimal difference in the growth rates between boys and girls, both gain height at around 5-7cm per year and weight at around 1.5-2.5kg per year (55-57).

The growth rate of girls increases at around 10 years, and reaches a peak of 10.5cm per year around the age of 12. Growth rate decreases at about 15 years, when growth plates fuse, giving a total growth in puberty of about 25cm. During puberty, girls peak at a weight gain of 8.5kg per year at 12 years and boys 9.5kg per year at about 14 years (55-57).

For young males, the growth spurt starts at 12 years of age, reaching a peak of about 12cm per year at about 14 years and then drops in rate at approximately 17 years of age. Boys generally grow about 28cm during puberty (58-59).

Figure 2.3: Change in growth velocity during childhood and adolescence (60).

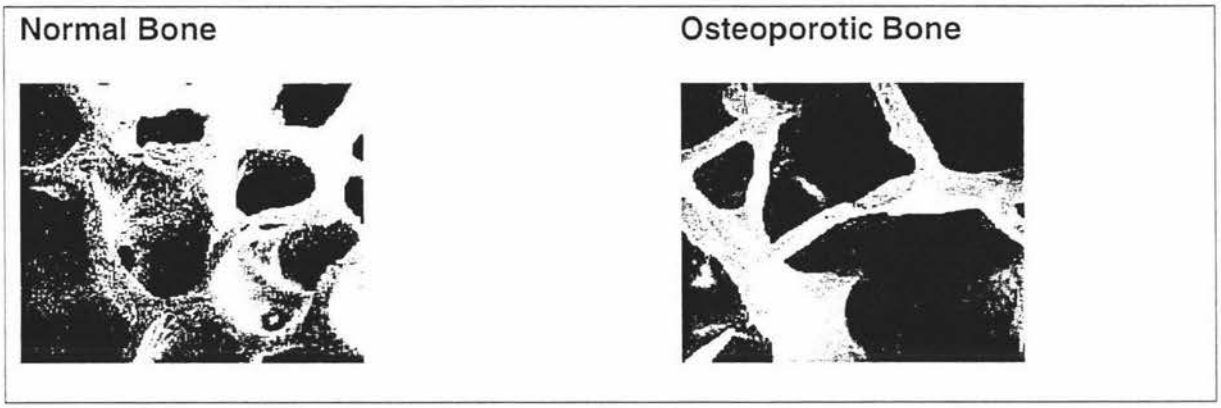


2.1.5 Osteoporosis

Osteoporosis is a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone. There is also a consequent increase in bone fragility and susceptibility to fracture (15).

Clinically, osteoporosis is defined in terms of the BMD that is below the age-adjusted reference range (15). An individual is defined to be osteoporotic if his or her BMD is 2.5 standard deviations or more below the young adult mean for bone density (15). At least one osteoporotic fracture confirms the diagnosis of osteoporosis. An osteoporotic fracture is defined as a non-traumatic fracture; that is sustained by a fall from standing height or less (61). Osteopenia is a condition of low bone mass in which the BMD is more than 1 standard deviation below the young adult mean, but less than 2.5 standard deviations below this value (15).

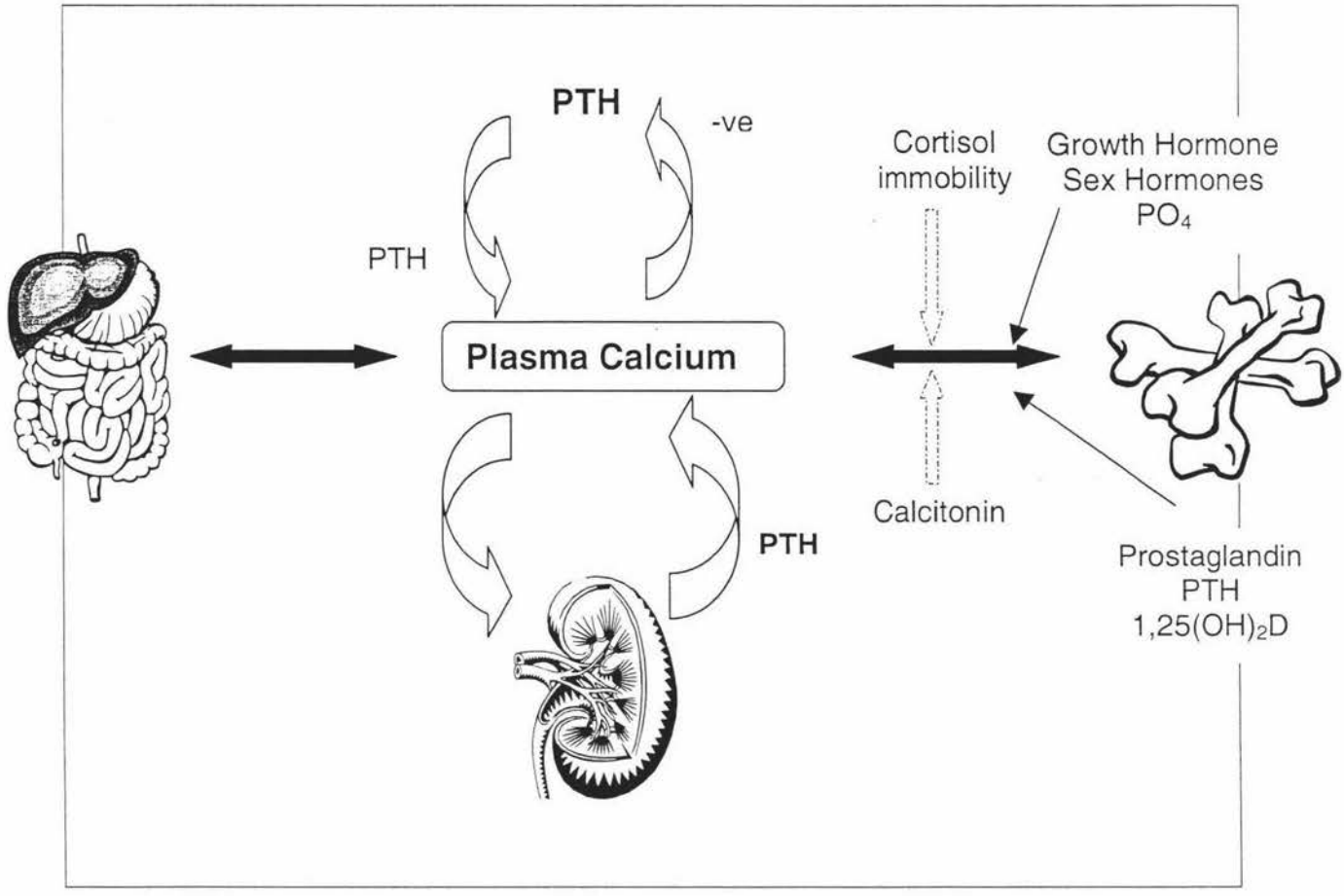
Fig 2.4: Photographic representation of normal bone matrix compared to osteoporotic bone matrix(1).



2.1.6 Calcium Homeostasis

Ninety-nine percent of calcium (~1 kilogram) in the body is within the bone, the other 1% in blood, body fluids and muscle (23). Non-skeletal calcium is used in intracellular processes, where it helps to regulate the transport of other ions across cell membranes, it is essential for muscle action and nerve to nerve, and nerve to muscle, reception and interpretation of nerve impulses. It also assists in maintaining blood pressure. The human skeleton retains the rather primitive function of serving as both a depot for the storage of excess calcium and as a reservoir, available to replenish calcium during times of deprivation (22). When the calcium reserve (the skeleton) is called upon to meet plasma calcium insufficiencies, due to diet, low circulating vitamin D levels, high levels of PTH or any other cause, bone strength may be compromised. However this depends on how much and how often skeletal calcium is mobilised.

Figure 2.5: The effect of the major hormones on calcium balance in the healthy adult. Parathyroid hormone (PTH) is suppressed by an increase in plasma calcium.



Calcium balance represents the balance between dietary intake of calcium, the amount of calcium absorbed from the intestine, and the amount excreted in the urine and the stools (62-63). The plasma calcium levels are tightly regulated by hormonal control (see figure 2.5). Hence, when negative balance occurs, due to low levels of circulating vitamin D or high levels of PTH, demineralization from the skeleton will follow. The dietary calcium requirement is based on the amount of dietary calcium, which will maintain calcium balance and optimal bone accretion rates.

Balance studies, that have looked at calcium ingested and compared it to calcium excreted, suggest there may be a threshold effect for calcium intake (62-63). This means calcium retention will increase up to a threshold, beyond which further dietary calcium does not result in increased calcium retention (62-63). Calcium absorption occurs in the small intestine. When there are low levels of calcium intake it occurs by active absorption, at higher levels by passive absorption. Calcium requirements are determined from balance studies at the point when calcium intake and losses are equal (see table 2.1). It is important to know, from these balance studies, at what level dietary intake of calcium has no further effect on skeletal mass. Weaver et al (64) have shown maximal calcium retention in white adolescent girls is achieved on calcium intakes of 1300mg/d. Matkovic and Heaney (65) estimated using a two-component linear-regression model to assess data from 133 calcium-balance studies in children aged 9-17 years, the calcium accretion plateaus to be 1480mg/d for this age group. Weaver et al (62) found that during adolescence females absorb more calcium from the gut and excrete less calcium in the urine and faeces than adults with the same calcium intake, this results in a significantly higher net calcium absorption (32.3 $\pm$ 8.8%) than adults (20.6 $\pm$ 11.1%).

The amount of calcium retained after obligatory losses from the various sites (digestive tract, skin, nails, hair, sweat and urine) and the amount that is finally incorporated into the skeleton compared to the amount ingested is called the calcium retention. There is a complex homeostatic control that determines this.

A recent longitudinal study in growing children documented calcium retention efficiencies of 33% for boys and 29% for girls (66). Higher calcium efficiencies appear to compensate for low dietary intakes of calcium. In another study in children aged 9-14 years, when calcium intakes dropped by 400mg/d, absorption efficiency rose to 50% (67). This is striking when compared with the calcium retention of 4-8% in the adult with skeletal deficiency (67). It, therefore, may only in cases of severe dietary restriction that bone mineral accrual is compromised.

In severe dietary restriction bone growth may proceed at a slower rate and bones are usually of normal shape and size but have lower than normal bone mineral mass.

Studies have shown calcium retention is also increased during pregnancy and lactation. Cross et al (45) found a significant increase in fractional calcium absorption during the 2^{nd} and 3^{rd} trimesters of pregnancy that was maintained during lactation in most subjects. Bone growth relates directly to the genetic and mechanical control of linear growth and periosteal and endosteal expansion (68).

Table 2.1: Review of the balance studies that have looked at calcium retention during adolescence

Study	Number of Subjects	Age of Subjects	Daily Calcium Intake	Daily Calcium Retention	% Calcium Retention
Abrams et al (67)	n=26	9-14 years	1310±82mg/d	193±134mg	14.7%
Matkovic and Heaney (65)	n=99	2-8 years	1390mg	246±126mg	17.7%
	n=133	9-17 years	1480mg	396±164mg	26.7%
Martin et al (58)	n=228	9½ - 19½ years	1172mg (males)	282mg	24.1%
			929mg (females)	212mg	22.8%
Jackman et al (69)	n=35	12-14 years	1300mg	473±114mg	36.4%

2.1.7 Recommended Dietary Intake

Determination of adequate calcium intake is compared to the Dietary Reference Intakes (DRI) for calcium. Dietary reference intakes are reference values that can be used for planning and assessing the diets for healthy populations. The DRIs encompass the estimated average intake (EAR), the recommended dietary allowance (RDA), the adequate intake (AI) and the tolerable upper limit (UL).

The EAR is the nutrient intake value that is estimated to meet the requirements defined for a specified indicator or adequacy in 50 percent of the individuals in a life stage and gender group (70). The EAR is expressed as a daily value averaged over time, and includes an adjustment for an assumed bioavailability of the nutrient. The EAR is used in setting the RDA.

The RDA is the average daily dietary intake that is sufficient to meet the nutrient requirements of nearly all (97.5%) individuals in a life stage and gender group (70-72). The RDA used in the USA and Canada has the same definition as the

recommended nutrient intake (RNI) used in the United Kingdom and the recommended dietary intake (RDI) used in Australia and New Zealand. If the standard deviation of the EAR is available and the requirement for the nutrient is normally distributed, the RDA is set at 2 standard deviations above the EAR (70). The RDA for a nutrient is a value to be used as a goal for dietary intake by healthy individuals. It is not intended to be used for assessing the diets of either individuals or groups or to plan diets for groups (70-72).

The AI is set instead of a RDA if there is insufficient scientific evidence to calculate an EAR (70). The AI is based on observed or experimentally determined estimates of average nutrient intake by a group (or groups) of healthy people. Although the RDA and the AI are used for the same purpose the RDA differs from the AI. If the EAR is unable to be determined then it is not known what percentage of individuals are covered by the AI. The degree to which an AI exceeds the average requirement is likely to differ among nutrients and population groups (70).

The UL is the highest level of daily nutrient intake that is likely to pose no adverse health effects to almost all individuals in the general population (70). As the intake increases above the UL, the risk of adverse effects increases. The UL for calcium in children from age 1 year to 18 years has been set at 2500mg/d (70). The main reason for this is with a diet high in calcium there is a risk of depleting other minerals from the diet like iron and zinc. There is no dose response data regarding these interactions in children or the development of adaptation to chronic high calcium intakes. When looking at supplementation intervention studies in children the safe

upper limit should be considered so as not to put them at risk of adverse health effects.

The dietary reference values for calcium in children in the United Kingdom have been calculated from a daily retention of 70mg/d at 1 year rising to 150mg/d at the age of 10 years. Absorption has been taken at 35% (72).

In the United Kingdom the recommended nutrient intake (RNI) is set at 550mg for children aged 7-10 years, and this increases to 1000mg in males and 800mg in females during puberty (11-14 years).

The current United Kingdom dietary calcium intakes for children of various ages are listed in table 2.2.

Table 2.2: The current United Kingdom recommended nutrient intakes for calcium in children (72).

	Females	Males
4-6 years	450mg/d	450mg/d
7-10 years	550mg/d	550mg/d
11- 14 years	800mg/d	1000mg/d

The Australian RDI that New Zealand has adopted has not been reviewed since 1991, however, the National Health and Medical Research Council (Australia) adopted the calcium RDI in 1985 (71). The Australian calcium RDIs are based on research carried out between 1959 and 1985, this suggests they are not current. When the working party developed the guidelines for children, they appear to only have examined review papers or extrapolated from studies in adults. It therefore

seems important that these recommendations are reviewed, since 1985 there have been a large number of studies that have examined the effect of calcium during childhood, both observational studies and clinical trials. In addition there have been a number of calcium balance and retention studies published.

The current Australian recommended dietary intakes (adopted by NZ) for calcium for children of various ages are outlined in table 2.3 (71,73).

Table 2.3: The current Australian recommended dietary intakes for calcium in children (71).

	Females	Males
4-7 years	800mg/d	800mg/d
8-11 years	900mg/d	800mg/d
12-15 years	1000mg/d	1200mg/d

Unlike the United Kingdom and Australia, the USA and Canada have set an AI for calcium. The Standing Committee on the Scientific Evaluation of Dietary Reference Values suggest there is insufficient scientific evidence to determine an EAR (70). The USA and Canadian values were reviewed in 1997, and have taken into consideration a much larger volume of scientific literature in their estimation. In light of this it is prudent to compare dietary intakes to the USA and Canadian AI as it is based on more recent evidence.

The current USA/Canada adequate intake levels for calcium are listed in table 2.4.

Table 2.4: The current USA/Canadian adequate intakes for calcium in children (70).

	Females	Males
4-8 years	800mg/d	800mg/d
9-13 years	1300mg/d	1300mg/d
14-18 years	1300mg/d	1300mg/d

There are four main areas to evaluate when developing a recommended intake for calcium; 1) calcium retention, 2) clinical trials measuring bone mineral content, 3) the factorial approach and 4) epidemiological evidence (or observational studies).

Calcium retention has been reviewed in section 2.1.6. The calcium retention studies used in developing the USA/Canada AI was based on information about peak velocity bone mineral content. Martin et al (58), found the average peak velocity of bone mineral content that occurs between the ages of 9.5 and 19.5 years was 320g/year in males and 240g/year in females. The assumption was made that bone is 32.3% calcium, the above values corresponded with a calcium retention of 282mg in males and 212mg in females. There are 2 main limitations of this study. There are no data on whether peak bone mineral accrual would be greater at higher calcium intakes than was consumed and the other limitation being that dietary calcium intake was analysed from 24-hour dietary recall.

Other calcium retention studies were pooled together using nonlinear regression to determine the calcium intake at which calcium retention of 282mg in males and 212mg in females could be achieved. A figure of 55mg/day was added to account for sweat loss, this had previously been determined by Peacock et al (74). The estimate of calcium intake that would result in a desirable level of retention was 1070mg/day for females and 1310mg for males.

Clinical intervention trials in children have suggested that by increasing dietary intake of calcium above habitual intake there is a positive effect on bone mineral accretion, this will be reviewed fully in section 2.6.

The factorial approach to determining recommended calcium intake is the traditional method used in children (70). This is the method used by the scientific committees in the United Kingdom and Australia (71-72). The variation in values comes about from the number of factors used and the primary research considered. When the USA/Canada Standing Committee estimated intake they used calcium requirements for growth, plus calcium losses (urine, sweat and faeces) and adjustments for absorption. Using this method the estimates they established were 1276mg for females and 1505mg for males (70).

The use of epidemiological evidence for this age-group has identified either a positive association or no association between calcium consumption and bone density in children. The studies that have shown a positive association tend to include a significant proportion of subjects with low calcium intakes resulting in an obvious bias (75-80). There have also been a number of retrospective studies that suggest a higher calcium intake during childhood results in a greater bone mass during adulthood (17,26,30-31).

In summary the true calcium requirement for children and adolescents probably lies between 1000mg and 1500mg per day. Also due to the large number of studies that

examine the effect of calcium intake in females only, it is difficult to determine the true gender differences at this age.

2.1.8 The Effect of Inadequate Calcium Intake

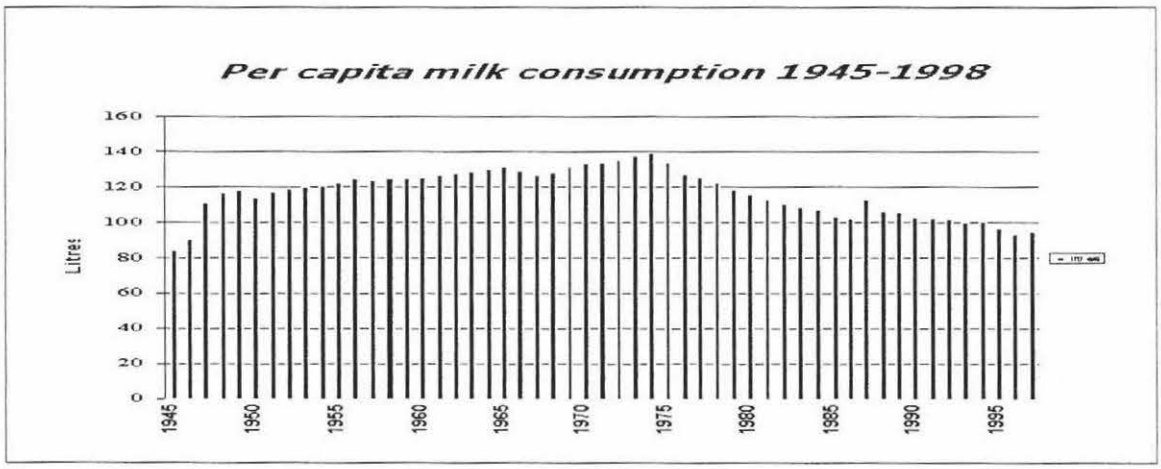
Chronic calcium deficiency resulting from inadequate intake or poor intestinal absorption is one of several causes of reduced bone mass and osteoporosis (refer to figure 2.5). A reduction in absorbed calcium causes the circulating ionized calcium concentration to decrease. This triggers an increase in PTH synthesis and release. Parathyroid hormone acts on three target organs to restore the circulating calcium concentration to normal (refer to figure 2.5). At the kidney, PTH promotes the reabsorption of calcium in the distal tubule. Parathyroid hormone also induces bone resorption, thereby releasing calcium into the blood. Thus, although PTH maintains a normal circulating calcium concentration during calcium deprivation, it does so at the expense of skeletal mass, which is of paramount importance due to the importance of the role of non-skeletal calcium in intracellular processes (23).

It is known that children with malabsorption problems have lower bone mineral density than their healthy counterparts. Boot et al (53) showed significantly lower BMD values for both the lumbar spine and total body measures in children with chronic inflammatory bowel disease. Children on long-term inhaled corticosteroids also have been shown to have a reduced BMD at the lumbar spine and total body when compared to healthy controls, as shown by Martinati et al (81). This suggests that children with malabsorption problems and/or those requiring corticosteriods have lower BMD than healthy children.

2.2 Trends in Consumption of Milk in New Zealand

The main source of calcium in the New Zealand diet (53%) is from dairy foods (82). In New Zealand the per capita consumption of milk has declined since 1976 (83) (see figure 2.6).

Figure 2.6: Milk consumption per capita in New Zealand (1945-1998) (83)



This is of concern because milk is such an important source of many nutrients, most importantly calcium. The 1997 National Nutrition Survey showed that New Zealanders over the age of 15 years obtained 37% of their calcium intake from milk (82). Wham et al (83) found at least one third of respondents in their surveys consumed less than 200mL of milk per day. In the youngest group surveyed (16 to 30 years) they tended to either not drink milk or be heavy consumers (drink more than 2 glasses or 400mL per day), with 9.4% of all young people consuming no milk at all. Young men appeared to be more attracted to soft drinks and lacked knowledge about the nutritional value of drinking milk. In comparison young women rejected milk on the grounds it is fattening. It was concluded there needed to be a

public health initiative with collaboration from the food industry and health agencies to encourage greater milk consumption and help improve the overall nutrition status of New Zealanders. One such intervention could be the reintroduction of the School Milk Programme.

2.3 The History of New Zealand's School Milk Programme

A recent review of the history of New Zealand's School Milk Programme has been published (84). This programme ran for 30 years between 1937 and 1967. It was instigated at a time when New Zealand had a surplus of milk and it was thought that malnutrition was a problem amongst New Zealand children. The object of the School Milk Programme was to make available 284mL of milk to every school child on each school day, this provided 324mg of calcium.

New Zealand's School Milk Programme was terminated in 1967, when it was costing the government £910,349 per annum. The School Milk Programme was thought to play an important role in the nutrition of school children, however there was no reported evaluation of efficacy. At its termination it was believed that it had outlived its usefulness. At the termination of the programme it was also thought that malnutrition was not, and had not been, a concern among New Zealand children. The most important lesson to be learnt from the original programme was if a programme like this was ever considered again, to serve the milk cold and in a variety of flavours. However the reported data does not mention consumption figures or whether milk was provided in different flavours, so this conclusion is difficult to substantiate.

A similar School Milk Programme was run in the UK from 1946 to the late 1970's, all school children were supplied with 190mL of milk a day, after 1968 secondary school children no longer received school milk and in 1971 it was restricted to children under the age of seven (85). A major difference between the NZ and UK programme was that in the UK the children could choose not to have school milk while in NZ it was compulsory. Cook et al (85) studied the nutritional impact school milk was having in school children in Kent, UK. Three hundred and twelve children were studied, the main aim was to compare the actual intake with the reported intake and determine if School Milk was having an effect on nutritional status. Eighty-five percent of boys and 73% of girls reported having school milk, when the actual intake was recorded 59% of the boys and 54% of the girls actually drank the milk. The children who drank milk at school had a mean daily intake of milk of nearly 500mL whereas children who did not take school milk drank about 300mL daily. There were no differences in school milk intake when socio-economic status, family size and whether the mother worked or not were considered. The children were examined for subcutaneous fat, muscle mass and general skin and hair condition and there were no differences in any of these variables. There was also no difference in height or weight of the children. When the recorded 5 day diets were analysed it was found there were significant differences in nutrient intake between the children that drunk school milk everyday compared to those that never drunk it. However, apart from increased intake of pyridoxine the difference in intake of other nutrients was not due to drinking 190mL of milk. The researchers also found that the children who drank school milk were also more likely to eat school meals and this was probably the major source of the variation in the nutrient intake between the milk and non-milk drinkers.

In 1967 McKenzie et al (86) attempted to encourage a greater number of children in the UK to consume school milk. An education programme was developed that included either 1) posters, a film and a lecture about the importance of milk in the diet or 2) a pamphlet about the importance of milk. The number of children who said they would drink milk increased, however, there was no significant difference in the number that actually did increase their consumption. It could be concluded from this study that either nutrition education had no effect on dietary behaviour or the nutrition education was not developed for the target market, as behaviour change is a multi-stage model (87).

2.4 Current New Zealand Calcium Intake

There are few data on the calcium intake of NZ children. A recent pilot study (88) designed to develop and pre-test the methods for the Children's National Nutrition Survey found in Auckland children the mean calcium intake for 8-10.9 year old boys was 666mg, this increased to 739mg between the ages of 11 and 14 years. In the girls the mean intake was 667mg in the younger age group and decreased in the 11-14 year old girls to 569mg. Studies in older age groups have found similar results to this. Turner et al (89) found in a group of NZ adolescent females with a mean age of 16.4 years, 60% of the group had a mean dietary calcium intake below 800mg. The 1997 National Nutrition Survey showed the average intake for males aged 15-18 years was 957mg and for females in this age group it was lower at 783mg (82). Merrilees et al (10) found in 15 year old adolescent girls the mean calcium intake to be 750mg, and 670mg three years later at age 18 years. This indicates in NZ

children a reduction in dietary calcium with increasing age, other studies in developed countries support this trend (12,90-91).

2.4.1 Calcium Intake in Other Developed Countries

Weaver et al (91), has reported the average calcium intake in the USA for females aged 9-13 years is 919mg/d and this decreases to 753mg/d in the 14-18 year olds. This is supported by Johnson (92) who reported 59% of USA girls aged 6-11 years and 86% of USA girls aged 12-18 years do not meet the recommended AI for calcium. Milk and dairy products provide the most important sources of calcium in USA children's diets, as they account for 75% of calcium in the US food supply. It must be remembered the AI for calcium in the USA is higher than NZ and Australia. Ireland has similar intake data with the average intake for females aged 15-18 years being 950mg/d (91). The British Children's Nutrition Survey reported that the older age groups (15-18 years) tended to have lower intakes of various minerals including calcium (93). While the Australian Nutrition Survey (94) found most of the calcium in the Australian diet comes from milk and dairy (50-66%). They found across the lifespan approximately 25% of males and 50% of females did not meet the RDI for calcium. Toddlers (2-3 years) had the highest intake of calcium, due to their higher milk intake.

2.5 Other Nutritional Factors that Affect Bone Health

2.5.1 Protein

Evidence suggests dietary protein may have an important influence on skeletal health, but the nature of its role has remained controversial. In 1968 Wachman and Bernstein developed the acid-base theory (95). They hypothesized that diets rich in protein will increase bone loss due to the hydrogen sulphate acid produced by protein metabolism, and the consequent need for calcium to leave the skeleton in order to buffer the elevated acidity of the extracellular fluids and plasma. Although there is indirect evidence for this deleterious effect of protein rich diets, epidemiological studies has pointed more strongly to a beneficial role for dietary protein in bone health (96-97).

A study by Heaney et al (98) of free-living middle-aged women demonstrated that urinary calcium was significantly positively associated with protein intake and that, accordingly, calcium balance was significantly negatively correlated. This study has been cited widely since it was published and has contributed to the popular belief that protein is harmful to bone. A main finding of the study that was widely ignored, was a positive correlation between calcium intake and calcium balance, that is, the higher calcium intakes offset the calciuric effects of protein (99).

Clinical trials in hip fracture patients have consistently observed patients who receive protein supplements experience significant improved recoveries and reduced bone loss (100). When the acid-base theory was examined in the Framingham Osteoporosis Study (101) a surprising result was that a higher protein intake was

associated with conservation of the bone, not lower as has been hypothesized. Researchers concluded that the role of protein is complex and is probably dependent on the presence of other nutrients available in a mixed diet.

In a study by Bonjour et al (102), in healthy children and adolescents a positive association between the amount of ingested protein and bone mass gain was observed in both sexes at the lumbar spine, the proximal femur and the midfemoral shaft. The association was significantly strong in the pre-pubertal children, this is similar to results seen with calcium and weight-bearing exercise. The possible mechanism for this could be explained by considering the effect of protein on insulin-like growth factor-1 (IGF-1). Low protein intake impairs both the production and action of IGF-1. Insulin-like growth factor-1 is an essential factor for longitudinal bone growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate, and also for bone formation (103). It can be considered as a key factor in the adjustments of calcium-phosphate metabolism required for normal skeletal development and bone mineralisation during growth.

A study by Dardevet et al (104), using a rat model, demonstrated that a protein intake of 0.7g/kg body mass (normal protein intake in rats is 1.9g/kg body mass) resulted in decreased plasma IGF-1 concentrations. This study found an increase in skeletal muscle IGF-1 and insulin binding proteins, the physiological consequence of an increase in these binding proteins would be to conserve muscle protein during periods of low protein, maybe at the expense of bone tissue. The duration of this study was 11 days, in contrast to Leili and Scanes (105) who studied chicken over 14 days. Initially there were similar results as seen in the rat model with an increase

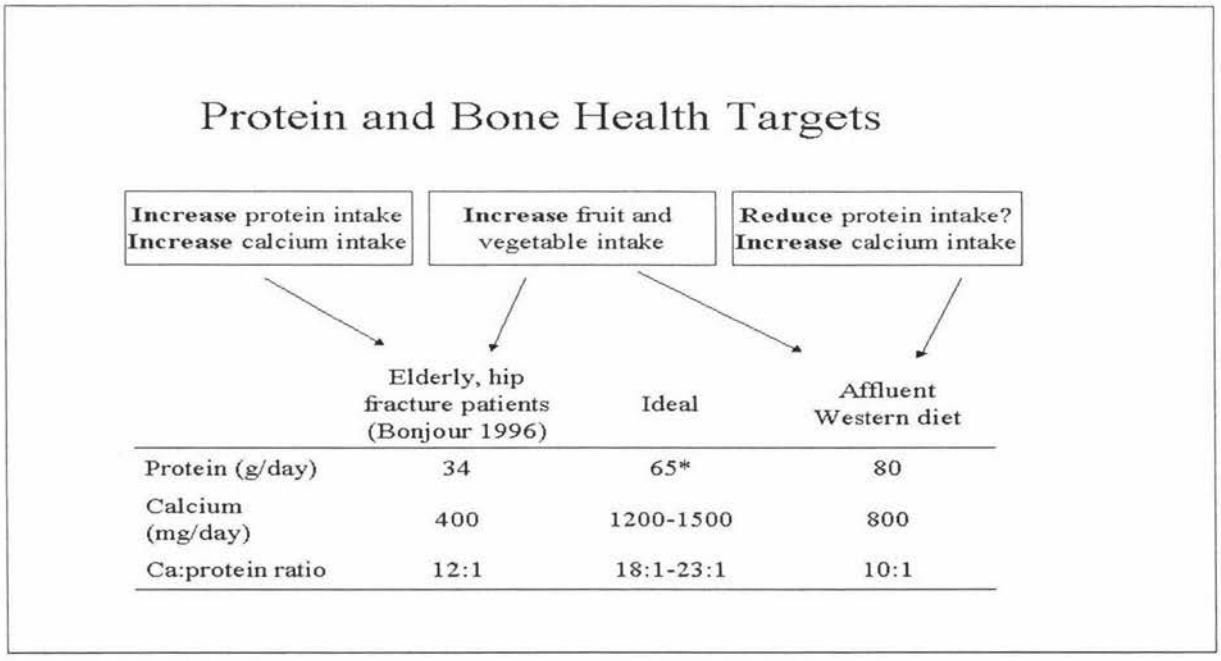
in the activity of the IGF-1 binding proteins following dietary protein restriction. However, after 10 days there was a decrease in the activity of the IGF-1 binding proteins that resulted in decreases in body and skeletal growth. Leili and Scanes (105) calculated the protein intake on percentage total energy, a 5% and 10% protein diets were compared to a 20% protein diet. These results are from animal models, which makes the results difficult to extrapolate to human subjects, especially with regard to how low a an animal low protein diet is. However, it is likely that a decrease in dietary protein impairs IGF-1 production and action.

Schette and Linkswiler (106) reviewed calcium retention in relation to protein intake. It was demonstrated with a normal phosphorus to calcium ratio (1:1.5), a calcium to protein intake ratio of 9:1 or higher resulted in the body remaining in positive calcium balance. At ratio levels less than this there was an increase in urinary calcium resulting in negative calcium retention. A study by Bonjour (100) examined the nutritional intakes of elderly hip fracture patients. While their calcium to protein ratio was 12:1, both their calcium and protein intakes were lower than elderly subjects who did not fracture. The ideal protein to calcium ratio is proposed to be within the range of 18:1 to 23:1. This would allow for maximal calcium retention especially during periods of bone accretion and accelerated bone loss, ie adolescence and post-menopause (see figure 2.7).

Recent studies have also looked at the role of fruit and vegetables on BMD, and support the Wachman and Bernstein theory on the role of the skeleton in acid-base homeostasis. New at al (107) used a cross-sectional study design to investigate the effect of current and previous nutrient and food intake on BMD and bone biomarkers.

They found long-term consumption of fruit and vegetables has a positive effect on bone health, this was attributed to the hypothesis that fruit and vegetables are alkaline-forming foods. The Dietary Approaches to Stopping Hypertension (DASH) study (108) found as a secondary finding a positive effect on calcium economy. When fruit and vegetable intake increased from 3.6 to 9.5 serves per day, urinary excretion of calcium decreased from 157mg/24hr to 110mg/hr. As it was a secondary finding biomarkers of bone turnover were not measured so it is not known what effect this has on long-term bone health. When the results of studies like this are compared to the studies of low protein intake, we can start to develop a picture of the ideal food intake for bone health.

Figure 2.7: How differing levels of dietary protein and dietary calcium affect bone health



	Elderly, hip fracture patients (Bonjour 1996)	Ideal	Affluent Western diet
Protein (g/day)	34	65*	80
Calcium (mg/day)	400	1200-1500	800
Ca:protein ratio	12:1	18:1-23:1	10:1

These reviewed studies have shown protein has a complex role in bone development, therefore any calcium supplement that also contains protein must consider the effect of protein as well as calcium on bone development.

2.5.2 Vitamin D

Vitamin D plays a critical role in the development, growth, and mineralisation of the skeleton during its formative years. Vitamin D deficiency in children results in the bone-deforming disease rickets. In adults, vitamin D insufficiency and vitamin D deficiency has a more subtle effect on the skeleton. The major source of vitamin D is not dietary; it is produced from 7-dehydrocholesterol (D_3) in the skin during exposure to sunlight. Vitamin D_3 is then hydroxylated in the liver to produce 25-hydroxy vitamin D (23). This is the major circulating form of vitamin D, having a half-life of about two months, and is used as a measure of long-term vitamin D status. Further hydroxylation in the kidney, catalysed by the 1α-hydroxylase enzyme, results in the formation of the active form of vitamin D, 1-25-dihydroxy vitamin D ($1,25(OH)_2D$). As the body becomes vitamin D insufficient, the efficiency of intestinal calcium absorption decreases from ~ 30-50% to no more than 15%. This results in a decrease in the ionized calcium concentration in the blood, which signals the calcium sensor in the parathyroid glands resulting in an increase in the synthesis and secretion of PTH (see figure 2.5). When dietary calcium is inadequate to satisfy the body's calcium requirement $1,25(OH)_2D$ in tandem with PTH, mobilizes monocytic stem cells in the bone marrow to become mature osteoclasts (22). The increased number of osteoclasts enhances the removal of calcium from the bone that enters the circulation to maintain normal serum calcium levels. Parathyroid hormone also increases tubular excretion of phosphorus causing hypophosphataemia. The net effect of vitamin D insufficiency and vitamin D deficiency is a normal serum calcium, elevated PTH and alkaline phosphatase and a low or low normal phosphorus (109-110).

Nutritional rickets is caused by a deficiency in vitamin D. The deficiency causes soft bones, which makes the child prone to bowing deformity of the lower limbs. When the child begins to walk weight-bearing makes the features of rickets especially apparent. In Northern Europe and the USA some dairy products are fortified with vitamin D and this may prevent rickets in some at risk populations. A search of the literature for studies looking at the efficacy of fortification on incidence of rickets could not be found, although one assumes that the incidence has decreased since fortification. It may be that this has not been examined and could be an important area of future research. However there has been published data on cases of hypervitaminosis D associated with over-fortification resulting in severe illness and death (111). Incidents like this highlight the importance of monitoring the fortification process and enforcing the upper limit for vitamin D addition to milk.

A combination of factors such as pigmented skin, inadequate sunlight, by widespread smog or covered skin, including sunscreens, breastfeeding with no supplementation or insufficient calcium by a breast-feeding mother and her infant may contribute to rickets in children.

If there is not enough production of active vitamin D, due to inadequate sunlight exposure then the body is only capable of absorbing 10-15% of all calcium consumed (112). A recent study in Kuwait found infants (mean age at diagnosis = 14.5 months) with rickets were started on weaning foods later and more were breast-fed than formula fed. The children with rickets were also likely to live in a multi-

storey block of flats and both the children and their mothers wore traditional dress (hijah)[3] (113). Similar findings have been reported in children who live in Delhi (114).

There are several studies that have demonstrated an increase in calcium intake of 800-1000mg/d with supplementation of ≥ 400-800 units of vitamin D daily will decrease the risk of vertebral and nonvertebral fractures and increase bone mineral density (46,115). It has been recognized since the late 1960s that vitamin D is associated with an increased risk of hip fracture in the elderly population. Aaron et al (116) in 1974 published a large study showing that 40% (n=59) of patients who were admitted to hospital for an acute hip fracture were vitamin D deficient, the main cause of the deficiency was due to inadequate sunlight exposure and decreased dietary intake.

Vitamin D is important due to its role in mineralisation of the skeleton and may also be a confounding factor in interpreting study data if taken in a supplement where other nutrients are hypothesized to have an effect. It may also be difficult to control for Vitamin D status because of varying sun exposure.

2.5.3 Phosphorus

About 85% of phosphorus is found combined with calcium in crystalline structure in the bones and teeth.

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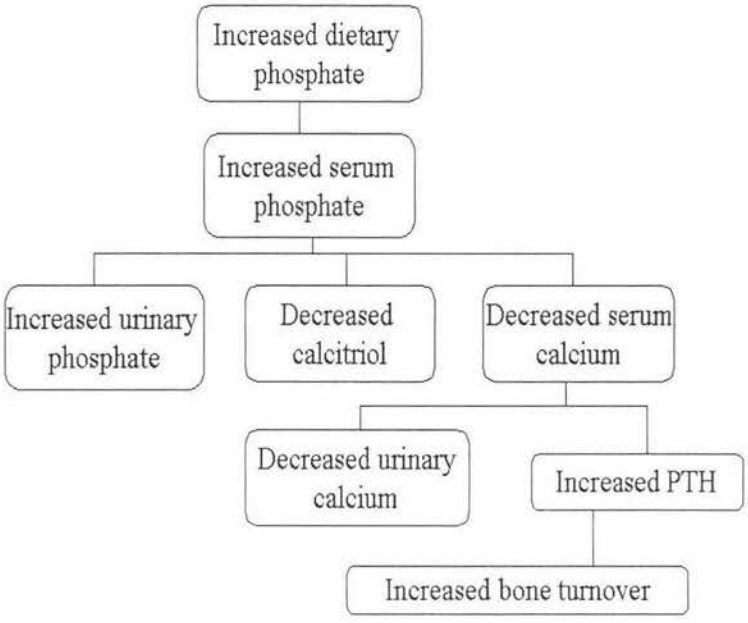
[3] Hijah is traditional dress worn by Kuwait women, it covers all of their body and includes a head scarf

There is concern a high phosphorus intake can affect calcium homeostasis. Eastell et al (117) have proposed the following model of adaptation to a high phosphate diet (refer fig 2.8).

Figure 2.8: Proposed model of adaptation to a high phosphate diet (117)



The effect of a high phosphate diet has been studied in a number of calcium studies (118-121). There was no reported effect on calcium balance, although a decrease in urinary calcium and increase in endogenous faecal calcium was reported for young women (122). Matkovic et al (24), has reported an effect of phosphorus intake on calcium balance in adolescent girls, and it is possible phosphorus intake could affect calcium balance at times of high calcium requirement. Further, an association of high phosphate diet and fracture rates has been observed in girls aged 8-16 years (123). Eastell et al (117) studied the effect of changing from a low to a high dietary phosphate intake and found little effect on bone turnover in young men, despite an

increase in PTH. From these studies it appears high dietary phosphate may affect peak bone mass in females, but not males. The main concern regarding phosphate intake is soft-drinks as these are one of the main sources of phosphate in the diet and these have displaced milk as the preferred beverage especially in adolescents.

2.5.4 Magnesium

Two-thirds of total body magnesium content is located in the skeleton. The magnesium in bone is not an integral part of the hydroxyapatite lattice structure, but appears to be located on the crystal surface. During times of deprivation, the kidney is primarily responsible for conserving magnesium. Magnesium is essential for the normal function of the parathyroid glands, vitamin D metabolism, and adequate sensitivity of target tissues to PTH, and active vitamin D metabolites. Hypocalcaemia is a common manifestation of moderate to severe magnesium deficiency, due to impaired PTH secretion (22).

In a community based study (124) in elderly men and women it was observed dietary magnesium intake was positively associated with BMD. However, epidemiological studies relating magnesium intake to bone mass or rate of bone loss have been conflicting, and there have been limited studies on the effect of magnesium supplementation on bone loss (125). Two studies looking at the effect of magnesium supplementation on biomarkers of bone turnover in young adults had differing results. Dimai et al (126) studied 24 healthy males and found a reduction in blood ionized magnesium that results in a reduction in bone turnover, however the mechanism is unknown. In contrast Doyle et al (127) studied 26 healthy females

and found no effect from magnesium supplementation on bone turnover. These two studies suggest there may be a gender response, however more data is required before the mechanism for magnesium and bone health is fully understood.

2.5.5 Vitamin K

Vitamin K nutrition has been proposed as a modifiable risk factor for osteoporosis. At least 3 vitamin K dependent proteins have been identified in bone or cartilage, including osteocalcin, which is one of the most abundant noncollagenous proteins in bone (128). Most of the evidence supporting a role for vitamin K in age-related bone loss is based on reported associations between BMD or the bone fracture rate and biological markers of vitamin K status (128). Due to the difficulty in controlling for other dietary factors that contribute to bone these studies are often criticized. A recent study (129) examined the effects of vitamin K on bone formation and resorption in humans after phylloquinone (vitamin K) depletion and subsequent repletion with phylloquinone when other dietary factors were controlled for. There was an increase and subsequent decrease in measures of bone formation and resorption after dietary phylloquinone restriction and repletion. These results suggest when dietary factors are controlled for, vitamin K still has an effect on bone health and should be included as a confounding factor when looking at dietary intervention and bone measures.

2.5.6 Fluoride

Fluoride is mainly associated with calcified tissues due to its high affinity for calcium. In addition to the strong evidence that the addition of fluoride to drinking water supplies has decreased the incidence of dental caries there is also evidence it may stimulate bone formation and has been used as an experimental drug in the treatment of osteoporosis (130).

Phipps et al (131) have suggested communities with water fluoridation have higher BMD at the femoral neck, and lumbar spine but lower BMD at the distal radius. While there is no significant difference in the incidence of wrist fractures there was a trend towards an increased risk of wrist fracture in the fluoridated group. There was however a significant reduction in the risk of vertebral fractures (relative risk=0.73 p=0.033) and a significant reduction in the risk for hip fracture (relative risk=0.69 p=0.028). In a review of the use of fluoride as a treatment for osteoporosis it was concluded that although fluoride has the ability to increase BMD at the lumbar spine, it does not result in a reduction of vertebral fractures. There was an increase in the relative risk for non-vertebral fractures(relative risk=1.85) and gastrointestinal side effects (relative risk=2.18) after 4 years of treatment with fluoride compared to the control group.

A more recent intervention trial has examined the effect of sustained-release sodium fluoride in the treatment of established osteoporosis in the elderly (132). The use of sustained release sodium fluoride was used due to the gastrointestinal side effects and mixed results seen with high dose and/or continuous sodium fluoride. This

study found in elderly females (n=85) over the age of 65 years there was a significant reduction in vertebral fracture rate (relative risk=0.32, p=0.007). Of importance for patient compliance was that no significant differences were seen in adverse effects between the groups.

While the effect of fluoride on BMD in children has not been examined, it would appear that due to the effect it has BMD in the elderly, that it may be a confounder when looking at dietary factors influencing bone health in children.

2.6 Studies of Calcium Intervention and Bone Growth

There have been a number of observational studies that have tried to explain the effect of current or previous calcium intake on bone (75-80). These studies have been carried out in children, and in adults with relation to their childhood intake. There are difficulties in the interpretation of observational studies due to the methodology used to assess accurate calcium intake and the limited observed ranges of calcium intake.

Eight intervention studies have been conducted, examining the effect of calcium supplementation on bone mineral excretion in children (2-9) (see table 2.5). These studies, with the exception of the study by Cadogan et al (7), were all of a randomized double-blind placebo-controlled study design. This is a strong study design to prove causality, and allowed for each of the research teams to control for potentially confounding factors that are known to influence bone mass. Additional factors associated with bone mass are genetics, hormones, physical activity, lifestyle factors such as smoking, and nutrition. Johnston et al (2) and Nowson et al (8)

successfully eliminated the factor of genetics through working with identical twins, who share 100% of their genes. In these studies one of the twin pair received the calcium supplement, and the other an identical placebo tablet.

Four of the eight studies identified, assessed physical activity by either administering a physical activity questionnaire (2,7-8), or through subjects completing a 3-day activity record (4) at regular intervals. These studies all reported no difference in physical activity level between the experimental and control group; however, the measures utilized are unlikely to be sensitive to subtle differences between the groups. Thus, the role of physical activity in the findings presented cannot be fully eliminated.

The effect of hormonal changes associated with puberty on bone mass, was assessed in six of the identified studies (2,4-8). Four of these studies assessed stage of pubertal development in their subjects using the previously validated Tanner staging method (2,4-6). Cadogan et al (7) used a previously validated modified Tanner assessment and Nowson et al (8) classified them as pre and post menarche. Johnston et al (2) analysed the results from the pre-pubertal twins separately from the pubertal twins, and was able to find a greater increase in bone mineral density in the younger age group. Nowson et al (8) also analysed the results separately and found no difference between pre and post menarche. The remaining four studies did not identify differences between their pre-pubertal subjects with respect to bone mineral status (4-7).

Nutritional factors other than calcium also influence bone growth, including energy intake and protein (refer to section 2.5.1). The studies that used a calcium supplement eliminated this factor (2,4-6,8-9), so too did Bonjour et al (3) as they provided identical food products to both of the study groups with only the experimental group foods being fortified with additional calcium from a milk extract. However, Cadogan et al (7) provided fluid milk to those in the experimental group, while the control group was instructed to continue with their usual diet. Protein intake of the experimental group significantly ($p<0.01$) increased over the study period in direct association with serum concentrations of insulin-like growth factor. Insulin-like growth factor is a marker that is responsive to changes in protein intake, and has a synthesising effect on growing skeletal tissue such as bone (refer to section 2.5.1). Therefore, milk consumption may partly account of the increase in total body bone mineral content and density, and the effect of the protein cannot be excluded.

Generally, it is optimal that a food source is used to obtain required nutrients rather than a supplement form. Studies in the UK during the 1920s showed that children who drank milk grew taller compared with subjects without milk supplementation (133-134). Milk provides an ideal source of calcium as it is cheap, the lactose in milk aids in calcium absorption, and it provides nutrients to support growth and development in children. In the study by Bonjour et al (3) the percentage increases in bone increments were not as high as the earlier studies that used a calcium supplement. This lower percentage difference may be attributed to a lower calcium

Table 2.5: Calcium supplementation intervention trials in children: a comparative matrix

	Johnston (1992)	Lloyd (1993)	Lee (1994)	Lee (1995)	Cadogan (1997)	Bonjour (1997)	Nowson (1997)	Dibba (2000)
Subjects	n=90 Males & Females White American 45 pre-pubertal twin pairs	n=94 Females White American Pre-pubertal & pubertal	n=162 Males & Females Chinese Pre-pubertal	n=84 Males & Females HK Chinese Pre-pubertal	n=84 Females English Pre-pubertal & Pubertal	n=108 Females Caucasian Pre-pubertal	N=88 Females Australian 44 Pre-pubertal & pubertal twins	n=160 Males & Females Gambian Pre-pubertal & Pubertal
Initial Age (years) (mean +/- SD)	9.5 +/- 2.4	11.9 +/- 0.5	7.0 +/- 0.2	7	12.2 +/- 0.3	7.9 +/- 1.2	14.0 +/- 2.6	10.3 +/- 1.0
Method of assessment of dietary calcium intake	3 day food record	3 day food record	5 day food record	Modified quantitative FFQ	7 day weighed food record	FFQ	4 day food record	2 day weighed food record
Baseline Mean Daily Calcium Intake	901mg	975.5mg	279mg	567mg	746mg	≈ 898mg	734mg	≈ 338mg
Method of assessment of physical activity	Questionnaire	-	3 day activity record	-	Questionnaire	-	Questionnaire	-
Supplemental Calcium: **Given** **Taken** **Mean total intake (%RDI)#**	 1000mg 719mg 1620mg (203%)	 500mg 352mg 1327.5mg (166%)	 300mg 276mg 555mg (69%)	 300mg 246mg 813mg (102%)	 550mg 379mg 1125mg (141%)	 850mg 807mg 1705mg (213%)	 1000mg 830mg 1564mg (195%)	 714mg 714mg 1052mg (132%)
Method of Pubertal Assessment	Tanner questionnaire	Tanner questionnaire	-	Tanner questionnaire	Modified Tanner questionnaire	-	+/- Menarche	Tanner questionnaire
Study Duration	3 years	18 months	18 months	18 months	18 months	12 months	18 months	12 months
Type of Calcium Supplement	Calcium Citrate Malate	Calcium Citrate Malate	Calcium Carbonate	Calcium Carbonate	Fluid Milk	Food fortified with milk extract	Calcium lactate gluconate	Calcium Carbonate
Bone Measurement Method	Lunar SP2 absorptiometry & Lunar DP3 absorptiometry	QDR bone absorptiometry	Single-photon absorptiometry	Single-photon absorptiometry (radius) and DEXA	DEXA	DEXA	DEXA	Single-photon absorptiometry
Experimental-Control: % diff In bone increments **Midshaft Radius** **Distal Radius** **Lumbar Spine** **Femoral Neck** **Femoral Trochanter** **Total Body Bone Mineral Content** **Total Body Bone Mineral Density**	 5.1*** (BMD) 3.8*** (BMD) 2.8*** (BMD) 1.2 (BMD) - - -	 - - 3.1*** (BMD) - - - 1.83***	 3.14* (BMD) - - - - - -	 1.74 (BMD) - 4.61*** (BMD) 0.77 (BMC) - - -	 - - - - - 2.9** 1.1***	 1.6*** (BMD) 2.4 (BMD) 0.3 (BMD) 1.4 (BMD) 1.9*** (BMD) - -	 - - 1.53** 1.27*** - - - NB: Effect only seen at 6 months	 4.5* (BMD) 7.0** (BMD) - - - - -
Bone Formation Marker - Osteocalcin	Decrease * in experimental group	-	-	-	No change	No change	-	Decrease* in experimental group

* p<0.001
** p<0.01
*** p<0.05

New Zealand RDI (not country of study)

form of calcium. The short study duration of 12 months may also not have been sufficient time to establish if a greater difference between the experimental and control groups would exist. In may also be that the precision and accuracy of the DEXA technology used in Bonjour's study was superior to the technology used in the preceding studies and they may have overestimated the true percentage difference between the groups. Follow-up studies of the intervention studies conducted by Bonjour et al (3), Johnston et al (2) and Lee et al (135) support the use of a food source of calcium in preference to a calcium supplement. Skeletal benefits obtained by those subjects in the Johnston et al (2) and Lee et al (135) experimental groups were not maintained 18 months to two years after supplementation ended. The control groups achieved greater bone mineral accretion in the follow-up period compared with the experimental groups. A one-year follow-up of the study by Bonjour et al (2), which used a food source as calcium, reported that most of the absolute differences in bone mass were still detectable, and therefore had been maintained. It is possible that the follow-up period of the Bonjour et al (2) study was too soon after the intervention had ceased, and that similar results to the other follow-up studies may have been found with a longer follow-up period. However, a further follow-up study by Bonjour et al (136) has shown the effects of the supplementation still exist 3.5 years after finishing the supplementation. These results have been challenged (137) as the gains in bone variables have not been adjusted for gains in height or muscularity, and more girls in the supplemented group were further through puberty. Both of these factors are major confounders and may overestimate the true calcium effect.

A previous study by Merrilees et al (10) found limited remaining effects 12 months after a 2 year supplementation with a dairy food product in teenage girls. The benefit of milk, therefore is supplying other nutrients to assist in bone development rather than a way of getting a more permanent result in peak bone mass.

2.7 Methods Used in Studies

When conduction a nutrition intervention study it is important that the methodology used will allow the researcher to elicit the information required, and be the correct method to look at the research outcome. There can be large variations when looking at dietary assessment so any research tool needs to be validated. In addition when looking at bone density the differences can be so small that there need to be a low co-efficient of variance to ensure you are observing a true change in bone density. The various methods used in this type of nutrition intervention research, with both the advantages and disadvantages are outlined below.

2.7.1 Dietary Assessment

The study of diets in adults poses methodological problems relating to the accuracy of assessment. Self-reporting of intake by the commonly used methods is prone to errors, including those attributed to memory, estimation of portion size, under-eating, under-reporting, over-reporting and socially desirable responses (13,138). Dietary studies in children have an additional dimension of difficulty because children's cognitive ability to record or remember their diets, as well as their limited knowledge of food and food preparation, must be addressed. In addition, the age of the group

being studied influences the study methods used and who records the diet (138-139).

As shown in previous studies, dietary records, 24 hour recalls, and Food Frequency Questionnaires (FFQ) all have strengths and weaknesses (see table 2.6). The dietary record, considered the gold standard for dietary assessment, provides an accurate quantitative account of a person's diet during a specific period. However, due to the requirement for the person recording to be motivated and literate, studies using this method often have a bias towards enrolling educated participants (13).

Depending on the nutrient being assessed, the period of observation may vary from a few days to 2-3 weeks. For mineral nutrient estimate 14 days' recording has been proposed as an individuals validation standard, although this method cannot be defined as the 'gold standard' (13,140).

The 24-hour recall only examines a 24 hour period in a person's life, therefore, may not yield an accurate picture of their diet. It does, however, mean the person is not required to be literate or motivated as this method is often carried out in an interview situation. This method does provide population means for intakes of various nutrients (13).

The FFQ estimates a person's usual intake over a specified period. It can be self-administered, reducing the time and costs involved in administration. It does not retrieve unique details of an individual's diet unless specifically designed to do so. As a result, its principal advantage is in ranking the diet of an individual, not in

quantifying individual intake, it can also be used to look at group data in population and clinical trials. Validation studies (141-146) examining dietary calcium intake in 7-14 day food records compared with FFQ have found FFQ tend to overestimate calcium intake. However, they still show reasonable ability to correctly classify the majority of subjects into quartiles of intake, and they identify the subjects with very high and very low intakes.

Vuckovic et al (147) found through the use of focus groups that people record portion sizes on personal eating habits, experience, and their perception of where a food is used in a meal. Participants also disclosed that they simplified food intake to make recording easier. If researchers employ this method they should be aware the data collected may not realistically portray the variety or quantity of food usually eaten in the course of one day.

The methods used for children must therefore take into account the problems of the ability for the child to remember what they ate, how much they ate and when they ate, their limited knowledge about food types and how it was prepared. In addition often parents are responsible for recording the dietary data of children and they are often unaware of what their child consumes when they are not with them. The method best suited for children would therefore depend on the ability of the child to record what they ate or recall what they have had. Without parental help in older children this would most likely be the 24 hour recall or 3-4 day food record. Where the parent is able to assist most methods would provide information on the dietary intake of the child, in order to get the most accurate data an interview with the parent and child would provide the most information.

Biochemical markers of dietary intake are often used as an additional tool to validate dietary assessment methods due to the known errors of dietary assessment. Random measurement errors in urinary nitrogen and potassium are unlikely to be correlated with random errors of the dietary assessment methods. However, it is important to collect the 24 hour urine on a different day to the food diary, as any behaviour modification due to the recording process may also decrease urinary excretion. Studies have shown dietary biomarkers are more closely correlated with 7 day food records than FFQ's (148-149).

2.7.2 Measurements of Bone Accretion

Dual-Energy X-Ray Absorptiometry

The introduction of dual-energy x-ray absorptiometry (DEXA) has had a profound effect on studies of growth and on assessment of paediatric diseases. DEXA has allowed measurements of the regional bone and the total skeleton to be done with ease (122). Limited measurements of the central skeleton (spine and femur) are often done by paediatric researchers because of their importance for fractures in the elderly rather than because of demonstrated clinical value in children.

Several studies have validated the use of pencil beam DEXA in paediatric populations, and reference data are available for lumbar spine in healthy children (12). Reference data are not similar between machine manufacturers, however and differences in results obtained using paediatric versus adult software can be

Table 2.6: Commonly Use Methods for Collecting Dietary Data

Method	Description	Period of Food Intake	Advantages	Disadvantages
24h recall	Subjects describe foods consumed over the last 24hr or on a "typical day". Widely used in epidemiological research.	24 hours	Fast. Low subject burden. Interview can be structured around daily activities. Doesn't alter usual intake.	Relies on subjects honesty, memory and food knowledge. Requires trained interviewer. Day chosen may be "atypical"
Food Frequency Questionnaire	Subjects asked how often they eat foods from a number of groups on a standardized list.	From 24h period to open-ended (eg how often do you eat a certain food).	Self administered. Can be used to cross-check data obtained from other methods. Validated for ranking individual intake. Validated against 7 day weighed food record. Can be modified to target certain nutrients or populations.	Validity dependent on the food list and the quantification method.
Diet History	Open-ended interview concerning food use, food preparation, portion sizes, food likes/dislikes and a food checklist. Originally also incorporated 24h recall and food frequency techniques.	Open-ended or over a specified period.	Accounts for daily variation in food intake by investigating a typical day. Can target contrasts between seasons, week-days/weekends etc. Food models assist estimation of food serves.	Relies on responder's honesty, memory, food knowledge. Labour intensive and time consuming. Requires trained interviewer.
Written Food Record	Weighed/semiweighed (household measure). Considered the gold standard for dietary assessment.	One day Three days Seven days	More accurate quantification of foods	Relies on responder's honesty, memory, food knowledge. Time consuming for subjects Subjects often alter their diet to improve their intake or reduce the workload of recording. Requires checking by a trained person Needs standardized set of household measures. Relies on subject assessment of portion sizes.
Duplicate Portion	Subjects place exact duplicates of consumed food items into a container. The foods are then homogenized and analysed for nutrients. Subjects may also have kept food records as a back up	24h – open ended	Analysis is independent on food databases.	Relies on subject's honesty and memory. Large compliance burden for the subject. Food analysis expensive. Causes alteration in usual intake.

significant. Currently there is no consistent recommendation regarding the weight or age for which paediatric or adult software should be used in children.

Clinical use of paediatric DEXA software is problematic owing the lack of an adequate reference database for children. There has been a review of reference databases to determine whether different classification by each reference database affected the diagnosis of osteopenia (151). These investigators found inconsistent diagnostic classification of BMD results even among similar DEXA machines using similar software(12,151-152).

A difficulty in interpreting DEXA results in children stems from the fact that BMD is expressed as an areal density, or BMC per projected bone area (153). The calculation of BMD incorrectly assumes that BMC and bone area are directly proportional. This assumption is most untrue in the growing skeleton where the bone area is constantly changing and the BMC is not yet filled. A variety of mathematical methods have been suggested to adjust areal BMD by DEXA to obtain a volumetric BMD measurement. Direct measurement of true volumetric BMD is possible using quantitative computed tomography (QCT). However, high cost, limited accessibility, and relatively high radiation exposures have limited the use of QCT in paediatric populations. However the use of DEXA in healthy paediatric populations has led to significant increases in our understanding of the influence of dietary calcium on bone accretion in children (154).

A positive attribute of using DEXA is that it enables the researcher to precisely measure growth (155). Total body measurements are critical in evaluating growth

since both appositional and linear trends of bone growth can be observed; these can be determined along with changes in lean tissue and fat mass.

Bone Biomarkers

The other method for examining bone turnover and accretion in children is via biomarkers (156), they are currently used in clinical investigation, however, the use in everyday clinical practice is not advised. There are some practical problems associated with the measurement of urinary excretion of markers of bone resorption in children. It may be difficult to obtain a reliable 24 hour urinary collection and first morning volume (FMV) is often preferred. Excretion of markers in the 24 hour urine and in FMV are weakly correlated, which may result from poor compliance but may also reflect a real lack of correlation between variables (156). Table 2.7 below outlines the various bone biomarkers and their use in children.

Table 2.7: Bone Biomarkers and Their Use in Children (156)

Bone Formation Markers	
Bone Alkaline Phosphatase (BAP)	Increases during puberty to mid puberty then decreases in late puberty. During puberty BAP is approximately 10x higher than adult values
Osteocalcin (OC)	Circulating concentrations of osteocalcin vary with age and pubertal stage. Circadian rhythm in children, the highest level is in the morning. Correlates with height and height velocity in pubertal children. Concentration 10-20x higher in children compared with healthy pre-menopausal women.
Procollagen I carboxy-terminal propeptide (PICP)	Not specific for bone, important contribution comes from soft tissue. In healthy children, PICP correlates with previous and subsequent growth velocity. PICP is also positively correlated with bone mineral accrual.
Bone Resorption Markers	
Hydoxyproline (HPro)	During periods of rapid growth and bone modeling (infancy and puberty) urinary HPro levels exceeed adult values 20- to 30-fold. Not specific for bone. HPro excretion influenced by dietary protein intake
Collagen pyridinium crosslinks (pyridinoline (Pyr) and deoxypyridinoline (DPyr))	Pyr higher than DPyr in bone, however DPyr more specific for bone because Pyr also found in cartilage. Range of crosslink excretion in children large, especially during puberty. Age-related variation in urinary Pyr and DPyr (levels appear to decrease once start puberty). Circadian rhythm in children, highest levels in the morning. Levels correlate with growth velocity.
Plasma Tartrate-Resistant Acid Phosphatase (TRAP)	May be a convenient marker of bone resorption in children because in can be measured in the blood. Responds to plasma isoenzyme 5 and not bone specific. TRAP is not specific for osteoclasts, is unstable when frozen, has enzyme inhibitors in serum and plasma TRAP chelates with calcium. New method of analysis suggest levels may be 2x higher in children than pre-menopausal women, but also increased in post-menopausal women, therefore the role in the assessment of skeletal function is unclear.
Hydroxylysine and glycosides (Hyl)	Marker of collagen degradation during bone resorption. In adults Hyl increases with ageing and is negatively correlated with BMD. In children Hyl is higher than adults, but his depends on their age and pubertal stage. Levels correlate with growth velocity.

It therefore seems if a bone biomarker is used in clinical investigation a serum marker may be easier to collect and they have a smaller coefficient of variation and hence more precision. It is also important to control for the stage of puberty,

especially as females enter puberty before males, as puberty correlates with most of the bone biomarkers.

2.7.3 Measures of Acceptability of Food Products

When developing products for children in a clinical trial it is important the acceptability of the product in measured at regular intervals. This is most easily done with sensory testing using a product blinded to the children. Sensory testing in children involves special problems not encountered with consumer panelists of an older age. Some of these problems include 1) verbal skills (157), 2) short attention span (158), and 3) difficulty in comprehension of standard sensory tests by children (159).

The use of facial hedonic scales are popular in determining preferences. They are used primarily for children and people with limited reading or comprehension skills. Stone and Sidel (160) cautioned that young children may not have the cognitive skills to infer that face scales are supposed to indicate their internal response to the test product. Kroll (161) found children eight and older were able to use up to 9-point scales with verbal descriptors and were able to self-administer the questionnaire. Kimmel et al (162) found a 7-point hedonic scale anchored with descriptors was reliable and could be used consistently in children as young as 4 years.

2.7.4 Research Design

Intervention trials are the strongest study design to establish a cause and effect relationship. There are four features of these trials:

1. Prospective subjects are informed about the study and asked to consent.
2. Consented subjects are randomly assigned to either group.
3. One group receives the treatment protocol and the other group receives the placebo or control protocol.
4. Subjects are monitored at predetermined times and the results from the groups are compared to each other.

The degree in which a study group is representing a reference population determines whether the results can be generalized. A random method of treatment assignment is essential. The random method eliminates the selection bias that can occur if the subject or the investigator selects the treatment. It also mitigates non-intentional bias, or the chance formation. These may not be comparable because of differences in factors that affect the response to treatment, such as age or gender. The random method does not guarantee comparable groups, however, and a chance imbalance between groups is possible, especially if the sample size is small.

2.8 Summary

From this review of the literature it can be seen the period of most rapid skeletal growth starts when a child is pre-pubertal and continues through puberty. It therefore is important that lifestyle and nutrition interventions be carried out at this stage. Calcium continues to be the nutrient that is most researched in relation to bone, however other nutrients have also been shown to have an effect. Most

previous intervention studies in the pre-pubertal age group have studied females only or males and females but have not included a follow-up period to see if the effect persists. There does appear to be a threshold for calcium intake at which calcium has no further effect however both in New Zealand and overseas the habitual intake is well below this.

The best method for assessing the effect of calcium on bone health is DEXA in this age group, however it is important that the methods of assessing dietary calcium intake and compliance of the intervention are also accurate if the intervention is to show a significant difference

3. Aims of the Study

This study investigated the effect of a calcium enriched milk drink on bone density, bone mineral content and bone size in both male and female 8-10 year old pre-pubertal children.

This age group was chosen because previous studies have shown this is a period of most rapid skeletal development (11). Supplementation with both milk calcium and calcium salts have been shown to increase bone mineral density in this age group (2-9). There is also limited data on male pre-pubertal children and with the increase in osteoporosis in both males and females it is important to consider the effect nutrition may play on their bone development also.

The secondary aim was to determine whether a daily intake of a milk drink had an adverse effect on the body composition of these children.

Finally, we wanted to determine whether this milk product is acceptable to New Zealand children of this age.

4. Methodology

4.1 Subjects

Three local primary schools in Christchurch New Zealand, located close to the research centre were approached and asked to participate in the study. All three schools were from higher socio-economic areas and classified as decile 10 schools. Three hundred and ninety children, aged 8-10 years, who were pupils at the schools, were asked to attend an information evening with their parents. At the information evening the study was outlined, an information sheet was provided (see appendix 1) and the researchers were available to discuss any questions. There was the opportunity to consent at the end of the information evening or it was done at a later date at an arranged time with the principal researcher. Of the 211 who attended 159 met the inclusion criteria and volunteered for the study. The parents of the children were required sign the informed consent form (see appendix 2) before the child was asked to sign their copy of the informed consent form (see appendix 3). The exclusion criteria were an allergy to dairy products, any major disease states including significant psychological problems. If the child was on any medication that influences bone growth or metabolism, that is, steroids (inhaled or oral), anti-convulsants, thiazide diuretics or vitamin D they were also excluded.

4.2 Randomisation

The baseline bone density measures included a heel ultrasound (Lunar Achilles Ultrasound, Lunar Radiation Corp., Madison, Wisconsin) for the purpose of stratification for randomisation into the treatment or control group.

The children were ranked on the ultrasound stiffness value[4], this ensured there were an even spread in bone density measures in each group. In cases were there was more than one child from the same family, they were both allocated to the same group. Each school was ranked separately as they had different start dates. Randomisation occurred after the children had had their baseline bone density and anthropometric measures. Of the 159 randomised 5 children (2 controls and 3 treatment) did not complete the baseline questionnaires after randomisation and were excluded from the study.

The children were randomised to either receive a high calcium chocolate milk drink or a placebo chocolate milk drink. The high calcium milk drink provided 600mg of calcium per 40g serve or 1200mg additional per day. The control milk drink provided 200mg of calcium per 40g serve or 400mg additional per day, this is equivalent to two glasses of milk. (see appendix 4).

Table 4.1: Nutritional composition of 2 sachets (80g) of high calcium supplement compared to 2 sachets (80g) of the placebo supplement.

	Treatment	Placebo
Energy	1529 kJ 364 kcal	1650 kJ 393 kcal
Protein	10.2 g	10.6 g
Carbohydrate	34.7 g	34.7 g
Fat	21.4 g	24.5 g
Vitamin A	656 µg*	656 µg*
Vitamin D	336 IU	320 IU
Vitamin C	52 mg	58 mg
Calcium	**1200 mg**	**400 mg**
Phosphorus	776 mg	320 mg

**= µg retinol equivalents*

[4] Lunar Corporation has developed a measure that combines the broadband attenuation of the ultrasound wave and the speed of sound of the ultrasound wave and called it stiffness.

4.3 Funding and Ethics Approval

The study was funded by New Zealand Milk, a division of the New Zealand Dairy Board.

The study was approved by the Southern Regional Health Authority Ethics Committee, (Canterbury) New Zealand (1997) (see appendix 5).

4.4 Protocol

All the children (and their parents) attended the research centre for measurements or bone mineral density, height and weight measurements, with the research technician and study co-ordinator. The first was at baseline and then every 6 months for the 18 months of supplementation. The supplementation period was chosen as previous studies (2-9) had found significant differences in bone density over a 12-18 month period. The children were followed up at 30 months, 12 months after they had completed the supplementation. This time frame was chosen as Bonjour et al (3) had found the difference in bone density persisted for the following year when a food-based supplement was used. Data was recorded on the visit sheet and stored in the child's notes (see appendix 6).

Bone density was measured by the study technician, using dual-energy x-ray absorptiometry (DPX-IQ; Lunar Radiation Corp., Madison, Wisconsin), at baseline, 6 months, 12 months, 18 months and 30 months; height and weight was also recorded at these times. In addition at these time points the children completed a calcium food frequency questionnaire (see appendix 7) to determine habitual dietary calcium intake. This questionnaire had been

previously validated in older adults (142) and young children (141). A medical questionnaire was completed at baseline and 30 months to check medication use, medical history, previous fractures, family history and caffeine intake; for the females menarche history was also detailed (see appendix 8). Pubertal stage was assessed by the previously validated self-administered Tanner questionnaire (see appendix 9)(163-164).

4.5 Dietary Compliance and Acceptability

The children had sachets of the product delivered to them either fortnightly at school or monthly at home over the 18 months of the supplementation. Each child was required to have two sachets of the product per day mixed with hot or cold water as a drink.

The children completing a tick sheet after they had consumed the drink, and the study coordinator collected them, with the used sachets, at the end of each month to measure compliance. It was advised either a parent or teacher supervise the consumption of the milk, however this did not always happen.

The study product was assessed for acceptability with the children prior to starting the study and every six months while they were on the supplement. A 5 point pictorial hedonic scale with descriptors, using words developed by the study children, was used (see appendix 10).

The study product was initially chocolate flavoured, however other flavours were introduced to maintain compliance.

4.6 Procedures

Bone mineral density, BMC and body composition was determined by dual-energy x-ray absorptiometry. The total body, lumbar spine and total hip were measured for bone mineral density and bone mineral content; lean muscle mass and fat mass were also measured from the total body scan. The bone size was measured by examining the bone height, width and area in the lumbar spine. The research technician followed the quality assurance document developed for the study (see appendix 11). The scans were analysed using the Lunar database (version 4.7a), on high resolution medium 3000mA and total body depending on body thickness. The coefficient of variation for repeated scans of the lumbar spine is 1%, the femoral neck 2.5% and 0.5% for the total body. The coefficient of variation for repeated scans of lean muscle mass is 1.1% and 1.9% for total fat mass.

Height was measured using a calibrated wall mounted stadiometer (Harpendin) (see appendix 11 pg 16), the children were measured 3 times to within 4mm and the average obtained. Weight was measured on electronic scales (Salter), to within 500g, the scales were calibrated twice a week with a known 5kg weight. All measurements were undertaken by the same trained assessor, who was blinded to the treatment each child was receiving. All procedures where carried out in accordance with the standard protocol (see appendix 11)

4.7 Measurement of Nutrient Intake

Dietary calcium intake was calculated from a modified calcium food frequency questionnaire using data from the 5th edition of the New Zealand food composition tables (Crop and Food Research, Palmerston North, New Zealand)(165). Serving sizes were based on the serving sizes provided in the food composition tables.

At baseline the children were asked to complete a 3-day weighed food record, however this was optional. The main reason for making it optional was respondent burden at baseline. A proportion of the children chose to complete the 3-day weighed food record (n=91) for the purpose of validating the FFQ. The children and their parents were asked to record everything the children ate and drank for three days prior to starting the supplementation. Whenever possible food was to be weighed, or compared to the photographs in the back of the food diary (see appendix 12). The study co-ordinator collected these in from the families at the baseline visit and clarified any foods that didn't have weights or sizes recorded. The three-day food diaries were analysed and the dietary calcium intakes used to validate the baseline calcium intakes from the food frequency questionnaire.

4.8 Statistical Analysis

Comparisons between the two groups at baseline were made using independent *t* tests. ANOVA for repeated measures was used to compare the changes over time between the two groups. When ANOVA indicated a significant interaction between time and treatment group, comparisons

between groups at individual times were made using Fisher's least Significant Difference test. Analyses were performed on an intention to treat basis. For the validation of the food frequency questionnaire Pearson's correlation was used in addition to a cross-classification of both methods of dietary analysis. The statistical package used was Statistical Package for Social Sciences (SPSS) 10.0.1 for Windows (1999). Statistical advise and assistance was sought from Dr Chris Frampton (Christchurch School of Medicine).

5. Results

5.1 Physical Characteristics and Demographic Data

The children in the study were all residents in Christchurch, New Zealand. The schools whose children participated in the study were all from higher socio-economic suburbs in the city, as evident in Table 5.1. The socioeconomic indicators are from Statistics New Zealand, 2001 census data (166).

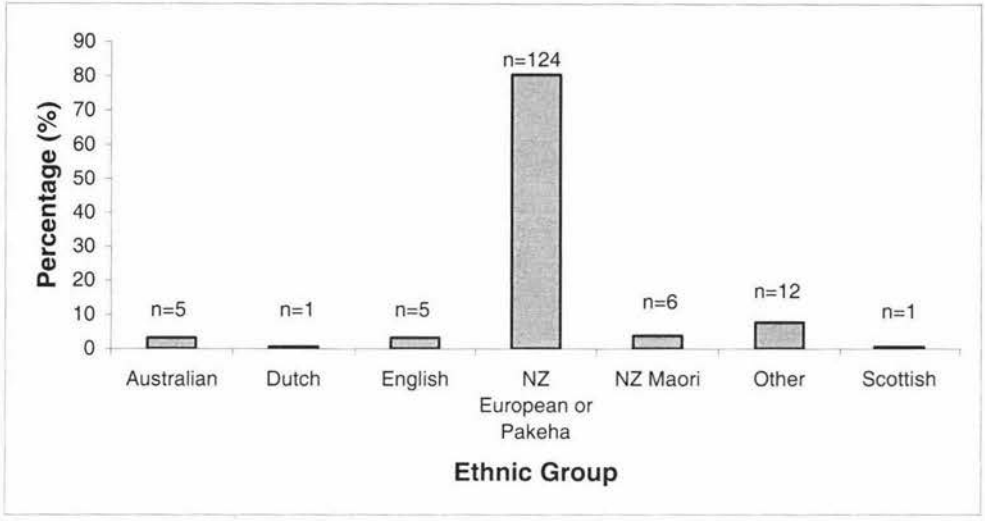
Table 5.1: Socioeconomic indicators for the suburbs where the children and participating schools resided in Christchurch, compared to the National and Christchurch values.

	NZ	Christchurch	Redcliffs	St Martins	Cashmere
% less than 15 years old	22.7	19.3	16.4	19.8	19.9
% Maori in the population	14.7	7.1	3.2	4.5	2.1
% of households earning more than $30,000pa	30.7	28.1	42.4	34.0	42.3
Decile[5] rating of the school			10	10	10

Of the children randomised into the study 51% were female and 49% were male. There was a mixture of ethnic identities represented in the study population, however the majority of participants identified themselves as NZ European or Pakeha (n=124). Other nationalities were Australian, Dutch, English, NZ Maori, Scottish and Other (see Figure 5.1).

[5] Decile rating based on the socio-economic rating of the school as determined by the Education Review Office, Ministry of Education.

Figure 5.1: Ethnic nationalities of the children participating in the study.



The groups were very well matched at baseline. The baseline characteristics of the two groups are shown in Table 5.2.

Table 5.2: Baseline characteristics of both groups, mean values (± SEM)

	Treatment (n=74)	Control (n=80)	p-value
Age (years)	9.4 (0.1)	9.4 (0.1)	0.952
Height (cm)	135.1 (0.8)	135.4 (0.8)	0.767
Weight (kg)	31.5(0.7)	32.4 (0.9)	0.399
Ratio males to females	36:38	39:41	
Dietary Calcium Intake (mg)	934 (44)	985 (53)	0.461
Total Body BMD (g/cm^2)	0.881 (0.006)	0.881 (0.007)	0.951
Total Body BMC (g)	1152 (23)	1172 (29)	0.603
L1-L4 Spine BMD (g/cm^2)	0.742 (0.009)	0.730 (0.009)	0.329
L1-L4 Spine BMC (g)	23 (1)	23 (1)	0.800
Total Hip BMD (g/cm^2)	0.813 (0.014)	0.792 (0.015)	0.305
Total Hip BMC (g)	18 (0.4)	17 (0.5)	0.727
Total Fat Mass (kg)	5.8 (0.4)	6.3 (0.5)	0.428
Total Lean Mass (kg)	23.8 (0.3)	24.3 (0.4)	0.357
Tanner 1 (breast/genital)#	1 (1-2)	1 (1-2)	
Tanner 2 (pubic hair)#	1 (1-2)	1 (1-2)	

 # = median and range

5.2 Medical History

5.2.1 Family History of Osteoporosis

Sixty-six children (42.8%) reported having a maternal or paternal grandmother that had lost height or had a rounded/stooped back. There were 90 children (58.4%) who reported that their mother and/or a paternal or maternal grandmother had had a fracture.

5.2.2 Previous Facture

Twenty eight children had had a fracture before they started the study and another 14 children experienced a fracture during the study. The fracture rate was evenly matched between each group at baseline ($n_{treatment}$ = 13, $n_{control}$ = 15) and at follow-up ($n_{treatment}$ = 6, $n_{control}$ = 8). All the fractures experienced by the children were classified as traumatic. The definition of traumatic was given when the fracture occurred as the result of a fall higher than waist height or when it occurred as a result of contact with another object (other than the ground). Fractures ranged from broken fingers and toes to a fracture of the femur or forearm.

5.2.3 Medications

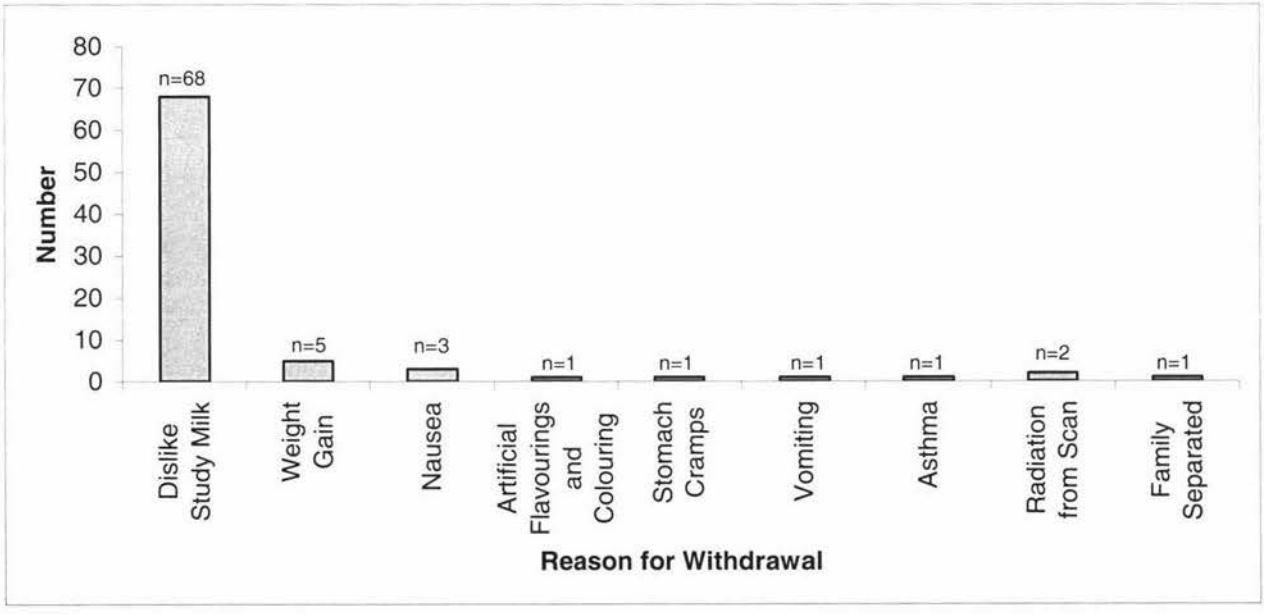
Eight children had previously taken prednisone, (an oral steroids), or continued to take them during the study as often as required (PRN) medication. In addition another 10 children were using inhaled asthma medication, of the 18 children taking asthma medication 8 were in the placebo group and 10 were in the

treatment group. No-one was taking continuous asthma medication on randomisation into the study. Four children were taking fluoride at baseline but stopped prior to starting the supplement and one child was taking calcium supplements and he also discontinued this before starting the supplement.

5.3 Retention of Subjects

The withdrawal rate was high in this study, 83 (53.9%) withdrew from taking the supplement. The main reason for withdrawal was a dislike of the study supplement (n=68), the other reasons are outlined in Figure 5.2 below.

Figure 5.2: Reasons for withdrawal from taking the milk supplement.



Of the children who withdrew 58 (66.3%) continued to be monitored at each data collection point. The data points for the children who did not finish the supplement, but were continued to be monitored, are included in the analysis, as

an intention to treat analysis. It was not decided to follow-up the children who stopped taking the supplement until after the 6 month data had been collected in two of the schools and 12 month data had been collected in the other school (they started earlier). Both groups were evenly matched in terms of withdrawals from the study ($n_{control} = 39$ $n_{treatment} = 45$).

Table 5.3 shows the change in subject numbers from baseline in each group. The change in subject numbers is due to the high withdrawal rate early in the study, followed by the decision to continue monitoring the children in an intention to treat analysis. At 6 months there were slightly more children who had withdrawn from the treatment group than the control group. However, once the children where asked to continue being monitored the numbers were matched between the two groups.

Table 5.3: Difference in numbers per group from baseline.

	Baseline	6 months	Diff	12 months	Diff	18 months	Diff	30 months	Diff
Treatment	74	45	29	56	18	60	14	58	16
Control	80	58	22	60	20	65	15	65	15

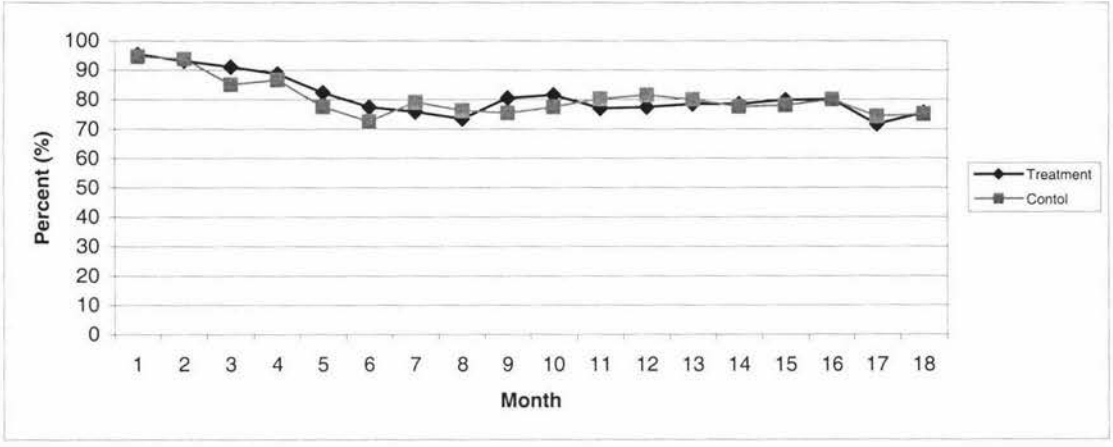
Four children self reported having a dairy allergy at baseline and still had it at the completion of the study. This was not reported to us until after the consent and randomisation had occurred, all four children wished to participate knowing the supplement was dairy based. Three of these children withdrew from the study and the other child had the supplement for the 18 month period. There were also

2 children who reported having a dairy allergy at 30 month follow-up who did not report having one at baseline.

5.4 Compliance

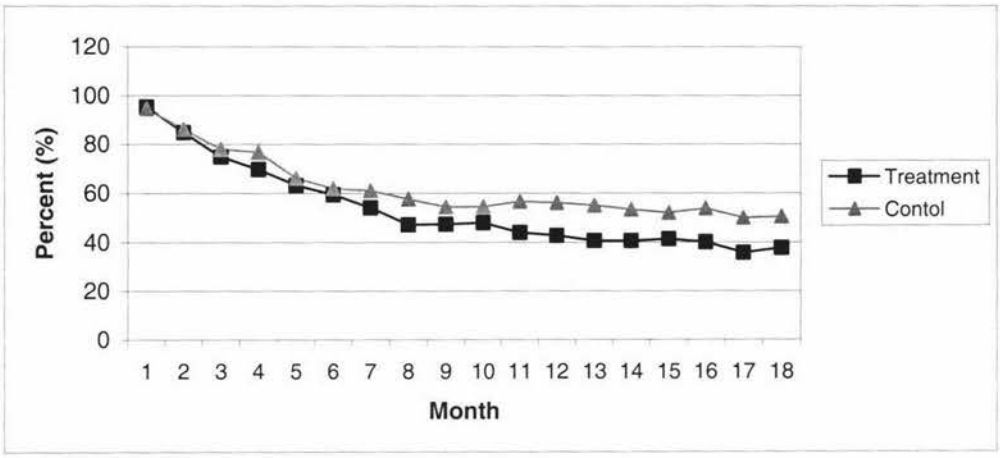
Figure 5.3 shows the mean dietary supplement compliance for those who completed the supplementation was 80.6%, and there was no difference between the groups.

Figure 5.3: Mean percentage compliance of the supplement for the children who completed the supplementation period.



When the children who stopped from taking the supplement are included the compliance rate drops to around 45%, see figure 5.4.

Figure 5.4: Mean percentage compliance for the milk supplement if the children who withdrew are included.



5.5 Acceptability

The majority of the children thought the milk was really cool or quite yummy and most of them felt they could drink one or more glasses at one time.

Figure 5.5: What the children thought of the milk supplement throughout the study.

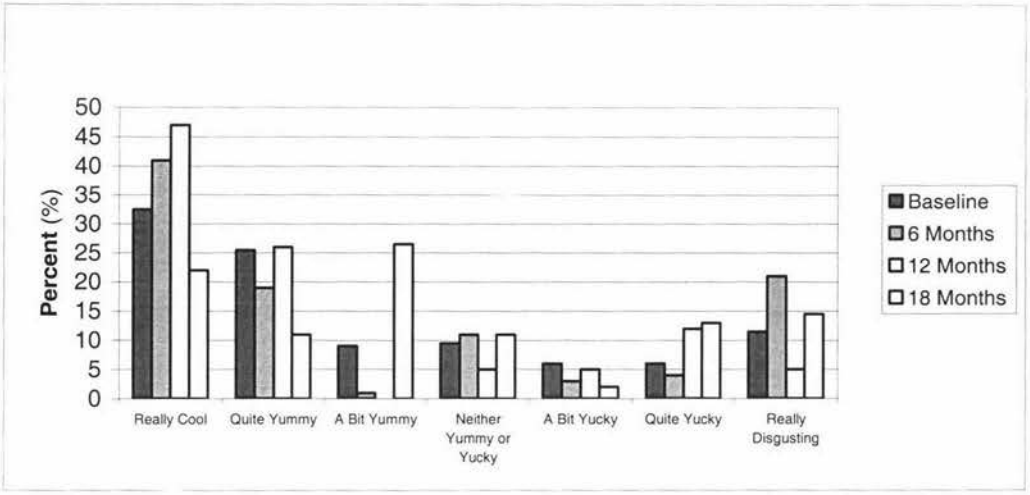
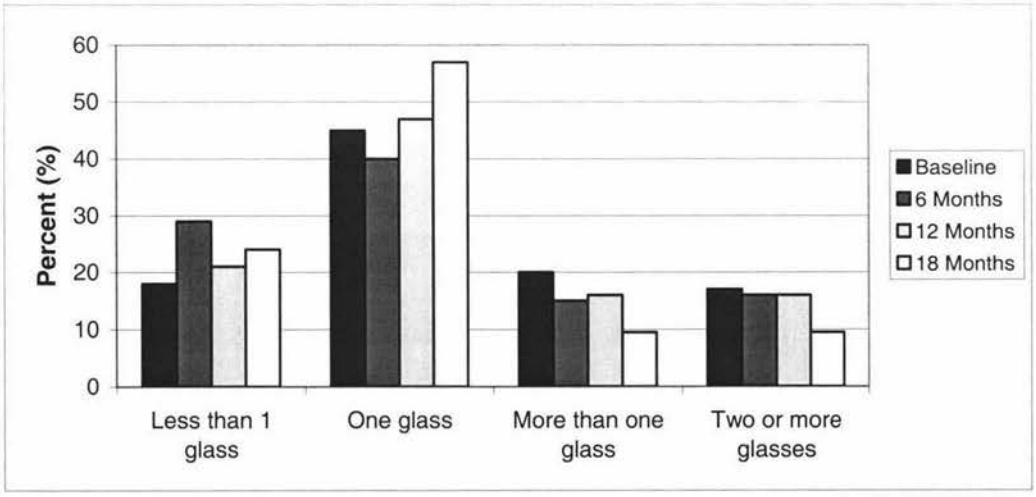


Figure 5.6: How many glasses of the milk supplement the children thought they could drink at one time during the supplementation period.



5.6 Anthropometric Indicators

No significant differences were seen in anthropometric values for either group, see table 5.4. Throughout the study the mean value for both the males and females in both groups remained between the 50th and 75th percentile for both height and weight (NCHS growth curves for children) (167).

Table 5.4: Height, Weight, Total Body Lean Mass and Fat Mass for the Treatment and Control group at baseline, 6, 12, 18 and 30 months (SEM)

	Baseline	6 Months	12 Months	18 Months	30 Months
	(n_{txt}=74, n_{con}=80)	(n_{txt}=45, n_{con}=58)	(n_{txt}=56, n_{con}=60)	(n_{txt}=60, n_{con}=65)	(n_{txt}=58, n_{con}=65)
Height (cm)					
Treatment	135.1 (0.8)	138.4(1.2)	141.6 (1.0)	144.3 (1.0)	151.0 (1.0)
Control	135.4 (0.8)	138.9 (1.0)	142.8 (1.0)	145.3 (1.0)	151.8 (1.1)
Weight (kg)					
Treatment	31.5 (0.7)	33.5 (1.1)	36.3 (1.0)	38.4 (1.0)	42.9 (1.2)
Control	32.4 (0.9)	33.8 (1.0)	37.4 (1.2)	39.0 (1.2)	44.0 (1.4)
Lean Mass (kg)					
Treatment	23.8 (0.3)	25.0 (0.5)	26.5 (0.5)	27.8 (0.5)	31.0 (0.6)
Control	24.3 (0.4)	25.3 (0.5)	26.9 (0.5)	28.1 (0.6)	31.2 (0.7)
Fat Mass (kg)					
Treatment	5.8 (0.4)	6.8 (0.6)	7.9 (0.7)	8.4 (0.7)	9.5 (0.7)
Control	6.3 (0.5)	6.8 (0.6)	8.3 (0.8)	8.6 (0.8)	10.3 (0.9)

Txt = treatment group
Con = control group

There was no difference in the change in pubertal stage for the children between groups. Fifteen girls started menstruating during the study duration.

Figure 5.7 shows the stage of pubertal development for the control and treatment groups at baseline and 30 months.

Figure 5.7: Stage of pubertal development at baseline and 30 months.

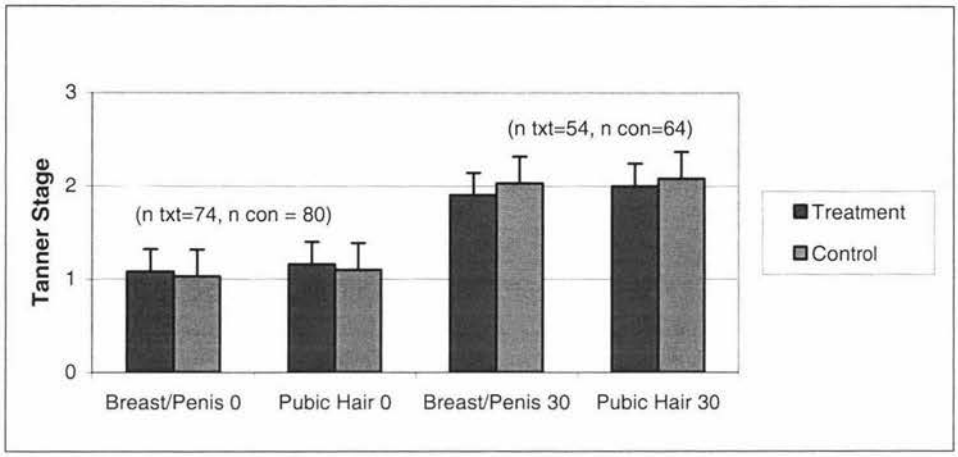


Figure 5.8: NCHS Growth Curves for Weights for Boys aged 2-18 years showing mean weight during the study.

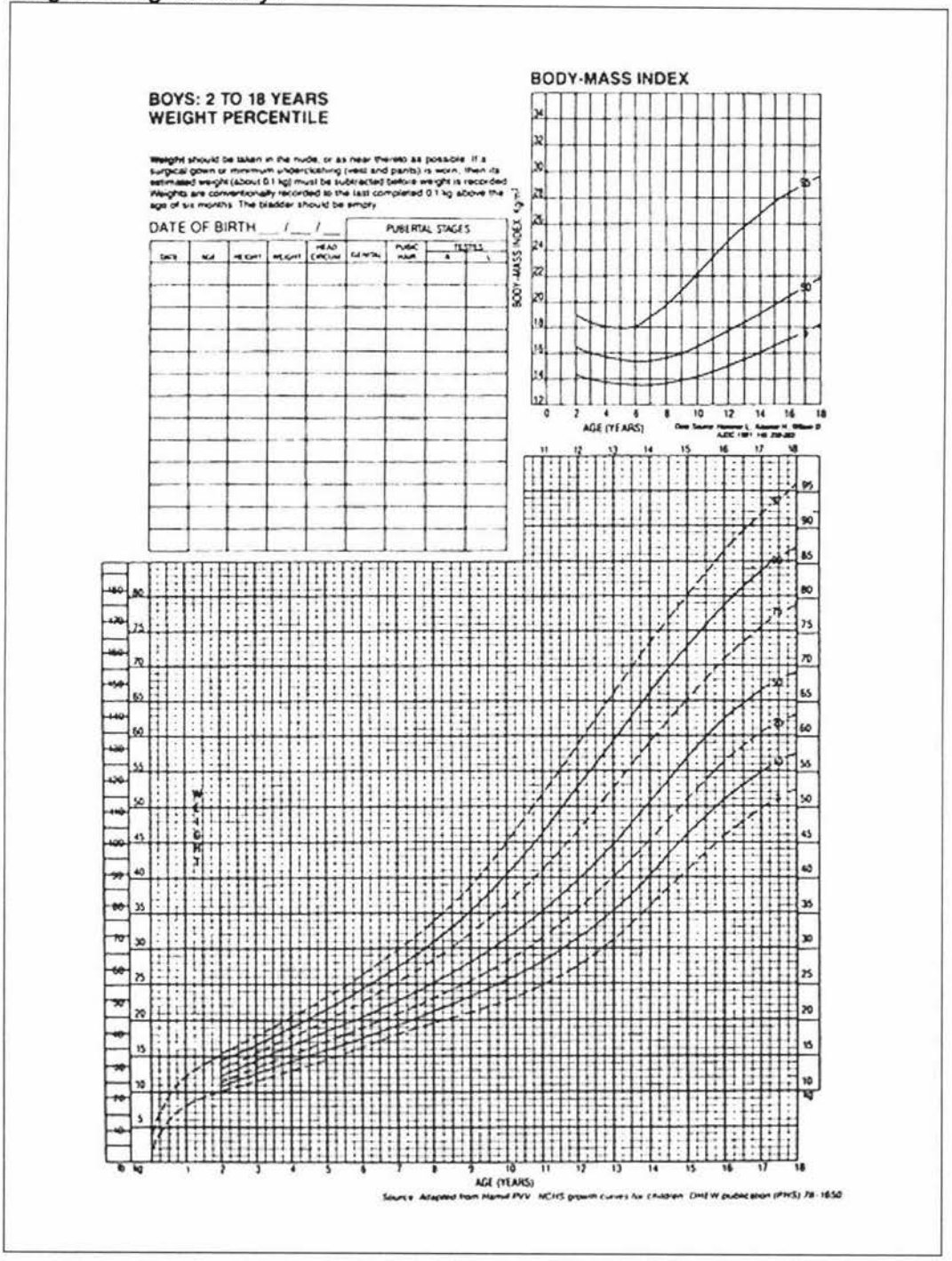


Figure 5.9: NCHS Growth Curves for Heights for Boys aged 2-18 years showing mean height during the study.

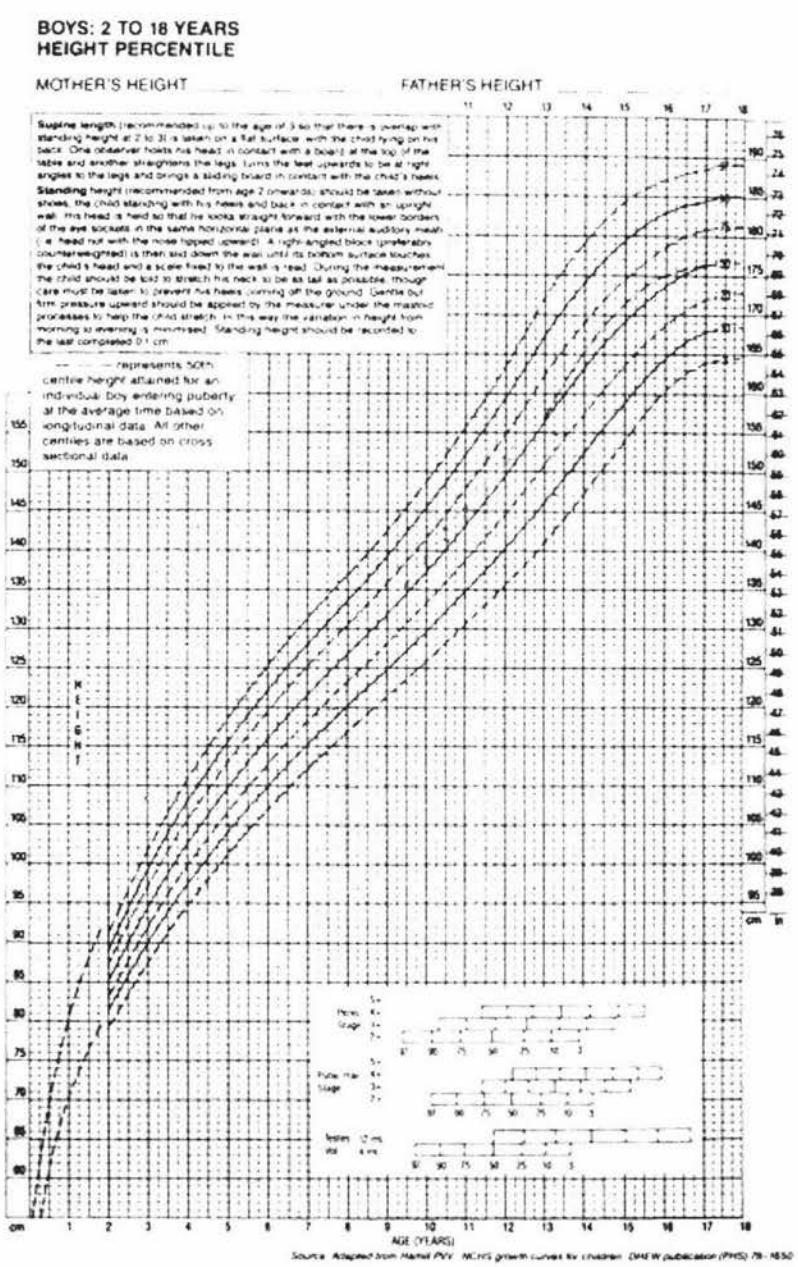


Figure 5.10: NCHS Growth Curves for Weights for Girls aged 2-18 years showing mean weight during the study.

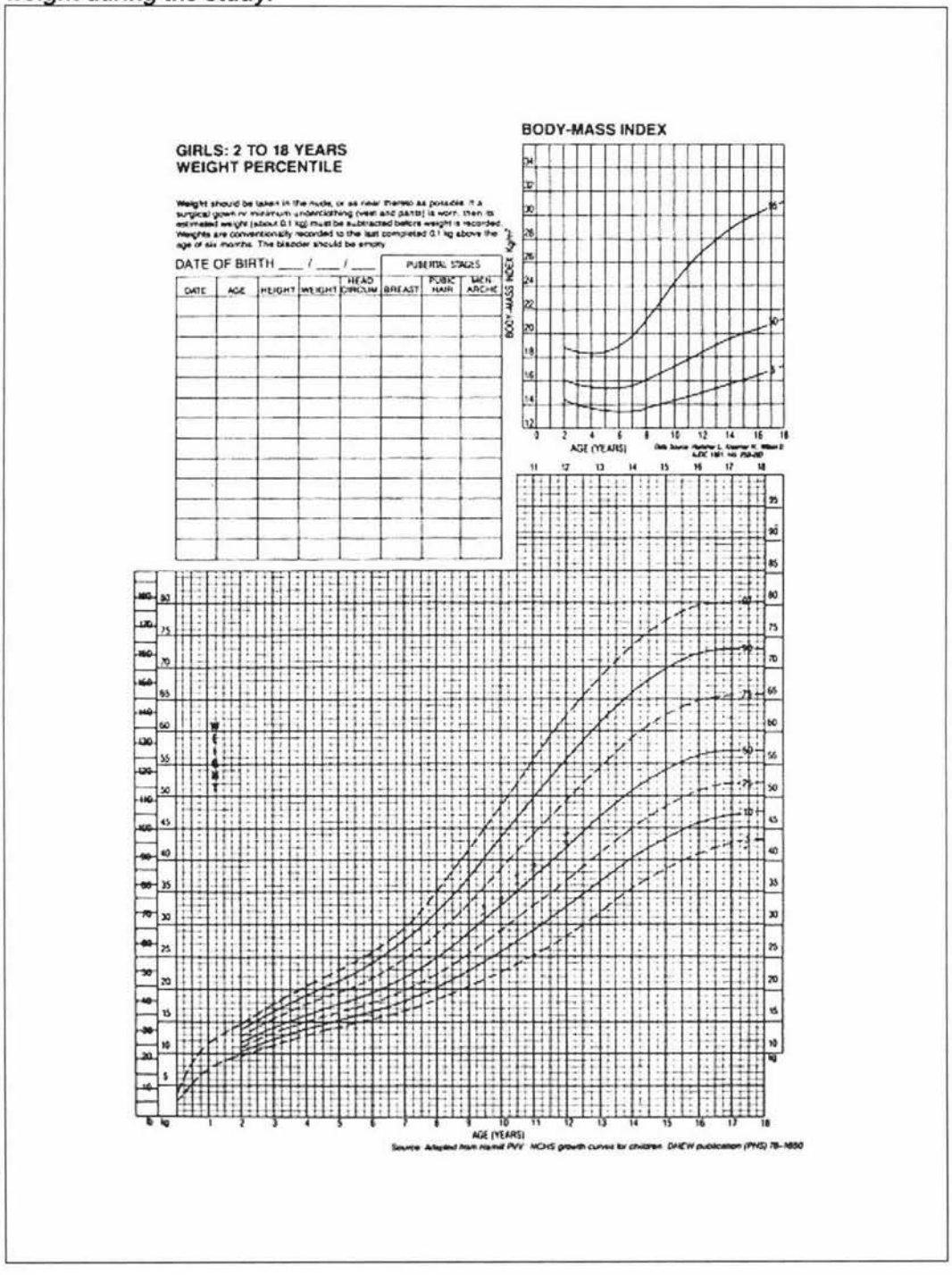
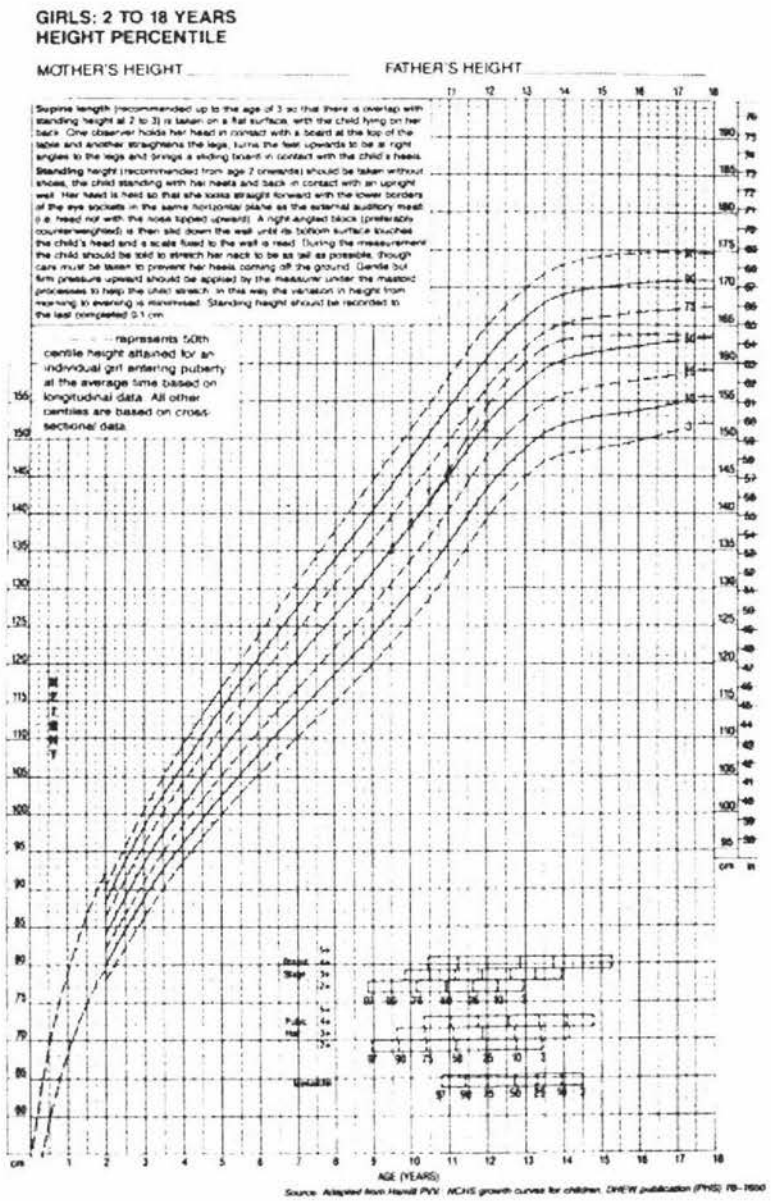


Figure 5.11: NCHS Growth Curves for Heights for Girls aged 2-18 years showing mean height during the study.



5.7 Bone Mineral Density

There were no significant differences in the bone mineral density between the groups, although there were trends in a higher BMD at the total hip, and trochanter in the supplemented group, refer to table 5.5.

Table 5.5: Percentage Change from Baseline for the Total Body, Lumbar Spine, Total Hip, Trochanter and Femoral Neck Bone Mineral Density for the Treatment and Control groups at 6, 12, 18 and 30 months (±SEM).

	6 Months	12 Months	18 Months	30 Months	p-value (0-30)
	(n_{txt}=45, n_{con}=58)	(n_{txt}=56, n_{con}=60)	(n_{txt}=60, n_{con}=65)	(n_{txt}=58, n_{con}=65)	
Total Body (%)					
Treatment	1.6(0.9)	4.4(0.9)	5.1(0.9)	9.4(1.0)	0.737
Control	1.6(0.9)	3.3(1.0)	4.3(1.0)	8.9(1.1)	
L1-L4 Spine (%)					
Treatment	2.3(1.6)	5.9(1.5)	8.4(1.5)	16.3(1.9)	0.616
Control	4.1(1.5)	5.8(1.5)	8.6(1.5)	16.8(2.1)	
Total Hip (%)					
Treatment	1.0(2.1)	4.8(1.7)	6.8(1.6)	14.0(1.9)	0.081
Control	2.4(2.0)	3.9(1.8)	5.4(1.6)	12.4(2.0)	
Trochanter (%)					
Treatment	0.9(2.1)	6.2(1.9)	8.6(1.9)	15.8(2.2)	0.088
Control	2.8(2.2)	5.1(2.0)	7.5(2.0)	14.9(2.2)	
Femoral Neck (%)					
Treatment	0.7(2.1)	6.7(1.7)	8.9(1.9)	15.4(1.9)	0.447
Control	3.4(1.9)	7.0(1.8)	8.2(1.7)	15.3(1.7)	

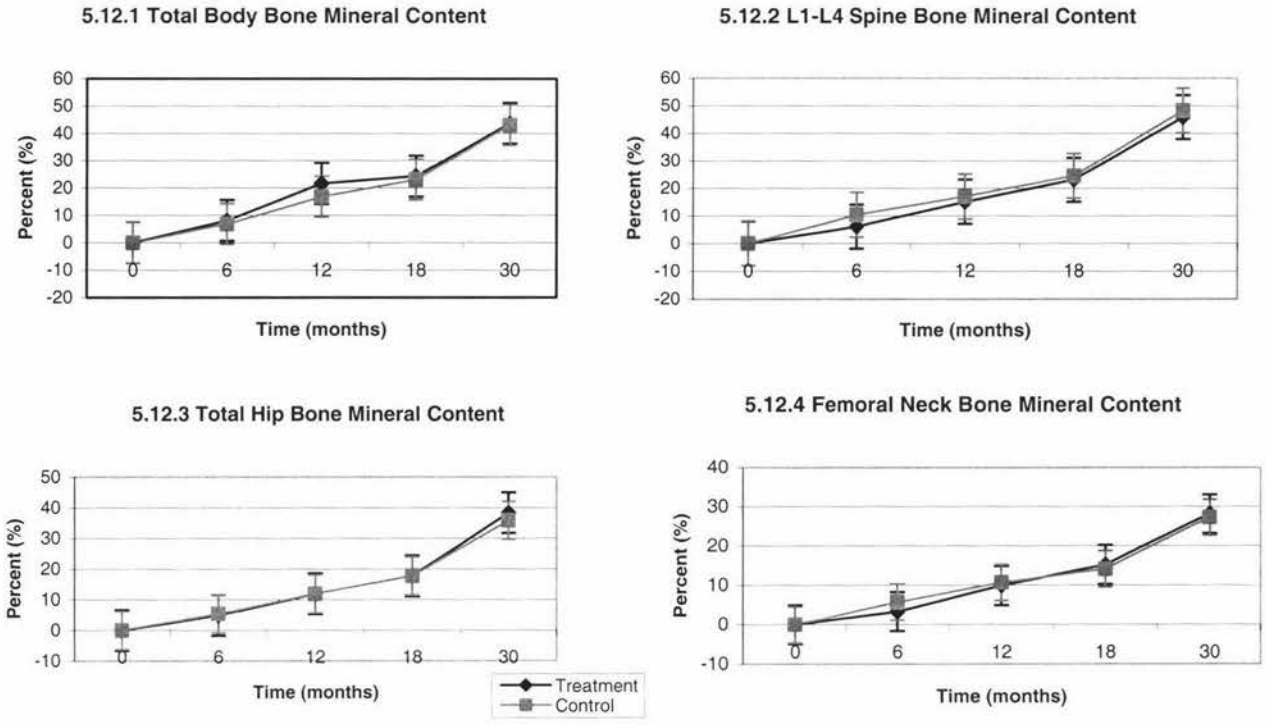
Table 5.6 shows the absolute values for BMD at the total body, L1-L4 spine, the total hip, trochanter and femoral neck.

Table 5.6: Bone Mineral Density Values (g/cm^2) for the Total Body, Lumbar Spine, Total Hip, Trochanter and Femoral Neck for the Treatment and Control groups at baseline, 6, 12, 18 and 30 months (±SEM).

	Baseline	6 Months	12 Months	18 Months	30 months
	(n_{txt}=74, n_{con}=80)	(n_{txt}=45, n_{con}=58)	(n_{txt}=56, n_{con}=60)	(n_{txt}=60, n_{con}=65)	(n_{txt}=58, n_{con}=65)
Total Body					
Treatment	0.881(0.006)	0.895(0.009)	0.920(0.008)	0.926(0.008)	0.964(0.009)
Control	0.881(0.007)	0.895(0.008)	0.908(0.009)	0.919(0.009)	0.959(0.010)
L1-L4 Spine					
Treatment	0.742(0.009)	0.759(0.012)	0.786(0.011)	0.804(0.011)	0.863(0.014)
Control	0.730(0.009)	0.760(0.011)	0.772(0.011)	0.793(0.011)	0.853(0.015)
Total Hip					
Treatment	0.813(0.014)	0.821(0.017)	0.851(0.014)	0.868(0.013)	0.927(0.015)
Control	0.792(0.015)	0.811(0.016)	0.823(0.014)	0.835(0.013)	0.890(0.015)
Trochanter					
Treatment	0.676(0.011)	0.682(0.014)	0.718(0.013)	0.734(0.013)	0.783(0.015)
Control	0.651(0.011)	0.669(0.014)	0.684(0.013)	0.700(0.013)	0.748(0.014)
Femoral Neck					
Treatment	0.805(0.013)	0.811(0.017)	0.859(0.014)	0.877(0.015)	0.926(0.015)
Control	0.784(0.014)	0.811(0.015)	0.839(0.014)	0.847(0.013)	0.904(0.013)

5.8 Bone Mineral Content

Figures 5.12: Percentage Change from Baseline for Total Body, L1-L4 Lumbar Spine, Total Hip and Femoral Neck Bone Mineral Content at 6, 12, 18 and 30 Months (I =SEM).



There was no difference in percentage change from baseline in bone mineral content between the groups.

Table 5.7 shows the absolute values for the bone mineral content during the study period. There was no difference between the groups for total body BMC as the total increase from 0 to 30 months is 503g in the treatment group and 504g in the control group (p=0.760).

Table 5.7: Bone Mineral Content Values (g) for the Total Body, Lumbar Spine, Total Hip, Trochanter and Femoral Neck for the Treatment and Control groups at baseline, 6, 12, 18 and 30 months (±SEM).

	Baseline	6 months	12 months	18 months	30 months
	(n_{txt}=74, n_{con}=80)	(n_{txt}=45, n_{con}=58)	(n_{txt}=56, n_{con}=60)	(n_{txt}=60, n_{con}=65)	(n_{txt}=58, n_{con}=65)
Total Body					
Treatment	1152(23)	1245(36)	1401(52)	1432(35)	1655(43)
Control	1172(29)	1252(34)	1369(42)	1441(43)	1676(53)
L1-L4 Spine					
Treatment	23.30(0.51)	24.73(0.77)	26.83(0.70)	28.69(0.75)	34.00(1.01)
Control	23.12(0.50)	25.25(0.64)	27.06(0.73)	28.80(0.82)	34.28(1.18)
Total Hip					
Treatment	17.58(0.43)	18.44(0.55)	19.68(0.47)	20.69(0.49)	24.32(0.56)
Control	17.34(0.50)	18.28(0.60)	19.40(0.56)	20.41(0.57)	23.54(0.68)
Trochanter					
Treatment	4.24(0.14)	4.60(0.16)	5.34(0.18)	5.84(0.18)	7.25(0.24)
Control	4.10(0.17)	4.72(0.24)	5.28(0.25)	5.84(0.27)	7.13(0.31)
Femoral Neck					
Treatment	3.03(0.05)	3.13(0.08)	3.33(0.08)	3.49(0.08)	3.88(0.09)
Control	2.98(0.06)	3.15(0.07)	3.30(0.08)	3.40(0.08)	3.79(0.09)

5.9 Bone Size

There was no significant difference in percentage change of the indicators of bone size between the groups, see table 5.8.

Table 5.8: Percentage Change from Baseline in Parameters of Bone Size; L1-L4 Lumbar Spine Width, Area, Height and Volumetric Density for 6, 12, 18 and 30 Months (±SEM).

	6 Months	12 Months	18 Months	30 Months	p-value (0-30)
	(n_{txt}=45, n_{con}=58)	(n_{txt}=56, n_{con}=60)	(n_{txt}=60, n_{con}=65)	(n_{txt}=58, n_{con}=65)	
L1-L4 Width (%)					
Treatment	2.3(1.4)	4.9(1.3)	7.2(1.2)	12.1(1.2)	0.676
Control	2.6(1.1)	5.5(1.2)	7.2(1.1)	12.1(1.3)	
L1-L4 Area (%)					
Treatment	2.9(2.4)	8.1(1.7)	13.7(1.8)	25.0(2.0)	0.511
Control	4.9(2.0)	10.4(1.9)	14.3(2.0)	25.8(2.4)	
L1-L4 Height (%)					
Treatment	1.9(0.8)	3.8(0.7)	6.2(0.7)	10.1(1.6)	0.293
Control	2.7(0.7)	5.0(0.8)	7.1(0.8)	12.1(1.1)	
L1-L4 Volumetric Density (%)					
Treatment	6.5(5.6)	19.6(4.9)	28.3(5.0)	54.3(6.5)	0.603
Control	14.0(5.3)	20.9(5.2)	32.6(5.6)	60.5(7.3)	

Table 5.9 shows there are no significant differences in the absolute values for the indicators of bone size. The L1-L4 area increased at the same rate between the treatment group (7.79cm^2) and the control group (8.12cm^2), p=0.511.

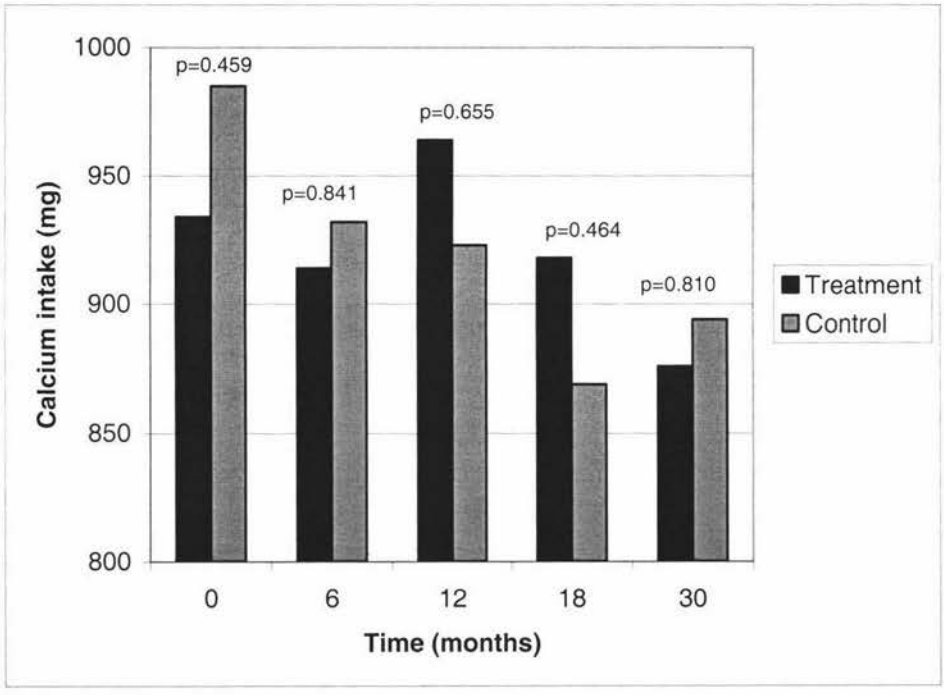
Table 5.9: Parameters of Bone Size; L1-L4 Lumbar Spine Width, Area, Height and Volumetric Density for 0, 6, 12, 18 and 30 Months (±SEM).

	0 months (n_{txt}=74, n_{con}=80)	6 Months (n_{txt}=45, n_{con}=58)	12 Months (n_{txt}=56, n_{con}=60)	18 Months (n_{txt}=60, n_{con}=65)	30 Months (n_{txt}=58, n_{con}=65)
L1-L4 Width					
Treatment	3.05(0.02)	3.12(0.04)	3.20(0.04)	3.27(0.04)	3.42(0.04)
Control	3.09(0.02)	3.15(0.03)	3.24(0.04)	3.29(0.04)	3.44(0.04)
L1-L4 Area					
Treatment	31.19(0.42)	32.08(0.73)	33.70(0.54)	35.45(0.55)	38.98(0.63)
Control	31.51(0.43)	33.04(0.52)	34.80(0.59)	36.02(0.61)	39.63(0.74)
L1-L4 Height					
Treatment	10.18(0.06)	10.38(0.08)	10.58(0.07)	10.82(0.07)	11.22(0.16)
Control	10.18(0.06)	10.47(0.07)	10.69(0.08)	10.91(0.08)	11.43(0.11)
L1-L4 Volumetric Density					
Treatment	46(2)	49(3)	55(2)	59(2)	71(3)
Control	43(2)	49(2)	52(2)	57(2)	69(3)

5.10 Calcium Intake

Throughout the study habitual calcium intake did not change, indicating the children where supplementing their diet not substituting the supplement into the diet at the expense of something else (mean $_{treatment}$ 30 months = 876mg, mean $_{control}$ 30 months = 894mg), refer to figure 5.13.

Figure 5.13: Comparison between the habitual calcium intake in the treatment and control groups throughout the study.



5.10.1 Comparison of calcium intakes from FFQ and 3-day food record

Table 5.10 shows the mean calcium intakes calculated from the FFQ and the 3-day food records at baseline.

Table 5.10: Comparison of calcium intake assessed by 3-day food record and food frequency questionnaire at baseline (n=91).

	Mean ± SD (mg)	Range (mg)	Pearson's Correlation	p-value
FFQ	905 ± 373	(339-1905)	0.395	0.001
3-day food records	794 ± 365	(177-2173)		
Mean difference (FFQ-FR)	111 (14.0%)			

A significant ($p<0.01$) Pearson's correlation coefficient of 0.395 was obtained comparing between the two dietary assessment methods. Sixty-six (73%) of the subjects had calcium values in the same or adjacent quartile for each method. There were 6 subjects who were grossly misclassified, in that, the two values were 3 quartiles apart (Table 5.11).

Table 5.11: Cross-classification analysis for calcium intake from the three day food record (3DFR) and the calcium food frequency questionnaire (FFQ)

	3DFR quartiles				
FFQ quartiles	<574	574-765	766-952	>952	TOTAL
<651	9	4	5	4	22
651-826	6	9	3	5	23
827-1120	5	6	6	6	23
>1121	2	4	9	8	23
TOTAL	22	23	23	23	91

When these 6 outliers are excluded from the data, the Pearson's correlation improved to $r = 0.503$ ($p<0.001$).

If the validity of the FFQ is calculated by the same methods as used by Taylor and Goulding (141). The specificity of the calcium FFQ (the % of subjects with intakes less than the NZ RDI of 800mg for 8-11 year old boys on the basis of the average 3 day food record values, who also had a FFQ intake of less than 800mg) was 57%. The sensitivity of the FFQ (the % subjects greater than 800mg who also had a FFQ intake above 800mg) was 68%. Eight hundred milligrams was chosen as the reference value as it is the recommended dietary intake for 8-11 year old boys and it was better to use one value, than one for the boys and another for the girls.

6. Discussion

This study was designed to investigate:

- The effect of a calcium enriched milk drink on bone density, bone mineral content and bone size in both male and female 8-10 year old pre-pubertal children.
- To determine whether a daily intake of a milk drink had an adverse effect on the body composition of these children.
- To determine whether this milk product is acceptable to New Zealand children of this age.

This study shows that there is no beneficial or detrimental effect of taking a high calcium dairy supplement on bone mineral density, bone mineral content or bone size. There was no difference between the two groups after intervention, although trends between the groups in BMD at the total hip and trochanter were evident.

This age-group was chosen as previous studies have shown that this is the period of most rapid skeletal development. It is therefore important to provide the nutrients that are involved in skeletal development. Calcium supplementation intervention trials using elemental calcium supplements and high calcium foods have previously shown that bone density increases with supplementation, but the increase is transient and disappears when the intervention is withdrawn. Although a study by Bonjour et al (136) suggests there may be evidence for a maintained effect when a high calcium food is

used as the supplement. A recent study by Dibba et al (168), has shown that 12 and 24 months after completing a calcium intervention trial there is lasting increases in BMD and BMC at the mid-shaft radius, but not at the distal radius. This is in contrast to what has been previously reported by Johnson (2) and Lee (135), and is the first study using a supplemental form of calcium to have shown a lasting effect following supplementation.

6.1 Physical Characteristics and Demographic Data

All the children resided in and attended schools in a higher socio-economic area, these results therefore can only be generalised to other New Zealand children of this age from this socio-economic group. However this could be questioned, as the sample was not random, the schools were asked to participate, and the children and parents self-selected to participate. A major problem with the selection of the subjects in this trial was that they where asked to consume milk for 18 months. The subjects who liked milk were the ones who volunteered to participate. This caused an obvious selection bias and the subjects that volunteered were the ones that may not have required the intervention, as they already drank milk. Therefore, when studying the effect of specific nutrients like calcium, it may be preferable to use a supplemental tablet form or fortify foods that a number of children like, as has been previously done in the study by Bonjour et al (3).

However this study looked at the effect of a food on indicators of bone health; as the children were required to take a milk based product for 18 months they had to like milk, or tolerate a product that they did not like. While the study

product and the placebo were controlled for nutrients that may have an effect on bone, with the exception of calcium, the product was a powder that was mixed with either hot or cold water. The Christchurch City water supply contains 12mg/L of calcium (169), so an additional 2.4mg of calcium was in each prepared drink, this is unlikely to have an effect on the overall results. In some towns in NZ the use of water would have meant the product also contained fluoride from the municipal water supply, however in Christchurch, NZ the water is not fluoridated and therefore this is not a confounding factor in the results.

The study group was selected because the schools were near the research centre, which made accessibility for the researchers easier. It also decreased travel time for the parents who brought their child in at each visit.

We included both females and males, as osteoporosis had been shown to be a concern in elderly men as well as elderly women in New Zealand (1). Other studies that have looked at this age-group have included females only (3,7-9) or males and females (2,4-6).

Most of the children in the study identified themselves as New Zealand European or other European descent. Being of European descent puts you at higher risk for osteoporosis than being from African American descent (27). It seems likely that Maori and Pacific Peoples are at less risk also.

A NZ Ministry of Health report into the priorities for Maori and Pacific health (170) did not mention the individual risk of osteoporosis, however compared to European peoples the relative risk for fractures resulting from falls was 0.5. A more recent document from the NZ Ministry of Health (171) titled "An Indication of New Zealanders' Health" reported on the rate of fall-related hospitalisation rates for Maori and Pacific people, over the age of 65 years, during 2000. It was shown the rates for Maori were 0.6 and Pacific people 0.7 compared to a European rate of 1.0. While these figures do not look directly at the rates of osteoporosis, it does suggest there are less fractures resulting from the falls that are occurring and that there may be a reduced incidence of osteoporosis.

6.2 Medical and Family History

Genetics is known to be the major risk factor in osteoporosis. It is thought up to 80% of bone mineral density may be genetically determined (10). In this study it was found 43% of the children reported they had a maternal or paternal grandmother that had lost height or had a rounded/stooped back. Fifty eight percent of the children reported their mother or grandmother had had a fracture at some point during their life. From the reported histories it seems likely that some of the children have the genetic factors that will predispose them to osteoporosis in later life.

Low bone density is known to be a risk factor in children with forearm fractures (172). Other studies have also suggested forearm fractures in childhood may be indicative of vertebral fractures in elderly women (21,173).

In this study 28 children had previously fractured prior to starting the study and another 14 fractures were sustained during the study. While these children were not separated from the rest of the group they may have lower bone density than their peers and it may put them at increased risk of osteoporosis in later life.

Some medications are known to interfere with bone metabolism, the most common ones are long-term use of steroids (inhaled and oral), anticonvulsants, thiazide diuretics and vitamin D. The main medication children in this study were using was steroids for the treatment of asthma. All the children who were using asthma medication had mild asthma and most were taking it when necessary. Studies have shown moderate asthma medication does not alter BMD or bone growth in children (174-175).

6.3 Retention of Subjects

The retention of subjects in a long-term nutrition intervention can be difficult. It may be even more difficult in children as their enthusiasm for participating decreases. In this study there was a high number of withdrawals, mainly due to the child no longer liking the study product. Most withdrawals occurred in the first six months of the intervention period. In many cases the parents did not insist that the child took the drink, so the child was asked to take it voluntarily or withdraw. The high withdrawal rate (54%) in this study reduced the chance of seeing a statistically significant result. At the spine if there was a 4% increase in BMD in the placebo group there would have needed to be a 7% increase in BMD in the supplemented group due to the reduction in

numbers to see a significant difference between the groups (see ethics application appendix 5).

Other studies have also reported high dropout rates due to a dislike of the study product (2-3,8), although not as high as the numbers in this study. In the other studies it is not reported whether it was an immediate dislike of the product or a dislike over time, in this study most withdrawals due to dislike of the study product were within the first 6 months. In the cases of Johnston et al (2) and Nowson et al (8) their rates of attrition were high because of using twins, meaning if one child wished to withdraw the other also had to.

6.4 Compliance

In order to test nutrition interventions compliance must be monitored. If compliance is not monitored systematic bias can develop that may effect the results and hence the conclusions drawn. When two products are used, like in this study, there is the chance that one may be liked more than the other and this may lead to a higher rate of compliance in this group. In this study there was no difference in the rates of compliance between the groups. The other important factor to consider is how and when compliance is measured. If it is recorded retrospectively from memory then the recorded value is likely to be higher than the actual value. In this study the subjects ticked a sheet after they had drunk the milk product and also kept the empty sachets for collection by the study coordinator.

Maintaining compliance is difficult in an intervention of this length. Compliance in the children who completed the intervention was high. This may be due to supervision from either the parents or the teacher in the classroom. If the children who withdrew from taking the supplement are included the compliance rate decreases to around 45% and this may be a major reason that there is no difference in BMD, BMC or bone size. The table below outlines the calcium intake when compliance is considered.

Table 6.1: Mean calcium intake (mg) when compliance is considered during the supplementation period.

	Dietary Intake	+ supplement	+ 45% compliance
Treatment	934	2134 (1200)	1474 (540)
Control	985	1385 (400)	1165 (180)

The introduction of a variety of flavours was important for maintaining compliance and reducing the rate of withdrawals. Previous calcium intervention studies have reported higher levels of compliance with the lowest rate reported by Lloyd et al (9), at a range of 64-77% and the highest by Dibba et al, at 100% (6), both of these studies used a calcium tablet. In studies using a food based supplement Bonjour et al (3) had a compliance rate of 75%. Cadogan et al (7) calculated the dietary calcium intake in each group every 3 months throughout the study to monitor compliance, one subject was excluded as they did not comply. Results from previous studies have suggested there is a decrease in dietary calcium intake from childhood to adolescence (12,90-91). By developing dietary habits that include the frequent intake of milk during childhood and adolescence it may lead to a higher calcium intake in later years. Studies that look at compliance during

supplementation are important to determine whether children can achieve the recommended daily calcium intake from diet.

6.5 Acceptability of the Product

In this study a dairy calcium food product was used to supplement the subjects. Two previous studies (3,7) studying this age group have also used a calcium-containing food product to supplement their experimental group. It is optimal that a food source is used to obtain calcium rather than a supplement in most cases, as food also supplies energy, protein and other nutrients that enhance bioavailability of the milk. Fluid milk provides an ideal source of calcium as it is cheap, the lactose in milk aids in calcium absorption, and it provides nutrients to support growth and development in children (27). Studies have compared the bioavailability of milk in comparison to fortified soy beverages, and calcium supplements like calcium carbonate (175-176). It has been shown that the bioavailability of calcium carbonate is as good as the milk calcium, however it does not provide any other nutrients to support bone growth and development (176). In comparison the calcium from fortified soy beverage is absorbed at only 75% the efficiency of calcium from cows milk, and a much larger volume would need to be consumed to meet the daily calcium requirements (175).

A recent feasibility study (177) examining efficacy of the introduction of a school milk programme in Wellington NZ, found if children were to drink milk it would need to be flavoured. The children in Wellington were asked if they

would drink milk or a soft drink, most thought they would choose a soft drink. Johnson (92) found similar results in children in the USA.

If a product is to be used in an intervention study it must be acceptable to the study population. In this study we measured acceptability with a 5-point hedonic questionnaire using descriptors developed by the children. Previous studies have found 5 year old children have the cognitive ability to rank acceptability on a 7-point scale (178). We chose a 5-point scale so not to excluded any children with cognitive difficulties. Kimmel et al (162) found children can reliably distinguish between preferences and discriminate between taste stimuli.

This study found that most of the children liked the milk product, and could drink 1 glass at a time. It was important to introduce more flavours throughout the supplementation period to maintain compliance and provide variety to the children.

6.6 Anthropometric Indicators

There is a common perception that dairy products are high in fat and consequently should be limited in the diet, or excluded. They are often thought to be the major cause of weight gain and undesirable changes in anthropometric measures. While there is little data to support these theories there have been a number of studies that have examined the nutritional intake in overweight children and adolescents (179-181). In most of these studies the calcium intake is below the recommended intake and this suggests dairy

products are probably not a part of the dietary intake in overweight children and adolescents. In this study total lean mass, total fat mass, height and weight were measured at baseline, 6 months, 12 months, 18 months and 30 months. There were no adverse changes in the anthropometric measures between the groups (see growth charts in results section 5.6). This is consistent with previous findings in pre-pubertal girls (7) and teenage girls (10). More recent work now suggests a link between increasing dietary calcium intake in those with a low intake and weight management in humans. Davis et al (182) has shown calcium intake is negatively associated with body weight; for every 100mg increase in calcium there was a 0.82kg per year decrease in body weight in young women who have a low dietary intake or calcium. This effect has not been studied in children, although Cadogan et al (7) reported non-significant trends towards higher lean body mass and lower fat mass in pre-pubertal girls. However, cross-sectional data examining body weight in relation to BMC and bone area in obese and overweight children has suggested there is a mismatch between body weight and bone development during growth. The BMC and bone area, in obese and overweight children, relative to their body weight were 2-5-10.1% ($p<0.05$) lower than what was predicted for their height and age (183). This suggests with increased adiposity the skeleton is not developing to support the increased weight of the body, this could be due to decreased calcium intake from poor food choices or an inability for the skeleton to develop quickly in response to the increased weight.

Historical data from Japanese children has shown nutrition has a major effect on growth and development (184). With an increase in the food supply following World War 2, the peak height age (the age when the speed in height growth is at its peak) decreased and consequently the children increased in height and are taller at age 17. Early studies into milk consumption in the UK, in children found children who drank milk were taller than their peers (133-134). It is important to ensure children are receiving sufficient nutrients to meet their requirements for growth and develop a strong skeleton to support them.

Pubertal stage is a known determinant of bone mass, using subject groups closely matched in this respect reduces the likelihood that differences in pubertal progression would obscure interpretation of bone measurements. The two groups in our study were matched at baseline and still matched at the end of 30 months. It was also important to have the same ratio of males to females in each group, due to earlier commencement of puberty in the females.

It has been suggested that the pubertal stages where most of the bone mass is accumulated (37%) is between stages 2 and 4 (24). This is of importance when looking at the results from this study as the subjects moved from pubertal stage 1 to pubertal stage 2. While the pre-pubertal period is one of most rapid bone growth, it seems likely the bone mass accumulation may not happen until later in puberty and hence the subjects in this study may have been too young to see a major increase in their bone mass.

6.7 Bone Mineral Density and Bone Mineral Content

Bone mineral mass of each individual follows a trajectory corresponding to a specific percentile from the mean (136). This is regarded as the expression of genetic factors that eventually determine inter-individual variability in peak bone mass. The aim of this study and several others has been to shift the trajectory of bone mineral mass to a different percentile in response to a higher calcium intake. However, there may be a limit at which calcium supplementation has a positive effect on bone mineralisation. In the control group the mean intake during supplementation was 1385mg per day, and the treatment group was higher at 2143mg per day (see table 6.1). Weaver et al (64) has shown through calcium balance studies that maximum retention of calcium in adolescent girls occurred at a level of 1300mg per day, this has since become the recommended intake in the USA for this age group. This group has estimated that an increase in calcium intake from 918mg to 1300mg per day could increase skeletal mass by 4% per year. This is consistent with the annual increase in bone mineral density we saw in this study group, both treatment and control. Matkovic and Heaney (65) also suggested there is a calcium threshold, in a review of 99 studies in children 2-8 years and 133 studies in 9-17 year olds they found the threshold level of the children was 1390 ± 246mg per day and 1480 ± 396mg per day. Bonjour et al (3) also supports the theory of a calcium threshold, they suggested from their findings that an intake of less than 800-900mg per day is not sufficient for optimal bone mass accrual in 7-9 year olds. However, they found the effect of calcium on bone mass is mild when the habitual consumption of dietary

calcium is above 900mg. It would therefore be useful to compare this study population with age-matched peers who are not high milk consumers. In this study the habitual calcium consumption was above 900mg and this has been shown to be near threshold level. In previous studies examining the dietary intake of NZ children the habitual calcium intake has been lower (550mg-750mg). Therefore, if the study was carried out in this group it may be easier to see a positive result on bone accrual.

The previous studies that have looked at the effect of calcium supplementation on pre-pubertal bone health have suggested the appendicular skeleton, particularly the regions of compact bone, appear more sensitive than the axial skeleton to effects of calcium supplementation. This is because of increases in height and long bone length. Bone growth during this period occurs along the longitudinal axis (24). The main target areas for increases in bone mineralisation have been the radial and femoral diaphysis(3), the midshaft radius (2,4-6), total hip (8), and leg and pelvis (7). However some studies have also found significant differences in the spine (5,8-9) and the total body (7,9). The results from this study suggested a trend towards a higher BMD at the total hip and trochanter that would support the theory of the appendicular skeleton being more sensitive to calcium supplementation.

6.8 Bone Size

No difference between the two groups was observed in bone size during the supplementation period or during follow-up. This is different to results found

by Bonjour et al (3) where they found an increase in bone size between the supplemented and control groups. However they compared the results of the children below the median for dietary calcium intake and the children above the median and only found a significant difference in the low habitual calcium intake. This has previously been confirmed from historical data (133-134,184) that a low dietary calcium intake has an effect on bone size and height. The other calcium intervention trials that studied the effect of a calcium salt on bone size found no difference between the supplemented group and the control group (see table 2.5) (2,4). This suggests the subjects in these studies were well nourished and had an adequate calcium, energy and protein intakes, in contrast to the low calcium intakes in the study by Bonjour et al (3). Previous studies (4) have suggested that using a supplement that is not a calcium salt also acts as a confounder, as food supplements also contain energy and protein for growth. This study and the study by Bonjour et al (3) controlled for this by using a placebo product that was matched in energy and protein content, unlike other studies where milk products were compared to the usual diet (7,10).

6.9 Dietary Calcium Intake

Our baseline calcium intake was in line with the recommended intake of 800-900mg per day. However the method used to determine habitual intake may not have been sufficient as we only used a single food frequency questionnaire. The most accurate method of individual dietary assessment is a weighed duplicate food collection and then assays to determine calcium mineral content. However even the favourable methods of having the

subjects complete a food record requires a record of at least 15 days for children aged 6-11 to obtain "reasonable accurate" estimate (within 20% of the true value) of their calcium intake (14). Thus, the values we obtained for habitual individual calcium intake may potentially be either over or under estimated.

In comparison to previous studies that have looked at the calcium intake of New Zealand children the results in this study were higher than have been found in Auckland school children aged 8-14 years (88) and higher than what had been found in adolescents (10,89). They are comparable to studies in the USA where they have determined the average calcium intake to be 919mg/d for females aged 9-13 years (91-92) and in Ireland where the average intake is 950mg/d in 15-18 year olds (91). It has been found in Scotland, that school children from higher socio-economic areas tend to have a higher intake of micronutrients (185) and this also may be a contributing reason for the higher habitual calcium intake in this study. Results from the Australian National Nutrition Survey suggest that adults from higher socio-economic status groups were more likely to eat a wider variety of foods, and obtained higher nutrient intakes than adults from lower socio-economic status groups (186).

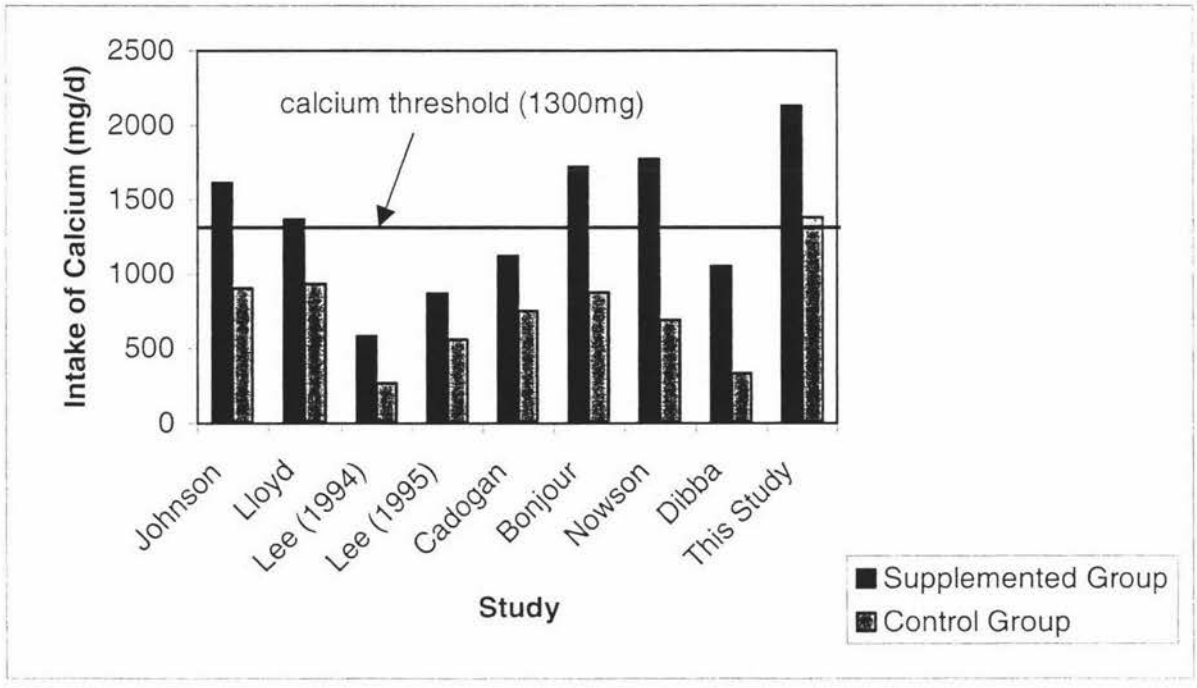
Of surprise was that the habitual calcium intake remained high throughout the study, even when the children had to drink an additional 2 glasses of high calcium milk each day. It appears they did not substitute this supplement for other calcium containing foods. It would have been interesting to have asked

the subjects to record their dietary intake with a 7 day food record during the study to determine what was being substituted for the supplement, in their habitual diet. In a previous study where dairy products were used as the supplement it was found cakes and biscuits were substituted for the supplement, so the girls tended to snack on flavoured milk or yoghurt instead of cakes and biscuits (187).

The previous studies that have looked at the effect of calcium supplementation on bone health have reported varying levels of habitual calcium intake (see figure 6.1). Three studies had comparable baseline calcium intakes to this study (2-3,9), in that they were approximately 900mg/d, three studies (6-8) had intakes between 600mg/d and 750mg/d and the other two studies had low intakes of around 300mg/d (4-5). The big difference in the studies with the high intake when compared to this study was that the placebo product we used still contained an additional 400mg/d of calcium and this lifted the control group to around 1300mg. This was the only study to lift both the control group and the supplemented group over the "threshold" level for dietary calcium. This is an obvious flaw in the study design and would need to be addressed if this study was repeated. The main reason for the control group receiving calcium in the supplement was that the ethics committee at the time felt there was sufficient scientific evidence that calcium has an effect on bone health and that it is unethical to not provide some calcium to the growing skeleton. In addition the researchers were not aware how high the baseline calcium would be, as other studies into calcium intake in NZ children and adolescents have reported much lower levels than this

study. This may be one of the main reasons no differences were seen in indicators of bone health.

Figure 6.1: Differences in the calcium intakes in the intervention trials that have been carried out in pre-pubertal children.



6.10 Validation of Dietary Calcium Intake

The FFQ may be more useful for classifying individuals by rank, identifying groups at extremes of intake, and monitoring trends in dietary patterns over time. However, the advantages of low respondent burden, simplicity, and ease of administration make the FFQ an attractive option for collecting dietary data on the habitual or usual consumption in studies of relatively large populations especially studies of the long-term effects of diet on health status. The dietary tool will only be as useful as the validity of it; in the case of the FFQ it is important to compare the method with one or more reference methods that are assumed to have better validity. A major flaw in comparing

a FFQ with a food record is the major sources of error in the FFQ (memory, accuracy of the food composition data and perception of serving size) are likely to be duplicated in the food record. However, without using nutrient biomarkers or direct calorimetry this is the accepted method for validation (14). In this study a three day weighed food record was used as the validation comparison.

If the specificity and sensitivity are looked at in this study we can calculate 22 children (24%) who actually had low calcium intakes would be missed for intervention purposes (specificity error) and 13 children (14%) would be targeted for nutrition advice when it was not required (sensitivity error).

Other studies have compared calcium FFQ with varying lengths of diet records. Montomoli et al (143) compared their FFQ with a 14 day food record and reported very high correlation (r=0.90) and high specificity (87%) and sensitivity (89%). In comparison Chee et al (144) and Taylor et al (141) compared the FFQ to a 3 day and 4 day food record respectively. They found similar levels of correlation (r=0.563 and r=0.52) to the results of this study and similar levels of specificity (60% and 68%) and sensitivity (70% and 79%). All three studies (141,143-144) have found the FFQ tends to overestimate calcium intake as the results from this study also demonstrate. Correlation coefficients of the order of 0.5 to 0.7 appear to be typical for validity of nutrient intakes and while they may seem low they are similar to other epidemiologic measurements in population studies (14).

This FFQ was able to correctly identify 78% of subjects in the same or adjacent quartile for intake; it grossly misclassified 6 subjects. This is comparable with previous studies (141,143-144,146).

6.11 Limitations of the Study

One of the limitations of this study was that subjects were drawn from higher socio-economic areas. The justification for using these schools was that they were closer to the research centre making access easier for both the parents and the researcher. However, this may have reduced the chance of seeing a significant difference between the intervention groups. It means that these results cannot be generalised to all NZ children, but only to children from high socio-economic areas and schools.

The subjects all had high intakes of habitual dietary calcium and other nutrients. The habitual calcium intake was above the level previously found in NZ children (88). This is most likely due to the higher socio-economic status of the children and the method of subject selection. The children from the schools were asked to volunteer for the study and this may have excluded subjects that do not like milk and dairy products because a food-based supplement was used. While it is better to promote nutrition from a food perspective, as food is more than a single nutrient, the children who needed the calcium may have been missed. It may be that children who do not like milk and dairy products have lower BMD, due to the difficulty in achieving adequate calcium intakes without these foods.

Another limitation was the use of a placebo product that contained calcium. This made it more difficult to see a difference between the two intervention groups as the control group had a supplement that contained 400mg of calcium in addition to the 900mg they were getting from their diet. At the time of the study development the ethics committee required the control group to be given calcium as well as it was felt that it was unethical to not provide calcium to growing bones when it was known it may help achieve a higher PBM, although in this study subjects had good dietary intakes of calcium. The 400mg was decided on for the placebo product as it was equivalent to 2 glasses of standard milk and it was also one third of the high calcium milk so there would still be sufficient difference in calcium intake between the groups.

The high number of withdrawals from taking the supplement was also a limitation in the ability to see a difference. If any product is to be taken by children for a significant period of time then it needs to be acceptable. A large number of the children did not like the supplement and stopped taking it; this impacted on the percentage difference between the groups and on the overall compliance of the groups. It may have been better to over-select and have a pilot run in period of 6 months and then randomise, as the children who did not like the supplement would have withdrawn, however this does not get around the bias of only having children in the study who like milk.

Physical activity was not measured in this study and this is a major confounder for measurements of bone health. It was decided that the number of questionnaires and measurements taken on the study group needed to be

keep to a minimal to reduce respondent burden. This means it cannot be ruled out that the supplement did actually cause a significant difference but the control group may have been more active and this prevented a significant difference being observed. It could also be suggested the trends seen at the total hip and trochanter were the result of exercise and not nutrition as this is a major area where exercise has an impact on bone development (33).

While the FFQ is the most widely used in longitudinal studies of nutrition intervention there are limitations with using this method for collecting habitual calcium intake. FFQ are known to overestimate calcium intake (141-146), but are useful for ranking subjects and identifying subjects at the extremes of intake (141). There have been many validations studies (141-146) comparing them with other more accepted methods of dietary assessment. However all methods of dietary assessment are known to contain many inherent errors. In addition the use of a FFQ that focused on calcium containing foods prevented the researcher from identifying other nutrients that may have had a confounding effect on the measurements of bone. It is well documented that many nutrients are involved in bone development in addition to calcium (see section), and it may be self-limiting to only examine this nutrient, especially when using a food-based supplement. In addition the nutrient analysis from foods is only as accurate as the food database used, the accuracy of the portion sizes and the skills and knowledge of the researcher. In this study there was no indicator of portion size on the questionnaire, the subjects were asked how many serves they had. This meant the database serving size was used as the portion for analysis. This would have introduced many errors,

including the use of adult portion sizes for children. The database is also limiting in that when a food is not available the researcher needed to substitute or omit that food.

The final limitation of this study was the use of DEXA only for the assessment of bone health. When calcium intervention studies are looking at changes over time DEXA is the best method for seeing the change, however it does not give a full picture of the bone kinetics and changes in the remodelling process that could be seen from also looking at bone biomarkers. While it was decided not to take blood or urine samples from these children, the use of biomarkers would have given a picture into the process of bone modelling not seen by only examining BMD or BMC.

6.12 Recommendations

Some recommendations can be made based on the information from the literature review combined with the data from the study.

1. To ensure strong bones in children it is important to develop public health messages aimed at children with low calcium intakes, there does not appear to be any benefit when habitual calcium intake is already high. This is probably due to the calcium threshold. While it is difficult to encourage children to increase their PBM now to prevent osteoporosis in later life, there is increasing evidence that low BMD may also increase the risk of childhood forearm fractures (172) this

could be one way of developing a public health message targeted to this age group.

2. If intervention studies of this length are carried out the product needs to be acceptable to the study population. For a milk based product it has been demonstrated that it needs to be flavoured when used with children (84,178). It could also be argued the intervention period was not long enough, the period of most bone mass accrual is during pubertal stages 2 to 4 and in this study the supplement was stopped at stage 2. In order to see a difference the supplementation may need to continue not only through the period of rapid growth in stature but also during the period of most bone mass accrual.

3. When educating children about bone health it is important to ensure their diet provides all the necessary nutrients from a variety of foods, as many nutrients are now known to play a part in the development of the skeleton. While calcium intake is still the major nutrient researched in relation to bone health, in children with an adequate intake there appears to be a threshold intake. It is important that the other nutrients are included to ensure adequate bone health and normal growth and development.

4. Finally if this study was to be repeated it would be important to:
    - Choose a population that had a lower habitual calcium intake in addition to the population that was studied, this would provide a

greater range in calcium intakes that may lead to measurable effects in bone health.

- Use bone biomarkers to examine the kinetics of the bone remodelling, to determine the method of bone mass accumulation and assess whether it is a transient of persistent effect.
- Use a more comprehensive dietary assessment tool in addition to the FFQ to be able to examine the correlation between bone health and other nutrients. The other option for using a FFQ for the study would be to validate it in the study population prior to the commencement of the study. This could be done using a weighed food record of between 3 and 7 days in length.
- Measure the exercise levels of the study participants to control for the possible confounding effect of exercise on bone mineral density.
- Have available either calcium fortified foods or a calcium supplement in addition to the milk-based supplement, this would ensure the children who did not like milk or dairy products still had the opportunity to participate in the study. By using the milk-based supplement only, the subjects who need calcium the most may be excluded from the study.
- Recruit a larger population sample, and include a pilot period with randomisation occurring after 6 months to allow for any withdrawals from the study. The larger study population would also allow for a larger number to be followed up and for longer

after the intervention, as currently there are conflicting results with regard to the lasting effect of calcium supplementation on bone health.

7. Conclusions

It is known calcium intake is related to parameters of bone health and incidence of fracture. However, it remains unclear whether a high calcium intake for a short period of time during childhood or adolescence has an effect during later life when there is a higher risk of osteoporotic fracture, and whether there is a threshold point where calcium supplementation has no further effect. The current literature suggests the answer is probably that a high calcium intake needs to be continued throughout childhood, adolescence and during adulthood as well (11,17-18,27-28), and that there is probably a threshold level of intake at each age (62-65). In this study it was shown that there was no beneficial or detrimental effect in pre-pubertal children from taking a high calcium drink when their habitual calcium intake was already high.

The results from this study suggest a trend for the BMD at the total hip and the trochanter to be higher in the treatment group than the control group, however this was not statistically significant. Previous results in this age group have found the appendicular skeleton to be more responsive to calcium supplementation than the axial skeleton (2-8) and these results support this.

In order to carry out an intervention study using a food based product it must be acceptable to the study population. In this study a large number of children withdrew early into the intervention because they did not like the study product. This limits the results due to a low compliance rate and a

reduced ability of seeing a difference due to lower numbers actively taking supplementation.

The method of dietary assessment, chosen and used, for determining habitual dietary calcium intake will determine how accurate the dietary intake is assessed. In this study a modified food frequency questionnaire was used and this was validated from 3 day weighed food records. The sensitivity and specificity errors were similar to those from other calcium validation studies (141,143-144). As with the other calcium FFQ's that have been validated this FFQ tended to overestimate calcium intake. However, the questionnaire used was found to be valid for estimating habitual calcium intake in 8-10 year old children if used in an intervention trial for a study population.

Finally, these results suggest calcium supplementation in children needs to be targeted in those children with low habitual dietary calcium intake and probably from a lower socio-economic region.

Limitations of this study included:

- The study population were from high socio-economic areas.
- The study population had high habitual calcium intakes.
- The use of a food-based milk supplement may have biased only milk consumers to volunteer to participate in the study.
- The placebo product contained calcium, which when added to the high habitual calcium intake, increased the control group to the calcium threshold level also.

- The product was unacceptable to a number of children, which lead to a high incidence of withdrawals from the intervention.
- Physical activity was not measured and this is a major confounder in bone development.
- The FFQ was the only method of collecting dietary information.
- DEXA was the only method of bone assessment; if bone biomarkers had also been used then the process of bone remodelling could have also been examined.

Future research is needed to:

- Develop an acceptable product that allows children to receive adequate calcium while their skeleton is developing. This would need to be a public health collaboration between the food industry, public health educators and scientists.
- Determine at risk populations for low indicators of bone health and establish suitable interventions to increase their parameters of bone health as literature suggests there is a calcium threshold.
- Examine the process of bone remodelling in the developing skeleton using bone biomarkers in tandem with DEXA. This will help to explain whether the appendicular skeleton is more responsive to calcium supplementation and the possible mechanism.
- Examine the influence of other nutrients on the parameters of bone health, especially protein as this appears to also play a major influence.

It is important to consider the nutritional requirements during period of rapid growth, as it is the nutrition at this stage that will determine the health during later life. We know the incidence of osteoporosis is increasing in the elderly NZ population, but by looking at the nutrition of the children we may be able to reverse this health statistic in future years.

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Appendix 1

Invitation to participate (information sheet)

Information Sheet

Project Title

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo (dummy) supplement

Investigators: **Dr Nigel Gilchrist**
Dr T Wilkinson
Dr J Turner
Dr J Elliot

Venue of Study: **The Princess Margaret Hospital, Christchurch**

Contact Telephone Numbers:

Dr Nigel Gilchrist	**3377899**
Rachel March	**3377752**
After Hours	**025 360 141**

Study Personnel:

Dr Nigel Gilchrist is the principal investigator for the study.

Rachel March is the principal nurse for the study. She or one of the other nurses (Pat, Marie or Penny) can be contacted at any time on one of the above numbers.

Pat Maguire is the bone density technician and she can be contacted at telephone 3377821.

Megan Merilees is the dietitian involved in the study.

Study Introduction:

Your child is invited to take part in a new study. The study is being sponsored by the NZ Dairy Board. This study involves 130 children from two Christchurch schools and compares the effect of a dairy (calcium) supplement with a placebo (dummy) supplement. The supplement is a powder which is taken daily dissolved in a glass of water.

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

Each child is randomly (by chance) assigned to receive either the active or placebo supplement. This process is called randomisation. Your child will have a 1 in 2 chance of being given the placebo (dummy) supplement.

The aim of the study is to assess whether increasing a child's calcium intake has an effect on the thickness and size of their bones.

The size and thickness of bones is important because osteoporosis (thin bones) is an important health problem in older people.

We would like to see if this problem can be prevented by increasing the amount of bone gained in childhood.

Following the completion of this study your child will probably be asked to participate in further follow up studies.

Study Procedures:

This is a 18 month study with a follow up visit 12 months later. During the study period visits occur 6 monthly.
If you agree to your child taking part in the study the following will happen:

1. We will ask you to complete a medical questionnaire about your child.

2. A Dietitian will ask questions about your child's diet.

3. Your child will have his/her height and weight measured and be asked to identify (by the use of pictures) his/her stage of physical development.

4. You child's bone density will be measured by a machine called a DPX-IQ and by ultrasound. Information about how these measurements are done is attached to this information sheet.

The table below identifies the study procedures and when they occur
All the assessments required for each visit will be performed at the one time at The Princess Margaret Hospital.

	Baseline	6 months	12 months	18 months	follow up
Height	x	x	x	x	x
Weight	x	x	x	x	x
Medical questionnaire	x				x
Dietary assessments	x	x	x	x	x
Assessment of physical development	x				x
Bone density	x	x	x	x	x

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

We estimate each visit will take about 2 hours.

Risks:

The DPX bone density measurement involves a small dose of radiation. The approximate total effective dose is 20 microsieverts for the complete study.

As a perspective, the effective dose received by all persons living in New Zealand each year from natural background radiation is about 2000 microsieverts.

If your child has an allergic reaction to the supplement you should not give your child any further supplement. Contact either the investigator or your General Practitioner.

Qualifications:

Your child cannot take part in this study if he/she has:

1. An allergy to dairy products
2. Any **major** current illness
3. Is taking certain medication such as steroids (oral or inhaled) , anti-convulsants, Vitamin D or Thiazide diuretics.

Your child should not take part in any other study while involved in this one.

Confidentiality:

Records from the study that identify your child will be confidential, except they may be inspected by the sponsor of the study (NZ Dairy Board), the regulatory authority of New Zealand, or other external auditors and will not otherwise be released except by law. We will inform your child's General Practitioner of his/her participation in this study if you are agreeable.

Information:

If you wish to get more information or answers to your questions about the study do not hesitate to contact us.(details page 1). You will be told of any significant new information, either good or bad, that may be learned about the treatment used in this study.

If you have any concerns about your child's rights as a participant in this research you may contact *a Health and Disability Services Consumer Advocate, telephone* (03) 377 7501.

Costs and Payment:

The supplements used in this study and all procedures and medical care called for by the study protocol will be provided to you at no cost.

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

If you choose to go to The Princess Margaret Hospital with your child reimbursement of travel costs is available.

Stopping the study:

Your child's participation in the study is voluntary. You may withdraw him/her from the study at any time. If your child does not take part your family's health care will not be affected in any way.

Your child's participation in the study may be terminated by the investigator or the sponsor without regard to your consent if he/she needs certain medications, does not adhere to the study plan, experiences a study related injury, or for administrative reasons.

The study has received ethical approval from the Canterbury Ethics Committee.

Compensation:

There may be compensation available to your child if he/she is injured by taking part in this trial.

The Canterbury Ethics Committee has certified that this clinical trial is being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which this trial is being carried out. This means that if your child suffers injury as a result of his/her participation he/she will not be eligible for ARCIC (ACC) cover. Compensation however, will be provided by the New Zealand Dairy Board in accordance with Guideline 13 of the Guidelines for Biomedical Research Involving Human Subjects.

This guideline is only a guideline and until your claim is assessed by the insurers of the New Zealand Dairy Board, it cannot be said with any certainty exactly what type or amount of compensation you will receive if your child suffers injury as a result of his/her participation or exactly what type of injury will be covered. However, the guideline requires that compensation must be provided where your child suffers significant deterioration in health or well being or any adverse reaction which is caused by his/her inclusion in the trial. The guideline also requires that the compensation your child receives must be appropriate to the nature, severity and persistence of his/her injury. This means that your child will be likely to receive some compensation from the New Zealand Dairy Board unless his/her injury is minor or temporary. However, he/she will not receive compensation from the New Zealand Dairy Board if his/her injury or deterioration in health or adverse reaction was caused by an existing condition which you knew about before taking part in the trial. For example, if you know your child is allergic to dairy products and he/she develops an allergy caused by participating in the trial, you will not receive compensation.

Dr Nigel Gilchrist 3377899

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

The Ethics Committee has give its approval on the understanding that an acceptable level of compensation will be paid by the company in the event of an injury caused by participation in the trial.

Dr Nigel Gilchrist 3377899

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

Information Sheet for Children

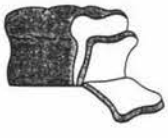
We would like to ask you to drink a special drink every day for the next one and a half years. This drink is a powder that is dissolved in water.

The drink contains **calcium.** Calcium is important for strong bones. We usually get calcium from what we eat and drink especially from dairy products.

We are interested in finding out if this will help your bones to become bigger and stronger than those of other children who do not have this special drink. We hope this will help stop you breaking a bone when you are older.

Some of you will get the drink containing lots of calcium and some of you will get a drink that contains little calcium. We need to do this so we can compare the group of children who have the real drink with the other children who do not. No one will know until the end of the study which drink you are having.

We would also like to ask you some questions about your health and what foods you eat four times over the next year and a half and then again one year after you stop having the special drink.



At the same times that we ask you the questions we would also like to measure how tall you are, how much you weigh and test the thickness of your bones with two special machines. All you have to do is lie still on a bed for one of these and for the other you need to put one of your heels in a bath of warm water. These tests do not hurt.

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

All of the tests will be done at The Princess Margaret Hospital. You will go to the hospital with other children and your parents will know this and may come with you if they wish.

You can't be in the study if you take some medicines already, or if you are allergic to milk. Your parents and doctor will check this out.

We will write reports about the study, but we won't put your name in them or tell anyone you are taking part.

Appendix 2

Consent form for the parents of the children

Does increasing calcium intake by the use of a supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo (dummy) supplement

Consent statement by Parent

1. I have read and I understand the information sheet dated 23/3/98 for parents of volunteers taking part in the study designed to investigate whether giving a calcium supplement to children makes their bones bigger and stronger than those of unsupplemented children. I have had the opportunity to discuss this study . I am satisfied with the answers I have been given.
2. I understand that allowing my child to take part in this study is voluntary (my choice) and that I may withdraw my child from the study at any time and this will in no way affect his/her future health care.
3. I understand that my child's participation in this study is confidential and that no material which could identify my child will be used in any reports on this study.
4. I understand that the treatment, or investigation, will be stopped if it should appear harmful to my child.
5. I understand the compensation provisions for this study.
6. I have had time to consider whether to allow my child to take part.
7. I know who to contact if my child has any side effects to the study.
8; I know who to contact if I have any questions about the supplement or the study.
9. I agree to an auditor appointed by the sponsoring pharmaceutical company and approved by the Canterbury Ethics Committee reviewing my child's relevant medical records for the sole purpose of checking the accuracy of this information recorded for the study.
11. I wish to receive a copy of the results YES/NO

12. I consent to my child's GP being informed of participation in this study/the results of my participation in this study YES/NO

I ..... (full name) hereby consent to
my child..... .....(name of child) taking part in this study.

Signature:_____
Date:_____

Investigator Name_____

Signature:_____

Date:_____
In my opinion consent was freely given and the parent understands what is involved in the trial.
Witness Name:_____

Signature:_____
Date:_____

Appendix 3

Consent form for the children participating in the study

Dr Nigel Gilchrist 3377899

Does increasing calcium intake by the use of a dairy supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo
(dummy) supplement

I.....have read or an adult has read to me the information about the dairy calcium and bones study.

- My questions about what I have to do have been answered
- I know I can ask more questions if I want. I know I can ask my parents or telephone Rachel or Marie at 3377821.
- I know it is OK for me to change my mind about taking part at any time and that if I do there will not be any trouble at home or at school.
- I know that my parent/s/ guardian are happy for me to choose whether or not to take part in this study.

I.....agree to take part in this study.

Signed (child)..... Date:.....

Signed (Parent/guardian)..... Date:.....

Signed (Investigator):..... Date:.....

Appendix 4

Nutritional analysis of the supplement, both high calcium and placebo

MINISTRY OF AGRICULTURE
TE MANATU AHUWHENUA

MAF

Report No HB8017861

16 Mar 1998

NEW ZEALAND DAIRY BOARD
PO BOX 417
WELLINGTON

PLACEBO

Attention: E WILLIAMS

Order No PSR29268

Report of Analysis - REGD No 9999

Product Buttermilk Powder
Cypher GI17
Date samples received 06/03/98

Specification No 7800

Analysis	Sample	Result
Protein	221-53389	13.9 %m/m
	221-53468	13.7 %m/m
	221-53546	13.6 %m/m
Fat Roese Gottlieb	221-53389	31.1 %m/m
	221-53468	31.5 %m/m
	221-53546	31.6 %m/m
Moisture KF	221-53389	1.8 %m/m
	221-53468	1.7 %m/m
	221-53546	1.6 %m/m
Lactose Anhydrous	221-53389	18.2 %m/m
	221-53468	18.1 %m/m
	221-53546	18.4 %m/m
Calcium	221-53389	0.64 %m/m
	221-53468	0.49 %m/m
	221-53546	0.45 %m/m
Phosphorus Total	221-53389	0.53 %m/m
	221-53468	0.46 %m/m
	221-53546	0.43 %m/m
Vitamin A HPLC	221-53389	815 ug/100g
	221-53468	825 ug/100g
	221-53546	875 ug/100g
Vitamin D	221-53389	406 iu/100g
	221-53468	390 iu/100g
	221-53546	429 iu/100g
Vitamin C	221-53389	64 mg/100g
	221-53468	63 mg/100g
	221-53546	65 mg/100g

Comments
EX DAIRY PACKERS PSR 29268 217800 NO CALCUIM

A Conder

Anne Conder
Customer Services

MAF Quality Management
Lynfield, 131 Boundary Road, Blockhouse Bay, PO Box 41, Auckland 1, New Zealand.
Telephone 0-9-626 6026, Facsimile 0-9-627 9750.

+FAX +64 4 471 [illegible]

MINISTRY OF AGRICULTURE
TE MANATU AHUWHENUA

MAF

Report No HB8017858

15 Mar 1[illegible] 8

NEW ZEALAND DAIRY BOARD
PO BOX 417
WELLINGTON

Attention: E WILLIAMS

Order No [illegible]

Report of Analysis - REGD No 9999

STANDARD

Product Buttermilk Powder
Cypher EH21
Date samples received [illegible]

Specification No [illegible]

Analysis	Sample	Result
Protein	221-53547	13.2 %m/m
	221-53628	13.0 %m/m
	221-53709	13.1 %m/m
Fat Roese Gottlieb	221-53547	27.9 %m/m
	221-53628	27.[illegible] %m/m
	221-53709	27.[illegible] %m/m
Moisture KF	221-53547	1.[illegible] %m/m
	221-53628	1.[illegible] %m/m
	221-53709	1.[illegible] %m/m
Lactose Anhydrous	221-53547	57.[illegible] %m/m
	221-53628	58.[illegible] %m/m
	221-53709	57.[illegible] %m/m
Calcium	221-53547	1.[illegible] %m/m
	221-53628	1.[illegible] %m/m
	221-53709	1.[illegible] %m/m
Phosphorus Total	221-53547	1.[illegible] %m/m
	221-53628	1.04 %m/m
	221-53709	1.[illegible] %m/m
Vitamin A HPLC	221-53547	823 ug/100g
	221-53628	811 ug/100g
	221-53709	885 ug/100g
Vitamin D	221-53547	432 iu/100g
	221-53628	416 iu/100g
	221-53709	456 iu/100g
Vitamin C	221-53547	72 mg/100g
	221-53628	72 mg/100g
	221-53709	72 mg/100g

Comments
EX DAIRY PACKERS PSR 29268 MINIMUM CALCIUM

A Conder
Anne Conder
Customer Services

NUTRIENT	ANDEC (40g serve)		FERNLEAF BASE VARIANT (21g serve)	
	active	placebo	active	placebo
Protein (g / serve)	5.12	5.4	5.17	5.45
Fat (g / serve)	11	12.5	5.25	5.8
Vitamin D (IU / serve)	158	144	127	164
Phosphorous (mg / serve)	484	168	506	160
Calcium (mg / serve)	784	168	762	200
Magnesium (mg / serve)	not avail.	not avail.	29	15
Zinc (mg / serve)	not avail.	not avail	1.7	0.52

You stated that you were happy with the composition of the active blend in December. The vitamin D level of the placebo has matched the original ANDEC product more closely. I trust the difference in vitamin D levels between the active and placebo Fernleaf base variant product is not a problem. there is nothing I can do to match them more closely - as the amount of vitamin D added is so small.

I have made arrangements for these two powders to be sachet packed in Auckland and then proceed through final composition checks and microbiological clearance. I have no confirmed packaging date yet but can hopefully confirm this in our meeting at NZDB (meet at level 8 reception) on Monday 18th January.

Labeling requested as follows:

ACTIVE PRODUCT

Package in 21gm plain foil sachets.

Label 2 line code:

1st line: TIGER
2nd line: E: dd: mm: yr

PLACEBO PRODUCT

Package into 21gm plain foil sachets

Label 2 line code:

1st line: LION
2nd line: E: dd:mm:yr

Table 2: Compositional Analysis (NZDRI Chemistry Report No. 4179)

Component, % (w/w)	ANDEC - standard	ANDEC - placebo
Protein	13.40	12.95
Ash	3.45	7.45
Fat	31.0	27.4
Lactose*	15.7	15.4
Moisture content*	1.42	1.47
Calcium	0.40	2.06
Phosphorus	0.38	1.20

Note: * Lactose and moisture content determinations - different methods used by NZDRI and MAF, Lynfield

Vitamin D not determined. Paper by Rao & Mathur (1988)# indicates minimal loss under NZ storage conditions.

Sensory assessment:

Both samples were free-flowing, except that the standard-labelled powder showed slight clumping. The clumps broke up easily when touched. This clumping can be caused by the relatively high level of amorphous sucrose, and can be minimised by preventing the absorption and loss of moisture by the sugar crystals, *i.e.* avoiding any major shifts in relative humidity. Compression should also be avoided.

The samples reconstituted well in ambient and 40°C water. Informal tasting detected no off-flavours. It is possible that the chocolate flavour will mask any flavour deterioration (R. Lloyd, pers.comm., 1999).

Reference FAX 888 Page 1 of 2
Report No HB8080972

AgriQuality New Zealand Limited
Huarangi Rotorua

16 Nov 1998

WILLIAMS & ASSOCIATES
246A SUTHERLAND ROAD
LYALL BAY
WELLINGTON 6003
Fax 04 387 7010

Attention: EINIR WILLIAMS Order No E WILLIAMS

Report of Analysis - REGD No 5939

Product Powder Samples
Date samples received 06/11/98

Sample LION (placebo)

Analysis	Result	
Protein	26.0	%m/m
Fat Roese Gottlieb	28.1	%m/m
Moisture Karl Fischer	2.9	%m/m
Lactose Anhydrous	36.4	%m/m
(30 C) Aerobic Plate Count	200	cfu/g
Coliforms	Not Detected	/g
Coag Pos *Staphylococcus*	Not Detected	/g
Salmonella	Not Detected	/100g
Listeria	Not Detected	/63g
Bacillus cereus	<10	cfu/g
Bacillus cereus	Detected	/g
Clostridium perfringens	<10	cfu/g
Calcium	9700	mg/kg
Phosphorus Total	9000	mg/kg
Zinc	29	mg/kg
Magnesium	780	mg/kg
Vitamin A (Retinol)	950	ug/100g
Vitamin D	10.8	ug/100g
Vitamin C	56	mg/100g

920/100g x 17 = 164.9mg/ser
800/100g = 136mg/ser
2.9/100g = 0.49mg/ser
78/100g = 13.26mg/ser

Sample TIGER (active)

Analysis	Result	
Protein	24.0	%m/m
Fat Roese Gottlieb	24.1	%m/m
Moisture Karl Fischer	3.0	%m/m
Lactose Anhydrous	33.9	%m/m
(30 C) Aerobic Plate Count	120	cfu/g
Coliforms	Not Detected	/g
Coag Pos *Staphylococcus*	Not Detected	/g

Reference FAX_888 Page 2 of 2

Report No HB8080972

Page 2

16 Nov 1998

Analysis	Result		
Salmonella	Not Detected	/100g	
Listeria	Not Detected	/48g	
Bacillus cereus	10	cfu/g	
Bacillus cereus	Detected	/g	
Clostridium perfringens	<10	cfu/g	
Calcium	4450	mg/100g	756.5mg/serv.
Phosphorus Total	2900	mg/100g	493mg/serv.
Zinc	11.2	mg/100g	1.90mg/serv.
Magnesium		mg/100g	2?.?mg/serv.
Vitamin A (Re.. ol)	1100	ug/100g	
Vitamin D	10.5	ug/100g	
Vitamin C	49	mg/100g	

Comments

The Ministry of Health Revised Microbiological Guidelines for Water October 1995, suggests the following limits for Infant (Foods (Section 5.20c):

Aerobic Plate Count	<1000	cfu/g
Bacillus cereus	10	cfu/g
Clostridium perfringens	<1	cfu/g
Coagulase producing (stet) Staphylococcus	<1	cfu/g
Listeria monocytogenes	Abs	/25g
Presumptive Coliforms	<1.8	
Salmonella	Abs	

FX SACHET PACKAGING LTD

Original Report Signed by
Anne Conder
Customer Services

Appendix 5

Ethics Application

BUILDING STRONGER BONES

It has been well established that skeletal mass doubles through childhood, puberty and adolescence (Madivic V, New England Journal of Medicine 327: 119-120, 1992). At younger age groups the increase in bone mass can be in the order of 7 - 8% per year (Madivic V, The Journal of Rheumatology 19 (Suppl. 11133) 54-59, 1992) and the highest bone density gain in girls appears to be between the age of 10 to 14 (Theintz G et al, Journal of Clinical Endo and Metabolism 75: 1060-1065, 1992).

Interesting data has been presented in a younger age group showing significant effects in young pubertal girls in bone density and body composition (Chan et al, Journal of Paediatrics 126 (4) 551-556, 1995). Similar results have also been presented by ourselves and Margo Barker in abstract form at the International Workshop in Bone Health in Relationship to Dairy Products in Utrecht, 1995.

Work published by a Swiss group (Bonjour et al Clinical Investigation Vol 99; 6:1287-1294, 1997) has reported very interesting work in 149 pre-purbertal girls aged 8. They were randomised to received calcium enriched food or placebo food in which the calcium content had been removed. This study was a 24 month study and demonstrated an increase in bone density, bone mineral content and bone size at the wrist, femoral neck and lumbar spine. The best results were achieved with girls whose spontaneous pre-study calcium intake was low. This was accompanied by an increase in bone mineral content, bone size and statural height. These results were very encouraging and suggested a possible effect of calcium supplementation on skeletal growth that was observed 12 months after the cessation of the study. The additional information that has been obtained from the new ultrasound bone measurement technique has enabled the use of non ionising radiation to measure and follow bone density. This has been demonstrated by Jaworsai et al "Ultrasound bone measurement in paediatric subjects", Calc Tis Int 1995; 56:368-371. It is proposed in this study to examine in male and female school aged children the effect of a calcium enriched, cocoa flavoured product (ANDEC) on bone density and bone growth and size. It is also hoped to access the acceptability of such a programme in schools.

Title:

An 18 month placebo controlled calcium supplement study in 130 male and female school children ages 8 to 10; with a 12 month follow up study

Investigators:

NL Gilchrist

JG Turner

T Wilkinson

JR Elliot

Aims:

1 Acceptability of chocolate milk based powder as a calcium supplement.

2 That this provides enough calcium per day for growing children.

3 Promotes strong bone growth.

We have approached two primary schools, Redcliffs and Halswell, who have agreed, after consultation with the Board of Trustees of the schools, to be involved in this study. The classes at the school will be approached in February, 1998 with structured meetings with the teachers, pupils and parents to discuss the aims and methodology of the study. Informed consent will be then obtained from the parents or guardians.

Methodology:

Two classes in both schools are anticipated to take part in this study. It is anticipated that after informative meetings and signing of informed consent study procedures will be commenced.

1 Bone Densitometry:

Bone density will be performed by the DPXL or DPXIQ on five occasions at: 0, 6, 12, 18 months and then 12 months after the cessation of the study.

Sites to be scanned will be the total body, total hip, femur, lumbar spine and also an ultrasound of the heel bone (calcaneus) will be performed.

The radiation dose is minimal and advice on risks and exposure has been sought from the National Radiation Laboratory, Wellington. (See enclosed letter).

2 Anthropometric Data:

Height and weight will be measured at baseline then again at 6, 12, 18 and 30 months. Medical questionnaire will be administered at baseline and at the follow up study (see attached questionnaire). Purbertal status will be access using a self administered questionnaire (see enclosed).

3 Calcium Intake and Compliance:

The calcium intake will be assessed via a calcium Food Frequency Questionnaire at baseline, 6, 12, 18 months and at follow up. A compliance measurement will be undertaken by the children and will be situated in the classroom. This will comprise of a clipboard which will be ticked off when the child takes his or her supplement in the morning and afternoon. The dietitian's roll will be education and recruitment at school, dispensing and monitoring of the product, administrating and compiling a modified calcium food frequency questionnaire, monitoring compliance and administrating and collating the acceptability questionnaire (see enclosed).

4 Administrating of the calcium/placebo supplement:

The supplement, ANDEC (see enclosed nutritional information), is supplied in a powered form. It is anticipated that it will be reconstituted with water and kept in a cooler dispenser in an area of the classroom that would be dedicated to bone health and healthy living. The volume would allow for 400mg of calcium per polystyrene container to be administered to the child in the morning and the afternoon. This would give a total daily intake of 800mg of calcium. Individual sachets would be provided for weekend and holiday periods. Normal use of calcium containing foods would not be restricted in either the supplement or the placebo group. It is planned that a general talk on healthy food and eating habits will be given to the four classes by the dietitian.

5 Study Procedures:

All measurement procedures will be performed by research nursing staff at The Princess Margaret Hospital. It is anticipated that the classes will be bused to The Princess Margaret Hospital where their study procedures can be performed. It may also be possible to involved the children in a general health education visit of various areas of the hospital when they attend. Research nurses will be involved in the coordination of the visits and data collection, medical questionnaires and measurement of height and weight, signing of informed consent as a witness and performing bone mineral density.

6 Inclusion Criteria:

Healthy boys and girls whose parents/guardians are able to give informed consent.

7 Exclusion Criteria:

Those children with significant medical health problems, milk allergy, medication that would affect bone growth, (inhaled and oral steroids, vitamin D, anticonvulsants and thiazide diuretics). Any child whose parent/guardian is unable to give informed consent and any child who is thought to have significant psychological problems which would preclude them from entering an 18 month long study.

8 Study Conduct:

The study will be conducted according to Good Clinical Practice Guidelines. I have included our study personnel and our standard operating procedures.

9 Study Flow Diagram:

	Baseline	6 months	12 months	18 months	30 months
Height	√	√	√	√	√
Weight	√	√	√	√	√
Medical Questionnaire	√				√
Calcium intake assessment	√	√	√	√	√
Compliance questionnaire	√	√	√	√	√
Acceptability	√	√	√	√	√
Purbertal status	√				√
Supplement	← ---------- →				Follow up
Bone density:					
Spine	√	√	√	√	√
Total Hip	√	√	√	√	√
Femur	√	√	√	√	√
Total body	√	√	√	√	√
Ultrasound	√	√	√	√	√

Statistics:

Statistical advice is being sought from Dr Chris Frampton. Using the similar data from Bonjour's group using an alpha = 0.05, beta = 0.20, ie a power of 80 percent.

1 At the formal neck the placebo change of 2 percent per annum and the supplemented group change 4 percent per annum.

To show a significant improvement in favour of the supplemented changes 3 percent per annum we need 252 per group, if supplemented increases 4 percent we need 64 per group, if 5 percent change is observed we need 29 per group.

2 At the trochanter if the placebo is 3 percent per annum and the supplemented change is 4 percent.

If we see a 4.5 percent increase in the supplemented group we will need 113 per group, 5 percent difference we will need 64 in each group and if a 6 percent change is needed we will need 29 per group.

3 At the spine the placebo change is 4 percent and the supplemented change is 4 percent.

If we are to show a 5.5. percent increase in the supplemented group we will need a 113 per group, however, if there is a 6 percent increase in the supplemented group we will need 64 subjects per group and if the increase is 7 percent we will only need 29 per group.

Looking at these results it is predicted that the minimum number of people we would need per group is between 60 and 70, taking the total number of subjects up to 130.

Statistical Analysis:

Comparison between two groups at baseline will be made using independent *t* tests, ANOVA for repeated measures will be used to compare the changes over time between the two groups. When the ANOVA indicates a significant interaction between time and group, a'posteriori

comparison between groups at individual times will be made using less significance difference test. Correlation between the changes in bone mineral densities, bone mineral content and dietary calcium intake at baseline and during the study, total body fat and lean muscle mass will be made using Pierson correlation coefficient.

Budget:

A budget for the study is enclosed and a break down on GST is also included. It is anticipated that this grant be paid in three separate payments; a start up grant of a third at the beginning of the study, a further third at 12 months and the final third at the end of the follow up study.

Start Time and Reporting Schedules:

1 It is anticipated that this proposal, along with the Ethics Committee application (see enclosed) will be submitted to the New Zealand Dairy Board and Ethics Committee by the beginning of December.

2 The first payment will be made available to the Canterbury Geriatric Medical Research Trust by January, 1998.

3 Six monthly reports on the study will be prepared.

4 An end of study report will be prepared to enable it to be published in an internationally refereed journal and also to be presented at local and international scientific meetings.

National Application form for Ethical Approval of a Research Project

INFORMATION FOR APPLICANTS:

Note- Researchers should complete this form in conjunction with the HRC Guidelines for Researchers.

1. Applications should be typewritten or word processed using the same format as this form. The response to questions should be in a different font to the questions.

2. **The original and 12 copies** should be forwarded to:
The Administrator, Southern Regional Health Authority Ethics Committee (Canterbury), Fourth Floor, 144 Kilmore Street, P O Box 3877, Christchurch.

   do not staple application forms.

3. All questions must be reproduced and either completed or marked not applicable. Copies of information sheets, consent forms, questionnaires and other relevant documentation enclosed.

4. All clinical trial applications must be accompanied by the appropriate Accident Rehabilitation and Compensation Insurance Corporation (ARCIC) statutory declaration form.

   A clinical trial has been defined by the Ministry of Health and ARCIC as "*... any research in human subjects conducted to gain new knowledge into mental and physical health and disease. It would exclude research based on the analysis of secondary sources of health information. Clinical trials involve a wide range of health professionals with different qualifications, skills and expertise and would usually be conducted in hospitals, other health care settings, the community and academic host institutions.*"

5. In the case of pharmaceutical trials please enclose two copies of the sponsoring company's protocol as well as completing the relevant sections of our application form. It is not acceptable to refer to the company's protocol instead of completing the appropriate sections of the application form.

6. Each page should be numbered.

7. A copy of the application form is available on disk from all accredited ethics committees.

CHECKLIST FOR APPLICANTS

1. All relevant sections of application form completed with N/A sections marked
2. Consent forms, information sheets and questionnaires enclosed
3. Part IV signed by applicant, Head of Department or Dean, or CEO/Manager
4. If required, Accident Rehabilitation and Insurance Corporation Declaration correctly witnessed (Form A)
5. If required, a copy of the compensation provisions by the sponsoring company
6. If required, two copies of pharmaceutical company protocol enclosed
7. Investigators brochure (2 Copies) enclosed
8. Standing Committee on Therapeutic Trials (SCOTT) Approval attached if drug unregistered
9. National Radiation Laboratory Approval if relevant
10. Is Gene Therapy Advisory Committee approval required?
11. Is National Ethics Committee on assisted Human Reproduction (NECHAR)

Consultation/Approval required?

12. Copies of notices and/or advertisements for participants

If you have any questions in regard to your application, or if you require any assistance, please contact Sally Cook, telephone 372 1000

NATIONAL APPLICATION FORM FOR ETHICAL APPROVAL OF A RESEARCH PROJECT

PART I : BASIC INFORMATION

It is proposed that data from this page will be entered on to the National Register

1. Full Project Title
   An 18 month placebo controlled calcium supplementation study in 130 male and female school children aged 8 to 10; with a 12 month follow up study, to assess the influence of increased calcium intake on the acquisition of peak bone mass and bone growth
2. Short Project Title (lay title)
   (Use a description readily understandable by lay persons. This title is also to be used on consent form and information sheet.)
   Does increasing calcium intake by the use of a supplement in 8-10 year old children make their bones stronger and bigger than those of another group of children of the same age who take a placebo (dummy) supplement
3. Principal Investigators Name and Position:
   (If this is supervised work then the student should be listed as the principal investigator)
   Dr Nigel Gilchrist
4. Department/location of Principal Investigator (All correspondence will go to this address)
   Third floor, The Princess Margaret Hospital, PO Box 731, Christchurch
   Phone Number:*3377820*
   Fax Number:*3377857*
   E-mail Address:*enquiries@gm-research.org.nz*
5. Co-investigators Name and Position:
   (Include the names and qualifications of all persons who will be conducting the research)
   Dr J Turner Medical Director, Nuclear Medicine Department, Christchurch Hospital
   Dr T Wilkinson Senior Lecturer/ Consultant, Older Person Health, TPMH
   Dr J Elliot Consultant, Older Persons Health, TPMH
6. Supervisors Name and Position (where this is supervised work):
   N/A
7. Proposed Starting Date:
   (Note: this should not be prior to the notification of ethical approval)
   February/ March 1998
8. Proposed Finishing Date:
   January 2000
9. Duration of Project:*18 months with an 12 month follow up period = 30 months*
   (Note: a final report will be required within 3 months of completion of the study)

10. **Proposed and/or Required Number of Participants:** *130 boys and girls*

11. **Is this a multicentre project? NO**
(If YES, please complete question 1. in Part II)

12. **Do you request a fast track procedure? NO**
(This procedure will be used only for certain projects. See Guidelines/Explanatory Notes)

PART II : PROJECT SUMMARY

Please reproduce all of the questions and provide answers in a different typeface. Those questions which do not apply to your project should be marked N/A (Not applicable)

1. MULTICENTRE PROPOSALS

1.1 Is this a multicentre study? NO

1.1.1 If yes, please provide name and address of the principal investigator in New Zealand, if any, and local contact name and address

1.1.2 Please list all other New Zealand centres involved in the study

Note: **If there is a principal investigator in New Zealand, protocols will be dealt with by the principal investigator's local Ethics Committee who will correspond with other Ethics Committees as necessary. Please see details of the multicentre process appended.**

1.2 Has the protocol been reviewed by any other Ethics Committee in New Zealand? NO *(If yes, please name and enclose copies of any relevant correspondence)*

2. FUNDING

2.1 What is the proposed source of funding?*A grant to cover study costs will be provided by NZ Dairy Board*

2.2 Give name(s) of proposed funder(s) and date when result of funding application will be known *December 1997 NZ Dairy Board*

3. SCIENTIFIC ASSESSMENT

Has this project been scientifically assessed by independent review?
Yes
If yes:
By whom? (Name and Position) *Associate Professor I Reid, Department of Medicine, Auckland Medical School, Auckland*
Dr Chris Frampton has been asked for his opinion.
On reviewing all the data it was decided that 130 subjects would be studied over 18 months with 6 monthly investigations. Statistical method- comparisons between the two groups will be made by independent T-tests and ANOVA for repeated measures.
(A copy of the report should be enclosed.)
If **no**:
Is it intended to have the project scientifically assessed and by whom?

4. SUMMARY

Give a brief summary, not more than 200 words, of the study
(Please write in language which will make the project comprehensible to lay persons)

This Study will involve a total of 130 male and female school students at two Christchurch schools. The age of the students will be 8-10 years and because of this written informed consent will be given by parents. This is a placebo controlled study in which the children will be randomised to receive either placebo or active Andec calcium supplement.

Following the acquisition of parental consent students will undergo the following procedures: Height, weight, medical questionnaire (parents), dietary questionnaires (with parental involvement), assessment of pubertal status (self administered questionnaire), and bone mineral density measured by DPX-L at the total body, spine, total hip and femur using paediatric software. Ultrasound bone density measurements will also be performed at the heel.

Dietary questionnaires, height, weight and bone density measurement will be repeated 6 monthly for the 18 month period .

At the end of the 12 month follow up period all initial assessments will be repeated.

No blood or urine tests are planned.

PART III : PROJECT DETAILS

Please reproduce all of the questions and provide answers in a different typeface. Those questions which do not apply to your project should be marked N/A (Not applicable)

A SCIENTIFIC BASIS

1. AIMS OF PROJECT

Note: The description of the project should not use jargon. References accessible to the Committee should be given.

1.1 What does the project aim to investigate? *To assess the influence of increased calcium intake on the acquisition of bone mass and size in children aged 8-10 years*

1.2 Is it based on specific hypotheses? (If so, state them briefly)

Studies carried out by this group and a similar group in Switzerland have shown a beneficial gain in bone density in children and teenagers who are given dairy products containing calcium. This effect was sustained at 12 months after ceasing the dairy calcium supplement which is not seen with calcium tablets. Furthermore a beneficial increase in the size of bone was seen, indicating not only increase in density but bones of a greater size and possibly strength.

1.3 What is the potential significance of this project for improved health care for the community, and for the advancement of knowledge?

I increasing calcium intake is proven to influence the acquisition of bone mass in this age group it could lead to recommendations for calcium requirements in children

1.4 Is this project to be used to formulate policy?

An increasing amount of data on the benefits of dietary calcium on bones in children and young teenagers is appearing. This will lead to definite daily dietary calcium recommendations and to increased education in schools as part of their curriculum.

2. RESEARCHER QUALIFICATIONS

2.1 What experience do the researchers have in this type of research? (Please include a brief curriculum vitae, and details of recent publications.)

3. RESEARCH METHODS

(The following 2 sections should be described in lay terms)

3.1 What is the method of analysis? If this is (wholly or partly) quantitative research, please give the following:

- Describe the statistical method that will be used

Comparisons between the two groups at baseline will be made using independent T-tests, ANOVA for repeated measures will be used to compare changes over time between the two groups. When the ANOVA indicates a significant interaction between time and group, a-posteriori comparisons between the groups at individual times will be made using Least Significant Difference Test. Correlations between the changes in BMD's and dietary calcium intake, total body fat and lean muscle mass will be made using Pearson's correlation co-efficient.

- Has specialist statistical advice been obtained? If so, from whom? *Dr C Frampton*
- A brief statistical report should be included, if appropriate.
- What is the proposed power of the study?

Power analysis for the calcium supplementation study using results from Bonjour's paper and using alpha=0.05, beta=0.20 i.e. power of 80%

a) *Femoral neck*

Placebo change 2.0% per annum, SD change 4.0%; to show a significant improvement in favour of supplement if supplemented change is 3% per annum; need n = 252 per group; if supplemented is 4.0% need n = 64 per group; if 5% need n = 29 per group.

b) *Trochanter*

Placebo is 3% per annum, SD change 4.0%; to show supplement at 4.5% per annum need n = 113 per group; supplement 5.0% need n = 64 per group; if 6.0% need n = 29 per group.

c) *Spine*

Placebo is 4%, SD change 4.0%; to show 5.5% supplement need n = 113 per group; to show 6.0% need n = 64 per group; to show 7.0% in supplemented need n = 29 per group.

3.2 If the research methods are (wholly or partly) qualitative, give a brief description of their theoretical basis:

3.3 Describe the study design. Include diagrams and charts to illustrate if necessary.
This is an 18 month study with a 12 month follow up. The study is placebo controlled. See attached flow chart

4. PROCEDURES

4.1 What procedures will be carried out? Include all tests to be carried out on samples.

1.Medical and dietary questionnaires
2. Anthropometric measurements

3. *Bone mineral density measurements both DPX-L and ultrasound*

4.2 How many visits/admissions of participants will this project involve? Give also an estimate of total time involved for participants.

5 visits are required.2 hours per visit

4.3 Describe any interview methods involved and attach copies of any questionnaires being used.

Medical and calcium questionnaires i.e. Food Frequency Questionnaire, Medical questionnaire and Tanner self assessment of pubertal status (A parent or guardian will be present during this assessment)

4.4 If blood, tissue or body fluid samples are to be obtained, state type, use, access to, frequency, number of samples, total volume, means of storage, length of proposed storage and method of disposal. *none*

4.5 Will any drugs be administered? If so, then the attached Drug Administration Form for trials involving the administration of drugs, MUST be completed.

A dietary supplement or matched placebo will be administered daily. This is a sachet of powder which is dissolved in water and is not a drug.

5. RISKS AND SAFETY

5.1 Who will carry out the research procedures? *Dr Nigel Gilchrist and study staff*

5.2 Where will the research procedures take place? *The Princess Margaret Hospital, Christchurch*

5.3 Is there scientific evidence of any physical or psychological risks? *No*

5.4 What arrangements will be made for monitoring and detecting adverse outcomes?

All participants and their parents will be able to contact the study staff at any time.

5.5 Will any potential toxins, mutagens or teratogens be used? If so outline the justification for their use. *No*

5.6 Will any radiation or radioactive substances be used?

Note: If any form of radiation is being used please answer the following:

5.6.1 Under whose license is the radiation being used? *Dr J Turner*

5.6.2 Has National Radiation Laboratory (NRL) approval been sought to use radiation in this study? *YES*

If *yes*, please enclose a copy of the approval, and contact name and phone number. *enclosed*

If *no*, please explain why:

5.7 What facilities are there for dealing with emergencies? *The study is carried out at The Princess Margaret Hospital where all standard procedures for emergencies exist.*

B BUDGET AND USE OF RESOURCES

6. BUDGET

6.1 Please supply a budget for this study, including a description of all financial support to be received by the researchers, such as fees or expenses.

6.2 Does the researcher, the host department or the host institution, have any financial interest in the outcome of this research? *No*

6.3 Will there be payments according to the number of participants recruited? If so, please specify. *No*

Note: Investigators are entitled to adequate and reasonable reimbursement for their own time on the project. Funds arising additional to actual expenses for the Investigators own time, should be paid to a specified Trust Account.

7. RESOURCE IMPLICATIONS

7.1 Does the study involve the use of healthcare resources? *No*

7.2 What effect will this use of resources have on waiting list times for patients i.e., for diagnostic tests or for standard treatments? *none*

7.3 What are the likely benefits to participants? *Improved knowledge of subjects and school on bone and bone health.*

C PARTICIPANTS

8. SAMPLE

8.1 How will potential participants be identified? *Through their school*

8.1.1 Where will potential participants be approached? For example in an outpatient clinic? (If appropriate describe by type e.g. students.)

Written information will be sent to parents

8.1.2 Who will make the initial approach to potential participants?

Dr Gilchrist and research staff and school staff.

8.1.3 What relationship, (if any) will participants have to the researchers? *None*

8.2 How many participants is it intended to recruit?*130*

8.3 What are the inclusion/exclusion criteria?

Inclusion:
Healthy 8 - 10 year old boys and girls

Exclusion
Allergy to dairy products
Drugs that influence bone ie steroids oral or inhaled, anti convulsants, thiazide diuretics, Vitamin D
Major disease states
Parent/ Guardian unable to consent
Significant Psychological problems

8.4 How will participants be recruited, eg. advertisements, notices

Through two primary schools. The Headmasters and Boards of Trustees of both Halswell and Redcliffs primary schools have been approached for their potential involvement in the study.

***Copies of any advertisements/notices to be included with this application.**

9. FINANCIAL COSTS AND PAYMENTS

9.1 Will there be any financial cost to the participant? Give examples. *No*

9.2 Will the study drug/treatment continue to be available to the participant after the study ends? If yes, will there be a cost? *The supplement will be commercially available*

9.3 Will any payments be made to participants or will they gain materially in other ways from participating in this project? *No*
If yes, please supply details:

9.4 What are the additional benefits to the participants from participating in this project? *It is anticipated that the Canterbury Geriatric medical Research Trust will donate to the school resources that will benefit the participants and the school.*

10. COMPENSATION OF PARTICIPANTS

Is this a clinical trial as defined in the ARCIC Guidelines? YES
If yes, please answer the following:

10.1 Is the trial being carried out principally for the benefit of a manufacturer or distributor of the drug or item in respect of which the trial is taking place? *Yes*

10.2 a) If the answer to 10.1 is NO please complete Statutory Declaration Form A (and provide participants with an explanation of the ARCIC requirements for and level of compensation)

b) If the answer to 10.2 is YES, please complete Statutory Declaration Form B and answer questions 10.3, 10.4 and 10.5.

Note: This information is also to be included in the Patient Information Sheet

10.3 What type of injury/adverse consequence resulting from participation in the trial has the manufacturer or distributor undertaken to cover:

a) any injury (mental or physical) *yes*
b) only serious or disabling injuries. *no*
c) only physical injuries *no*
d) only physical injuries resulting from the trial drug or item, but not from any other aspect of the trial. *no*
e) physical and mental injury resulting from the trial drug or item, but not from any other aspect of the trial. *no*
f) any other qualification *no*

10.4 What type of compensation has the manufacturer or distributor agreed to

pay?

a) medical expenses *yes*
b) pain and suffering *no*
c) loss of earnings *n/a*
d) loss of earning capacity *n/a*
e) loss of potential earnings *no*
f) any other financial loss or expenses *no*
g) funeral costs *no*
h) dependants' allowances *no*

10.5 Exclusion clauses:

a) Has the manufacturer or distributor limited or excluded liability if the injury is attributable to the negligence of someone other than the manufacturer or distributor? (such as negligence by the investigator, research staff, the hospital or institution, or the participant). *no*
b) Has the manufacturer or distributor limited or excluded liability if the injury resulted from a deviation from the study protocol by someone other than the manufacturer or distributor? *no*
c) Is company liability limited in any other way?

11. INFORMATION AND CONSENT

Note: Principal Investigators should make themselves familiar with the provisions of the Code of Health and Disability Services Consumers' Rights, obtainable from the office of the Health and Disability Commissioner.

Consent should be obtained in writing, unless there are good reasons to the contrary. If consent is not to be obtained in writing the justification should be given and the circumstances under which consent is obtained should be recorded. A protocol should be attached, indicating the form of words to be used on the Consent Form.

11.1 Who will explain the project to potential participants? *Dr Nigel Gilchrist and staff will explain the study to parents and students*
11.2 Is there any special relationship between the person explaining the project, or any of the investigators and the participants (e.g. teacher/student; doctor/patient)?*No*
11.3 When and where will the explanation be given? *At a meeting*
11.4 Will a competent interpreter be available, if required? *Yes if applicable*
11.5 How much time will be allowed for the potential participant to decide about taking part? *As much as they require to make an informed decision*
11.6 Will the participants be capable of giving consent themselves? *No*
- If not, to whom will the project be explained and who will give consent? *Parental consent will be obtained*

11.7 In what form (written, or oral) will consent be obtained? If oral consent only, state reasons why. *Written*

Note: Copies of consent form and of written information to participants must be appended.

12. CONFIDENTIALITY AND USE OF RESULTS

Note: Principal Investigators should make themselves familiar with the requirements of the Privacy Act (1993), and the Health Information Privacy Code (1994).

12.1 How will data be handled to safeguard confidentiality (both during and after completion of the research project)?*Participants will be identified only by initials and study number on all study documents*

12.2 How long will the data from the study be kept and who will be responsible for its safe keeping? *As required by the NZ Interim guidelines for Good Clinical Practice*

12.3 Who will have access to the raw data and/or clinical records during, or after, the study? *Only study staff and regulatory agencies*

12.4 If recordings are made, will participants be offered the opportunity to edit the transcripts of the recordings? Yes/No *No recordings*

12.5 What will be done with the raw data when the study is finished? If audio or video tapes are used how will these be stored and disposed of? *N/A*

12.6 Describe any arrangements to make results available to participants, including whether they will be offered their audio tapes or videos. *N/A*

12.7 Is it intended to inform the participants' GP of the results of the investigations, if the participant consents? If NO, outline the reasons. *Yes*
(NOTE: Specific consent to inform the GP should be included on the consent form. If it is regarded as essential to inform the GP, then refusal of a participant should constitute an exclusion criterion)

12.8 Will any restriction be placed on publication of results? If yes, please supply details.
No restriction will be placed on results

13. CULTURAL ISSUES

It is important that issues regarding cultural safety are addressed when research involves participants from various ethnic groups. This issue should be addressed even when a minority of participants from other cultural groups are involved. Where a particular nationality is the principal subject of the research, consultations must be undertaken with appropriate parties and this process outlined in the application. Note: Include research non-specific to Maori but involving Maori participants.

13.1 Are there any aspects of the research which might raise specific cultural issues? NO

13.1.1 The following checklist has been devised to assist researchers complete their research proposals where Maori participants/resources are party to the process:

1. Does your project impact on Maori Health? NO
2. If the response to Question 1 is NO, outline the reason to support this. *The number of Maori students in both schools is very low.*
3. Outline the consultation process undertaken prior to methodology being consolidated.

13.1.2 Does your research involve other ethnic or cultural groups? NO
If YES, what consultation has taken place with the relevant communities
Who was contacted: Phone No.:
How was the process undertaken:
What results were achieved:
What advice was given:

14. OTHER ETHICAL ISSUES

14.1 Do you see any other ethical issues arising from this project, other than those already dealt with in your answers?

No

Thank you for your assistance in helping us assess your project fully. Please now complete the declaration's page (Part IV) and then enclose a completed Drug Administration Form (if applicable), and ARCIC Declaration (if applicable)

PART IV: DECLARATIONS

1. DECLARATION BY PRINCIPAL INVESTIGATOR

The information supplied in this application is, to the best of my knowledge and belief, accurate. I have considered the ethical issues involved in this research and believe that I have adequately addressed them in this application. I understand that if the protocol for this research changes in any way I must inform the Ethics Committee.

Name of Principal Investigator (please print): DR N GILCHRIST

Signature of Principal Investigator:

Date: 28/11/97

2. Declaration by the Head of the Department or Service Manager in which the principal investigator is located**

I have read the application and believe it to be scientifically and ethically sound. I approve the Research Design. I give my consent for the application to be forwarded to the Ethics Committee.

Name of Head of Department or Service Manager (please print): DR N MILLER DR NIGEL D. MILLAR

Signature of Head of Department or Service Manager:

Date: 28.11.97.

** (Note: Where the head of department is also one of the investigators, the head of department declaration must be signed by the appropriate Dean, or relevant senior officer)

3. Declaration by the General Manager of the health service in which the Principal Investigator is being undertaken (If Applicable)

I have reviewed the proposal for cost, resources, and administrative aspects and issues regarding patient participation and staff involvement. The proposal has my approval subject to the consent of the Ethics Committee.

Name of General Manager (Please Print): BEV O'CAIN

Signature:

MINISTRY OF HEALTH
MANATU HAUORA

NATIONAL RADIATION LABORATORY
108 Victoria Street
P O Box 25099
Christchurch
New Zealand
Phone 64-3-366 5059

FACSIMILE COVER PAGE

Ref. No: 17/2

To: Dr Nigel Gilchrist

Date: 26 November 1997

Company: The Princess Margaret Hospital Christchurch

Fax Number: 337 7857

Return Fax No: 64-3-366 1156

From: John Le Heron

Internet Address: johnleh@nrl.moh.govt.nz

No. of Pages: 1
(including cover sheet)

Subject: DEXA doses and risks in proposed study

Message

Dear Nigel

Thank you for the information concerning the use of radiation in the proposed study of children. I have estimated the effective doses for the proposed DEXA procedures.

The estimated effective doses for a child (aged 8 to 10) undergoing scans with a Lunar DPX IQ for the following procedures using the protocols indicated in your letter are: total hip, 2 μSv; femur, less than 0.5 μSv; AP spine, 1.5 μSv; total body, 0.01 μSv. This gives an approximate total effective dose of 20 μSv for the complete study - 5 lots of investigations.

Using a lifetime risk factor of 14% per sievert for cancers and hereditary effects for children gives an estimated lifetime risk of about 3 in 1 000 000 for the DEXA procedures in the proposed study.

As a perspective, the effective dose received by all persons living in New Zealand each year from natural background radiation is about 2000 microsieverts.

If the information in this fax is included in your submission, the National Radiation Laboratory would ratify the proposal as correctly presenting the dose and risk from the use of radiation for the Ethics Committee's consideration.

If you have any questions please do not hesitate to contact the Laboratory.

Yours sincerely

John Le Heron
Senior Scientific Advisor

Appendix 6

Visit sheet for data collection (stored in the child's notes)

School Study Bone Density Cover Sheet

NAME:.....

INTIALS:..... NUMBER: .....

Date of Birth:..... GP:.....

VISIT Baseline☐ 6 month☐ 12 month☐
18 months☐ Follow up☐

HEIGHT X3 :..... MEAN:.....

WEIGHT:.....

TOTAL BODY THICKNESS:.....
.....

AP SPINE THICKNESS:.....
.....

HIP THICKNESS:.....
.....

ACHILLES: SHIM LIGHT BLUE☐ MEDIUM BLUE☐ DARK BLUE☐

ANY REANALYSES: YES/NO

SPECIFY:.....

Appendix 7

Calcium Food Frequency Questionnaire

CALCIUM SUPPLEMENTATION STUDY

NAME:_____ Code Number: _____ Date: _____

What type of milk do you usually drink? ☐ Trim milk ☐ Whole milk
☐ Lite milk ☐ Soy milk
☐ Slim N Fit milk

How many cups of tea or coffee with milk do you usually drink? ...../day or ...../week
How many glasses of milk do you usually drink? ...../day or ...../week
How many other drinks with milk do you usually drink? ...../day or ...../week
How many slices of white or brown bread do you usually eat? ...../day or ...../week
How many slices of wholemeal bread do you usually eat? ...../day or ...../week
How many eggs do you usually eat? ...../day or ...../week
How many plain biscuits or crackers do you usually eat? ...../day or ...../week
How many chocolate biscuits do you usually eat? ...../day or ...../week

How often do you usually eat the following foods?

	twice or more a day	once a day	5 or 6 times a week	3 or 4 times a week	once or twice a week	once or twice a month	rarely or never
breakfast cereal with milk on it	☐	☐	☐	☐	☐	☐	☐
natural yoghurt	☐	☐	☐	☐	☐	☐	☐
fruit yoghurt	☐	☐	☐	☐	☐	☐	☐
frozen yoghurt	☐	☐	☐	☐	☐	☐	☐
hard cheeses (eg cheddar)	☐	☐	☐	☐	☐	☐	☐
cream cheese	☐	☐	☐	☐	☐	☐	☐
cottage cheese	☐	☐	☐	☐	☐	☐	☐
soft cheeses (eg Camenbert)	☐	☐	☐	☐	☐	☐	☐
ice-cream	☐	☐	☐	☐	☐	☐	☐
milkshakes (including McDonald's)	☐	☐	☐	☐	☐	☐	☐
flavoured milk	☐	☐	☐	☐	☐	☐	☐

How often do you usually eat the following foods ?

	twice or more a day	once a day	5or 6 times a week	3 or 4 times a week	once or twice a week	once or twice a month	rarely or never
white meat (eg. chicken)	☐	☐	☐	☐	☐	☐	☐
red meat (eg. beef, lamb)	☐	☐	☐	☐	☐	☐	☐
soybeans or tofu	☐	☐	☐	☐	☐	☐	☐
tinned salmon	☐	☐	☐	☐	☐	☐	☐
tinned sardines	☐	☐	☐	☐	☐	☐	☐
prawns or shrimps	☐	☐	☐	☐	☐	☐	☐
fresh fish	☐	☐	☐	☐	☐	☐	☐
battered fish	☐	☐	☐	☐	☐	☐	☐
fish cakes	☐	☐	☐	☐	☐	☐	☐
shellfish	☐	☐	☐	☐	☐	☐	☐
cake	☐	☐	☐	☐	☐	☐	☐
green leafy vegetables	☐	☐	☐	☐	☐	☐	☐
dried fruit (eg. figs, raisins)	☐	☐	☐	☐	☐	☐	☐
nuts	☐	☐	☐	☐	☐	☐	☐
muesli bars	☐	☐	☐	☐	☐	☐	☐
roll ups	☐	☐	☐	☐	☐	☐	☐
porridge made with milk	☐	☐	☐	☐	☐	☐	☐
muesli	☐	☐	☐	☐	☐	☐	☐
allbran cereal	☐	☐	☐	☐	☐	☐	☐
desserts made with milk	☐	☐	☐	☐	☐	☐	☐
milk-based sauces	☐	☐	☐	☐	☐	☐	☐
soups made with milk	☐	☐	☐	☐	☐	☐	☐

Appendix 8

Medical Questionnaire

The information obtained as part of this study is strictly confidential and will be used for research purposes

MEDICAL

QUESTIONNAIRE

MEDICAL QUESTIONNAIRE

The information obtained as part of this study is strictly confidential and will be used for research purposes

A: GENERAL INFORMATION

1. **Your Child's Name**

.....
First Names Surname

2. **Gender:** Male Female (Circle One)

3. **Date of Birth**

_____/_____/_____
Day Month Year

4. **What is your child's ethnic background? (please mark the relevant box) Tick as many circles as you need to show which ethnic group(s) your child belongs to:**

- ✝ NZ M♠ori
- ✝ NZ European or P♠keha
- ✝ Other European _____ Which of these groups?
  - ✝ English
  - ✝ Dutch
  - ✝ Australian
  - ✝ Scottish
  - ✝ Irish
  - ✝ Other ——
- ✝ Samoan
- ✝ Cook Island Maori
- ✝ Tongan
- ✝ Niuean
- ✝ Chinese
- ✝ Indian
- ✝ Other _____

Print your ethnic group(s)
.....
.....
.....

5. **HAS YOUR CHILD EVER HAD ANY ALLERGY TO DAIRY PRODUCTS ?:**
YES NO (Please circle one)

The information obtained as part of this study is strictly confidential and will be used for research purposes

B: FAMILY HISTORY

1. Has your child's mother or grandmother (on father's or mother's side) broken or fractured a bone? (please answer for your child's natural mother OR grandmother)

† yes † no † don't know

If yes please write below each bone broken (for example "wrist" or " spine") , the approximate age when fracture occurred and how it happened.

Name(s) of bone (s) Broken	How did it happen?	Date occurred and age

2. Does (did) your child's grandmother have a rounded or stooped back and/or lose height.

† yes † no

The information obtained as part of this study is strictly confidential and will be used for research purposes

C: FEMALE HISTORY (IF APPLICABLE)
Please answer this section if your daughter has started her periods

1. What age did your daughter's periods start? .....years

How many periods does she have a year?
- ☨ 11-13 (approximately 1 per month)
- ☨ 3-7 (approximately one per 2 mths)
- ☨ one or less

What are her periods like?
- ☨ normal
- ☨ scanty
- ☨ heavy

The information obtained as part of this study is strictly confidential and will be used for research purposes

D:FALLS AND FRACTURES

Have your child ever fractured (broken) a bone ?

IF YES Please note below the names of all the bone (s) broken, the age when they occurred and how they happened.

Name of bone(s) broken	How did it happen ?	age

MEDICAL QUESTIONNAIRE

The information obtained as part of this study is strictly confidential and will be used for research purposes

E:MEDICATION INCLUDING VITAMIN AND MINERAL SUPPLEMENTS

Has your child ever taken:

Oral Steroids	†	yes	†	no
Calcium	†	yes	†	no
Diuretics	†	yes	†	no

If you answered yes to any of the above please enter the details in the table below along with your child's current medication

Please note below any medications your child is taking now .

Name of medicine	Brand (for vitamin and minerals)	Strength if known ie 25mg	Dose - how many tablets taken each time	How often - number of times per day	Date Started	Stop Date	Why did your child take the medicine eg cold, asthma etc

Comments:

.....

.....

MEDICAL QUESTIONNAIRE

The information obtained as part of this study is strictly confidential and will be used for research purposes

F: MEDICAL HISTORY:

Has your child got now or had in the past any significant illnesses or operations.

Please include all conditions but not minor illnesses such as influenza

Disease Disorder or operation	Date started	Date recovered

Comments:

.....

.....

.....

.....

.....

.....

The information obtained as part of this study is strictly confidential and will be used for research purposes

G:HABITS

Caffeine

How many cups of tea, coffee or coca cola does your child drink each day? (total combined of all drinks)

† None

Daily † 1-3 † 4-5 † more than 5

or if less than one per day

Weekly † 1-3 † 4-5 † more than 5

If only occasionally please estimate number per month or year

.....

Date last drank caffeine containing beverages _____/_____/_____

or ongoing †

Appendix 9

Tanner Questionnaire for assessment of pubertal stage

Pharmacia & Upjohn
Pharmacia & Upjohn
P.O. Box 11-282
Ellerslie
Auckland
Freephone: (0800) 108 822
Fax: 276 5209

GIRLS 2-18

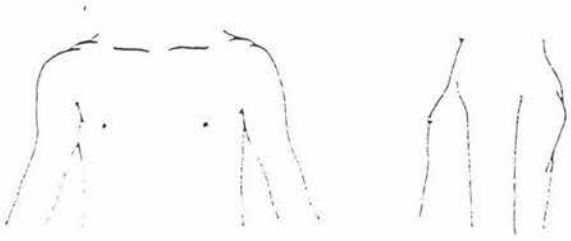
SURNAME _____

GIVEN NAMES _____

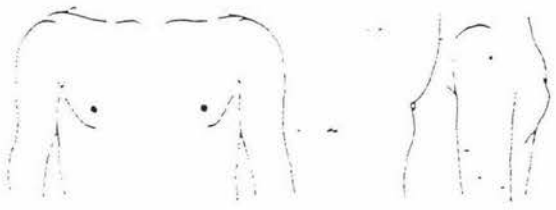
IDENTIFICATION NUMBER _____

DATE OF BIRTH _____

Breast Development

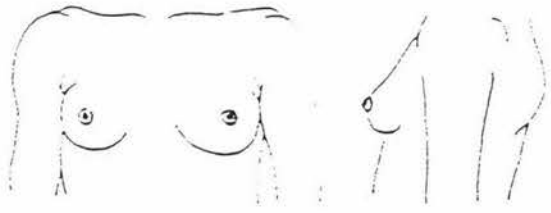


Stage 1 – prepubertal

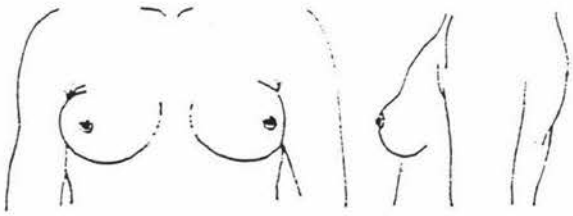


Stage 2 – elevation of breasts and papilla

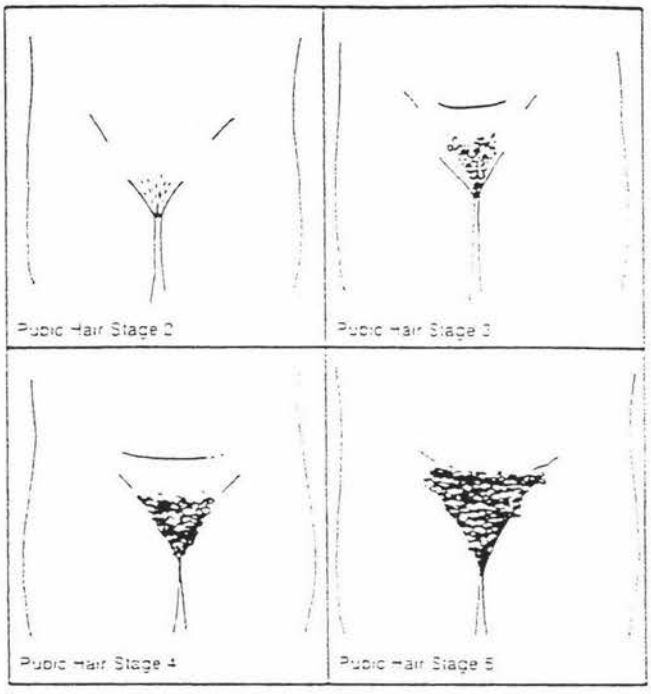
Stage 3 – further elevation and areola but no separation of contours



Stage 4 – areola and papilla form a secondary mound above level of the breast



Stage 5 – areola recesses to the general contour of the breast



STAGES OF PUBERTY

Ages of attainment of successive stages of pubertal sexual development are given in the height centile chart overpage. The stage Pubic Hair 2– represents the state of a child who shows the pubic hair appearance stage 2 but not stage 3 (see below). The centiles for age at which this state is normally seen are given, the 97th centile being considered as the early limit, the 3rd centile as the late limit. The child's puberty stages may be plotted at successive ages (Tanner, *Growth at Adolescence*, 2nd Ed., 1962).

Pubic hair:

Stage 1. Pre-adolescent. The vellus over the pubes is not further developed than that over the abdominal wall, i.e. no pubic hair.

Stage 2. Sparse growth of long, slightly pigmented downy hair, straight or slightly curled, chiefly along labia.

Stage 3. Considerably darker, coarser and more curled. The hair spreads sparsely over the junction of the pubes.

Stage 4. Hair now adult in type, but area covered is still considerably smaller than in the adult. No spread to the medial surface of thighs.

Stage 5. Adult in quantity and type with distribution of the horizontal (or classically 'feminine') pattern. Spread to medial surface of thighs but not up linea alba or elsewhere above the base of the inverse triangle (spread up linea alba occurs late and is rated stage 6).

Designed by the Department of Endocrinology, The Adelaide Children's Hospital, 1989

Pharmacia
&Upjohn

Pharmacia & Upjohn
ACN 000 185 525
P.O. Box 46
Rydalmere NSW 2116

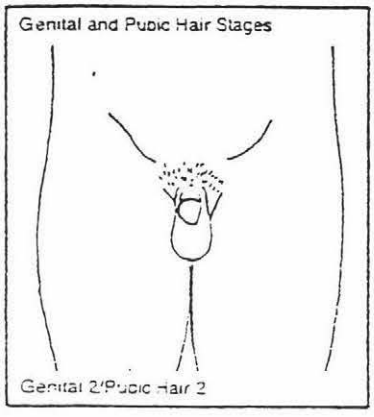
BOYS 2–18

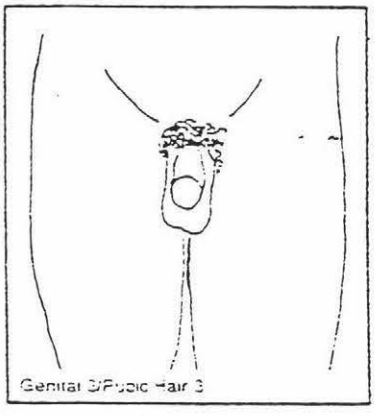
SURNAME _____

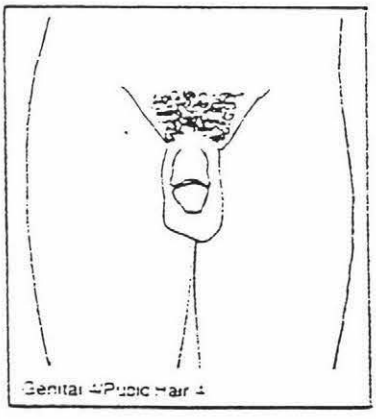
GIVEN NAMES _____

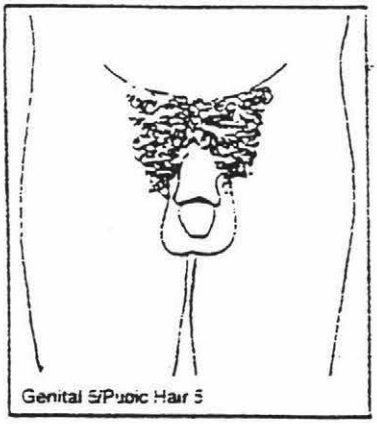
IDENTIFICATION NUMBER _____

DATE OF BIRTH _____









STAGES OF PUBERTY

Ages of attainment of successive stages of pubertal sexual development are given in the height centile chart overpage. The stage Pubic Hair 2+ represents the state of a child who shows the pubic hair appearance stage 2 but not stage 3 (see below). The centiles for age at which this state is normally seen are given, the 97th centile being considered as the early limit, the 3rd centile as the late limit. The child's puberty stages may be plotted at successive ages (Tanner, *Growth at Adolescence*, 2nd Ed., 1962). Testis sizes are judged by comparison with the Prader orchidometer (Zachmann, Prader, Kind, Haflinger and Budliger, *Helv. Paed. Acta*, 29, 61–72, 1974).

Genital (penis) development:

Stage 1. Pre-adolescent, testes, scrotum and penis are of about the same size and proportion as in early childhood.

Stage 2. Enlargement of scrotum and testes. Skin of scrotum reddens and changes in texture. Little or no enlargement of penis at this stage.

Stage 3. Enlargement of the penis which occurs at first mainly in length. Further growth of the testes and scrotum.

Stage 4. Increased size of penis with growth in breadth and development of glans. Testes and scrotum larger; scrotal skin darkened.

Stage 5. Genitalia adult in size and shape.

Pubic hair:

Stage 1. Pre-adolescent. The vellus over the pubes is not further developed than that over the abdominal wall, i.e. no pubic hair.

Stage 2. Sparse growth of long, slightly pigmented downy hair, straight or slightly curled at the base of the penis.

Stage 3. Considerably darker, coarser and more curled. The hair spreads sparsely over the junction of the pubes.

Stage 4. Hair now adult in type, but area covered is still considerably smaller than in the adult. No spread to the medial surface of thighs.

Stage 5. Adult in quantity and type with distribution of the horizontal (or classically "feminine") pattern. Spread to medial surface of thighs but not up linea alba or elsewhere above the base of the inverse triangle (spread up linea alba occurs late and is rated stage 6).

Designed by the Department of Endocrinology, The Adelaide Children's Hospital, 1989.

Appendix 10

Acceptability Questionnaire – to determine acceptability of the study product

ACCEPTABILITY OF MILK DRINKS

Name:_____ School:_____

We would like to know what you think of the milk drinks.

1. What did you think of the milk?

		Choc	Ban	Straw	Van	Caramel
☺	Really Cool	☐	☐	☐	☐	☐
	Quite Yummy	☐	☐	☐	☐	☐
	A Bit Yummy	☐	☐	☐	☐	☐
😐	Neither Yummy or Yucky	☐	☐	☐	☐	☐
	A Bit Yucky	☐	☐	☐	☐	☐
	Quite Yucky	☐	☐	☐	☐	☐
☹	Really disgusting	☐	☐	☐	☐	☐

2. How many glasses of this milk would you like to drink at one time?

Less than one glass	☐	☐	☐	☐	☐
One glass	☐	☐	☐	☐	☐
More than 1 glass	☐	☐	☐	☐	☐
Two or more glasses	☐	☐	☐	☐	☐

3. What do you think of the sweetness of the milk?

	Choc	Ban	Straw	Van	Caramel
Far to sweet for you	☐	☐	☐	☐	☐
A little to sweet for you	☐	☐	☐	☐	☐
Just right for you	☐	☐	☐	☐	☐
Not quite sweet enough for you	☐	☐	☐	☐	☐
Needs to be much sweeter for you	☐	☐	☐	☐	☐

4. What do you think of the flavour?

Much too strong for you	☐	☐	☐	☐	☐
A little too strong for you	☐	☐	☐	☐	☐
Just right for you	☐	☐	☐	☐	☐
Not quite strong enough for you	☐	☐	☐	☐	☐
Needs to be much stronger for you	☐	☐	☐	☐	☐

Thanks for your help
Megan

Appendix 11

Bone Densitometry Quality Assurance Manual Lunar DPX-IQ. Specifically prepared for the supplementation study in 8-10 year olds

BONE DENSITOMETRY QUALITY ASSURANCE MANUAL LUNAR DPX IQ

CANTERBURY GERIATRIC
MEDICAL RESEARCH TRUST

8-10 YEAR OLDS DAIRY
SUPLEMENTATION STUDY

VERSION 1
8 April 1998

BONE HEALTH SERVICES (NZ)
CHRISTCHURCH, NEW ZEALAND
Ph: 64 3 3377 762
Fx: 64 3 3377 757

TABLE OF CONTENTS

INTRODUCTION

THE CANTERBURY GERIATRIC MEDICAL RESEARCH TRUST IS CONDUCTING THIS STUDY IN CONJUNCTION WITH THE DAIRY ADVISARY BOARD.
An 18-month placebo controlled calcium supplementation study in 130 male and female school children aged 8-10; with a twelve month follow up study.

The primary skeletal endpoint is the assessment of BMD every six months to 18 months at the completion of the study and then again at 36 months (12 months after cessation of the study).
BMD will be measured at the femoral neck, Lumbar spine, Total body, and Achilles

Because these results are critical to the study special care must be taken to ensure the high quality of this data.
This Quality Assurance program is designed to enable the high quality measurement of bone density during the study.
In order to achieve this it is necessary for the implementation of a standardised procedure to be adopted for this study.
The instructions in this manual are a supplement to the LUNAR operators manual and must be followed by each site

During the course of the study questions regarding scanning procedures are to be directed to

Pat Maguire ph: 03 337 7821
During the course of the study questions regarding protocol are to be directed to

Dr Nigel Gilchrist ph: 03 337 7899
Cellphone: 025 323 046

STUDY SITE

Canterbury Geriatric Medical Research Trust
3rd Floor, Princess Margaret Hospital
Cashmere Rd, Christchurch, NZ

OBJECTIVES OF THE QA PROGRAMM

- To monitor the short term and long term performance of DEXA scanners used for the acquisition of bone density data
- To ensure the consistent acquisition and analysis of patient scans over the three year course of the study.
- To generate and maintain a central database of bone density results.

THE ROLE OF THE BONE DENSITY PERSONNEL

- To monitor machine performance
- To ensure that BMD measurements are skilfully acquired and analysed for each patient following the procedures in the LUNAR Operators Manual and in the following sections of this protocol.
- To take special care of all study data at site

This document is the manual for LUNAR users and is meant to supplement the LUNAR operators manual.
Some of the information from the LUNAR manual is repeated here for emphasis.
Clearly highlighted are the places where differences between the LUNAR manual and our instructions exist.

ON SITE QUALITY CONTROL MEASURES

Study personnel have the objective of ensuring the precise and stable performance of the scanner.
Monitoring of scanner precision and performance will continue throughout the duration of the study study.
If problems are detected it is the responsibility of the Study Centre to coordinate with LUNAR to correct a problem with hardware or software without delay.

DAILY CALIBRATION

Perform the daily calibration procedure outlined in the LUNAR Manual.
Check that the system passes all portions of the calibration tests.
If calibration fails, repeat calibration.
If it fails again do not scan patients and contact your service agent.
File copies of the daily calibration in a specified file for review and possible audit.

HOLOGIC SPINE PHANTOM

Scan the phantom a minimum of three times per week during the course of the study and always first thing on the days you are scanning study patients.

NB: If the following phantom data is already being acquired for a current study continue as you are doing for that study making sure that copies of QA data are made for these study records

Separate phantom database
Description: QUALITY ASSURANCE
Pathname: C:\PHANTOMS\

PHANTOM BIOGRAPHY

MANDATORY INFORMATION as per all current studies:

First name	1SPINE PHANTOM xxxx	(xxxx being the ID number)
Middle name		
Last name	HOLOGIC	
Birth date	01/11/1957	
Height	180cms	
Weight	70kg	
Sex	F	
Ethnic	W	

Optional Information

Facility ID	Phantom ID number
Dept ID	
Physician	Principal investigator
Comment 1	Initials of person acquiring scan
Comment 2	Initials of person analysing scan

ACQUISITION

Note:

- Ensure you are in the correct database
- Database utilities: Ensure ID is File name
- Scan Values: Mode medium
  Current 750uA
  Width auto 130
  Length manual 155 (scan all vertebrae but no air)
- Scan the phantom on the scan table
- Position phantom in the centre of the table with the smaller vertebrae towards the head of the bed.
- Use the localizer light along the centreline on the bed to ensure the phantom is centred and straight.
- Begin scanning from the middle of the dot just below the largest vertebral body.

You need about 10 lines before the vertebra is scanned.

- The program should stop the scan automatically

ANALYSIS

- Analyse the L1-L4 region taking care to use the histogram for careful placement of markers on Baseline scan.
- There will be 102 lines from the top of L1 to the bottom of L4.
- Use the COMPARE SCANS feature of the LUNAR software for all subsequent analyses after the baseline. Your BASELINE scan will be the first scan
  Filename HOLOG100.Sxx (xx being the extension of your scanner)
- Bone results: L1-L4
- Printout the analysed scan, sign and store printout in file
- Archive

- QA Spine Phantom Form
  Record the following information
  Filename
  Date
  BMC, AREA, BMD

File this form with the phantom scan printouts

- QA Spine Phantom Graph
  Maintain a plot of L1-L4 vs time
  Note any unusual trends
  Look for most points to fall within + or - 1.5% of the average

BASELINE PHANTOM MEASUREMENT

*Perform the Baseline Phantom Measurement prior to staring the study:

Scan your Hologic Phantom ten times on the same day without repositioning.
Analyse these scans (L1-L4) using COMPARE SCANS

Calculate the mean BMD for the QA Spine Phantom as follows-
Add the BMD results together and divide by 10= Mean BMD result
Say this is 1.180
Multiply 1.180 by 1.5% = .017
This means the BMD scan values for the phantom should fall within
1.180 + .017 and 1.180 - .017
ie Between 1.197 and 1.163

You are now ready to set up your QA Spine Phantom form and Graph

CONTINUOUS PHANTOM MONITORING

Once you have performed your baseline measurements you have started to collect your QA data for the study period.
QUARTERLY this data is to be reviewed
This is required to track the longitudinal precision of the scanner over time.
Scan the phantom each morning a patient is scheduled for a scan.
The phantom must be scanned at least three times per week even if no patients are scheduled.
Use the same position each time and compare scans
Maintain your specified file for these hardcopies

DEXA SERVICE RECORD FORMS

This form covers each three months and is for recording all work or problems associated with your scanner ie: repairs, calibrations, software changes

SOFTWARE VERSION CHANGES

No software changes are to be implemented during the study as this may alter bone density results.

SCANNER REPAIR

Routine maintenance should not alter performance of the scanner but can sometimes do so.
It is important to describe any problems, repairs, and or recalibrations of the scanner.
When maintenance is performed, scan and analyse the spine phantom 10 times to re establish the baseline before resuming patient scanning.

CHANGE IN DEXA TECHNOLOGIST

There should be no changes of Technologist over the course of the study unless it is absolutely unavoidable as this has a direct affect on precision and reproducibility of scans.

CARE OF DATA AT STUDY SITE

THE DATA AT SITE IS THE SOURCE DATA FOR THE STUDY

1. Archive all study data including phantom data
2. A back up all study data should be performed
3. Store electronic data securely in labelled containers at site
4. File copies of all correspondence
5. Hardopies to be kept in an orderly fashion in file provided
6. Throw nothing out.
7. If you reanalysed any scans keep old analyses in patient folder clearly marked .

SCANNING PROCEDURES

BMD MEASUREMENTS

	Baseline	6mths	12mths	18mths	30mths
Spine	x	x	x	x	x
Total Hip	x	x	x	x	x
Femur	x	x	x	x	x
Total Body	x	x	x	x	x
Ultrasound	x	x	x	x	x

Note If patient discontinues study > 6mths since last visit rescan patient before dropout

GENERAL GUIDELINES FOR PATIENT SCANNING

DPX-IQ

- Speed Scans and Currents

AP Spine	Total Femur
Hi-res Medium 3000uA	Hi-res Medium 3000uA

Total Body

Children < 22cm thick by the end of the study	Fast detail 150uA
Children >22cm thick by the end of the study	Fast 150uA

Ultrasound

Standard scanning procedure using the correct paediatric for the size of the foot

If an error is made and a patient is scanned in the wrong mode or current at baseline this same mode must be continued throughout the study

An incorrect follow up scan mode will require a repeat scan

- Be consistent with positioning
  An incorrectly acquired scan will require a repeat scan
  Use your visit log to record information helpful for follow up scans
- Follow up scans must be acquired at the same mode and current as baseline
- Have the hardcopy of the baseline scan to refer to when positioning, acquiring and analysing follow up scans
- Allow plenty of appointment time for study patients. This enables accurate positioning and analysis with difficult scans.

PATIENT BIOGRAPHIES

NOTE: ALL PATIENTS CAN BE PRE LOADED INTO THE DATABASE USING THE F5 PRELOAD OPTION IN THE F1 MEASURE SCREEN.

IT IS VERY IMPORTANT THAT THE BIOGRAPHICAL INFORMATION FOR THE FIRST SCAN BE ENTERED CORRECTLY
This information determines the Filename in the LUNAR database for all of this patients scans. The filename cannot be altered once established

BASELINE Visit 1
The patient will be allocated a study number at this visit.
Enter the patient biographical information as follows

Enter the patient biographical data as follows
FIRST NAME : Enter CGM ,Then the patients allocation number eg 013

ie the first name is CGM013

note: Do not leave blank spaces
a bank space is considered
a number

MIDDLE INITIAL leave blank

LAST NAME	Enter patients initials (first, middle, last) If no middle initial use a dash "-" (eg A-G)
BIRTH DATE	Enter date of birth (day, month, year)
SEX	F
HEIGHT	Enter to the nearest cm (>.5 round up)
WEIGHT	Enter weight to the nearest kg (>.5 round up)
ETHNIC	Enter patient ethnicity from list on screen

Optional information

PHYSICIAN :	Enter the name of your Investigator
COMMENT 1:	Enter the initials of the Technologist acquiring the scan
COMMENT 2:	Enter the initials of the Technologist analysing the scan
FACILITY ID:	Enter CGM Primary
SOCIAL SEC:	Enter Visit, baseline,6mths etc

PRE SCAN PROCEDURE

Ensure plenty of time allotted for visit, 60 minutes suggested
Explain procedure to patient
Ensure no metal in scan area
Measure height 3 times using the Harpendens Stadiometer
Measure weight
Find correct study database
Database Utilities-Pt ID : Change to Filename
Enter particulars in patient biography
Round up if >.5cm, Round down if = or < .5cm for height and weight
Record exact weight and heights on visit sheet

FEMUR SCAN

GOAL: Correct scan values used
Maximum exposure of the femoral neck with adequate separation from the ischium

VISIT 1

FEMUR ACQUISITION

Scan values

- Mode @ Current Hi res Medium 3000uA
- Width Auto 150
- Length Auto ~~170~~ 200

The left femur is to be scanned unless contraindicated
Position patient on table
Ensure pt centered on table, pillow under head
Rotate femur by rolling the thigh inward until the foot is angled to the brace, the long axis of the left femur to be parallel to the centre line of the bed
Strap legs firmly in LUNAR foot brace
Position the three rice bags around the hip
Instruct the patient to remain still during the scan
Position the localizer beam 23cm below iliac crest in the midline of the thigh.

Review the following during the scan
? shaft straight and centered
? are there at least 20 lines of shaft before the ischium appears
? lesser trochanter visible but not too large
? is any artifact visible
? is there adequate space for the femoral neck ROI box
If the answer is no to any one of these questions abort the scan and restart
The scan should continue until it automatically stops at about 25 scan lines (3cm) above the trochanter. Continue the scan if it stops too soon or manually stop the scanner if it scans beyond the required length.

VISIT 1

FEMUR ANALYSIS

GOAL :Analysis performed in a systematic and reproducible manner

Verify values

Auto analysis

Step 1: Place the femoral neck ROI box perpendicular to the long axis of the femoral neck

If your adjustment is within 3 degrees of the softwares placement do not change the angle

*Flag on the printout that you have changed the angle

Step 2: Place the femoral neck ROI box as high in the femoral neck as possible without touching the head of the femur, pelvis, or trochanter

Step 3: Make sure there is soft tissue in all four corners of the ROI box, but not necessarily equal amounts

Step4: F9 Search. Check the final placement of the neck ROI

If dissatisfied with the placement repeat the above steps

NOTE: Short neck of Femur

Do not reduce the size of the ROI box

A small amount of ischium in one corner of the of the ROI box in this case is acceptable * Flag on printout

THIS IS YOUR BASELINE SCAN

FEMUR FOLLOW UP ACQUISITION

Retrieve the hardcopy of the BASELINE SCAN

Keep this beside you for comparison

Check the visit sheet for information regarding positioning and acquisition

Enter weight and height if changed, note exact recording on visit sheet

Check that you are in the correct database

Database Utilities- Filename for ID

Ensure the same scan values are being used

Review the following during scanning

? is the starting point the same as baseline

? is the femur positioning the same

If the answer is no to any of these questions abort the scan and restart

After completion of the scan visually check that the scan and baseline scan are virtually identical. If not repeat the scan.

FEMUR FOLLOW UP ANALYSIS

Perform auto analysis as described for BASELINE scan

Positioning identical to screening scan should result in a very similar angle and position of ROI box.

* Flag any scans on which you have changed the angle or had any other difficulties

Type 1 Printout, two copies

AP SPINE SCAN

VISIT 1

AP SPINE ACQUISITION

GOAL: Straight spine with maximum vertebral separation

Scan values

Mode @ Current	Hi res Medium 3000uA
Width	Auto 175
Length	Manual 200

Centre patient on bed, pillow under head
Elevate legs on LUNAR block to flatten lumbar lordosis.
Position localizer beam in line with iliac crest
3 rice bags (1 either side, 1 across abdomen)

Review during first 15 lines of scan:
?patient centered
?Started in the middle of L5
?Crests visible and heights equal
? 10-15 lines of crest
If the answer is no to any of these questions abort the scan and restart

Review during scan:
? is the spine straight
? are there any artifacts
? are the ribs visible
If the answer is no to any of these questions abort the scan and restart
Stop the scan in the middle of T12

VISIT 1

AP SPINE ANALYSIS

Verify values
Auto analysis
Profiles:
Correct any misplaced bone edges using F3 recalculate edges

Spaces @ Labels

- Use the spine image to locate the disk space then use the Histogram to identify the shortest bar. The shortest bar is to be included in the vertebra above the marker.
- ie The bottom of each vertebrae corresponds to the shortest bar on the histogram
- Do not rotate markers as this affects precision
- Check that vertebrae are labelled correctly
- Bone Results
  Choose L1-L4 region

Printout
Type 1, 1 copy
Record placement of intervertebral markers manually on hardcopy
Archive

THIS IS YOUR BASELINE SCAN

AP SPINE FOLLOWUP ACQUISITION

Retrieve the hardcopy of the baseline scan and have this beside you for reference
Ensure the you are in the correct database
Database utilities ID= Filename
Alter weight and height if necessary
Ensure scan values the same as baseline
Check visit sheet for information regarding positioning and acquisition
Review the following during scanning
? scan started at the same point as baseline scan
? spine positioned same as baseline
If the answer is no to any of these questions abort the scan and restart
After completion of the scan visually check that the scan and baseline scan are virtually identical. If not repeat the scan.

SPINE FOLLOWUP ANALYSIS

Verify values
Check visit sheet for information regarding analysis
Auto analysis as for BASELINE scan (repeated here for emphasis)

TOTAL BODY SCAN

VISIT 1

TOTAL BODY ACQUISITION

GOAL: Straight torso.

Scan values

Mode @ Current	Fast Detail 150Ua or Fast depending on thickness
Width	Auto 576
Length	Auto 1958

Centre patient on bed,
Position head just below line at top of scanner bed
Review during first lines of scan and as scan progresses:
?patient centered
If the answer is abort the scan and restart

VISIT 1

TOTAL BODY ANALYSIS

Verify values
Analyse as per Lunar protocol adjusting cuts as required

Printout
Type 1, 1 copy
Archive

THIS IS YOUR BASELINE SCAN

TOTAL BODY FOLLOWUP ACQUISITION

Retrieve the hardcopy of the baseline scan and have this beside you for reference
Ensure the you are in the correct database
Database utilities ID= Filename
Alter weight and height if necessary
Ensure scan values the same as baseline
Check visit sheet for information regarding positioning and acquisition
Review the following during scanning
? Positioning same as baseline
If the answer is no abort the scan and restart
After completion of the scan visually check that the scan and baseline scan are virtually identical. If not repeat the scan.

TOTAL BODY FOLLOWUP ANALYSIS

Verify values
Check visit sheet for information regarding analysis
Auto analysis as for BASELINE scan

SCANNING PAPERWORK

HARDCOPIES OF PATIENT SCANS

- Type 1 Printouts
- Print 1 copy of all scans
- Ensure all copies have FILENAME as ID, initials and allocation number visible as name. If not then reprint hardcopy
- Write Visit Number manually at the top of all hardcopies
- If you have reanalysed a scan write re-analysis on bottom, reason for reanalysis, sign
- Keep all patient scan hardcopy files in an orderly fashion divided by visit number
- Label BASELINE Scan clearly

VISIT SHEET

This sheet is to be the front page for the set of scans for each visit
On this sheet all helpful information pertaining to acquisition and analysis can be recorded for future visits.
Also record the exact weight and height for that visit.

BONE DENSITOMETRY LOG SHEET

Use this sheet to record all patients and scans performed at each visit
Flag those scans that you had difficulty with or would like the QA Centre to check
At the end of the visit period the Principal Investigator for the Study will sign it.

SUPPLEMENT TO THE LUNAR PROTOCOL

Stadiometer Calibration Instructions
Instructions for using phantom compare scans over time

HARPENDEN STADIOMETER

(STANDARD OPERATING PROCEDURES)

The Stadiometer is a counter recording instrument. The purpose of the standard operating procedures is to assure consistency in use of the instrument and hence more consistent results.
This document covers the weekly calibration procedures and the procedures for measurement.

WEEKLY CALIBRATION

- Stand the metal calibration rod vertically between the headboard and the floor
- Record the measurement in a Weekly QC Logbook, dated and initialled
- If the counter does not record the correct length take the following steps to reset the Stadiometer.

RESETTING THE STADIOMETER

- Loosen the stadiometer by undoing the two metal retaining screws and pull it away from the main fibre cog of the carriage
- Turn the metal cog of the counter to the true length of the metal rod while in this position
- Press the counter back against the backplate so that the teeth of the counter cog engage and tighten the retaining screws
- Move the headboard up and down a few times to check that the counter continues to give an accurate reading. If not the counter must be replaced
- Remeasure the rod and record in the log book the action taken, date, initial

MEASUREMENT OF THE PATIENT

- Preferably measure the patient dressed only in a gown or at least with minimal clothing
- Remove shoes and socks
- Instruct the patient to stand upright against the stadiometer such that their heels, buttocks, and scapulae are in contact with the backboard, and the heels are together.
- Check positioning is as described above starting with the feet and moving up
- Check shoulders are relaxed by running your hands over them and feeling the relaxed trapezius muscle.
- Check that the arms are hanging loosely at the sides.
- The head should be positioned in the" Frankfurt Plane" ie the head is tilted not too far backwards or forwards. Move the headboard down in contact with the skull.

To ensure this plane is achieved grip the head gently with open hands and pivot it backwards and forwards observing the counter at the same time. The counter should register the greatest height when the head is tilted not too far forwards or backwards.

- It is advisable to place/press a weight of about 0.5 kg on the headboard to flatten any hair.
- Once correctly positioned instruct the patient to take a deep breath and stand tall (This straightens out any kyphosis or lordosis and produces the greatest unaided height.)
- At this point apply pressure to the mastoid process, not to physically raise the head but to hold it in the position that the patient has lifted it to by breathing deeply.
- Instruct the patient to relax, let the air out , drop the shoulders.
  (The shoulders are naturally raised when a deep breath is taken thus increasing tension in the spinal muscles and preventing total elongation of the spine.)
- Stature is read to the last completed unit (do not round up to the nearest unit as this produces statistical bias)
- Repeat all the above steps 3 times for Protocol M22008. If the three measurements differ by more than 4mm repeat a further 2 times.
- Record all of these measurements in the spaces provided on the BMD visit sheet.

HOLOGIC SPINE PHANTOM
LUNAR INSTRUCTIONS RE USE OF COMPARE SCANS OVER TIME

The Hologic phantom is scanned as if it is a patient
The LUNAR software allows a maximum of 100 scans per patient

The following instructions provide a solution enabling compare scans to be used continuously

Each 100 scans the Hologic phantom must be entered as a new patient
With a systematic naming convention (1spine phantom, 2spine phantom etc)

All biographical and optional data must entered **EXACTLY** the same as the "original" baseline phantom data

Assume that the baseline phantom biography is :
First name: 1SPINE PHANTOM
Last name: HOLOGIC
This will result in DOS filenames beginning with HOLOG1

Assume that your phantom database is C:\ PHANTOMS\
NOTE xx is where you put insert the extension number of **your** scanner

The following sets of instructions are performed in DOS
F10 exit to DOS

SETTING UP A SUBDIRECTORY TO CONTAIN THE BASELINE SCAN FILE

1. Type cd\

2. Type mkdir c:\compare

3. To check, type cd\compare

4. To return to the root directory type cd\

COPYING THE BASELINE FILE SCAN

If your baseline phantom scan is archived:

1. Insert your archive disk containing the baseline phantom scan

2. Type a:

3. Type cd\backup

4. Type copy HOLOG100.Sxx c:\compare\ *.*

5. To check that the file is in the directory

6. Type c:

7. Type cd\compare

8. Type dir

9. To return to the root directory Type cd\

If your baseline phantom scan is on the hard drive:

1. Type copy c:\ PHANTOMS\HOLOG100.Sxx c:\compare*.*

2. To check that the file is in the directory type cd\compare

3. Type dir

4. To return to the root directory type cd\

When analysing scans:

select "compare scans"
then select "change directory"
type c:\compare

You now have your baseline scan file in the correct location but it will need to be renamed every 100 scans to match the next patient entry for the phantom

RENAMING THE BASELINE SCAN FILE

(ie : New phantom patient is in this case HOLOG2)

Type cd\compare

Type rename HOLOG100.Sxx HOLOG200.Sxx

Enter

To check that renaming has been successful: Type dir

To return to the root directory Type cd\

Appendix 12

3 Day Diet Record Booklet – used in validation of the calcium FFQ

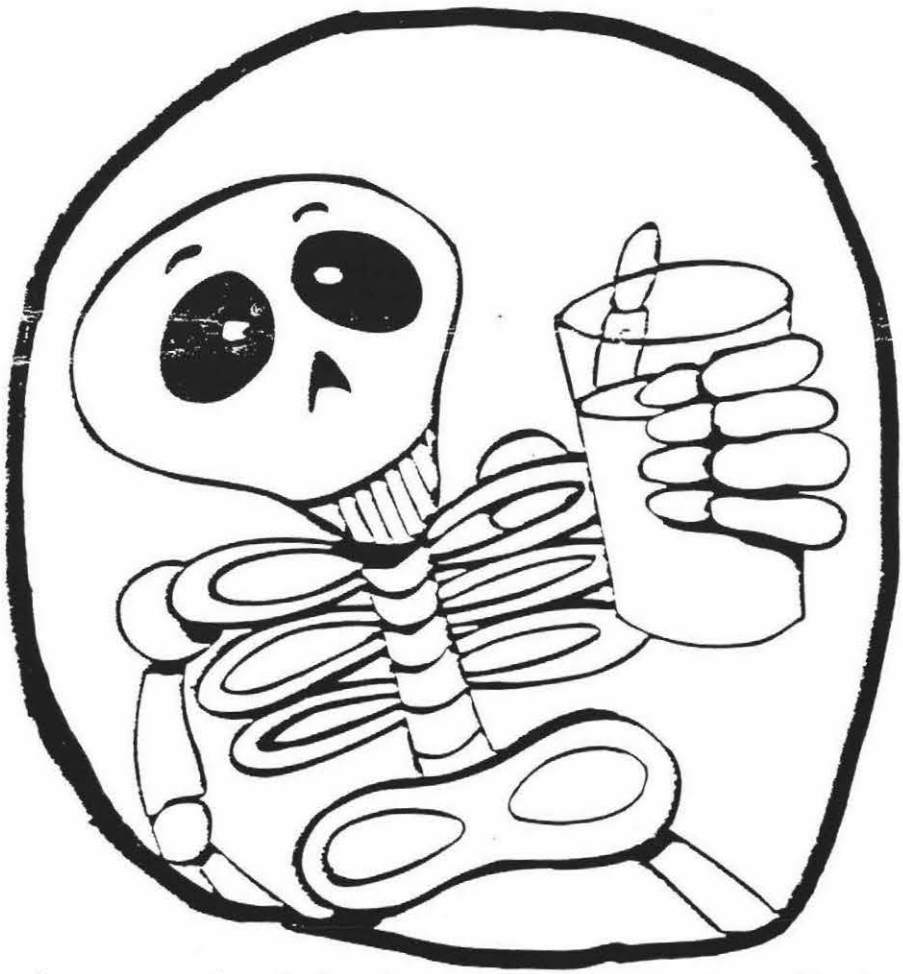
CALCIUM SUPPLEMENTATION STUDY

Name:_____

Code Number:_____

THREE DAY DIET RECORD BOOKLET

Day 1:_____
Day 2:_____
Day 3:_____



When you have completed the three days, please return this booklet in the stamped self addressed envelope provided to:

Megan Merrilees
C/- Dr N Gilchrist
The Princess Margaret Hospital
P O Box 731, Christchurch

INSTRUCTIONS FOR KEEPING A DIET RECORD AND PHOTOGRAPHS

RECORD SHEET

PLEASE READ THESE IMPORTANT INSTRUCTIONS CAREFULLY

* Please record ALL food and drinks consumed

* Please record the food at the time of eating and NOT from memory at the end of the day

* You should include all meals & snacks, plus sweets, drinks (including water) etc.

*Remember to include any additions to foods already recorded such as: sauces, dressings or extras e.g. gravy, salad dressings, stuffings, sugar, honey, syrups etc., butter or margarine (e.g. added to bread, crackers, vegetables).

*If you do not eat a particular meal or snack, simply draw a line across the page at this point. This will show that you definitely have not eaten anything.

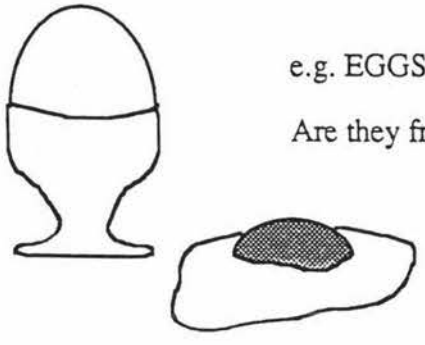
DESCRIBING FOOD AND DRINK – GUIDELINES

1. Please give details of the method of cooking all foods (e.g. fried, grilled, boiled, roasted, steamed, poached, stewed.

2. Give as many details as possible about the **type** of food that you eat e.g. brand name of food where applicable (e.g. Miracle margarine);

type of: Breakfast cereal (e.g. Weetbix)
milk (e.g. whole milk or 'trim milk')
cake or biscuit (e.g. fruit cake, wheatmeal biscuit)
fruit (e.g. fresh, canned, dried, stewed)
soft drink (e.g. regular or low calorie)

3. Name the type of cheese, fish or meat (e.g. cheddar, cod fillet, loin of pork)



e.g. EGGS

Are they fried, boiled, poached or scrambled?

RECORDING THE AMOUNTS OF FOODS THAT YOU EAT

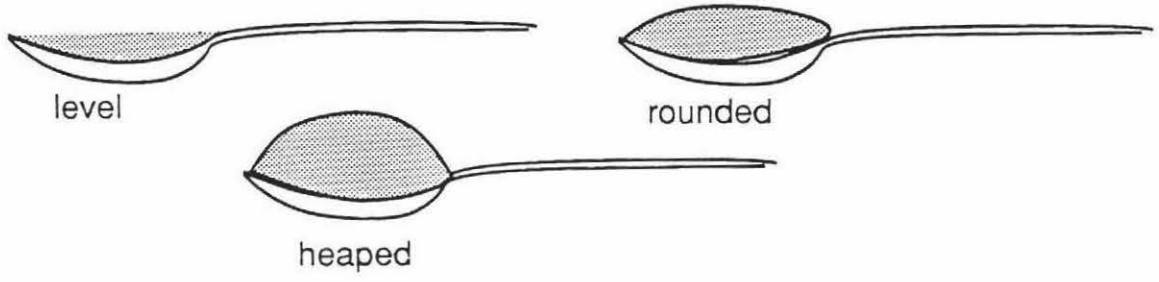
It is also very important to record the quantity of each food and drink you consume.

Here are some suggestions on how to record amounts:

- IN HOUSEHOLD MEASUREMENTS

  For many foods such as vegetables, cereals and canned or stewed fruit, a household measurement is adequate.

  e.g. STATE THE NUMBER OF TEASPOONS (t), TABLESPOONS (T), CUPS etc. State whether spoons are level, rounded or heaped.



  Butter and margarine can be measured in teaspoons or tablespoons if you find this an easy method.

- WEIGHTS MARKED ON PACKAGES

  All convenience foods have their weight marked on the packaging and this can be quoted e.g. half a 425g can of baked beans.

- BREAD - indicate the size of the slices (e.g. sandwich, medium, toaster).

- CHEESE, MEAT & FISH

  If at all possible, it would be very helpful to weigh your portions of these foods.

  If this is not possible, please **use the pictures on the attached sheets** to indicate what sort of portion sizes you eat e.g. you might have 1 portion of spaghetti size A, 1 portion of meat size B or 2 slices of cheese size C.

- USE COMPARISONS for describing portion sizes where this is easier e.g. potato - size of a hen's egg, cheese - size of a matchbox.

IT IS VERY IMPORTANT THAT YOU DO NOT ADJUST WHAT YOU EAT AND DRINK BECAUSE YOU ARE KEEPING A RECORD. THIS IS VERY EASY TO DO, BUT REMEMBER, WE ARE INTERESTED IN YOUR EATING HABITS, NOT THE PERFECT DIET!!!

UTENSIL SIZES

Please measure your home serving utensils and put the sizes here:

- Glass _____mls

  _____mls

- Bowl _____mls

  _____mls

- Mug/coffee cup _____mls

  _____mls

- Tablespoon _____mls

- Serving spoon _____mls

EXAMPLE OF HOW TO FILL IN A RECORD SHEET

DAY 1 - Date 22 May 1998

- Record **ALL** food and drink consumed during the day including sweets, snacks, 'nibbies', sauces and dressings.
- Please record: METHOD OF COOKING (e.g. *boiled* pasta)
  TYPE OF FOOD (e.g. boiled *wholegrain* pasta)
  QUANTITY OF FOOD (e.g. *6 heaped T* boiled wholegrain pasta)

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
EARLY MORNING	1 glass	water	
BREAKFAST	2 1/2 cup 1 tsp 1 toasted slice thin spread thick spread 1 glass	Weetbix blue milk (on weet-bix) sugar (on weet-bix) wholemeal toast butter jam Cerebral	
DURING MORNING	1 bottle 1 (size C)	Drink bottle of water apple	
MIDDAY	1 1 small plt Size A Size A 1 cup	Sandwich - 2 slices of bread - cheese + vegemite - margarine raisins Piece of cake Banana Fruit juice.	

DAY 1 - Date .....

- Record **ALL** food and drink consumed during the day including sweets, snacks, 'nibbles', sauces and dressings.
- Please record: METHOD OF COOKING (e.g. *boiled* pasta)
  TYPE OF FOOD (e.g. boiled *wholegrain* pasta)
  QUANTITY OF FOOD (e.g. *6 heaped T* boiled wholegrain pasta)

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
EARLY MORNING			
BREAKFAST			
DURING MORNING			
MIDDAY			

DAY 1 - continued

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
DURING AFTER-NOON			
EVENING MEAL			
DURING EVENING/ BEDTIME SNACK			

DAY 2 - Date .....

- Record **ALL** food and drink consumed during the day including sweets, snacks, 'nibbles', sauces and dressings.
- Please record: METHOD OF COOKING (e.g. *boiled* pasta)
  TYPE OF FOOD (e.g. boiled *wholegrain* pasta)
  QUANTITY OF FOOD (e.g. *6 heaped T* boiled wholegrain pasta)

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	LEAVE BLANK
EARLY MORNING			
BREAKFAST			
DURING MORNING			
MIDDAY			

DAY 2 - continued

LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
DURING AFTER-NOON			
EVENING MEAL			
DURING EVENING/ BEDTIME SNACK			

DAY 3 - Date .....

- Record **ALL** food and drink consumed during the day including sweets, snacks, 'nibbles', sauces and dressings.
- Please record: METHOD OF COOKING (e.g. *boiled* pasta)
  TYPE OF FOOD (e.g. boiled *wholegrain* pasta)
  QUANTITY OF FOOD (e.g. *6 heaped T* boiled wholegrain pasta)

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	LEAVE BLANK
EARLY MORNING			
BREAKFAST			
DURING MORNING			
MIDDAY			

DAY 3 - continued

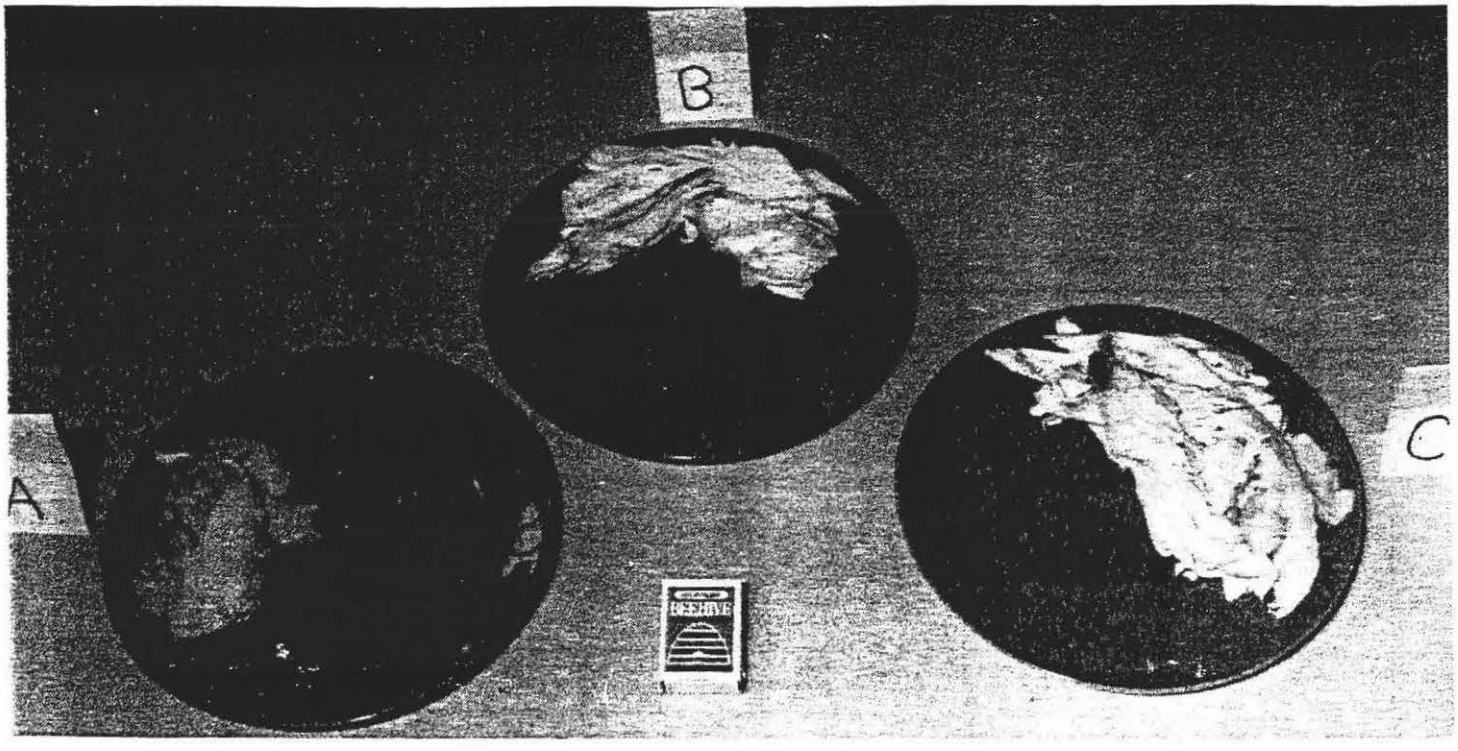
LEAVE BLANK

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD AND DRINK	
DURING AFTER-NOON			
EVENING MEAL			
DURING EVENING/ BEDTIME SNACK			

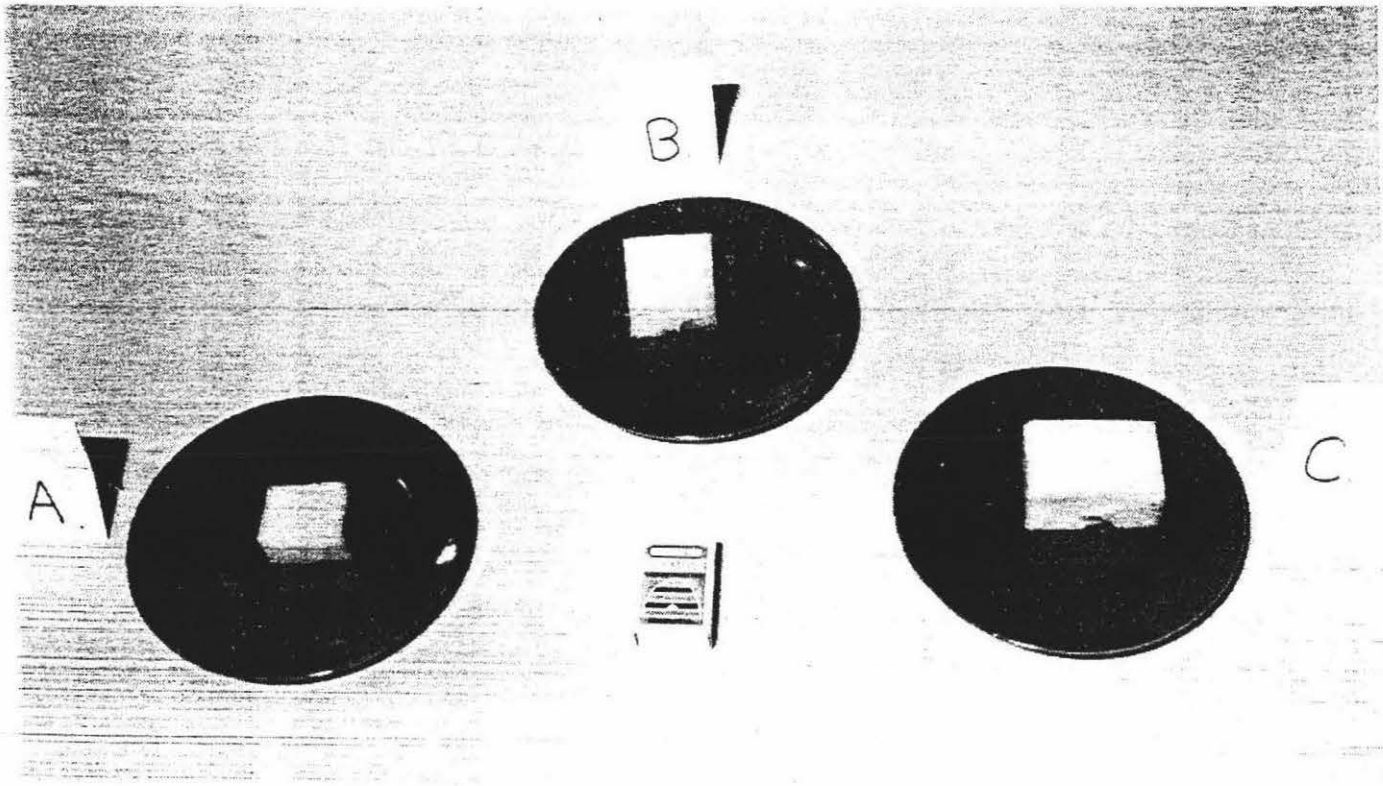
PHOTOGRAPHS FOR ESTIMATING THE SIZE OF THE PORTION OF FOOD THAT YOU EAT.

ALL PHOTOGRAPHS SHOW FOOD ON 22cm DIAMETER PLATES, UNLESS OTHERWISE STATED.

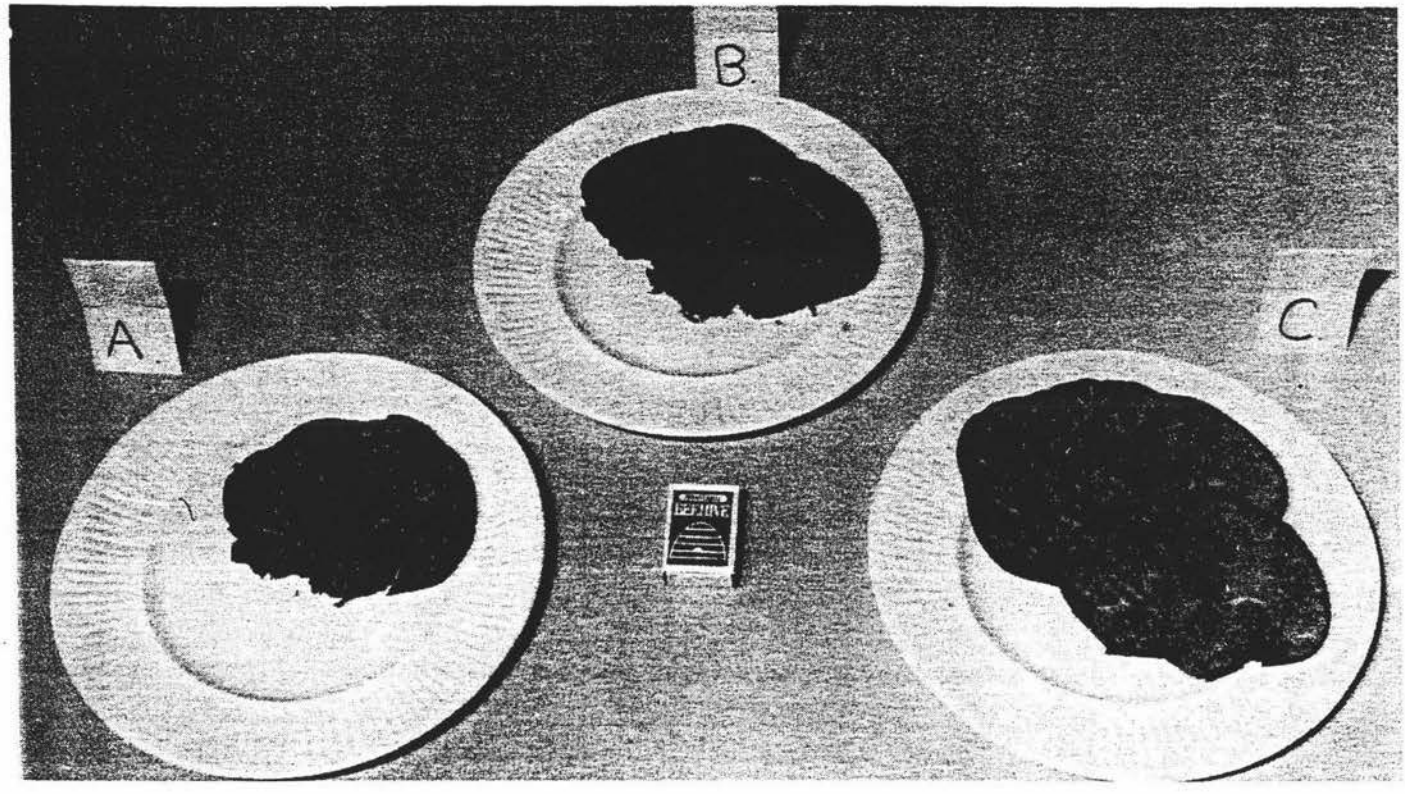
PHOTOGRAPH 1 CHICKEN



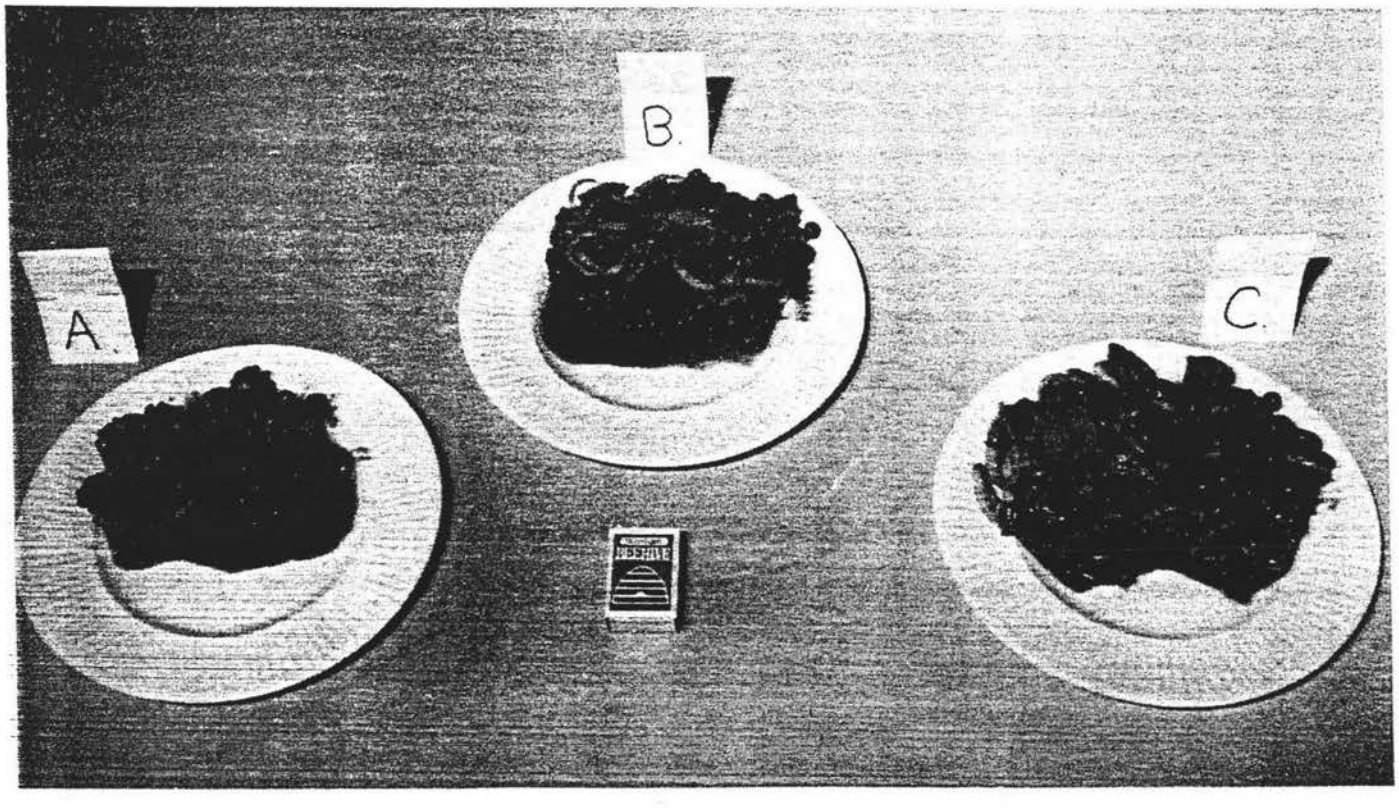
PHOTOGRAPH 2 CHEESE 18cm diameter plates



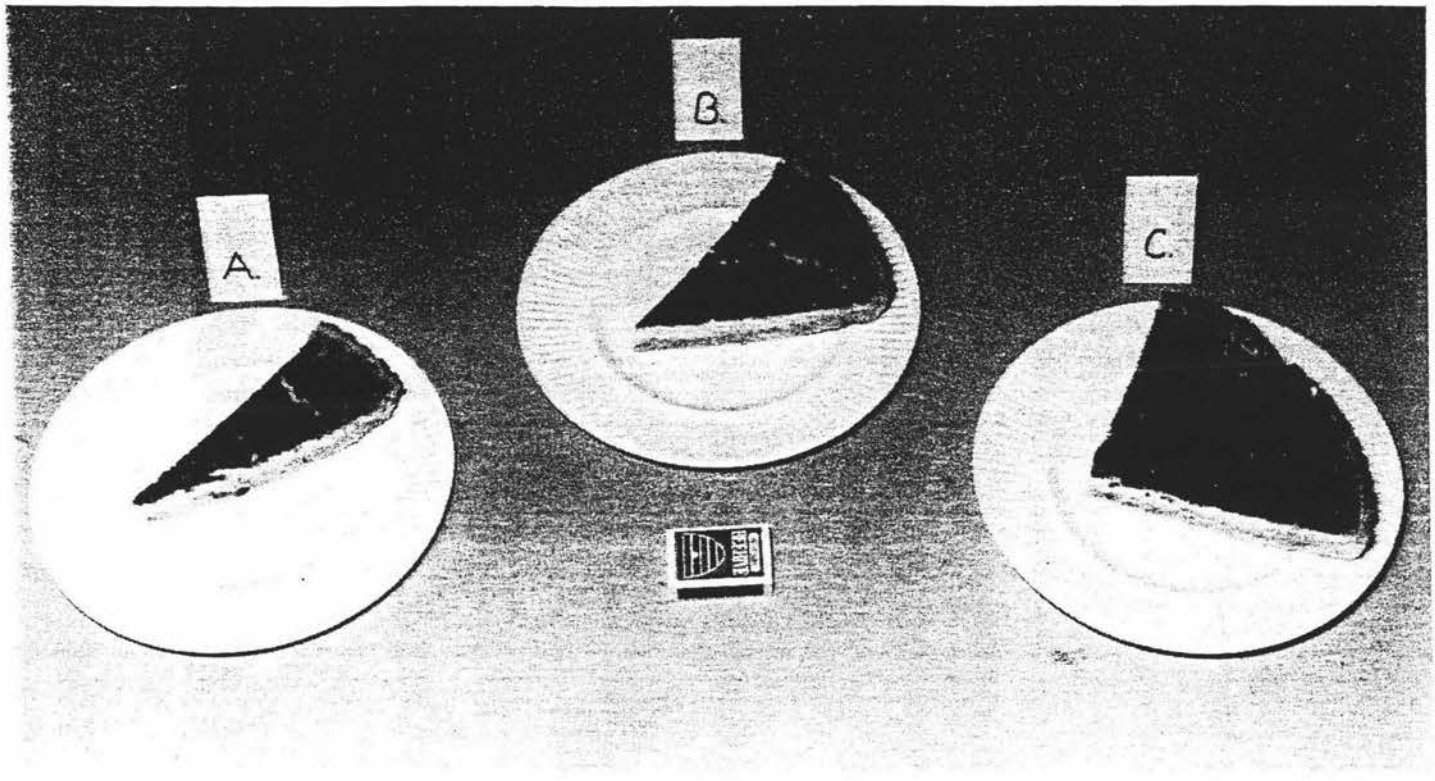
PHOTOGRAPH 3 ROAST MEAT 25cm diameter plates



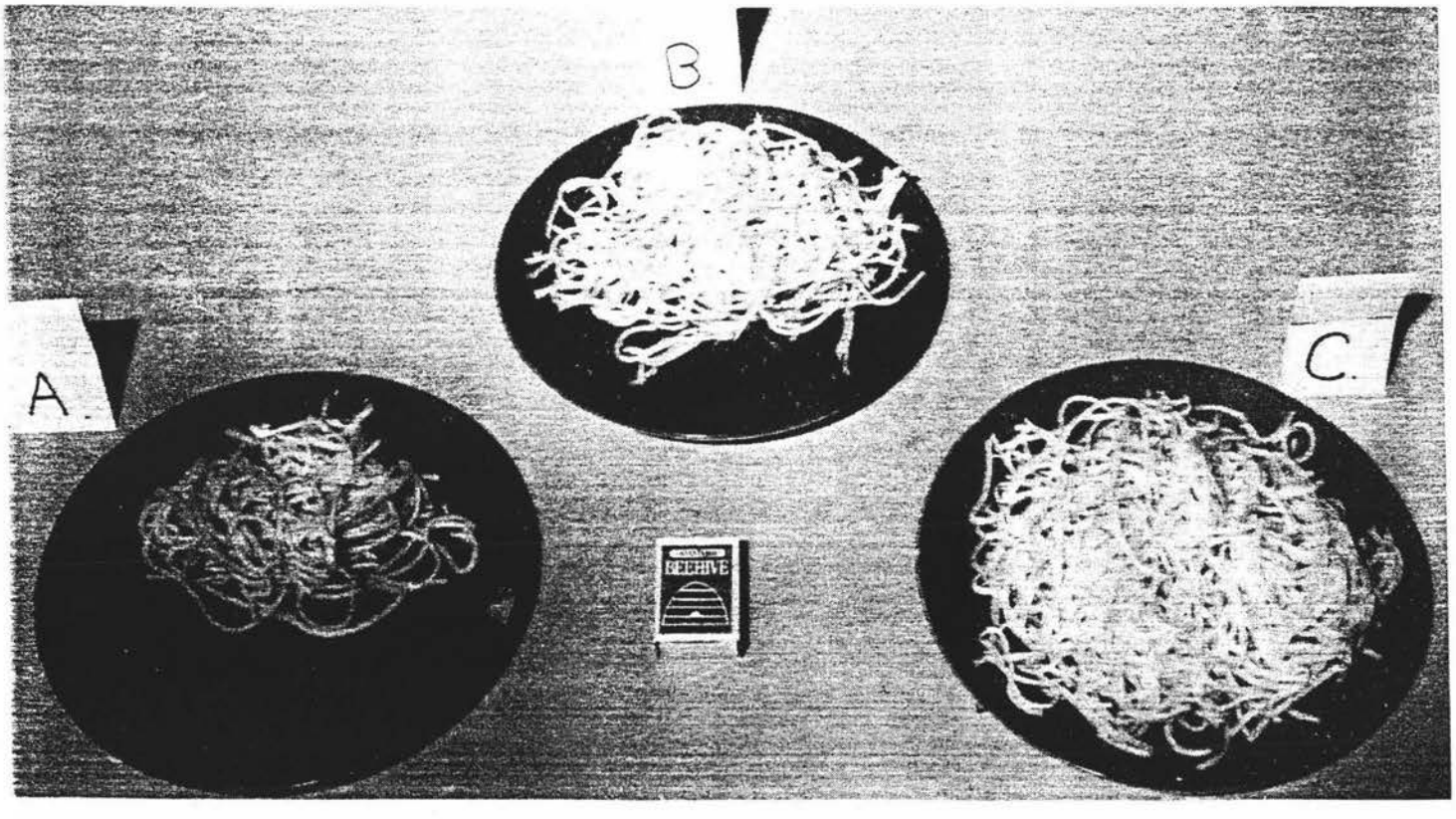
PHOTOGRAPH 4 VEGETABLE OR MEAT STEW



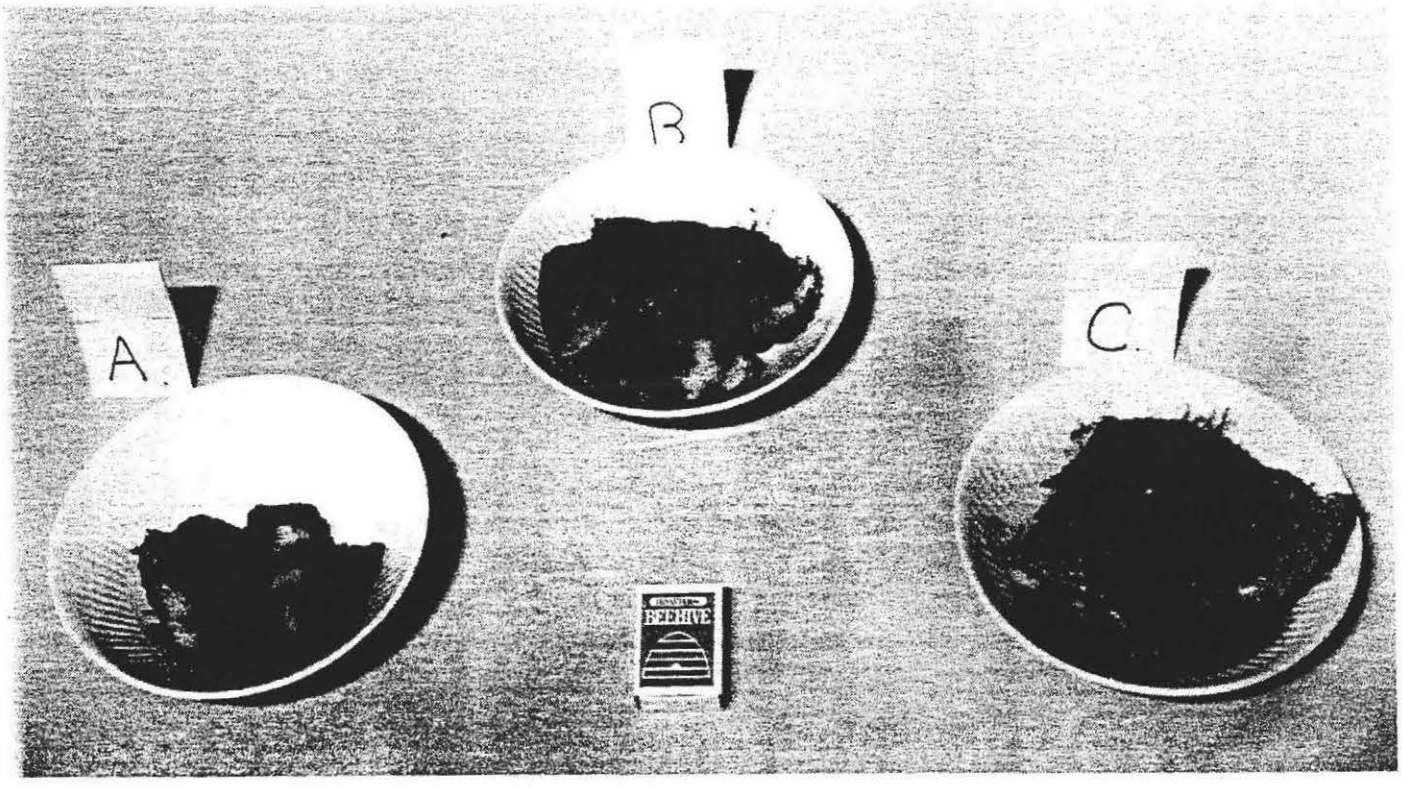
PHOTOGRAPH 5 VEGETABLE PIE



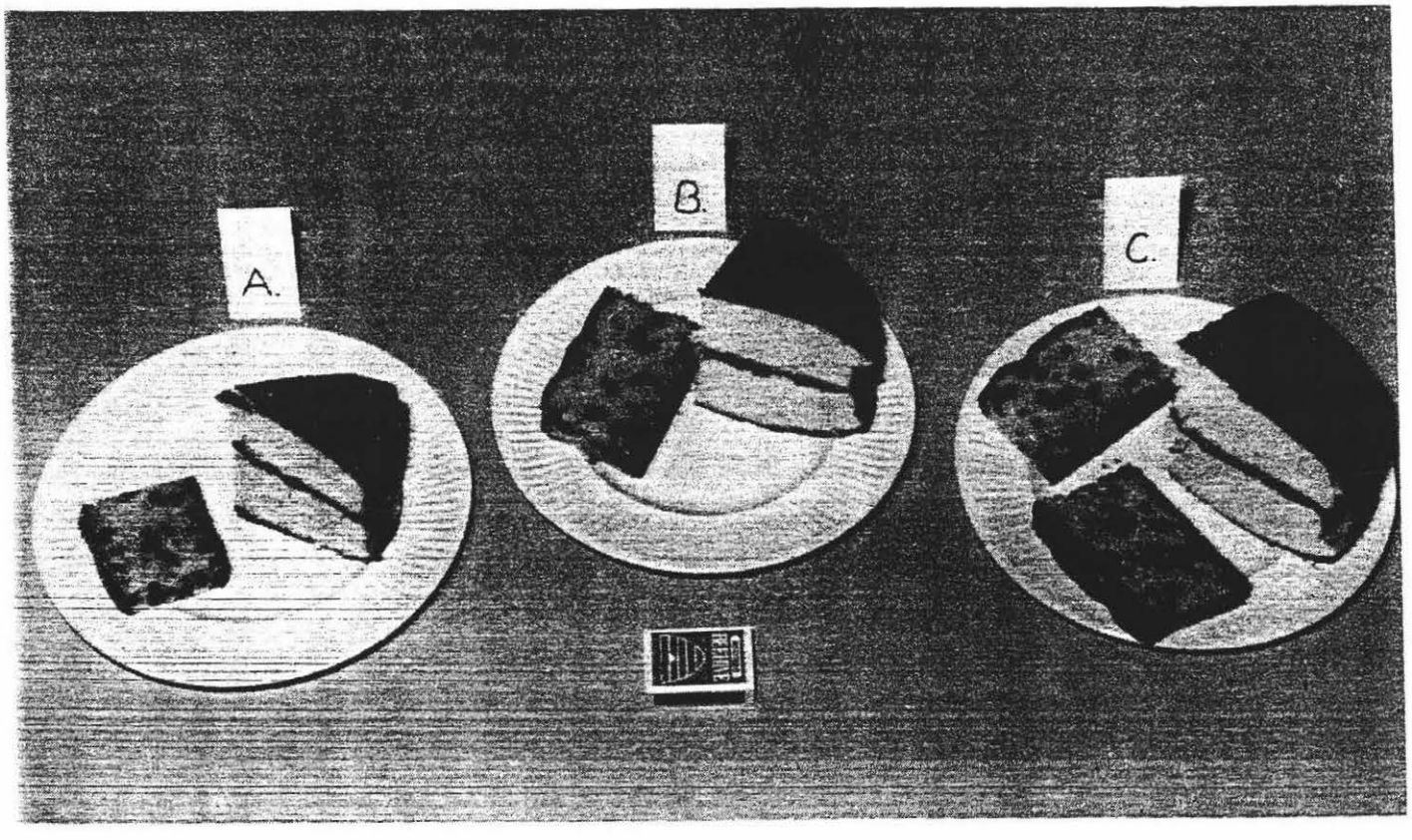
PHOTOGRAPH 6 . SPAGHETTI



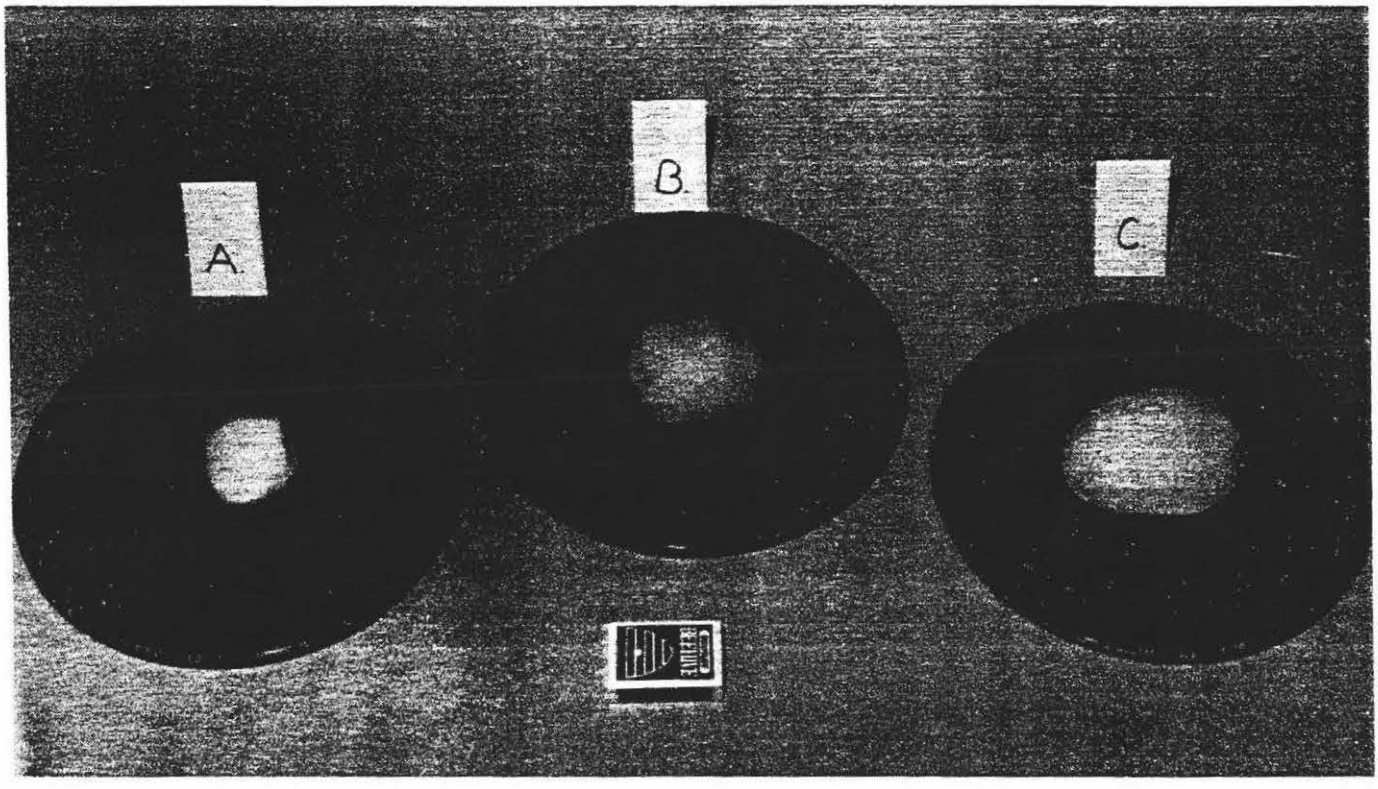
PHOTOGRAPH 7 PUDDING



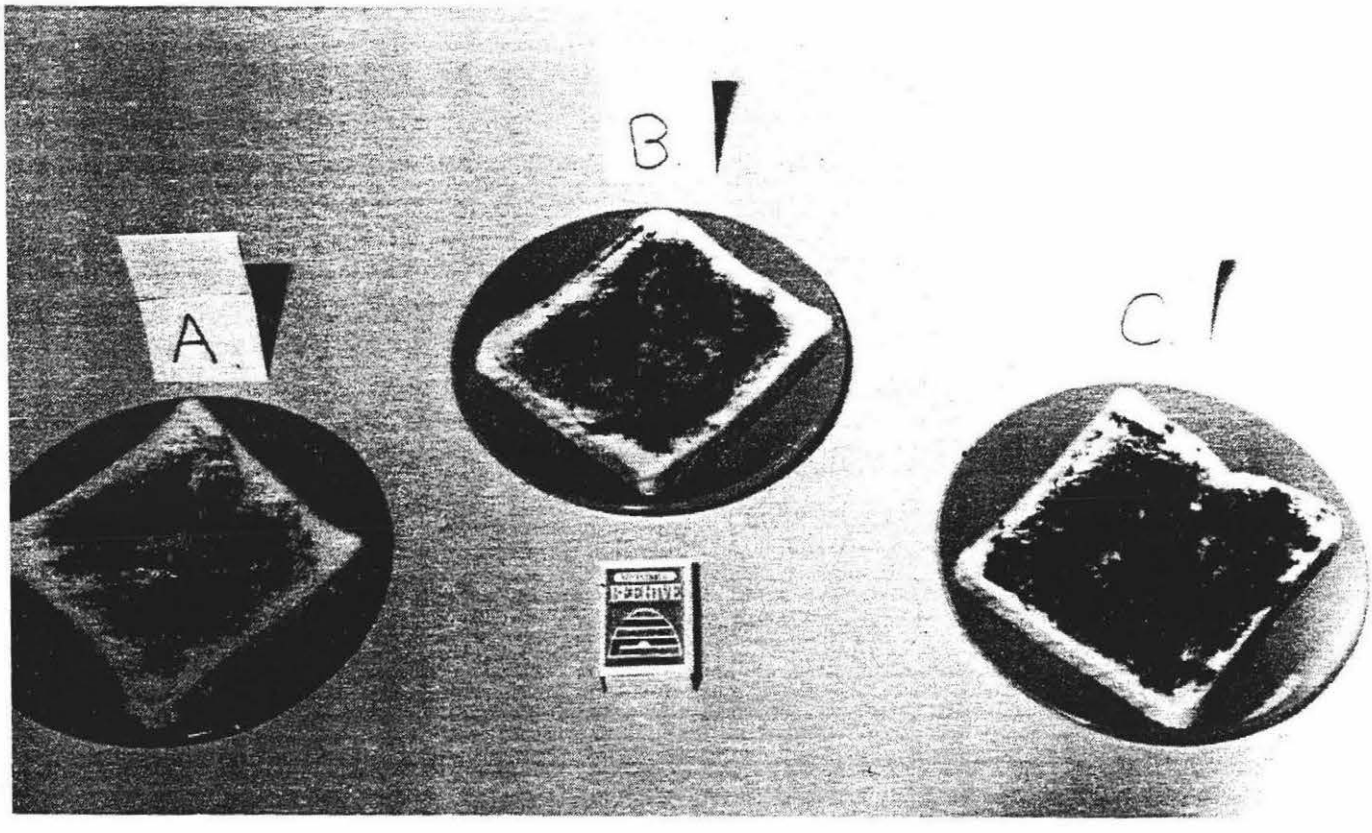
PHOTOGRAPH 8 CAKE



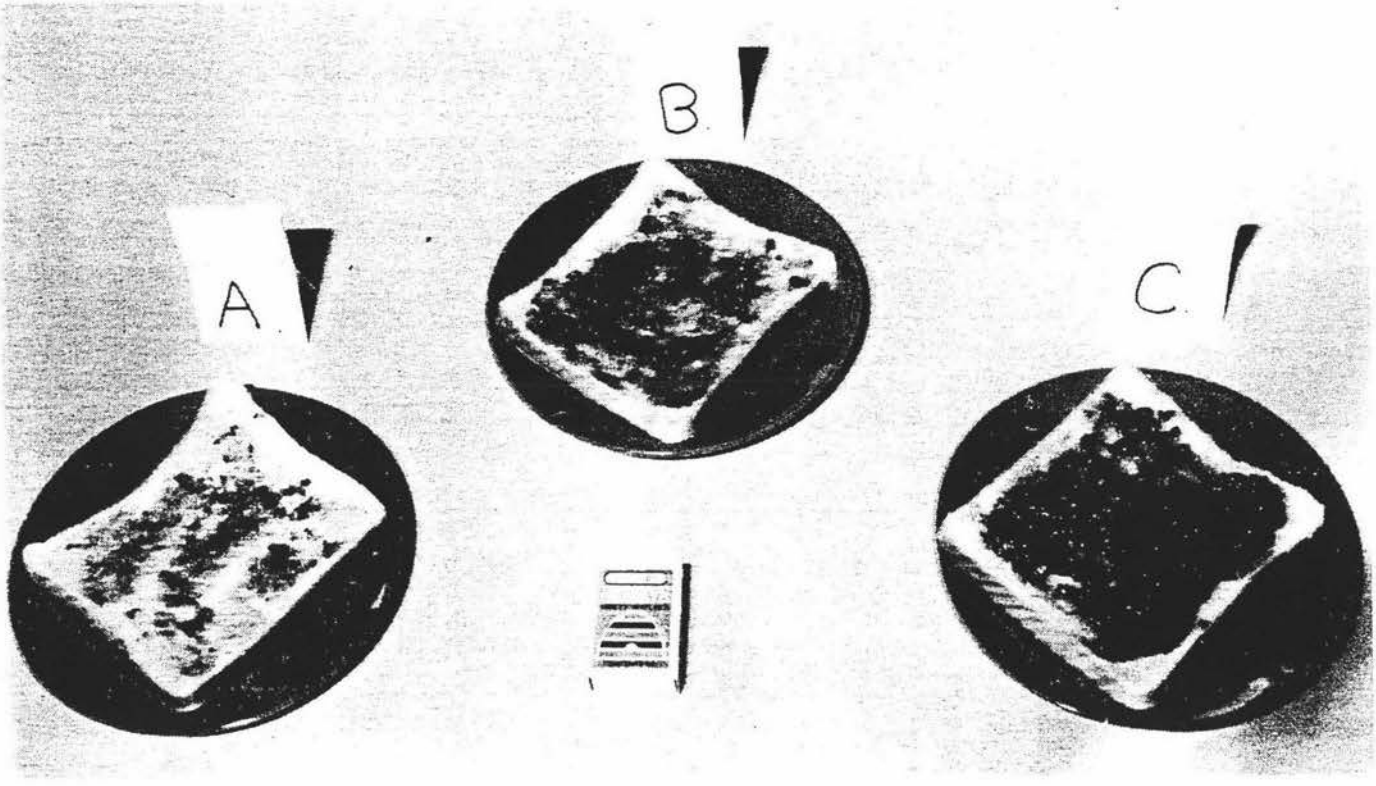
PHOTOGRAPH 9 POTATO



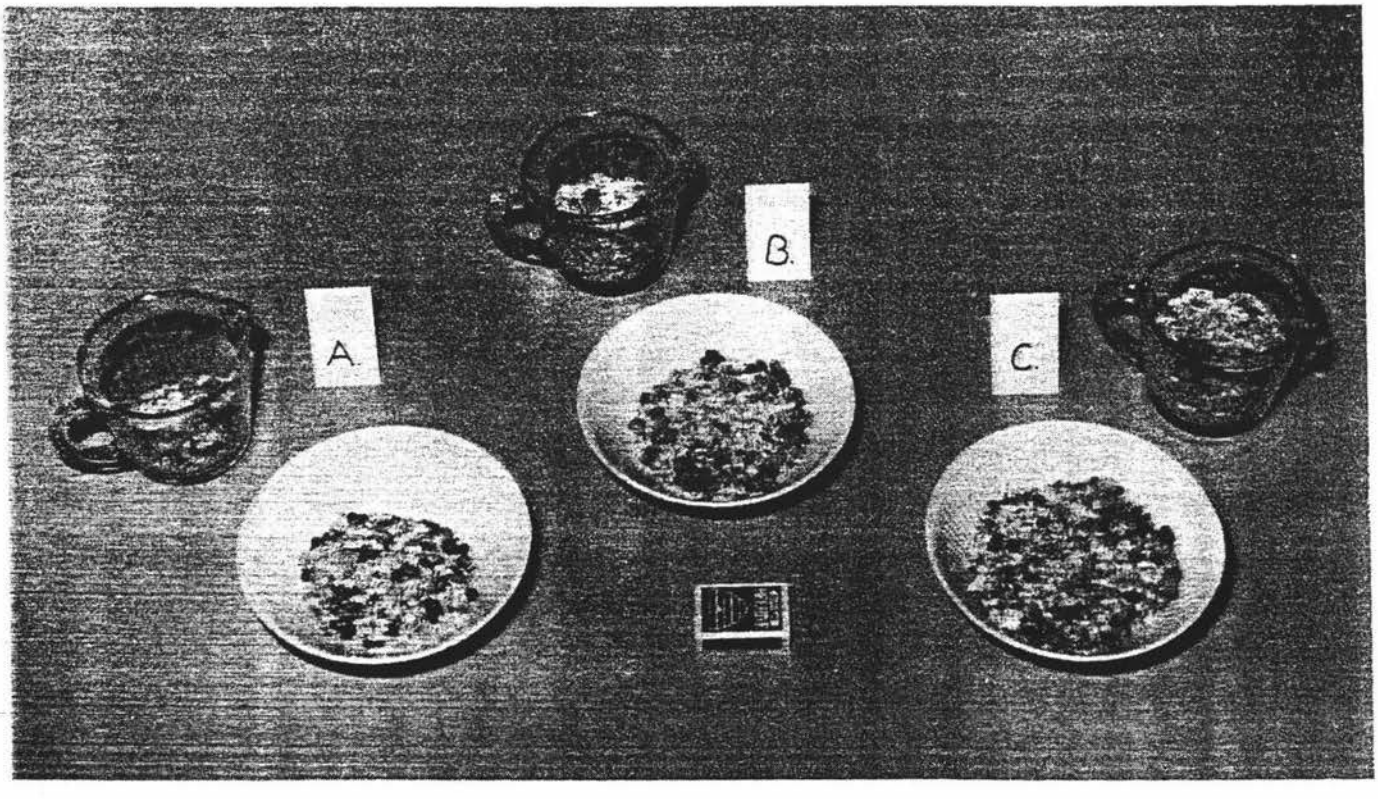
PHOTOGRAPH 10 MARMITE OR VEGEMITE



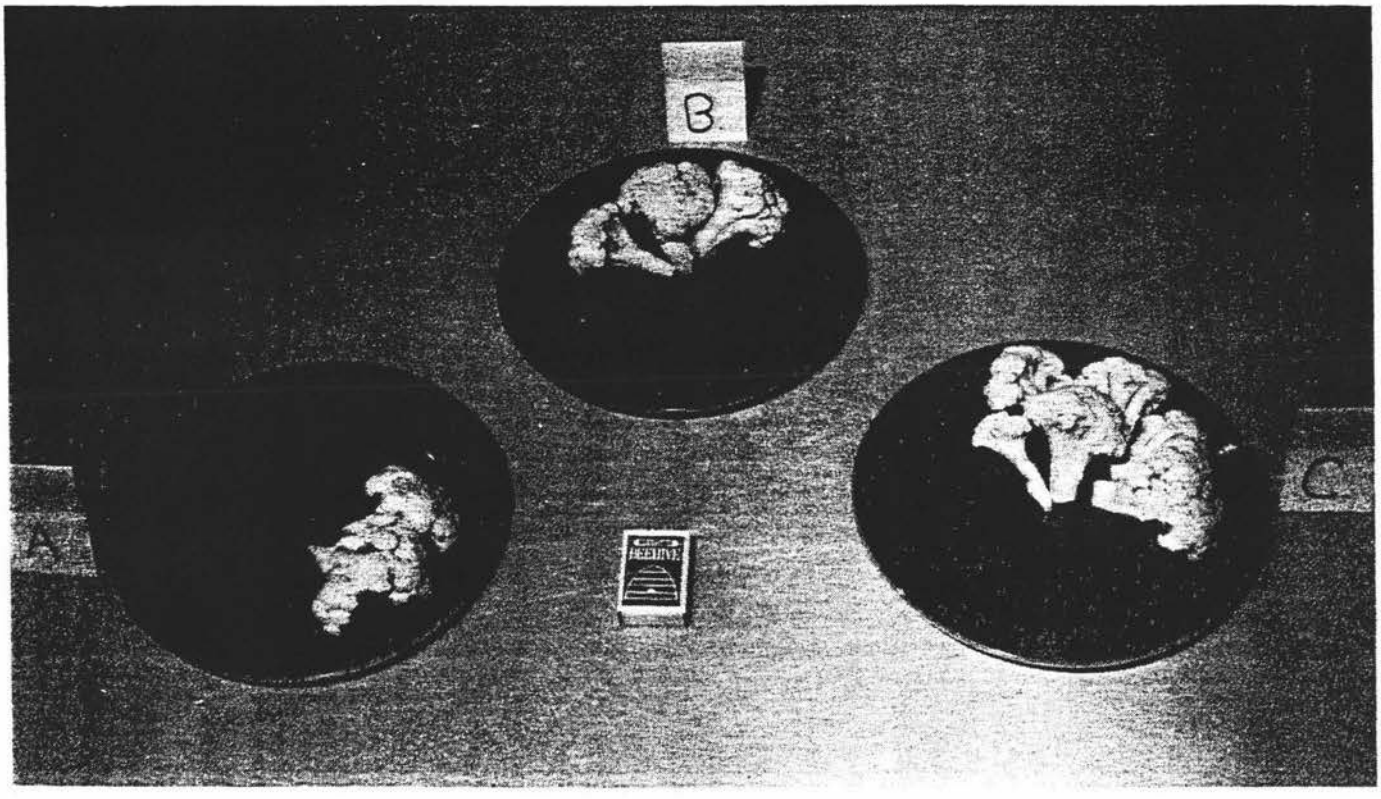
PHOTOGRAPH 11 JAM



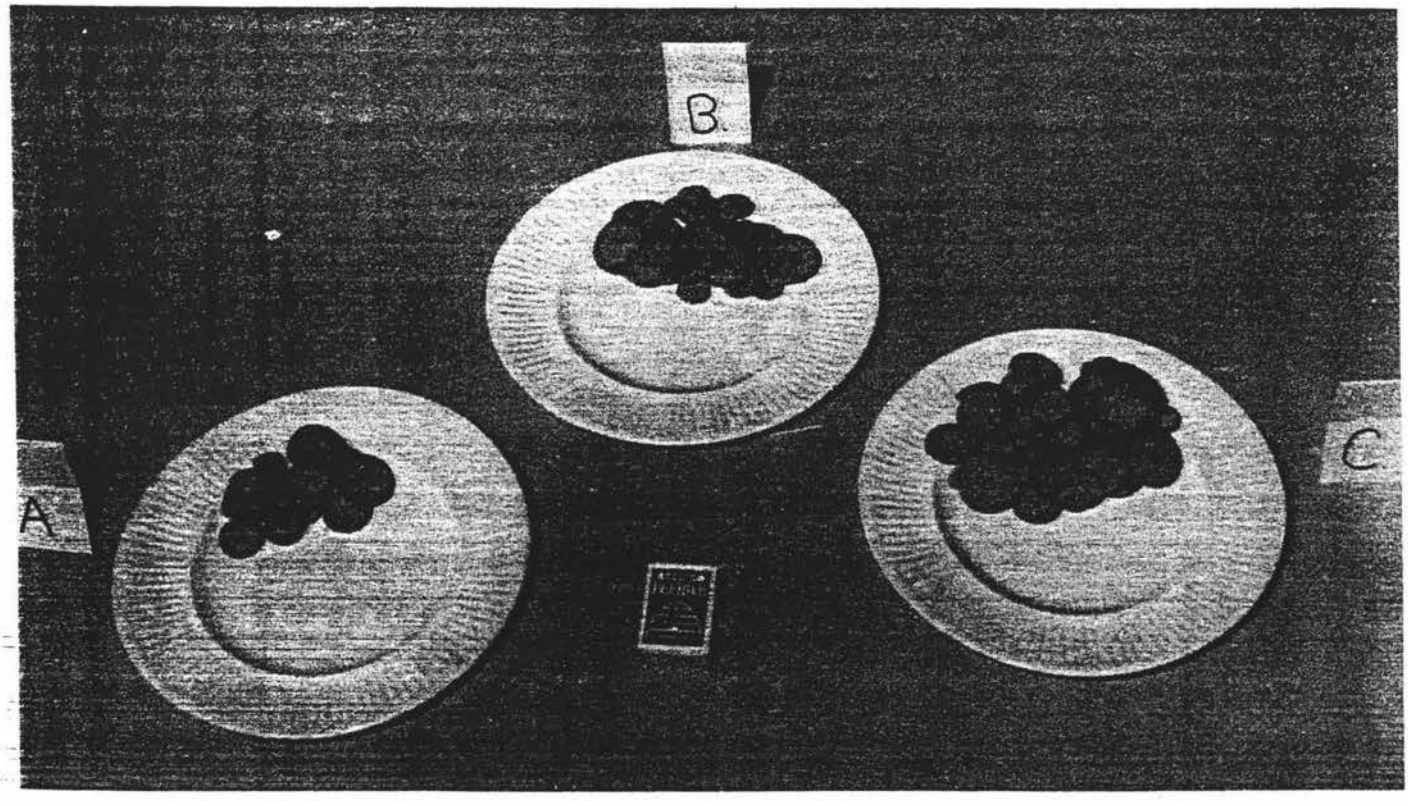
PHOTOGRAPH 12 MUESLI (1/4, 1/2 & 3/4 cup)



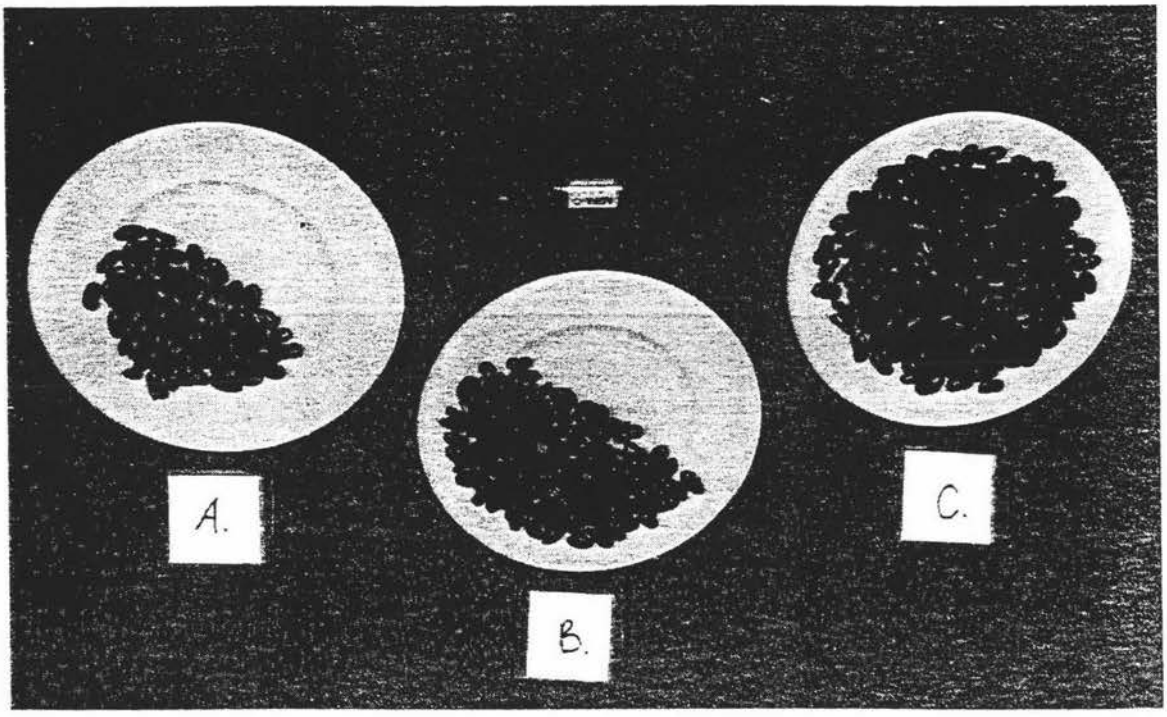
PHOTOGRAPH 13 CAULIFLOWER



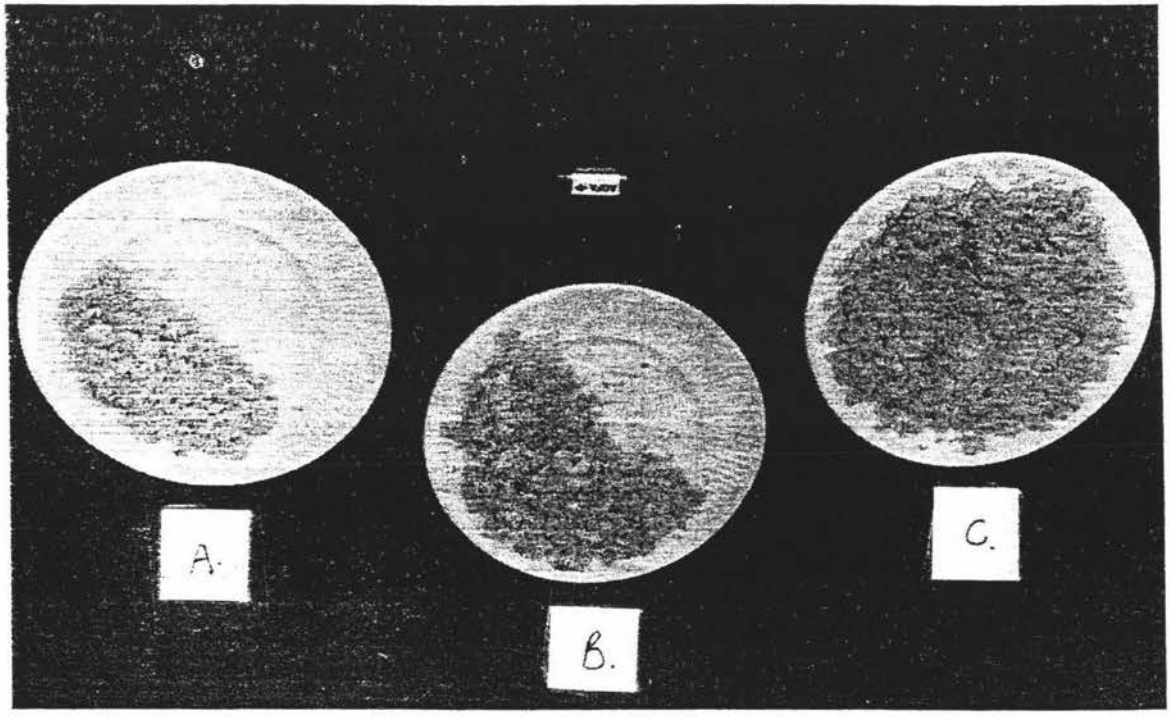
PHOTOGRAPH 14 CARROTS



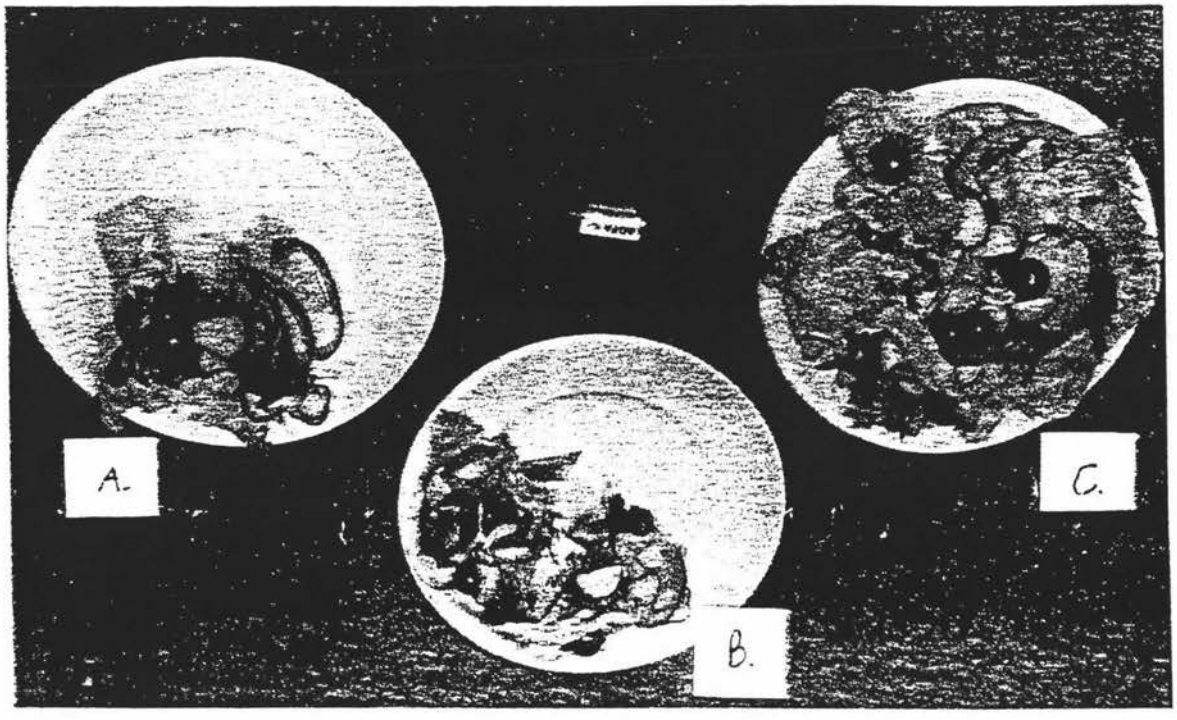
PHOTOGRAPH 15 BEANS



PHOTOGRAPH 16 RICE

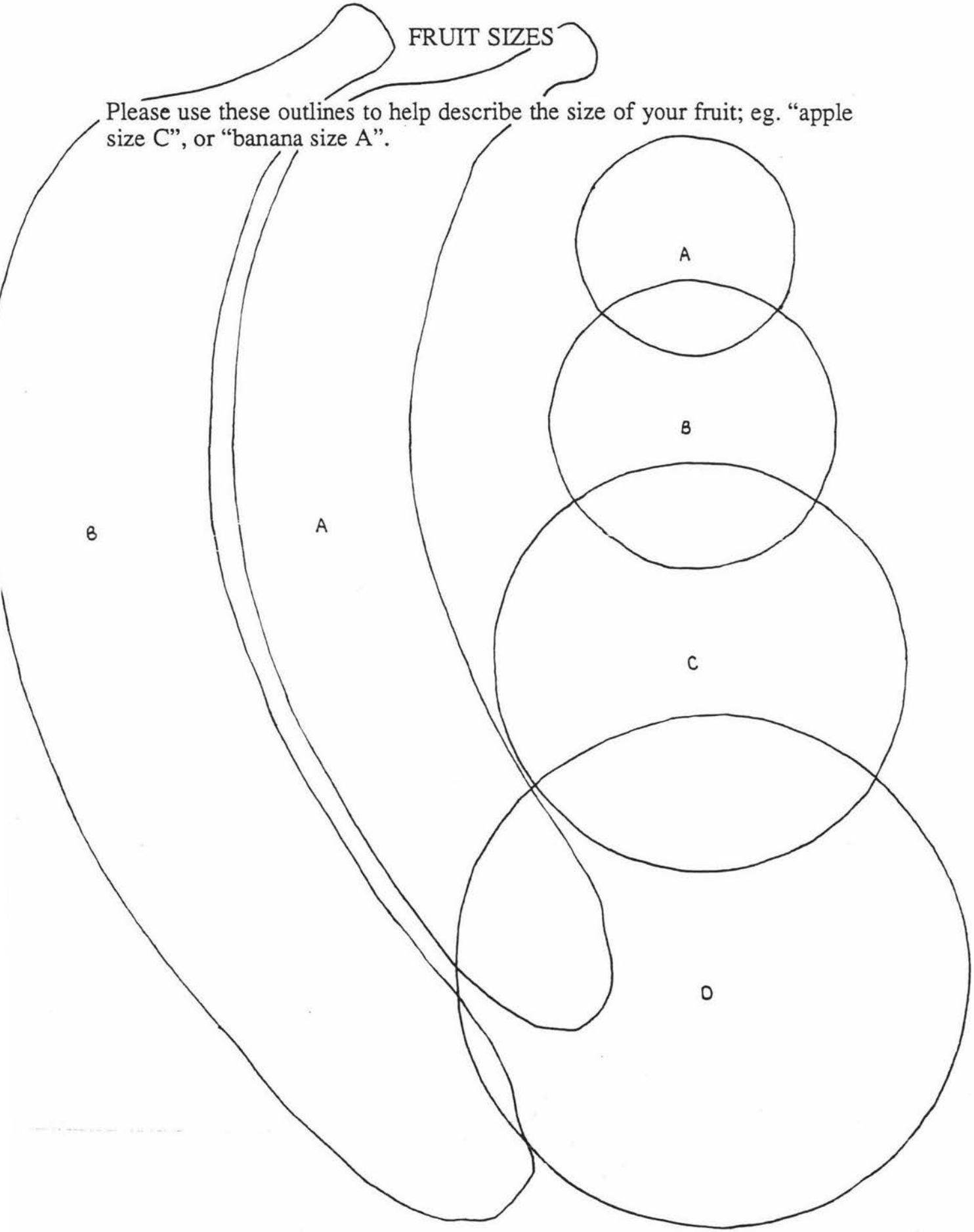


PHOTOGRAPH 17 LETTUCE SALAD



FRUIT SIZES

Please use these outlines to help describe the size of your fruit; eg. "apple size C", or "banana size A".



ADDITIONAL INFORMATION

If you have made any major **changes** to your eating habits during the past few months, please describe them here:

On completion of this question, please keep this questionnaire until we collect it.

THANKYOU FOR YOUR CO-OPERATION IN FILLING OUT SUCH A DETAILED FORM. WE ARE VERY GRATEFUL FOR YOUR TIME AND EFFORT.

If you have any comments or suggestions, please write them below.