BONE GROWTH DURING PUBERTY AND THE EFFECTS OF EXERCISE AND CALCIUM ON BONE MASS ACCRUAL

A DOCTORAL THESIS

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2001

SCHOOL OF HUMAN MOVEMENT, RECREATION AND PERFORMANCE
VICTORIA UNIVERSITY OF TECHNOLOGY

Dedication

No thesis can be completed without the support and encouragement of all those around you. This thesis is dedicated to everybody that has played a part in its creation, from editing through to moral support.

Acknowledgments

The following are acknowledged in no particular order for their contribution to this thesis.

Royal Childrens Hospital
- Kay Gibbons for her nutritional expertise in the development of the calcium supplementation component of the intervention.
- Robyn and Sue for cooking the foods used in the program.

Murray Gouldburn Co-op
- Dr. Wayne Sanderson and Michael Phillips for providing the milk minerals and the T-shirts for all the participants.

University of Saskatchewan
- Professor Don Bailey and Dr. Bob Mirwald for allowing me to work with them on their pediatric bone mineral accrual study and exposing me to the most significant learning experience of my life.
- The staff of the College of Physical Education for making me feel so welcome during my time in Canada.
- Susan Whiting for collaborating on the dietary calcium intake paper.

Milgate primary school
- The principal Ms Christine Lister for her foresight in seeing the value in the program and promoting its implementation at the school.
- The staff at Milgate Primary School who so willingly embraced the program and incorporated it into the school.
- All the girls who participated in the study – the hugest THANKS, and I hope they all enjoyed it.

Research team
- Dr Geraldine Naughton for believing in my vision when I first approached her with the research idea.
- Professor John Carlson for adopting me so willingly, and providing me with his honest and respected feedback.
- Dr. Shona Bass for her direction and attention to detail to ensure the project was of the highest standard.
- Leanne Saxon for being the other half of the project team, and without whom the project would not have succeeded.
- Deb Oliver who was pivotal in the running of this program and without her enthusiasm and wonderful rapport with the staff and students the program would not have been as successful as it was.

Supporters
- My husband Scott for his wonderful patience as I toiled over the thesis.
- Family and friends for living through the thesis with me.
- My friend Donna for proof-reading the thesis.

Awards

- Iuliano-Burns, S. – Roger Melick Young Investigator Award – Winner (2000)
  Australia and New Zealand Bone & Mineral Society 10th Annual Scientific Meeting, Hamilton Island, QLD, Australia, Nov 4 - 7.
  (Chapter Six)

Publications

- Iuliano-Burns, S., Whiting, S.J., Faulkner, R.A., Bailey, D.A. (1999)
  Levels, Sources and Seasonality of Dietary Calcium Intake in Children and Adolescents enrolled in the University of Saskatchewan Pediatric Bone Mineral Accrual Study.
  Nutrition Research 19:1471-1483.
  (Chapter Four)

- Iuliano-Burns, S., Mirwald, R.L., Bailey, D.A. (2001)
  The timing and magnitude of peak height velocity and peak tissue velocities for early, average and late maturing boys and girls.
  Amer. J. Human Biol. 13(1):1-8.
  (Chapter Five)

Presentations

- Whiting, S.J., Iuliano-Burns, S., Faulkner, R.A., Bailey, D.A. (1999)
  Levels, Sources and Seasonality of Dietary Calcium Intake in Children and Adolescents enrolled in the University of Saskatchewan Pediatric Bone Mineral Accrual Study (Poster).
  Canadian Federation of Biological Societies, Winnipeg, Manatoba, Canada, June 2-5
  (Chapter Four)

- Iuliano-Burns, S., Mirwald, R.L., Bailey, D.A. (1999)
  The timing and magnitude of peak height velocity and peak tissue velocities for early, average and late maturing boys and girls.
  Australia and New Zealand Bone & Mineral Society 9th Annual Scientific Meeting, Cairns, QLD, Australia, June 30 – July 2.
  (Chapter Five)

- Iuliano-Burns, S., Saxon, L., Oliver, D., Naughton, G., Bass, S. (2000)
  Weight bearing exercise and increased dietary calcium intake have a synergistic effect on bone mass accrual at the femur in pre- and early-pubescent girls.
  Australia and New Zealand Bone & Mineral Society 10th Annual Scientific Meeting, Hamilton Island, QLD, Australia, Nov 4 - 7.
  (Chapter Six)

- Saxon, L., Iuliano-Burns, S., Naughton, G., Bass, S. (2000)
  The osteogenic response to different mechanical loading regimes in pre and early pubertal girls.
  Australia and New Zealand Bone & Mineral Society 10th Annual Scientific Meeting, Hamilton Island, QLD, Australia, Nov 4-7. (Poster)
  (Chapter Six)

- Saxon, L., Iuliano-Burns, S., Naughton, G., Bass, S. (2000)
  Exercise and increased calcium intake: A synergistic effect at the loaded sites in pre and early pubertal girls.
  Special pre-American Society Bone & Mineral Research Meeting on Pediatric Bone. MacMaster University, Hamilton, Canada, October.
  (Chapter Six)

- Saxon, L., Iuliano-Burns, S., Naughton, G., Bass, S. (2000)
  Exercise and increased calcium intake: A synergistic effect at the loaded sites in pre and early pubertal girls.
  American Society Bone & Mineral Research Meeting, Toronto, Canada, October.
  (Chapter Six)

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Abstract

Childhood and adolescence are time periods characterised by the rapid growth and development of the skeletal system. The timing and magnitude of skeletal development varies considerably between individuals however, environmental factors may make significant contributions to the variance in bone mass. To optimise the development of bone it is important to identify and promote factors that may have a positive effect on bone mass accrual. Two lifestyle factors that may enhance bone mass accrual are calcium and exercise. The focus of this thesis was to investigate bone mass accrual in children and adolescents and to determine the effect calcium and exercise have on bone mass accrual. The research involved three studies. The first study compared calcium intakes in older (aged 13-18 yrs) and younger (aged 8-12 yrs) boys and girls, and to determine if calcium intakes demonstrated seasonal variations. The findings from this study revealed that older girls consumed significantly less calcium (and calcium from fluid milk) than younger girls (951 $mg.day^{-1}$ v 1021 $mg.day^{-1}$, $p < 0.01$). In contrast, older boys reported greater calcium intakes than younger boys (1386 $mg.day^{-1}$ v 1179 $mg.day^{-1}$, $p < 0.001$). Seasonal variations in calcium intake were reported for younger girls, who consumed less calcium (1001 $mg.day^{-1}$ v 1076 $mg.day^{-1}$, $p < 0.05$) and calcium from fluid milk (501 $mg.day^{-1}$ v 568 $mg.day^{-1}$, $p < 0.05$) in winter compared to summer. The second study compared peak height and tissue velocities in early versus late maturing boys and girls. The findings from this study revealed that males reached peak height and tissue velocities significantly later than females (mean difference 1.5 years, $p < 0.001$) and demonstrated significantly greater magnitudes at peak ($p < 0.001$). The age of peak height velocity (PHV) was negatively correlated with the magnitude of PHV in both sexes ($r = -0.4$, $p < 0.05$). Late maturing males had accrued more bone mineral (1779 g v 1493 g, $p < 0.05$) and lean mass (41.8 kg v 36.6 kg, $p < 0.05$), and were taller (168.1 cm v 161.6 cm, $p < 0.05$) by the age of PHV in comparison to early maturers. The third study determined the effects of 8.5 months of high impact exercise and calcium supplementation on bone mass accrual in pre-and early-pubescent girls. The findings from this study revealed that calcium supplementation enhanced bone mass accrual in the arms (13.6% v 10.4%, $p < 0.05$ relative to placebo), and high impact exercise enhanced bone mass accrual predominantly at the legs (16.7% v 14.4%, $p < 0.05$ relative to low impact exercise). A combined effect of exercise and calcium was reported at the femur. No effects of either calcium or exercise were detected at the lumbar spine. The results from these studies highlight the need for adequate calcium and weight bearing exercise during growth to enhance bone mass accrual. In spite of this, many children, in particular older girls in general and younger girls in winter are not consuming sufficient calcium. The effect of these variations in calcium intake on bone mineral content is yet to be determined. The enhanced need for calcium during periods of rapid skeletal growth, as reflected by the elevated recommended calcium intake levels during adolescence, may vary both between and within the sexes due to the variations in the timing and magnitude of these skeletal events. In conclusion the promotion of an adequate calcium intake and weight bearing exercise in this young population may serve to enhance bone mass accrual to optimise skeletal integrity.

Chapter One

Introduction

Sub-headings

1.1 Introduction

1.2 Purpose

1.3 Rationale

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1.6 Definitions of Terms

1.1 Introduction

Osteoporosis is a disease characterised by "low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" [WHO, 1994]. The disease has recently come to the fore as a major medical, social and health concern. Exuberant costs are involved in the treatment of those afflicted with osteoporosis. In 1992 it was estimated that in Australia the annual cost of treating osteoporotic fractures in the 60+ age group was $779 million Australian [Commonwealth Dept. Health & Family Services, 1997]. Fractures may not only cause permanent disabilities they may also be fatal, with one in four sufferers of hip fractures dying within a year of the fracture [Commonwealth Dept. Health & Family Services, 1997]. The problem is projected to escalate in the future as the Australian population ages [Wark, 1996]. Preventative strategies aimed at promoting bone mineral accrual during growth or reducing bone loss in later life may be salient in attenuating this potentially 'epidemic' disease. Deciding on the most effective time during the lifespan to implement preventative strategies may determine the success of the strategies.

Although osteoporotic fractures manifest in old age, osteoporosis may have its origins in youth [Bonjour, 1994]. Bone mass and subsequent fracture risk in the elderly results from the amount of bone accrued by skeletal maturity, and the rate of bone loss in later life. Johnston and Slemenda (1995) suggest that low bone mineral density (BMD) may result from one or both of these factors. Low peak bone mass (PBM) or the failure to maximise bone mass accrual during growth is considered by some investigators to be the most important determinant of bone mass and fracture risk in old age [Bonjour, 1994]. Entering adulthood with maximal bone mass may mean that for a given rate of bone loss in later life, more time would be needed before bone mass would be reduced to the point where skeletal integrity may be compromised and fractures may ensue.

The timing and magnitude of skeletal development varies considerably both between and within the sexes [Tanner, 1989]. The impact of these variations on adult bone mass is not clearly defined. It has been estimated that up to 80% of the variance in bone mineral content may be genetically determined [Seeman, et al., 1996]. The remaining variance however, may be influenced by environmental factors. Identifying and modifying these factors may alter bone mass accrual. Influences on bone mass accrual are multi-faceted and include hormonal status, in particular estrogen, diet, exercise, alcohol, smoking, some diseases, and the use of some drugs and medications [Valimaki et al., 1994]. Two important lifestyle factors, which are relevant to children, are diet (in particular dietary calcium) and exercise [Barr & McKay, 1998]. The ability to influence bone mass through the modification of diet and exercise may not, however be uniform throughout the lifespan.

Once adult stature has been attained the ability to significantly enhance bone mass through modifications to diet and exercise appear limited. Calcium or exercise intervention studies involving adults have reported no effect or small improvements in BMD [Dawson-Hughes et al., 1990; Kelly, 1998a]. In contrast calcium [Bonjour et al., 1997; Johnston et al., 1992] and exercise [Bradney et al., 1998; Morris et al., 1997] intervention trials involving children have demonstrated positive effects on measures of bone mass accrual. Evidence from bilateral, cross sectional and retrospective studies in children further support the claim that childhood may be a more opportune time than adulthood, to influence bone mass [Bass et al., 1998; Haaspasalo et al., 1994; Kannus et al., 1995: Ruiz et al., 1995]. Haaspasalo et al. (1994) reported greater side-to-side differences between the playing and non-playing arms of elite female tennis players when compared to non-players. More importantly, the side-to-side difference was greater in players who commenced playing before menarche compared to those who commenced after menarche. Kannus et al. (1995) further investigated the side-to-side difference in BMC of the humerus of elite squash and tennis players and reported a greater side-to-side difference in girls who commenced playing > 5 years before menarche (20-23%) compared to those who commenced playing > 15 years after menarche (9-10%). The findings reported by Haaspasalo et al. (1994) and Kannus et al. (1995) however, may be challenged as differences in loads experienced at differing stages of pubertal development were not quantified.

Independently, calcium and exercise intervention studies have reported positive effects on bone mass accrual. The existing evidence however, indicates that dietary calcium and exercise may act upon different constituents of bone and influence different aspects of the growth process. Calcium is reported to influence the remodelling process, by slowing bone turnover [Johnston et al., 1992], while exercise is believed to exert a greater influence on the modelling process [Bailey, 1995]. These observations raise the question as to the interactive effect of exercise and calcium on bone mass accrual. Limited data exist describing the combined effects of exercise and calcium on measures of bone mass in adults [Prince et al., 1995]. To date no data are available describing the combined effects of calcium and exercise on bone mass accrual during growth. For example, it is not known if the combined effects of calcium and exercise are synergistic or cumulative. Furthermore, there is a need to determine if exercise when combined with calcium would provide a greater osteogenic benefit to growing bone than the independent effects of exercise or calcium alone.

1.2 Rationale

The timing of skeletal maturity demonstrates large variance both within and between the sexes. The effect of these temporal differences on the magnitude of bone and tissue accrual has not been investigated using longitudinal analysis. Calcium and exercise are two important and modifiable lifestyle factors that may enhance bone mass accrual. Existing dietary intake data indicates that dietary calcium intake, especially in females may not be meeting recommended levels. It is not known what dietary habits are contributing to this failure to meet recommended calcium intake levels. Based on available research evidence, modifying childhood calcium intakes and exercise levels may be more beneficial to bone mass than similar strategies in adults. The rationale for this study is threefold. Firstly, there is a need to determine if calcium recommendations are being met, and if not which sex or maturity group are at the greatest risk and why. Information of this type may assist future educational or promotional campaigns aimed at ensuring an adequate calcium intake in our younger populations. Secondly, details of the differences in bone mass accrual between early, average and late maturing children may provide insight into the most appropriate means to meet skeletal needs during this period of rapid skeletal development. Finally, there is a need to understand the effects of calcium and exercise on bone growth, and to determine the independent and combined effects calcium and exercise may have on bone mineral accrual. The minimal amount, and type of exercise, or the level of calcium intake that may exert a positive effect on bone mineral accrual however, has not been clearly defined. There is a need, therefore to identify the nature and quantity of exercise and calcium that may provide an osteogenic benefit to growing bone.

1.3 Purpose

The purpose of this thesis was to measure calcium intakes in children both seasonally and at different maturity levels, to investigate bone mass accrual in children of differing age of maturity, and to determine the effects of increased calcium intake and high impact weight-bearing activity on bone mass accrual in pre- and early-pubertal girls. The specific objectives were:

1. To determine and compare total dietary calcium intake and sources of dietary calcium for younger and older boys and girls and to determine if differences are apparent between sexes, maturational groups and seasons.
   It was hypothesised that calcium intake would decrease with age in girls but not boys and that milk intake would decrease in the colder month.

2. To describe and compare the timing and magnitude of peak height and tissue velocities in early, average and late maturing boys and girls.

It was hypothesised that late maturing children would have lower peak height and tissue velocities than early matures due to growth occurring over a longer period of time.

3. To determine whether 8.5 months of calcium supplementation using calcium-enriched foods resulted in greater increases in the bone mineral content of pre- and early-pubertal girls when compared with pre- and early-pubertal girls not receiving supplementation.
   It was hypothesised that calcium supplementation would result in enhanced bone mass accrual.

4. To determine whether twenty minutes of high impact weight-bearing activity, three times a week for 8.5 months resulted in greater gains in the bone mineral content of pre- and early-pubertal girls when compared with girls who participated in a similar program involving low impact activities.
   It was hypothesised that high impact exercise would result in enhanced bone mass accrual at weight bearing sites.

5. To determine whether the combined effect of calcium supplementation and high impact weight-bearing activity on the bone mineral content of pre- and early-pubertal girls was greater than the individual effects of calcium supplementation or high impact weight-bearing activity.
   It was hypothesised that a combined effect would be evident at weight bearing sites.

Study one consisted of 226 participants aged 8 to 19 years involved in a six year longitudinal study examining skeletal development and lifestyle factors that may influence bone mass accrual. Dietary intake was determined by serial 24-hour recalls with each participant providing up to four recalls per year. Three thousand one hundred and thirteen diet records were assessed.

Study two consisted of a group of 60 male and 53 female adolescents measured annually over a six-year period using standard measuring procedures and dual-energy x-ray absorptiometry (DXA). Peak height and peak tissue velocities were determined using a cubic spline curve fitting procedure of the individual data.

Study three involved an 8.5 month school-based activity program incorporated into the regular physical education curriculum. It also included calcium supplementation with calcium-enriched foods. A 2 x 2 (repeated measures) design was adopted for this study. The girls were randomly

assigned to either the high- or low-impact exercise group, and to receive either the calcium-enriched or non-enriched foods.

1.4 Limitations

The recognised limitations associated with studies one and two were:

1. **The group of 8 to 14 year old boys and girls who initially enrolled in the study were not representative of children of this age in the total population.**
   A representative sample in a study of this nature would demand a nation-wide random sample of children. The logistics of these demands mean that it would not have been feasible to use a representative sample of children.

2. **The normal calcium intake of the participants was monitored at intervals throughout the study period.**
   For practical purposes, calcium intake was determined using 24-hour recall up to 4 times a year. Due to the large number of participants and the multiple number of data collection times it was impractical to use weight food records. However, it is acknowledged that weighed food records are the gold standard by which to record nutrient intake.

The recognised limitations associated with study three were:

1. **The group of 7 to 10 year old girls who participated in this study was not representative of 7 to 10 year old girls in the total population.**
   A representative sample in a study of this nature would demand wide scale co-operation from a random sample of schools. Furthermore, it would intrude on a large number of school curriculum policies and programs, as the intervention would have to have been incorporated into the physical education curriculum. The logistics of these demands mean that it would not have been feasible to use a representative sample of girls.

2. **The environmental conditions in which the program was undertaken could not be controlled.**
   They included different ground surfaces (eg. grass or asphalt), or climatic conditions. Weather changes may have resulted in the program changing locations, such as moving indoors. The ground surface on which the program was conducted may have influenced the

nature of the impact and the subsequent osteogenic responses of the participants to the variable impacts.

3. **The motivational levels of the participants may have varied during the program.**
The amount of motivation may have influenced the amount of effort by the participants. This may in turn, have influenced the amount of impact incurred by participants.

4. **The normal activity levels and calcium intakes of participants outside of the program were monitored but not controlled.**
High levels of physical activity or calcium may have masked the potential influence of the program. In addition, participants may have adopted lifestyle changes during the intervention period. These changes may have altered their response to the intervention.

5. **Participants were of Caucasian and Asian decent.**
Although racial differences in bone mass have been reported, adjustments were made to baseline data during the analysis to minimise the potential for discrepancies associated with racial differences in response to the intervention.

1.5 Delimitations

The recognised delimitations associated with this study were:

1. **Females only were included.**
The existence of sex-specific differences in the response to calcium and exercise are unknown. A single sex group was used therefore, to eliminate the potential for sex-related differences.

2. **All girls were pre- or early-pubertal.**
Hormonal changes associated with advanced puberty may obscure changes as a result of the intervention. Seven to ten year old girls were selected as this helped to ensure that the participants were pre- or early-pubertal at the commencement of the intervention period, and remained pre- or early-pubertal for the duration of the intervention period.

3. **Participating girls were delimited to girls of similar socio-economic backgrounds.**
Socio-economic status may influence bone mineral accrual. To reduce the possible effect of socio-economic differences participants were selected from one socio-economic group.

4. **Participating girls were randomly allocated to the study groups.**

This type of allocation was made to reduce the potential for selection bias, as girls more suited to exercise may have self selected to be in the high impact group.

5. **A calcium supplementation level of 450 mg.day^{-1} was administered.**

This level was to increase calcium intake levels to be above or near the recommended levels for children of this age.

6. **No one methodology describes the impact of exercise on human bone in vivo.**

The activity levels of the girls within the confines of the program therefore, were monitored using the following techniques. The number of impacts during the program was determined by video surveillance. The average landing forces and peak magnitude of impacts of various movements performed during the sessions were determined using a pedar mobile system on a subset of girls. The amounts and type of activity that may have been effectual on bone, therefore could be estimated.

7. **The duration of the intervention period was delimited to 8.5 months.**

This duration was selected as it could be undertaken and completed within one school year. The duration however, was long enough to allow a potential osteogenic response to be observed.

8. **Baseline dietary calcium intake and physical activity levels were determined before, during and after the intervention period.**

The determination of dietary calcium intake was delimited to a 3-day food record. The determination of physical activity was delimited to the use of a pediatric specific questionnaire, which was validated with video surveillance. These methodologies were employed to determine if differences between the groups were apparent in baseline measures, which may have confounded the effects of the intervention.

9. **Changes in bone mineral measurements and body composition were delimited to detection via DXA.**

In agreement with ethical guidelines DXA measurements were only conducted annually. This method exposes participants to relatively low radiation doses, while providing sufficient accuracy to enable the apparent changes in bone mineral measurements to be observed.

10. Calcium supplementation was delimited to calcium-enrichment of food products.

Calcium supplementation may be in mineral form or of food sources, however, only supplementation via the enrichment of food sources has demonstrated residual benefits to measures of bone following the cessation of supplementation.

11. Blood and urinary measures were not taken

Due to the age group of the participants in the study, the inclusion of these measures would have severely reduced the number of girls who volunteered.

1.6 Definition of Terms

The following terms were defined with respect to their usage in this thesis

BMC *Bone mineral content*
The amount of bone mineral contained within the skeletal envelope.
Expressed in grams (g).

BMAD *Bone mineral apparent density*
Bone mass divided by the volume of the region scanned.
Expressed as grams per centimetre cubed (g/cm^3).

BMD *Bone mineral density*
Bone mass divided by the projected area of the area scanned.
Expressed in grams per centimetre squared (g/cm^2).

DPA *Dual photon absorptiometry*
Method of measuring bone mass and mineral density using low dose radiation.

DXA *Dual energy x-ray absorptiometry*
Method of measuring bone mass and mineral density using low dose radiation.

ECF *Extra cellular fluid*
Fluids located outside of the body's cells.

GH *Growth hormone*
Hormone released from the pituitary gland that is the primary mediator of linear growth.

IGF-1 *Insulin like growth factor*
Secreted in response to GH, IGF-1 stimulates the differentiation and proliferation of many different cells including the cells (chondrocytes) within the growth plates of long bones.

HI *High impact*
Movements that are associated with ground reaction forces of 1.8 times body weight or greater.

LM *Lean Mass*
Total mass – (fat mass + bone mineral content).

MES_m *Minimal effective strain for controlling modelling drifts*
Strain magnitude at which bone modelling is initiated.

MES_r *Minimal effective strains for controlling remodelling units*
Strain magnitude below that which bone remodelling predominates and bone loss occurs.

MU *Mechanical usage*
Strain threshold at which bone modelling and remodelling are initiated.

PBM *Peak bone mass*
Maximal bone mass achieved by the time of skeletal maturity.

PBMCV *Peak bone mineral content velocity*
Maximal velocity of bone mineral content accrual.

PFV *Peak fat velocity*
Maximal velocity of fat mass accrual.

PHV *Peak height velocity*
Maximal velocity of height gain, which is used as a marker of maturity.

PLV *Peak lean velocity*
Maximal velocity of lean mass accrual.

PMV *Peak mass velocity*
Maximal velocity of body mass accrual.

PTH *Parathyroid hormone*
Hormone released from the parathyroid gland, which is involved in calcium homeostasis.

SPA *Single photon absorptiometry*
Method of measuring bone mass and mineral density using low dose radiation.

Chapter Two

Literature Review

Sub-headings

2.1 Overview of bone growth

Childhood and adolescence are time periods characterised by the rapid growth and development of the skeletal system. Growth occurs in length, in width and in the mineral content contained within the skeletal envelope. To optimise the development of bone it is important to identify and promote factors that may have a positive effect on bone mass accrual. Two lifestyle factors that can be easily modified to enhance bone mass accrual are dietary calcium intake and exercise.

Adolescence is a time of accelerated skeletal growth therefore calcium needs may be heightened during this time. In spite of the greater calcium need during puberty, calcium intake levels of many young people do not meet recommended intake levels [English, 1989]. Furthermore, calcium intakes in females are reported to decrease during puberty, often well below recommended intake levels [Albertson et al., 1997]. The effect of these apparent inadequacies in calcium intake on skeletal integrity is yet to be determined.

Animal- and human-based studies have shown that mechanical loads applied to bone during growth results in greater bone mass accrual. The promotion of appropriate weight bearing exercise during childhood and adolescence may assist in the optimisation of bone mass accrual. If the enhanced bone mass accrued is maintained beyond the growth years, then the benefits of exercise undertaken during childhood and adolescence may be continued into later life. Exercise during childhood and adolescence may help reduce the risk of osteoporotic fractures in the elderly by promoting the accumulation of a greater reserve of bone, which can be drawn from to accommodate age-related bone loss.

Calcium supplementation and high impact weight bearing activity are both reported to have a positive effect on bone mass accrual [Johnston et al., 1992; Morris et al., 1997]. The amount of calcium or level and type of physical activity that elicits a positive osteogenic response is yet to be determined. In addition there is a paucity of information regarding the combined effect of calcium and exercise.

The review of literature presented commences with a brief overview of the bone growth process highlighting the dimensional and bone mineral content changes that occur during growth. The following two sections highlight the roles of calcium and physical activity on bone mass in both children and adults. The calcium discussion covers the overall metabolism of calcium, the association of calcium with bone mass, and trends in calcium intake in children and adolescents. The specific impact of calcium supplementation on bone mass accrual and the residual benefits of calcium supplementation are discussed. Discussions in the physical activity section, focus on a brief explanation of the mechanical principals underlying the osteogenic effect of exercise on bone, the

relationship between exercise and bone mass, and more specifically the effects of school-based exercise interventions on bone mass accrual in children. The literature review concludes with a discussion of the combined effects of calcium and exercise.

2.2 Normal bone development in growth

Longitudinal growth and skeletal consolidation are dynamic processes that commence *in utero* and conclude some time in the third decade [Matkovic, 1991]. During this time the skeleton will approximately triple in length, and accrue over 40 fold its initial calcium content, to contain approximately 1200 g of calcium by adulthood [Heaney, 1991].

The processes that bring about growth and expansion of the human skeleton are very detailed, involving the complex interaction of genetics, hormones and environmental factors. The importance of each of these factors is acknowledged however, for the purpose of this section, the discussion will be on the dimensional and mineral content changes of endochondral bone (that which replaces an existing cartilage model) from the postnatal period to skeletal consolidation.

The skeleton reaches its adult state through the processes of growth, modelling and remodelling. All three process can occur simultaneously throughout the skeleton however, growth and modelling predominate during the growth phase [Bailey et al., 1996]. Growth is the process of skeletal enlargement and elongation. Modelling enables the shape of the bone to be altered and the mass of the bone to increase, as new bone is added without the prior resorption (removal) of existing bone. The process of remodelling involves the replacement of fatigue damaged bone, with the existing bone being removed as new bone is added [Mundy, 1999]. The growth of bone therefore occurs in length, in thickness and in mineral content [Bailey et al., 1996].

2.3 Linear growth of the human skeleton

The lengthening of long bones occurs at the epiphysis, the band of cartilage that separates the diaphysis (shaft of the bone) from the epiphysis (end of the bone). The cells within the metaphysis multiply, and elongate the cartilage band. The cartilage closest to the diaphysis is then replaced, initially by woven bone, then by lamellar bone, with the later being layered and structurally stronger [Solomon et al., 1990].

Linear growth does not occur at a constant rate, but varies throughout the growth period. Linear growth is characterised by accelerated phases during infancy and puberty, with a lesser rate of growth reported during childhood [Hagg & Taranger, 1991]. Prior to puberty little or no sex differences have

been reported in yearly rates of height gains. Hagg and Taranger (1991) reported growth rates of 25.6 $cm.yr^{-1}$ and 25.1 $cm.yr^{-1}$ for male and female Swedish infants, respectively. Tanner et al. (1966) reported growth rates of 5.7 $cm.yr^{-1}$ and 5.8 $cm.yr^{-1}$ for British male and female children ages 5-10 years, respectively. Sexual dimorphism in height gains occurs during the pubertal period [Matkovic 1992].

Sex differences are apparent in the timing and magnitude of peak height velocity (PHV), which is the acceleration of linear growth which coincides with puberty [Bailey, 1997]. The earlier age of PHV reported for females is consistent with the earlier onset of maturity in females compared to males [Malina & Bouchard, 1991]. The magnitude of PHV however, is greater in males than females. The timing and magnitude of PHV from previous growth studies are summarised in Table 2.1. Calculated from the data presented in Table 2.1 PHV occurs approximately 1.9 years earlier in females than males. The magnitude of PHV is approximately 1.3 $cm.yr^{-1}$ greater in males compared to females.

The majority of adult stature is achieved before the completion of puberty. Bailey (1997) determined that 90% of adult height in males and 92% of adult height in females was achieved by the age of PHV. Following sexual maturity, the rate of height gain slows considerably. Kroger et al. (1993) reported gains in height of less than 0.2 $cm.yr^{-1}$ after menarche in 37 females. Overall height results from lengthening of the legs and the trunk. During childhood, linear growth results from a proportionally greater gain in leg length compared to trunk length, while pubertal growth is characterised by an acceleration in the rate of gain in trunk length, and a slowing in the rate of growth of the legs [Bass et al., 1999].

2.4 Dimensional changes to bone with growth

Dimensional changes to bone result from differing rates of apposition and resorption from the inner (endosteal) and outer (periosteal) surface of bone. These differences result in changes to the total width of the bone, the width of the medullary cavity and to the thickness of the cortical wall (Figure 2.1). Sex-related differences are evident in relation to the dimensional changes to bone.

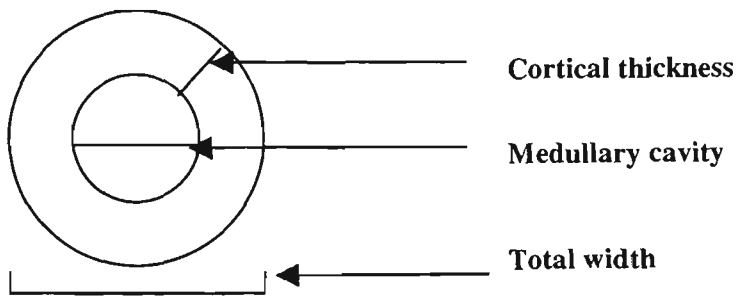

Figure 2.1: The dimensions of bone

Table 2.1: Peak height velocity data from previous growth studies.

Reference	**Study**	**Age PHV (yrs)**		**Magnitude PHV ($cm.yr^{-1}$)**	
		Females	Males	Females	Males
Beunen et al., 1992	Lueven Growth Study of Belgian Boys	-	14.1 ± 0.8	-	8.9 ± 2.1
Geithner et al., 1998	Warsaw Longitudinal study	11.8 ± 0.7	-	7.8 ± 1.8	-
Largo et al., 1978	Zurich Longitudinal Study	12.2 ± 1.0	13.9 ± 0.8*	7.1 ± 1.0	9.0 ± 1.1*
Lindgren, 1978	Random sample of Urban Swedish children	11.9 ± 0.9	14.1 ± 1.1*	8.3 ± 1.2	9.8 ± 1.4*
Lopez-Blanco et al., 1995	Caracus Mixed-Longitudinal Study	11.7	13.5	8.6	9.6
Martin et al., 1997	Saskatchewan Pediatric Bone Mineral Accrual Study	11.4	13.3	6.3	7.3
Tanner et al., 1966	British Standards	12.0	14.0	8.3 ± 1.1	9.5 ± 1.2
Zemel & Johnston, 1994	3rd Harvard Growth Study	12.2 ± 0.9	14.1 ± 0.9*	7.7 ± 1.1	9.1 ± 1.2*

* Sex comparisons not reported

Total bone width increases rapidly within the first year of life and again during the pubertal growth spurt, with moderate gains in bone width occurring during childhood. Tanner et al. (1981) reported gains of approximately 1 $mm.yr^{-1}$ in tibia width in pre-pubertal children. The accelerated gain in width which corresponds with the adolescent growth spurt occurs earlier in females than males however, the magnitude of the gains in bone width are greater in males than females [Malina & Bouchard, 1991]. Tanner et al. (1981) reported 13% greater bone width at the tibia and humerus in males compared to females after puberty. Overall, males have greater bone width due to; a later pubertal spurt (longer childhood growth period), a greater magnitude of gain during puberty, and the overall growth period of bone width expansion lasting longer in males compared to females [Garn, 1970].

Changes on the endosteal (inner) surface influence the size of the medullary cavity. Garn (1970) indicated that the endosteal surface of bone move from a phase of resorption, to a phase of apposition, then back to resorption again. Garn (1970) reported that resorption occurred at the endosteal surface rapidly during infancy, then more slowly until approximately aged 14 years in females and approximately age 18 years in males. Resorption commenced again at approximately age 40 in both males and females. The rate of resorption peaks at approximately age 45 in females. The findings by Garn (1970) are supported in part by work from others. Johnston and Watt (1969) reported a 16% reduction in the width of the medullary cavity of the second metacarpal in post-menarcheal girls compared to pre-menarcheal girls. Song et al (1994) reported a decrease in medullary diameter with increasing chronological and skeletal age in girls aged 12-18 years. Apposition of bone on the endosteal surface occurs at a greater rate in females compared to males, resulting in a smaller medullary cavity in girls compared to boys [Garn, 1970]. Tanner reported a 13% greater medullary width of the tibia in males than females after puberty. Sex-related differences are depicted in Figure 2.2. Following the period of apposition, resorption occurs at the endosteal surface during adulthood and older age in both males and females [Garn, 1970]. Ruff and Hayes (1988) reported no sex-related difference in the increase in medullary area of the tibia and femur in adults based on cross-sectional data.

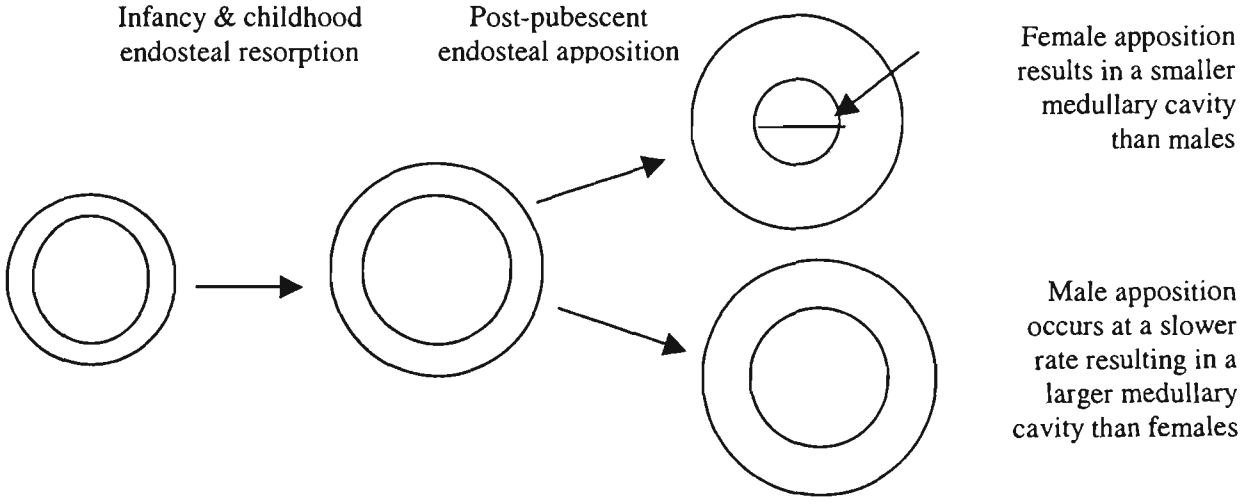

Figure 2.2 Endosteal resorption and apposition during the growth period

The differences in the timing and rates of resorption and apposition of bone on the inner and outer surface of bone will influence the thickness of the cortex. Cortical wall thickness as described by Garn (1970) generally increases from infancy to adulthood, with males generally demonstrating a greater cortical thickness compared to females. Tanner et al. (1981) reported similar gains in cortical thickness of the tibia in boys and girls during childhood. Gryfe and Exton-Smith (1971) reported that boys on average had a greater cortical area at the second metacarpal compared to girls. Sexual dimorphism in cortical area or thickness occurs during the pubertal period. Gryfe and Exton-Smith (1971) observed that the spurt in growth in cortical area of the metacarpal occurred 2 years earlier in females than males (at approximately 10 years of age). Garn (1970) noted that the increase in cortical thickness in females during puberty results from apposition of bone on both the endosteal (inner) surface and periosteal (outer) surface of bone. This observation is supported by the findings of Song et al. (1994). The authors reported an increase in cortical area and thickness of the second metacarpal with skeletal age in adolescent females. Cortical area increased form 36.4 mm^2 to 50.8 mm^2 between the ages of 12 to 18 years. Concurrently there was a significant decrease in medullary area and an increase in total area with skeletal age in this group of girls. Although during early adolescence the cortical thickness is greater in females than males, the period of apposition is longer in males compared to females [Garn, 1970], resulting in greater cortical thickness in males compared to females, by late adolescence. Tanner et al (1981) reported a 13% greater cortical area in males compared to females by the end of puberty.

Bouxsein et al. (1994) reported greater cortical area and thickness at the distal radius and ulna in younger women (n = 21, 20-30 years of age) compared to older women (n = 22, 64-84 years of age), indicating a decrease in cortical area with age in women. Ruff and Hayes (1988) described earlier made a similar observation. They observed that following adjustment for bone length, cortical area of the tibia decreased with age in women (aged 20 – 90 years), however remained relatively constant with age in similarly aged men. The authors suggest that the compensatory apposition of bone on the periosteal surface in males offsets the loss of bone at the endosteal surface (significant increase in medullary area) thus maintaining the cortical area and inturn the structural integrity of the bone.

In summary infancy and adolescence are periods of rapid linear growth and expansion of bone width compared to childhood. Sex differences in growth of the skeleton are less pronounced during childhood. Sexual dimorphism however, becomes evident during puberty, which is likely to be mediated by the release of sex hormones. Sex- related differences are also evident for bone mineral accrual during growth.

2.5 Bone mineral accrual and loss during the lifespan

Bone mineral is progressively accrued in bone up to the age of peak bone mass, which is reported to occur between the ages of 15 to 30 years [Matkovic et al., 1994; Ott, 1990; Theintz et al., 1992]. Following the attainment of peak bone mass, and a period of maintenance of bone mass during adulthood, calcium is progressively lost from bone [Seeman, 1997]. Although all individuals accrue and lose bone mineral content during their lifetime, the rates of accrual and loss are not constant throughout the lifespan, and vary depending on age and sex.

At birth the human body contains approximately 25 grams of calcium, which increases to approximately 1000-1200 mg by adulthood [Heaney, 1991]. The greatest rates of BMC accrual occur during early infancy [del Rio et al., 1994; Southard et al., 1991] and the pubertal growth spurt [Bailey, 1997], with lesser rates of accrual reported during childhood [Zanchetta et al., 1995]. Del Rio et al. (1994) used BMD as a measure of bone mineral content and size. Based on data collected for gains in lumbar spine BMD and BMC, an almost four fold increase in annual gain in BMD was observed in 2 year old children compared to 6-7 year olds (0.14 $g.cm^2$ v 0.03 $g.cm^2$).

Mean rates of total body BMC accrual in children (calculated from cross sectional data) have been estimated at between 100-150 $g.yr^{-1}$. Zanchetta et al. (1995) reported annual rates of total body BMC accrual of 104 $g.yr^{-1}$ and 105 $g.yr^{-1}$ for Argentine boys and girls aged 5 to 10 years, respectively. Molgaard et al. (1997) reported annual total body BMC accrual rates of 123 $g.yr^{-1}$ and 121 $g.yr^{-1}$ for 7 to 10 year old Danish boys and girls, respectively. Nelson et al. (1997) reported slightly higher rates of total body BMC accrual at 145 $g.yr^{-1}$ and 154 $g.yr^{-1}$ for 9-10 year old American boys and girls, respectively.

Based on cross sectional analysis it appears that prior to puberty, little sex-related differences occur in rates of bone mineral accrual. Nelson et al. (1997) described above noted no difference in total body BMC accrual between the 158 males and 130 females in their study. Bonjour et al. (1991) also reported no sex-related differences in rates of gains in BMD at the femoral neck, lumbar spine and femoral shaft between boys and girls aged 9–13 years. Sex-related differences in the timing and rate of bone mineral accrual appear during puberty.

During the pubertal period peak rates of bone mineral accrual calculated from cross-sectional data have been reported at between 200 $g.yr^{-1}$ to 400 $g.yr^{-1}$ [Martin et al., 1997]. Peak velocity for total body BMC occurs approximately two years earlier in females than males [Bailey 1997; Molgaard et al., 1997]. Molgaard et al. (1997) reported the greatest gains in total body BMC were between the

ages of 12-14 years in females and 14-16.5 years in males. Limited data are available that describe peak total body bone mineral accrual velocities during puberty. Bailey (1997) used longitudinal analysis to determine peak rates of total body bone mineral content (BMC) accrual in a cohort of over 200 Canadian children aged 8-14 years. Peak rates of total body BMC accrual were estimated at 320 $g.yr^{-1}$ for males and 230 $g.yr^{-1}$ for females. The absolute amount of BMC accrued during the pubertal period is greater for males than females [Bailey, 1997]. Bailey (1997) calculated that the total body BMC accrued during the two years before and two years after the age of PHV (over a four year period) was 1072 grams for males and 772 grams for females, which represented 36% of adult BMC.

Age related bone loss is greater in females than males. Perry et al. (1996) noted that approximately 230g grams of calcium is lost from the adult female skeleton from the time of peak bone mass through to old age, while males lose about 100g [Seeman, 1997]. As the female skeleton is generally smaller than the male skeleton, this absolute loss of bone mineral will result in a greater proportional loss of total bone mass.

From the growth data presented it is apparent that males tend to demonstrate overall greater bone mass than females. The sex-related difference results from greater height in males, due to a longer growth period, and greater magnitude of height gain at peak. Males also have greater bone width than females, which is also reflected in more bone mineral content. Males therefore, posses a larger and geometrically stronger bone than females. The greater bone mass achieved by males may be a contributing factor to their greater resistance to osteoporosis in later life when compared to females. Maximising peak bone mass may be a means of preventing low bone mass in later life.

2.6 Peak bone mass

Peak bone mass (PBM) is the maximal bone mass achieved by the completion of linear growth and skeletal consolidation. Discrepancies exist regarding the age at which PBM occurs which may, in part, be due to data presented being cross sectional. Initially, authors suggested that PBM occurred in the third and as late as the fourth decade of life [Ott, 1990]. Other authors suggest that PBM may occur earlier than was previously indicated. Lu et al. (1994) in their cross sectional assessment of bone mass in 136 males and 130 females aged 4 to 27 years reported that peak total body, lumbar spine and femoral neck BMD occurred at approximately 17.5 years of age in males. Peak total body and lumbar spine BMD occurred at approximately 15.8 years of age in females. Femoral neck BMD for females peaked earlier, at 14.1 years of age. Peak total body BMC occurred at 17.4 years of age in males and 15.7 years of age in females. Following the age of peak, the authors noted that there were no further age-related gains in BMC and BMD.

Matkovic et al. (1994) investigated the timing of PBM in 265 pre-menopausal Caucasian females aged 8–50 years using a cross sectional method of assessment. Using regression models, it was reported that most of the BMC at the lumbar spine, proximal femur, radius and for total body was accrued by late adolescence. The inflection in BMC accrual with age occurred between the ages of 17– 22 years depending on the site being assessed. It was noted that beyond the age of 18 years no differences were found between the younger and older pre-menopausal women for measures of bone mass at weight bearing sites.

Haaspasalo et al. (1996) evaluated lumbar spine, femoral neck, trochanter and distal radius BMC in 330 pre-menopausal females aged 7 – 47 years. Peak BMC values occurred at 21 years of age for lumbar spine, 19 years of age for the trochanter and femoral neck and 18 years of age for the radius. Peak values for BMD occurred between the ages of 18 - 21 years.

From the data presented, it appears that PBM occurs by late in the second decade or early in the third decade, which is much earlier than previously reported. Most of adult BMC and adult height therefore is accrued by late adolescence, which further emphasises the importance of the childhood and adolescent growth periods as significant contributors to adult bone mass. It is speculated therefore that the BMC accrued during growth may provide some protection against bone loss in later life, by providing a greater reserve of bone and bone mineral to draw from to cope with the natural bone loss process. If so, then the growth period may be a critical period in which to influence bone mass, strength and integrity. An understanding of the factors that effect BMC accrual therefore is imperative in maximising bone mass with the potential to prevent osteoporosis in later life. An adequate dietary calcium intake may be one lifestyle factor that influences bone mass accrual. The following section of the literature review will focus on calcium in relation to bone.

2.7 The role of calcium in the body

Calcium is a major inorganic element present in all living cells. At birth the human body contains approximately 25 grams of calcium. This amount increases to approximately 1000 - 1200 mg by adulthood [Heaney, 1991]. Ninety-nine percent of the body's calcium is found in bone (skeleton and teeth). The remaining 1% is found in extra cellular fluid, plasma, and in cell membranes [Kanis, 1994].

Calcium is an essential element, and performs both regulatory and structural roles in the body. In its regulatory role, calcium is necessary for blood clotting, muscle contractions, nerve transmission, hormonal function, and membrane transport [Gibson, 1990]. Calcium also has an important role in bone structure. Calcium is a major mineral component of bone representing approximately one third of the mineral in bone. Calcium is combined with phosphate to form the mineral hydroxyapatite, which is deposited into the collagen (protein) matrix that makes up bone tissue. The mineralisation of bone tissue contributes to its strength and hardness [Broadus, 1999]. The skeleton also serves as a reserve for this nutrient. Bone is the major tissue involved in the regulation of plasma calcium levels.

2.8 Regulation of plasma calcium levels

Despite large variations in dietary calcium intakes, plasma calcium levels are maintained within the relatively narrow range of 2.2 – 2.5 $mmol.L^{-1}$ [Arnaud & Sanchez, 1990]. The precision of calcium homeostasis results from the integrated response of the endocrine system. Principally, this involves the parathyroid hormone (PTH) which is released from the parathyroid gland, and calcitonin, which is released from the thyroid gland. Regulation is based on a negative feedback model, whereby PTH and calcitonin maintain plasma calcium levels by altering intestinal calcium absorption, renal calcium excretion, and the flux of calcium into and out of bone [Arnaud & Sanchez, 1990]. The relationship between these two regulatory hormones is demonstrated in Figure 2.3.

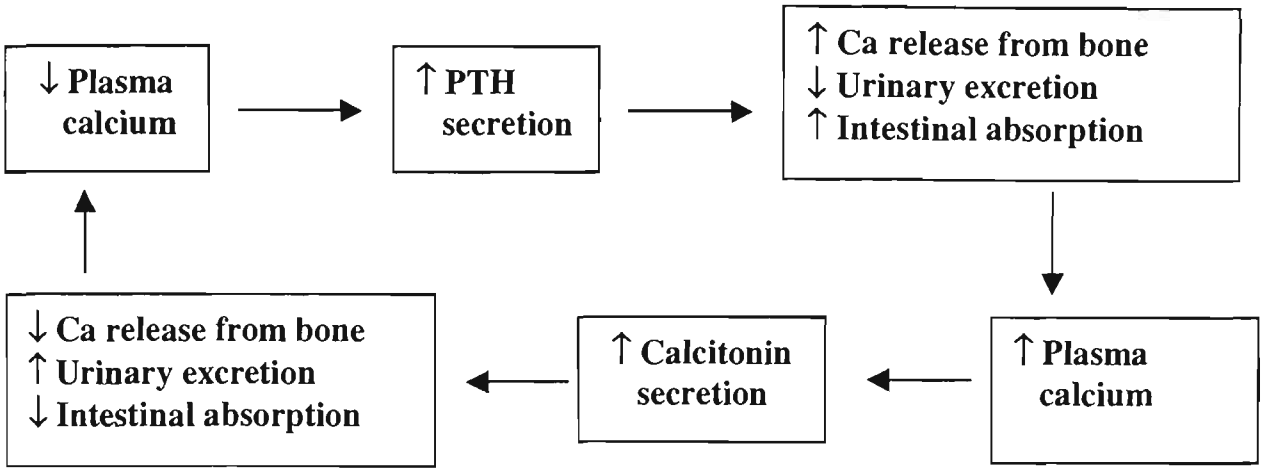

Figure 2.3. The relationship between hormonal and physiological responses to changes in plasma calcium levels.

2.9 Overview of calcium metabolism

Calcium metabolism can be divided into three components: intake, absorption, and excretion. In adults, approximately 25% - 30% of dietary calcium is absorbed in the gut [Passmore and Eastwood, 1986]. Calcium absorption during growth varies with maturity [Abrams et al. 2000]. The remaining calcium passes through the intestinal tract and is excreted in the faeces [Bueslau, 1996]. Absorbed calcium enters the extra cellular fluid, where it is either: involved in bone remodelling (or other metabolic processes such as blood clotting), filtered through the kidneys, or is lost via the skin. Urinary calcium excretion in adults approximates 100 $mg.day^{-1}$ – 350 $mg.day^{-1}$ [Passmore & Eastwood, 1986], with lower rates of excretion reported in children (20 $mg.day^{-1}$ – 200 $mg.day^{-1}$) [Bronner & Abrams, 1998]. Calcium sweat losses in adults were initially reported to be relatively low (approximately 15 $mg.day^{-1}$) however, dermal losses during heavy sweating in the heat or through intensive exercise may exceed 100 $mg.hour^{-1}$ [Passmore & Eastwood, 1986]. Dermal losses in active children have been estimated at 60 $mg.day^{-1}$ [Charles et al., 1983]. Prolonged, heavy sweating may, therefore contribute to substantial calcium losses if experienced regularly [Klesges et al., 1996]. Approximately 700 mg of calcium is turned over in the mature skeleton daily [Passmore & Eastwood, 1986]. Figure 2.4 summarises the aforementioned discussion on the metabolism of calcium.

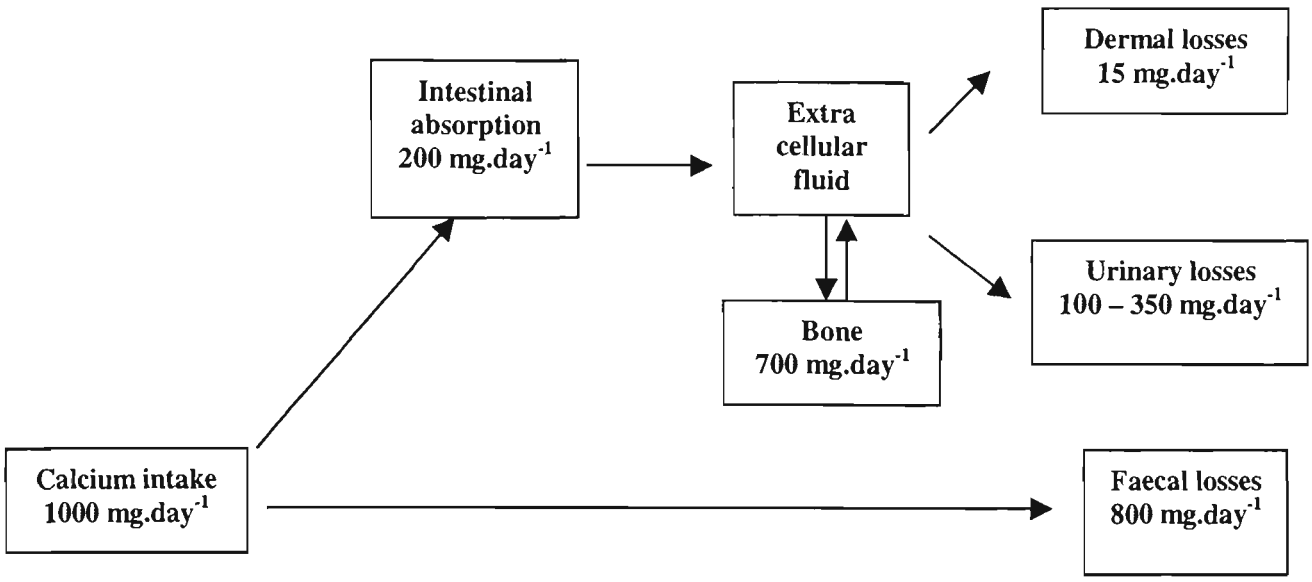

Figure 2.4: Calcium metabolism in the human body [Passmore & Eastwood, 1986].

2.10 Calcium balance

Calcium balance refers to the amount of calcium needed to maintain adult bone mass, or in the case of children, the amount needed to allow for skeletal development [Matkovic, 1991]. Adulthood is characterised by a zero calcium balance, as calcium enters and leaves the bone at equal rates. The equilibrium of skeletal calcium occurs via the process of remodelling. This process involves the coupling of the actions of osteoclasts (which remove bone) and osteoblasts (which replace bone) at discrete locations on the skeleton. The mechanisms that result in the coupling of these two contrasting actions are not clearly understood [Mundy, 1999]. The end result is maintenance of skeletal calcium. Calcium balance during childhood and adolescence is generally positive as new bone is added. Bone modelling occurs during childhood and adolescence and is responsible for the growth and re-shaping of bone. Unlike remodelling, during modelling bone formation is not immediately preceded by resorption [Baron, 1999]. The end result is the accrual of skeletal calcium. While researchers agree that a positive balance is necessary for skeletal growth, the degree of positive balance needed to maximise bone mineral accrual and achieve peak bone mass remains unknown [Matkovic, 1991].

The determination of calcium balance during adulthood is based on an assumed relationship between calcium intake and excretion using the following equation [Matkovic et al., 1990]:

$$Ca_{Balance} = Ca_{intake} - (Ca_{faecal} + Ca_{urinary})$$

Initial 'calcium balance' studies in adults used incremental increases in calcium intake until calcium intake equalled calcium excretion [Leitch & Aitken, 1959]. The authors in these earlier studies used a

standard rate of calcium absorption to establish the amount of dietary calcium needed to maintain zero balance.

These balance studies used to determine recommended calcium levels rely on the existence of a relationship between calcium intake and excretion. The rates of absorption and urinary excretion in the metabolism of calcium however, may be influenced by other dietary and regulatory factors, and vary independently of each other. For example calcium absorption varies with maturity [Weaver et al., 1995], and is influenced by the dietary fibre content of the diet [Heaney & Weaver, 1989; Levenson & Bockman, 1994]. Calcium excretion may be influenced by the protein content of the diet [O' Brien et al., 1996]. These factors challenge the accuracy of calcium requirements determined from balance studies.

Calcium balance has been linked to calcium intake [Matkovic et al. 1990; Matkovic, 1991] however, the relationship is not necessarily maintained for high calcium consumers, which led to the concept that calcium may be a threshold nutrient. Matkovic (1991) reviewed 487 balance studies spanning the period of skeletal growth to peak bone mass (0 – 30 years). From this analysis, Matkovic (1991) reported a positive correlation between calcium intake and calcium balance ($r = 0.589$, $p < 0.001$). The relationship was evident in all groups representing different stages of development (infancy, childhood, adolescence and adulthood). A further review of 34 published balance studies by Matkovic and Heaney (1992) reported a positive correlation between calcium intake and balance for children and adolescents aged 9-17 years. The intake of participants ranged from 225 to 988 mg of calcium a day ($n = 33$, $r = .803$, $p < 0.01$). No relationship was found for those participants with very high calcium intakes (1730 – 2721 $mg.day^{-1}$).

Matkovic and Heaney (1992) also calculated the level of calcium intake at which the relationship between intake and balance ceased. The inflection occurred at approximately 1500 $mg.day^{-1}$ for children and adolescents and 1000 $mg.day^{-1}$ for adults. Matkovic and Heaney (1992) postulated that calcium intakes greater than 1500 $mg.day^{-1}$ in young populations may not necessarily lead to substantially greater gains in bone mass compared to intakes up to 1500 $mg.day^{-1}$. Based on these findings, optimal calcium retention appears to occur at intakes in excess of the current recommended levels for children of between 700-1200mg per day [Briggs & Wahlqvist, 1988]. The concept of calcium being a threshold nutrient presented by Matkovic and Heaney (1992) was extrapolated from cross sectional data. Further research is needed to confirm or refute the occurrence of this threshold. Based on the mechanisms of calcium metabolism it may be suggested that a point of saturation may occur whereby further increases in calcium intake would not lead to substantial increases in bone mass as a measure of calcium balance.

Furthermore, the relationship between calcium intake and balance is likely to be confounded by variations in absorption and excretion during the metabolism of calcium.

2.11 Calcium absorption

Calcium absorption occurs via two mechanisms: active transport in the duodenum and passive diffusion throughout the small intestine [Breslau, 1996]. During active transport calcium is moved through the epithelial cells bound to the carrier protein calbindin. This process is Vitamin D dependent and is down regulated when calcium intake is sufficient, and up regulated when calcium intake is low [Bronner & Pansu, 1999]. Passive diffusion is not regulated [Breslau, 1996], and is the principle mechanism of calcium absorption when calcium intake is adequate or high [Bronner & Pansu, 1999].

Calcium absorption is reported to increase during times of high need such as rapid skeletal growth (infancy and adolescence), pregnancy, and lactation [Passmore & Eastwood, 1986]. Matkovic (1991) compared calcium absorption rates for participants with calcium intakes within a normal range (683 to 1297 $mg.day^{-1}$). The analysis involved studies including infants (0 – 1 year, n = 88), children (2 – 8 years, n = 82), adolescents (9 – 17 years, n = 117) and young adults (18 – 30 years, n = 159). Higher rates of calcium absorption were reported for infants (36% - 44%) and adolescents (40% - 41%), when compared with children (24% - 27%) and adults (20% - 24%).

Further studies have reported similar findings to Matkovic (1991). Weaver et al. (1995) compared the net calcium absorption from a controlled mixed diet, between 14 adolescent girls (13.1 ± 0.9 years) and 11 young adult females (22.2 ± 4.2 years) involved in a three-week balance study. A greater rate of calcium absorption was reported for the adolescent girls compared to the adult women (32.3% versus 20.6%, $p < 0.05$). Abrams and Stuff (1994) compared calcium absorption rates from self-selected diets in 51 females aged between 4.9 years to 16.7 years, who represented three stages of maturity. A greater rate of calcium absorption (34.4%) was reported in early-pubescent females (n = 13), when compared with pre-pubescent (absorption = 27.7%, n = 21, $p < 0.05$) and late-pubescent females (absorption = 25.9%, n = 17, $p < 0.01$). Thus in girls, the skeletal expansion that occurs during early puberty (eg. PHV) may be accommodated by greater rates of calcium absorption, which have been reported during this stage of development compared to the pre- and post-pubertal periods [Abrams et al., 2000]. These observed changes in rates of calcium absorption may serve to compensate for the reduction in calcium intake in females during adolescence [Ruiz et al., 1995: Sentipal et al., 1991].

The percentage of dietary calcium absorbed (fractional calcium absorption) was reported to increase with decreased calcium intake in healthy young girls, adult women, osteoporotic women, and girls with a family history of osteoporosis [Heaney et al., 1990; O'Brien et al., 1996; O'Brien et al., 1998]. Heaney et al. (1990) determined the fractional calcium absorption in 24 healthy women aged 29 to 45 years. Each woman consumed three of five prescribed calcium loads (15 mg, 36 mg, 86 mg, 205 mg, 500 mg) in a random sequence. The fractional absorption of calcium was inversely correlated with the logarithm of the load ($p < 0.001$). At the lowest calcium load, fractional absorption averaged 64.0%. In contrast, fractional calcium absorption averaged 28.6% at the highest calcium load.

O'Brien et al. (1996) found similar results to those reported above. These investigators evaluated calcium absorption efficiency in 11 girls (mean age 11.9 ± 2.4 years) after 10 days on a low calcium diet (7.05 ± 2.03 $mmol.d^{-1}$) and 10 days on a high calcium diet (35.30 ± 2.28 $mmol.d^{-1}$). Fractional calcium absorption was greater for the low calcium diet compared to the high calcium diet (58.2% ± 8.7% and 26.0% ± 6.8%, respectively, $p < 0.0001$). These data indicate that fractional calcium absorption appears to increase with decreased intake in females. However, if the calcium intakes are very low, in spite of the greater rate of absorption, the amount of available calcium may still be compromised.

Net calcium absorption is reported to increase with calcium intake [Matkovic et al., 1990]. For example, if 15% of 1000mg of dietary calcium is absorbed then the net Ca absorbed is 150mg. However, if 30% of 300 mg of dietary calcium is absorbed then the net Ca absorbed is 90 mg. This is supported by work presented in the literature. Heaney et al. (1990) reported that the net amount of calcium absorbed based on the calcium loads and fractional absorption rates were 10 mg, 102 mg and 143 mg, respectively for calcium loads of 15 mg, 300 mg and 500 mg. In addition, O'Brien et al. (1996) demonstrated that the actual calcium absorbed was greater for the young girls when they consumed the high calcium diet when compared with the low calcium diet (8.98 ± 2.65 $mmol.day^{-1}$ and 4.04 ± 1.00 $mmol.day^{-1}$, respectively, $p < 0.001$).

In summary, available data indicate that an improvement in calcium absorption efficiency occurs with a reduction in calcium intake. The improved absorption efficiency however, does not appear to be sufficient to overcome the total deficit in intake, and hence net absorption is lower than at higher calcium intakes. It may be suggested that if dietary intakes are very low, calcium balance may be compromised, but within the normal range of dietary calcium intakes, changes to the rate of absorption may accommodate the partial deficit in intake. Furthermore, these data presented have been obtained from studies employing short-term dietary manipulations. Therefore, results may not necessarily relate to

chronic or long-term reductions in calcium intake, where alternative adaptations may occur [Heaney, 1991].

Calcium absorption occurs within the context of a total diet. Constituents of other foods in the diet may influence the absorption of calcium. For example, calcium forms insoluble complexes with phytates in wholegrain cereal products, oxalates contained in green leafy vegetables, nuts and tea, and the uronic acid component of dietary fibre [Breslau, 1996]. The insoluble complexes reduces the solubility (and bioavailability) and subsequent absorption of calcium [Heaney & Weaver, 1989; Levenson & Bockman, 1994]. In addition, fatty acids, especially saturated fatty acids form insoluble soaps, which also hinder calcium absorption. Excessive phosphate and an alkaline environment may also decrease calcium absorption [Breslau, 1996]. Alternatively, some amino acids, lactose and an acidic environment in the intestine increase the bio-availability of calcium thus promoting absorption. Adequate vitamin D is also necessary for the absorption of calcium [Levenson & Bockman, 1994].

Dairy foods are often promoted as the most readily absorbable food source of calcium [Peacock, 1991]. Recent reviews of calcium absorption studies presented below however, indicate that calcium from other sources may be absorbed as efficiently as calcium from milk and milk products. Calcium absorption rates calculated from several studies indicate that calcium absorption from milk averages 33%, which compared less favourably with mean absorption rates of 37% for calcium chloride and 35% - 36% for calcium citrate maleate [Heaney & Weaver, 1989; Levenson & Bockman, 1994; Miller et al., 1988]. Calcium absorption rates less than those reported for milk were found for calcium oxalate (10% - 11%) [Heaney & Weaver, 1989], calcium carbonate (26% - 28%) [Miller et al., 1988], tricalcium phosphate (25%) and calcium citrate (22%) [Levenson & Bockman, 1994]. Similar rates of absorption to milk were reported for calcium lactate (32%) and calcium acetate (32%) [Levenson & Bockman, 1994].

The basis of the differences in absorption rates between calcium complexes is the solubility of the calcium complex (how easily the Ca^{2+} can dissociate from the compound and be absorbed). Intra-luminal factors may influence the availability of calcium from various sources. For example, an acidic environment will promote the dissociation of calcium from calcium carbonate, while other complexes (eg. calcium oxalate) are not as sensitive to pH levels [Breslau, 1996]. The availability of calcium from milk may be enhanced as the calcium is able to form more soluble complexes with the amino acids and lactose in the milk in place of the less soluble calcium phosphate complex that occurs naturally in milk [Breslau, 1996]. The absorption of calcium from milk minerals after being extracted from milk (contains limited lactose and protein) has not been extensively studied.

These data presented indicate that calcium absorption is greater during infancy and adolescents, compared to childhood and young adulthood. Also within the period of puberty in females, absorption appears to be greater during early puberty, compared to the rates of absorption observed during the pre- and post-pubertal periods. At a high calcium intake more absolute calcium is absorbed than at low intakes however, the proportion of calcium that is absorbed is less (smaller percentage). The form of the calcium, and other components contained in the diet may also influence absorption rates. In addition to calcium intake and absorption potentially influencing calcium balance, calcium losses may also influence calcium balance.

2.12 Calcium excretion

Calcium balance is determined from calcium intake and calcium excretion. As previously outlined, calcium losses may occur from dermal, faecal and urinary avenues. Dermal losses were estimated at approximately 15 $mg.day^{-1}$ however, losses from active children have been estimated in excess of 60 $mg.day^{-1}$ [Charles et al., 1983]. Faecal calcium losses are not regulated. These losses appear to be a function of intake and are reported to increase with increasing calcium intakes in children and young adults [Matkovic, 1991]. The principle means of regulating calcium excretion is via urinary losses. While earlier studies assumed a relationship between urinary calcium excretion and dietary calcium intake, other dietary constituents may influence this relationship.

Urinary calcium was reported to decrease with short-term reductions in calcium intake in 11 girls aged 11.6 ± 2.4 years who were fed a low calcium diet for 10 days [O'Brien et al., 1996]. Matkovic et al. (1995) however, reported that urinary calcium was not related to intake in 370 girls aged 8–13 years when consuming their usual diet. Matkovic et al. (1990) earlier reported that urinary calcium losses remained unchanged in spite of variations in calcium intake (270 $mg.day^{-1}$ – 1242 $mg.day^{-1}$) in 31 fourteen-year-old girls involved in a calcium supplementation trial. Jackman et al. (1997) reported that only 6% of the variance in urinary calcium excretion of 35 post-menarcheal girls could be accounted for by calcium intake.

Other dietary constituents may also influence urinary calcium losses. Both sodium and protein are purported to influence urinary calcium levels [Heaney, 1993; Matkovic et al., 1995; O'Brien et al., 1996]. Matkovic et al. (1995) found a positive relationship between urinary sodium levels and urinary calcium levels ($r = 0.419$), with urinary sodium being the strongest determinant of urinary calcium levels.

O'Brien et al. (1996) also reported a positive relationship between urinary sodium and urinary calcium levels ($r = 0.55$, $p < 0.0001$) in 89 girls aged 5 to 17 years, who consumed their regular diets.

While Heaney (1993) suggested that urinary calcium levels were influenced by protein intake, neither Matkovic et al. (1995) nor O'Brien et al. (1996) reported an association between protein intake and urinary calcium losses. The evidence presented by Heaney (1993) to support the effect of protein on urinary calcium losses was however, derived from adult data. Adult protein requirements have been shown to differ from those of children and adolescents during growth [Barr, 1995]. O'Brien et al. (1996) suggested the lack of association between protein intake and calcium losses may be due to heightened protein needs during periods of rapid such as during childhood and adolescence. Further research is needed to determine if protein influences calcium excretion differently in adults than children and adolescents.

From these data reviewed it appears that faecal calcium excretion is dependent of calcium intake. Urinary calcium excretion however, may be independent of calcium intake. The inability of all studies to report an association between calcium intake and urinary calcium excretion raises questions about the appropriateness of these two variables being used as the principle means of determining calcium needs in humans.

2.13 Calcium intake recommendations

Recommended intakes for calcium have been established to ensure an adequate intake for 95% of the population [National Research Council, 1989]. In Australia the recommended calcium intake levels range from 500 mg - 1200 $mg.day^{-1}$ depending on age [NHMRC, 1987]. Exact calcium needs remain uncertain, as minor deficiencies in intake are not necessarily clinically detectable. Even less certainty surrounds the requirements for children, with earlier recommendations for calcium intake for children in North America set at arbitrary levels extrapolated from adult data [Kanis, 1994].

Earlier recommendations for adults were based on balance studies with minimal requirements extrapolated from the point where intake and output were at zero balance [Leitch &Aitken, 1959]. From the data presented in the previous section it appears that the assumption of a relationship between calcium intake and output may be erroneous. The level of urinary calcium excreted may be independent of intake [Matkovic et al., 1995; Jackman et al., 1997] and other dietary constituents such as protein and sodium may influence calcium excretion [Matkovic et al., 1995; O' Brien et al., 1996]. Furthermore, adaptation

to prolonged reduction in calcium intake may occur. This may lead to a conservation of calcium and diminished urinary excretion [Heaney, 1991].

Recent North American calcium intake recommendations for children aged 4 – 13 years have been set at 1300 mg.day^{-1} and were based on bone mineral accrual studies [Inst. of Med., 1997]. These recommendations therefore, may accommodate calcium needs during growth more suitably than recommendations based on balance studies. These recommendations have been based on the assumption that the absorption of calcium is uniform, rate of accrual is standard, and all calcium that is absorbed is accumulated in the skeleton. Despite these assumptions, the present recommendations that were based on accrual studies provide an estimate of the amount of calcium needed for a given bone mass. The amount of calcium needed for optimal skeletal accretion however, remains uncertain. To date an update of the Australian RDI's have not been made.

Recommendations for calcium intake vary substantially around the world [Inst. of Med., 1997; Kanis, 1994]. Based on current recommended calcium intake levels however, inadequate intakes still appear to be a major nutritional concern in many developed countries. Calcium is one of the major nutrients most often consumed in insufficient amounts during childhood and adolescence, with the problem especially pertinent to females [Alberston et al., 1997; Amschler, 1999; English, 1989].

2.13.1 Trends in calcium intake in children of developed countries

Table 2.2 presents calcium intakes in children from various countries around the world. Mean calcium intakes are highest in Scandinavian and Northern European countries where intakes in excess of 1200 mg.day^{-1} have been reported [Bergstrom et al., 1993; Vandenbergh et al., 1995]. Considerably lower intakes are evident in Asian countries where mean calcium intakes as low as 244 mg.day^{-1} have been reported [Lee et al., 1993].

Available data from developed countries demonstrate sex and maturity related trends in intake. Boys tend to consume more calcium than girls do [Bergstrom et al., 1993; Crawley et al., 1997; English, 1989; Gunnes & Lehmann, 1996; Roma-Giannikou et al., 1997; Ruiz et al., 1995; Strain et al., 1994; Vandenbergh et al., 1995; Welten et al., 1994]. Boys have been reported to consume up to 250 mg.day^{-1} more calcium than girls in Australia [English, 1989], Sweden [Bergstrom et al., 1993], Scotland, Wales and England [Crawley et al., 1997], and Northern Ireland [Strain et al., 1994].

The differences in calcium intake between boys and girls may be as a result of the greater volume of foods consumed by boys. Greater energy intakes have been reported in boys compared to girls [Bergstrom et al., 1993; Strain et al., 1994]. Analysis of calcium densities (calcium per unit energy) revealed no substantial differences between the sexes. For example, mean reported calcium densities for Swedish [Bergstrom et al., 1993] and Irish [Strain et al., 1994] boys were 133mg.MJ^{-1} and 92 mg.MJ^{-1}, respectively compared with mean calcium densities of 143 mg.MJ^{-1} and 89 mg.MJ^{-1}, for Swedish and Irish girls, respectively. These data suggest that the greater calcium intake reported by males compared to females may be due to their greater energy intake (larger volume of food consumed), than to differences in the calcium densities of their respective diets.

Boys tend to increase calcium intake with increasing age [Bergstrom, et al., 1993; English, 1989; Ruiz et al., 1995; Shatenstein et al., 1996; Strain et al., 1994]. In contrast, girls tend to reduce their calcium intake with increasing maturity [Ruiz et al., 1995; Sentipal, et al., 1991] and age [Albertson et al., 1997; Bergstrom et al., 1993; English, 1989; Roma-Giannikou et al., 1997; Shatenstein et al., 1996; Strain et al., 1994; Young et al., 1995]. Therefore at a time of increasing skeletal need, females may not be adequately meeting the recommended calcium intake levels. The effect of this inadequacy on skeletal integrity remains unknown. A greater understanding of the primary sources of calcium and the food choices children and adolescents make may assist in understanding the apparent trends in calcium intakes.

Milk and milk products are the principle sources of calcium for children in developed countries, providing between 46% to 72% of total calcium intake [Bergstrom et al., 1993; Donovan et al., 1996; English, 1989; Fleming et al., 1994; Moynihan et al., 1994]. The second greatest calcium source is grain products, which provide between 10% to 30% of dietary calcium. Meats & eggs, and fruits & vegetables make minor contributions to calcium intake by providing between 3% to 10% of total dietary calcium intake. The contribution of 'other' foods to total calcium intake varies markedly, and ranges from 5% in Canadian girls [Donovan et al., 1996] to 42% in British girls [Moynihan et al., 1996]. This disparity however, may be due to the categorisation of foods. For example, the 'other' sources of calcium may include mixed dishes that contain milk or milk products as a major ingredient.

Table 2.2: Dietary calcium intakes in children from various counties.

References	Dietary Assessment Method		Number of Participants		Mean Calcium Intakes (mg.day^{-1})	
			Males	Females	Males	Females
Australia		10 yrs			865	716
English, 1989		11 yrs			883	724
		12 yrs			928	790
		13 yrs			1019	769
		14 yrs			1176	826
		15 yrs			1278	770
Australia	FFQ	11.9 yrs		114		1178
Young et al., 1995		18.3 yrs		116		1041
Britain	3 day food diary		184	195	786	763
Moynihan et al., 1996						
Canada	3 day weighed food intake	14-19 yrs: Vegetarian		78		733
Donovan et al., 1996		Semi-veg		15		760
		Omnivore		29		747
Canada	7 day food diary	5-6 yrs	12	19	857	938
Shatenstein et al., 1996		7-9 yrs	26	21	1183	1051
		10-12 yrs	15	28	1097	1047
		13-15 yrs	24	22	1114	951
		16-18 yrs	4	11	1396	1006
China & Hong Kong	5 day weighed food record	5 yrs: China	115		244	
Lee et al., 1993*		HK	128		542	
Finland	7 day food diaries	8-20 yrs				
Ussi-Rasi et al., 1997		Tanner 1		41		1018
		Tanner 2+3		54		1059
		Tanner 4+5		81		1231
France	FFQ	Tanner 1	33	16	862	800
Ruiz et al., 1995		Tanner 2	15	25	791	789
		Tanner 3	7	5	928	838
		Tanner 4	6	7	1063	832
		Tanner 5	9	28	1260	824
Greece	3 day food diary		1936			
Roma-Giannikou et al., 1997		6-7 yrs			899	819
		8-9 yrs			938	822
		10-11 yrs			963	851
		12-14 yrs			960	748

Netherlands Welten et al., 1994	FFQ	13-17 yrs	84	98	1110	941
Netherlands Vandenbergh, et al., 1995^	FFQ		653	706	1300	1200
Northern Ireland Strain et al., 1994	Diet history	12 yrs 15 yrs	251 252	258 254	1030 1180	830 790
Norway Gunnes & Lehmann, 1996	24 hour recall	8.2-16.5yrs			1070	913
Scotland, Wales/England Crawley et al., 1997	4 day unweighed food diaries	16-17 yrs Scotland England/Wales	 85 573	 133 824	 1010 1006	 768 723
Sweden Bergstrom et al., 1993	7 day food record	14 yrs 17 yrs	155 211	189 176	1279 1280	1061 966
USA Sentipal et al., 1991	4 day diet record	Tanner 1 Tanner 2 Tanner 3 Tanner 4 Tanner 5		10 9 9 10 11		1040 1060 1090 920 960
USA Katzman et al., 1991	Food recall	14.4±3.6 yrs		45		696
USA Alberston et al., 1992*	Food diary	2-10 yrs	1463		778	
USA Albertson et al., 1997	Multiple food diaries	11-12 yrs 13-14 yrs 15-18 yrs		73 109 169		781 751 602
USA Ilich et al., 1998	FFQ	8-13 yrs		456		956

* Data from both sexes are combined

The most significant single food source of dietary calcium is fluid milk. Fluid milk provides over 27% of the total dietary calcium for British children [Moynihan, et al., 1996] and between 51% - 56% for Australian children [English, 1989]. These data suggest that a reduction in fluid milk consumption may impact differently on total calcium intakes, depending on its importance as a calcium source. In the case of children in Australia, if fluid milk consumption ceased, calcium intake would be reduced by more than half.

It appears that the calcium intakes of males are greater than for females. Males also tend to increase their calcium intake with age, while the opposite has been reported in females. The effect of these trends in calcium intake on bone mineral accrual remains unknown. Milk and milk products are the principle sources of calcium in developed countries, with fluid milk the greatest single contributor to total calcium intake. A high calcium intake has been associated with greater BMD in adults and children in some [Boot et al., 1997; Ruiz et al., 1995], but not all studies [Vandenbergh, et al., 1995; Young et al., 1995]. The apparent association of calcium with BMD may be related to its effect on bone remodelling. An effect that is reported to remain as long as calcium intake remains high [Heaney, 1994].

2.14 The effect of calcium on bone remodelling

Calcium is considered a necessary component of bone, and is important for its structural integrity. At any given point in time, bone tissue and mineral is being removed (resorption) and added (formation). The coupling of formation and resorption may occur locally, at one bone site, or systemically, with formation and resorption occurring simultaneously, but at different bone sites in the body [Heaney, 1994]. In adulthood, the balance between these coupled events will determine the nature of the bone's dynamics. During growth, bone formation is not preceded bone resorption, therefore bone is added. However, during adulthood bone mass is maintained as bone formation and resorption are in balance. The bone loss associated with old age results from the rate of bone resorption exceeding the rate of bone formation [Seeman, 1997].

Bone mass is maintained in adulthood through the process of remodelling. The remodelling of bone follows a distinct sequence. The process consists of the excavation of a resorption cavity by osteoclasts, the synthesis of bone matrix in the cavity by osteoblasts and the mineralisation of the bone matrix [Kanis, 1994]. The process of matrix development and mineralisation continues until new bone is formed. The remodelling process from start to finish is reported to take approximately 3-4 months in healthy adults [Parfitt, 1994].

At any one point in time, remodelling sites are in various phases of the process. Not all sites therefore, are completely mineralised. The volume of bone that is not mineralised is termed the remodelling space. The frequency of activation of the remodelling sequence and the length of the sequence will influence the size of the remodelling space. For example, the greater the rate of activation and the longer the time span of the remodelling sequence, the greater the remodelling space that is created. A greater increase in the remodelling space results in an apparent decrease in bone mass [Heaney, 1994].

Calcium, biphosphates, calcitonin, estrogen, PTH and growth hormones are considered bone-active agents, and are reported to decrease bone turnover [Seeman, 1997]. Such variables have been shown to suppress the activation of the remodelling sequence. Suppression of the sequence results in an apparent increase in bone mass as the formation of new remodelling units is inhibited, while existing units are being mineralised. Heaney (1994) considered the phenomenon transient, as:

1. The apparent increase in bone mass will only persist for one remodelling cycle
2. The change in bone mass does not necessarily involve a change in balance between formation and resorption.

Therefore, it is believed that bone active-agents such as calcium, may bring about initial, but not permanent increases in bone mass. The improvement in bone mass can be attributed to the suppression of the remodelling process, which in turn leads to a temporary reduction in the remodelling space. Therefore, for a given unit of bone area, there is more fully mineralised bone while remodelling is suppressed when compared with bone undergoing remodelling at a normal rate. The gains in bone mass are maintained as long as bone turnover is reduced. The mechanisms for the apparent increase in bone mass are depicted in Figure 2.5. There is no net change to the rates of resorption and formation, therefore Heaney (1994) suggested that the benefits of an elevated calcium intake may be transient in nature, and bone mass may return to pre-treatment levels if the high calcium intake is not maintained.

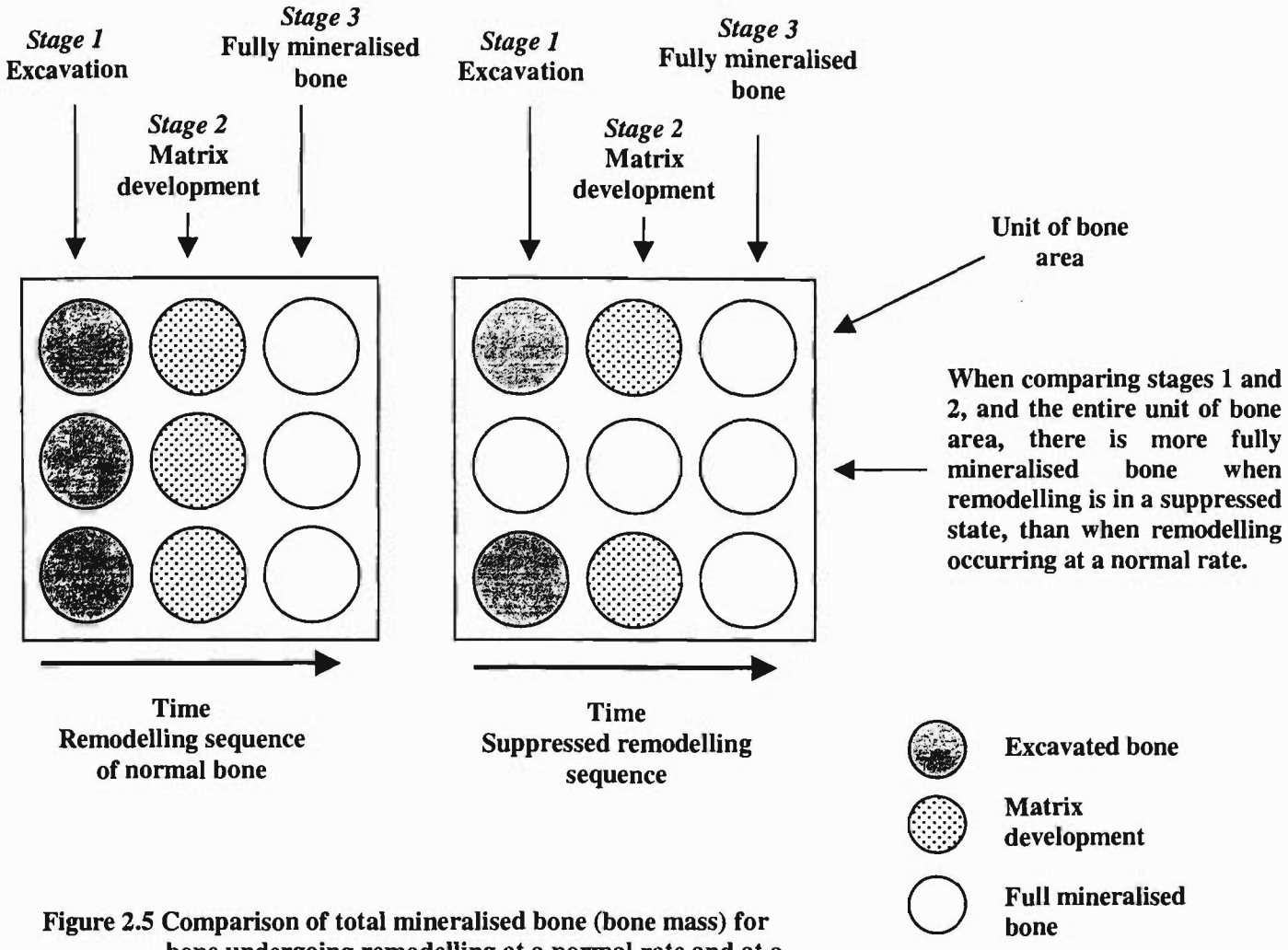

Figure 2.5 Comparison of total mineralised bone (bone mass) for bone undergoing remodelling at a normal rate and at a suppressed rate.

Enhancing calcium intake in children may also suppresses bone turnover, and result in a transient increase in bone mass [Johnston et al., 1992]. Providing the calcium intake remains elevated, the potential benefit calcium may offer to developing and mature bone may be maintained. It may be assumed therefore, that an association would be apparent between calcium intake and measures of bone mass.

2.15 Calcium intake and adult bone mass

There is some conflict in the literature in relation to the association between adult bone mass and current calcium intake. Methodological inconsistencies between studies however, may contribute to these conflicting findings. Welten et al. (1995) conducted a meta-analysis of the effect of calcium intake on bone mass in young and middle aged women, and reported a small but positive association between current dietary calcium intake and measures of bone mass in women. Twenty-four cross sectional studies

conducted between 1985 to 1994 were included in the meta-analysis. The mean correlation between calcium intake and BMD was r = 0.12 (range: -0.16 to 0.33). The partial correlation (PR), corrected for age, body weight (or BMI) height and physical activity was PR = 0.08. The r and PR values (weighted for sample size) were r = 0.13 and PR = 0.08, respectively ($p < 0.05$). These results indicate that calcium may have only a small effect on bone mass in pre-menopausal women as calcium accounted for less than 2% of the variance in BMD.

Low bone mass was initially considered a concern of elderly women, who were more susceptible to osteoporotic fractures [Jones et al., 1994]. Early calcium supplementation interventions therefore focused on post-menopausal women. Results from these studies demonstrate that calcium supplementation had a small, and generally positive effect on slowing bone loss in post-menopausal women (Table 2.3)

Dawson-Hughes et al. (1990) investigated the effects of 500mg of calcium from calcium citrate malate and calcium carbonate on bone mass in post-menopausal women. Three hundred and one participants were randomly assigned to receive calcium citrate malate, calcium carbonate, or a placebo for two years. Women were classified as early post-menopausal (mean age 54.5 ± 3.4 years and 3.2 ± 1.4 years since menopause) or late menopausal (mean age 59.9 ± 5.4 years and 13.0 ± 5.6 years since menopause). Lumbar spine, femoral neck, and radius BMD were assessed using dual photon absorptiometry (DPA). A significant difference in the rate of bone loss was only reported for radius BMD for the later post-menopausal women who were receiving calcium citrate malate ($p < 0.05$). These subjects demonstrated a 1.74 % reduction in rate of bone loss compared to their untreated counterparts. None of the reported reductions in rate of bone loss were significant for the early post-menopausal women. Furthermore, there were no differences in the rate of bone loss at the remaining sites in the late post-menopausal women.

Reid et al. (1993) conducted a two-year calcium intervention trial involving 122 women who were at least three years post-menopausal. Participants were randomly assigned to receive either 1000 mg of calcium from an effervescent tablet (calcium lactate-gluconate + calcium carbonate), or a placebo. A reduction in the rate of bone loss for the supplemented women was reported for total body (0.6%, $p = 0.005$), lumbar spine (1.0%, $p = 0.04$) and Ward's BMD (1.01%, $p = 0.04$). No differences were reported for BMD values at the femoral neck and trochanter between the treated and untreated women. The mean reduction in rate of bone loss with supplementation was 0.86 %.

A two-year calcium-exercise intervention trial involving women at least 10 years post-menopausal was conducted by Prince et al. (1995). Forty-two of the 168 women who were recruited for the overall study

received the calcium supplement (1000 mg of calcium from calcium lactate-gluconate) only. A group of forty-two women acted as controls. Slower rates of bone loss were reported for ultra distal ankle (0.82%, $p < 0.05$), trochanter (1.09%, $p < 0.05$) and intertrochanter BMD (0.98%, $p < 0.05$) in the supplemented women compared to the controls. No difference was reported between the two groups for lumbar spine, mid-tibial and femoral neck BMD. The mean reduction in the rate of bone loss with supplementation was 0.75% for the study period compared with a mean rate of bone loss of 1.13% for the control group.

Baseline calcium intake may influence the effect of calcium supplementation on rates of bone loss in older women. Dawson-Hughes et al. (1990) observed the effect of two years of calcium supplementation in late post-menopausal women and compared the response of those who had a low calcium intake (< 400 $mg.day^{-1}$) with those who had a higher calcium intake (between 400-650 $mg.day^{-1}$). No differences in rates of bone loss were reported between the supplemented and control women with a high calcium intake. In contrast, the mean reduction in the rate of bone loss for the low calcium consumers was 2.23%. For the low calcium consumers, the rates of bone loss were significantly less at the radius, spine and femoral neck for women supplemented with calcium citrate malate, and at the radius for women supplemented with calcium carbonate ($p < 0.05$) when compared with controls. These data support the notion that calcium supplementation may be more beneficial to those with low calcium intakes.

The results from the three calcium supplementation intervention studies conducted on older women indicate that calcium supplementation resulted in an average of less than 1% reduction in the rate of bone loss. However, greater benefits were reported in low calcium consumers. While calcium supplementation may have a small effect on reducing bone loss in the aged, it may also result in increasing bone gain during growth. Childhood calcium intake may also be associated with adult bone mass.

Table 2.3: Effects of calcium supplementation on bone loss in postmenopausal women

Reference	Participants	Intervention	Calcium Intake	Bone Measures	Results % difference in losses between groups	Significance P < 0.05
Dawson-Hughes et al., 1990	301 post-menopausal women	2 years with 500 mg of calcium from calcium citrate malate (CCM) or calcium carbonate (CC)	Control: Low Ca: 274 mg.day^{-1} High Ca: 513 mg.day^{-1} Intervention: Low Ca: 283 mg.day^{-1} High Ca: 530 mg.day^{-1}	Dual Photon Absorptiometry (DPA) Radius, lumbar spine & femoral neck	Early menopause LS, FN, Radius: mean = 0.76% Late menopause LS & FN: mean = 0.87% Radius: CC = 0.43%, CCM = 1.74%* Late menopause: Low Ca < 400 mg/day LS: CC = 0.4%, CCM = 2.6%* FN: CC = 2.2%, CCM = 2.8%* Radius: CC = 2.4%*, CCM = 3.0%* Late menopause: High Ca 400-650 mg/day LS: CC = 0.1%, CCM = 0.05% FN: CC = 0.6%, CCM = 0.9% Radius: CC = -1.0%, CCM = 0.8%	 NS NS p = 0.023* p < 0.05* p < 0.05* p < 0.05* NS NS NS
Reid et al., 1993	122 women at least 3 years post-menopausal	2 years with 1000mg of calcium from calcium lactate-gluconate & calcium carbonate (combined)	Control: 730 mg.day^{-1} Intervention: 760 mg.day^{-1}	DXA Total body, LS (L2-L4) & proximal femur	TB BMD = 0.6% LS BMD = 1.0% FN BMD = 0.9% Ward's BMD = 1.01% Trochanter BMD = 0.8%	p = 0.005 p = 0.04 p = 0.50 p = 0.04 p = 0.16
Prince et al., 1995	84 from a total of 168 women at least 10 years post-menopausal	2 years of 1000mg of calcium from calcium lactate-gluconate	Control: 787 mg.day^{-1} Intervention: 822 mg.day^{-1}	DXA LS (L1 – L4), distal tibia/fibula (ankle), hip	LS BMD = 0.6% Ultra distal ankle = 0.82% Mid tibial = 0.2% Trochanter = 1.09% Intertrochanter = 0.98% FN = 0.82%	NS p < 0.05 NS p < 0.05 p < 0.05 NS

* = significant difference between calcium treated and control groups

While acknowledging the limitation of using retrospective techniques to measure past calcium intakes two studies have reported an association between calcium consumption in youth and bone mass in adults. [Murphy et al., 1994; New et al., 1997]. Murphy et al. (1994) investigated the retrospective lifetime milk consumption in 284 women aged 44 to 74 years. Milk consumption was categorised into < 1 glass.week^{-1}, < 1 glass.day^{-1} and > 1 glass.day^{-1}. Following adjustment for age and BMI, it was revealed that milk intake between the ages of 0–25 years was positively associated with BMD at Ward's triangle, total hip and femoral neck ($p < 0.05$). Retrospective milk consumption was not associated with BMD at the spine, trochanter, or intertrochanter.

New et al. (1997) used a food frequency questionnaire to assess milk consumption during childhood and early adulthood (20 – 30 years) in 994 pre-menopausal women aged between 45 – 49 years. Milk consumption was categorised as low (< 284 ml.day^{-1}), medium (284 – 568 ml.day^{-1}) or high (> 568 ml.day^{-1}). Following adjustment for age, body mass, height, physical activity, smoking and social status, participants reporting a low intake of milk had lower BMD at the lumbar spine when compared with medium ($p < 0.01$) and high milk consumers ($p < 0.03$). Significant differences in BMD were also reported between low and high milk consumers at the femoral neck ($p < 0.01$) and femoral trochanter ($p < 0.01$). No difference was reported between the groups for BMD at the Ward's triangle. Milk intake from age 44 to their present age was not related to BMD at any of the measured sites.

Within the scope of using retrospective data, the dietary data may indicate general trends in calcium intake, without providing specific information. Longitudinal tracking of calcium intake from childhood to adulthood may reveal the impact of childhood milk (calcium) consumption on adult bone mass, and if milk (calcium) consumption patterns in childhood continue into adulthood.

2.16 Calcium intake and bone mass in children

Calcium is a major component of bone mass, and is necessary for skeletal development [Bailey, 1995]. The skeletal expansion that accompanies growth in children increases the demand for calcium as more bone is added. This need may be met with an increase in intake, improved absorption efficiency or both. The relationship between bone mass (expressed as density or content) and calcium intake in children childhood however, appears inconclusive. Chan et al. (1991) reported a small but positive relationship between calcium intake and midshaft radius BMC ($r = 0.18$, $p < 0.05$) in 88 males and 76 females aged 2 to 16 years. Kardinaal et al. (1999) reported a weak but positive association between dietary calcium intake and mid-distal (beta = 0.57 ± 0.22, $p = 0.03$) and ultra-distal (beta = 0.56 ± 0.22, $p = 0.01$) radius in

1116 girls age 11-15 years from five countries in Europe. Data were adjusted for age, height, weight, Tanner stage and bone area. The association was no longer significant however, following further adjustment for other predictors of BMD (eg. height and bone area), and total energy intake.

No relationships were reported between calcium intake and measures of bone for 1359 Dutch boys and girls aged 7 to 11 years [Vandenbergh et al., 1995], and 170 Finnish girls aged 8 to 20 years [Uusi-Rasi et al., 1997]. Mean calcium intakes in these studies were however, relatively high averaging approximately 1250 ± 440 $mg.day^{-1}$, and 1231 ± 565 $mg.day^{-1}$ for the Dutch and Finnish groups, respectively. If calcium is a threshold nutrient as proposed by Matkovic and Heaney, (1992) then the lack of association between calcium intake and measures of bone mass in the above two studies may be due to the calcium intakes of the participants being uniformly high and close to the threshold level.

No relationship was reported between calcium intake and measures of bone mass in 215 sets of female mono- and di-zygotic twins aged 10 to 26 years [Young et al., 1995]. Mean calcium intake was 1028 $mg.day^{-1}$ in this group of subjects. Katzman, et al. (1991) also reported no correlation between dietary calcium intake and bone mass in 45 healthy girls aged 9 to 21 years. Mean calcium intake for the group was 696 ± 417 $mg.day^{-1}$. In addition, Gunnes and Lehman, (1996) reported no correlation between calcium intake and changes in forearm BMD in 470 Norwegian boys and girls aged between 8.2 and 16.5 years. Mean calcium intakes were 1070 ± 362 $mg.day^{-1}$ for males and 913 ± 379 $mg.day^{-1}$ for females.

Boot et al. (1997) reported a positive relationship between total body BMD and calcium intake for 205 boys (beta = 0.003, $p < 0.009$), but not for 295 girls, aged between 4 and 20 years. Data were adjusted for age. Mean calcium intake for the total group was relatively high (1180 $mg.day^{-1}$), and did not differ between the sexes. Furthermore, calcium intake did not correlate with age. An expected increase in calcium intake with increasing age was not apparent in this group of children. Changes in BMD with growth were occurring therefore, with little change in calcium intake. The lack of association may have resulted from maturity related differences in calcium absorption [Abrams et al., 2000] or intakes being at or near the calcium threshold for all ages.

Ruiz et al. (1995) assessed the relationship between calcium intake and lumbar spine and femoral region BMD (femoral neck and head, trochanter and upper third of diaphysis) in 151 children aged from 7 to 15.3 years. The mean dietary calcium intake was 810 $mg.day^{-1}$ (range 157 $mg.day^{-1}$ to 2033 $mg.day^{-1}$). Dietary calcium intake was an independent determinant of BMD at the lumbar spine ($p = 0.05$) and femoral region ($p = 004$) in boys ($n = 70$) but not in girls. Dietary calcium intake was also an independent

determinant of lumbar spine BMD ($p = 0.02$) in children at Tanner stage 1 ($n = 49$). When data were expressed as z-scores (number of standard deviations from the population mean), dietary calcium was a determinant of lumbar spine BMD in both boys ($p = 0.03$) and girls ($p = 0.03$), but it was not a determinant of femoral region BMD in either sex.

Lee et al. (1993) reported no correlation between current calcium intake, and distal radius BMC in 128 five-year-old Chinese children. Cumulative calcium intake over the first five years of life however, positively correlated with distal radius BMC at age five ($r = 0.235$, $p = 0.013$). The correlation remained after adjustment for body mass, height, bone width, and accumulative intakes of energy, and protein ($r = 0.248$, $p = 0.011$). These findings indicate that long term calcium intake may be a better predictor of bone mass in children than current intake alone and may account for the lack of findings in the other studies.

Other factors also need to be taken into consideration when determining the relationship between calcium intake and bone mass. The relationship may be confounded by a higher incidence of mis-reporting of nutrient intake in females than males, which may be due to a greater pre-occupation with weight in girls, or a greater desire to be seen as eating the correct foods [Bandini et al., 1997]. The outcome of under-reporting is a decrease in reported nutrient intake. The relationship between rate of growth and calcium intake is not linear, and calcium intakes may be uniformly high and not change with age [Boot et al., 1997]. Furthermore, calcium intake may not necessarily reflect the calcium accrued in bone, as the rate of calcium absorption may alter during different phases of maturity [Weaver et al., 1995; Abrams et al., 2000].

The relationship between dietary calcium intake and measures of bone mass or density in children appears inconclusive. Five studies reported no relationship between calcium intake and bone mass in children [Gunnes & Lehman, 1996; Katzman et al., 1991; Uusi-Rasi et al., 1997; Vandenbergh et al., 1995; Young et al., 1995]. Two studies reported a relationship for males but not for females [Boot et al., 1997; Ruiz et al., 1995] while the remaining two studies reported a weak, but positive relationship [Chan et al., 1991; Kardinaal et al., 1999]. One study conducted serial measures and reported a relationship between cumulative calcium intake and bone mass, but not for current calcium intake [Lee et al., 1993]. Four studies reported no relationship between calcium intake and bone mass however, a number reported relatively high mean calcium intakes, which did not vary substantially between age groups despite differences in bone mass being apparent. Thus, the findings from Lee et al. (1993) indicate that serial measures may be more likely to detect changes in calcium intake that may have influenced prior bone mineral accrual, than current intake alone. Further longitudinal studies of calcium intake and its

relationship with bone mass that include participants with a larger variability in dietary calcium intakes may clarify some of the inconsistencies. Calcium intervention studies also provide information about the relationship between calcium and measures of bone mass.

2.17 The effects of calcium supplementation on bone mineral accrual in children

Historically, calcium supplementation was advocated to promote growth and skeletal development in poorly nourished children. Gains in stature of school children were reported as a result of supplementation with milk [Orr, 1928]. Milk consumption was subsequently promoted as a means of enhancing skeletal growth [Passmore & Eastwood, 1986]. The role of calcium in skeletal development again came to the fore in the 1970's and 80's, during which time investigations using mineral-based forms of calcium were popularised. The majority of investigations on the effects of calcium supplementation on bone mineral accrual in healthy children have appeared within the last decade and have included supplementation studies using foods, tablets and milk minerals.

Food-based calcium supplementation studies have been conducted with children. Chan et al. (1995) conducted a 12-month intervention trial involving 48 Caucasian pre-menarcheal females (mean age 11 years, range 9 – 13 years). Participants were randomly assigned to receive additional dairy foods to enhance calcium intake to greater than 1200 $mg.day^{-1}$ (mean calcium intake 1437 ± 366 $mg.day^{-1}$), or to consume their usual calcium intake (mean calcium intake 728 ± 321 $mg.day^{-1}$). Greater gains were reported for lumbar spine BMD (9.9%, $p < 0.001$) and total body BMC (6.6%, $p < 0.001$) in the supplemented group compared to the non-supplemented group. No differences were reported between the two groups for gains in height and weight or for radius and femoral neck BMD.

Cardogan et al. (1997) conducted an 18-month calcium supplementation study using whole or reduced fat milk. Eighty Caucasian girls (mean age 12.2 ± 0.3 years) were randomly assigned to the supplement group or the control group. The supplement group consumed milk products of their choice (mean calcium intake 1125 $mg.day^{-1}$) and the control group maintained their usual intake (mean calcium intake 728 $mg.day^{-1}$). The milk supplemented group gained more total body BMC (27.0% versus 24.1%, $p = 0.009$) and total body BMD (9.6% versus 8.5%, $p = 0.017$) compared to the controls. Regional analysis of the total body scan demonstrated that greater gains were evident at the pelvis (14.0% versus 11.6%, $p = 0.003$) and legs (10.4% versus 9.1%, $p = 0.005$) in the milk supplemented group compared to the control group. No differences were reported between the groups for gains in height, body mass, lean mass, and fat mass.

A difficulty encountered when interpreting results of food-based calcium supplementation trials is distinguishing the effect of the calcium from the effect of other nutrients contained in the food. For example, Chan et al. (1995) reported significantly greater intakes of protein, vitamin D, and phosphorus, in addition to calcium, in the supplemented group when compared to the control group. Cardogan et al. (1997) also reported significantly greater intakes of protein and phosphorus, as well as greater intakes of magnesium and zinc with supplementation when compared with controls. The individual, combined, or interactive effects of these nutrients on bone mineral accrual is not known.

Few randomised, placebo-control calcium supplementation studies have been conducted in children [Bonjour et al., 1997; Johnston et al., 1992; Lee et al, 1994; Lee et al., 1995; Lloyd et al., 1996; Nowson et al., 1997]. The results from these studies have indicated a positive effect of calcium supplementation on bone mineral accrual in children. Greater gains of between 0.7% to 6.0% per year in BMD or BMC have been reported in calcium supplemented children when compared to non-supplemented children. Table 2.4 summarises the most recent calcium supplementation studies involving children.

Johnston et al. (1992) conducted a three-year intervention study investigating the effect of 1000 mg of calcium from calcium citrate malate on bone mass accrual in children. Forty-five sets of monozygotic twins of both sexes (mean age 10 ± 2 years) were involved, with one twin from each pair randomly selected to receive the supplement. No difference was reported for height, weight, nutrient intake or physical activity between the groups at baseline. No difference was reported in gains in BMD between the supplemented and placebo groups. There was however, a trend towards greater gains in BMD with supplementation (p value not reported). The mean gain in BMD for the two radial sites, the lumbar spine and the three hip sites was 1.4% greater (range: 0.0% - 2.8%) in the supplemented twins when compared with the unsupplemented twins. No sex differences in gains in bone mass in response to supplementation were reported.

Lloyd et al. (1993) conducted an 18-month calcium supplementation study involving 94 girls (mean age 11.9 ± 0.5 years). Following stratification for BMI and lumbar spine BMD, participants were randomly assigned to receive 500 mg of calcium from calcium citrate malate, or a placebo. At baseline, no differences between the groups were reported for anthropometric measures, BMD, BMC, and urinary bone metabolism markers. Physical activity levels were not reported.

Following the intervention period, no differences in gains of height, weight, BMI, percent fat or in changes in maturity status were reported between the two groups. In relation to bone measures, greater absolute gains in lumbar spine BMD ($p = 0.03$), lumbar spine BMC ($p = 0.05$) and total body BMD ($p = 0.04$) were reported for the supplemented group when compared to the placebo group. No difference in absolute gains in total body BMC was reported between the two groups. When gains were expressed as a percentage, greater percent gains were reported for lumbar spine (2.9%, $p = 0.03$) and total body BMD (1.3%, $p = 0.04$) in the supplemented group when compared with the placebo group. No differences in percentage gain for lumbar spine and total body BMC were reported between the two groups.

Lee et al. (1994) conducted an 18-month intervention study involving 162 seven-year-old Asian children (87 males, 75 females) with low habitual intakes of calcium (mean intake 280 $mg.day^{-1}$). Participants were randomly assigned to receive 300 mg of calcium from calcium carbonate, or a placebo. Radius BMC; bone width (BW); and BMC/BW were measured using single photon absorptiometry (SPA). Greater gains were reported for BMC (2.5%, $p < 0.02$) and BMC/BW (3.2%, $p < 0.008$) in the supplemented group, compared to the control group. No difference was reported between groups for bone width measures.

Lee et al. (1995) repeated the calcium supplementation dosage from their previous study (Lee et al., 1994), to conduct a similar calcium supplementation intervention trial involving 84 seven-year-old Asian children. Children in the later study however, had higher baseline calcium intakes (563 $mg.day^{-1}$ v 269 $mg.day^{-1}$, respectively) when compared to the mean intake reported for children by Lee et al. (1994). In addition to radius bone measures (assessed using SPA), lumbar spine and femoral neck were also assessed using DXA. In contrast to the previous study, no differences in gains at the radius were reported between the two groups ($p > 0.05$). No differences were reported between groups for gains in bone measures at the femoral neck, or for lumbar spine BMD. Differences however, were reported for gains in lumbar spine BMC (4.7%, $p = 0.035$) and lumbar spine bone area (2.5%, $p = 0.049$).

Lloyd et al. (1996) conducted a 24-month calcium supplementation intervention trial involving 112 pre-menarcheal females (mean age 11.9 ± 0.5 years). Participants were randomly assigned to receive either 500 $mg.day^{-1}$ of calcium from calcium citrate malate, or a placebo. Greater percentage gains with supplementation were reported for pelvis BMD (4%, $p = 0.007$), BMC (4%, $p = 0.001$) and bone area (6%, $p = .007$), and total body BMD (6%, $p = 0.01$) and bone area (2%, $p = 0.005$), but not for total body BMC.

Table 2.4: The effect of calcium supplementation on measures of bone mass is children and adolescents

Reference	Participants	Intervention	Calcium Intake	Bone Measures	Results	Significance P < 0.05
Johnston et al., 1992	45 pairs of twins 10 ± 2 years old	3 year with 1 gm calcium from calcium citrate malate	Control: 908 mg.day^{-1} Intervention: 1612 mg.day^{-1}	Photon Absorptiometry Radius- mid & distal, LS, FN, GT & Ward's	Radius-midshaft: 2.5% - distal: 3.3% LS BMD: 0.7% FN BMD: 0.4% Ward's Triangle: 1.2% Greater Troch: 1.8% % difference in gain between groups	NS NS NS NS NS NS
Lloyd et al., 1993	94 girls 11.9 ±. 5 years old	18 months with 500mg.day^{-1} calcium from calcium citrate	Control: 935 mg.day^{-1} Intervention: 1370 mg.day^{-1}	DXA – LS & TB BMD & BMC	LS BMD: 2.9% LS BMC: 4.7% TB BMD: 1.3% TB BMC: 2.0% % difference in gain between groups	p < 0.03 p < 0.6 p = 0.05 NS
Lee et al., 1994	162 boys (87) and girls (75) 7 years old	18 months with 300 mg.day^{-1} calcium from calcium carbonate	Control: 269 mg.day^{-1} Intervention: 586 mg.day^{-1}	SPA Radius - midshaft	Radius: suppl. v control BMC: 16.5% v 14.0%, BW: 6.5% v 7.2%, BMC/BW: 9.5% v 6.3%, Difference in % gain from baseline	 p =0.02 NS p=0.0008
Lee et al., 1995	84 boys and girls 7 years old	18 months with 300 g of calcium from calcium carbonate	Control: 563 mg.day^{-1} Intervention: 811 mg.day^{-1}	SPA Distal radius DXA LS & FN	Radius: suppl. v control BMC: 15.9% v 15.0% BW: 7.6% v 8.6% BMC/BW: 7.7% v 6% Lumbar Spine BMC: 21% v 16.3% Area: 11.2% v 8.7% BMD 8.8% v 7.0%, Femoral neck BMC: 24.5% v 23.4% Area: 14.2% v 12.6% BMD: 9.0% v 9.6% Difference in % gain from baseline	 NS NS p = 0.08 p = 0.035 p = 0.049 NS NS NS NS

Lloyd et al., 1996	112 girls 11.9 ±. 5 years old	24 months with 500 mg.day^{-1} calcium from calcium citrate malate	Control: Not provided Intervention: Baseline not provided + 360 mg.day^{-1}	DXA TB, LS & Pelvis	Lumbar spine BMD: 23% v 19% Area: 22% v 20% BMC: 50% v 42% Pelvis BMD: 19% v 15% Area: 34% v 28% BMC: 59% v 47% Total body BMD: 12% v 10% Area: 24% v 22% BMC: 35% v 39% Difference in % gain from baseline	 p = 0.01 p = 0.09 p = 0.02 p = 0.007 p = 0.007 p = 0.001 p = 0.005 NS p = 0.01
Nowson et al., 1997	42 pairs of female twins 14 years	18 month with 1 g calcium from an effervescent tablet	Control: 692 mg.day^{-1} Intervention: >1600 mg.day^{-1}	DXA LS, hip & FN	6 months: LS BMD: 1.53% hip BMD: 1.27% FN BMD: 1.1% 12 months: LS BMD: 1.5% hip BMD: 1.0% FN BMD: 2.1% 18months: LS BMD: 1.5% hip BMD: 1.1% FN BMD: 0.6% % difference in gain between groups	p < 0.01 p < 0.05 NS p < 0.05 NS p < 0.01 NS NS NS
Bonjour et al., 1997	108 girls 7.9 ± x years	I year with 850 mg of calcium from calcium-enriched foods	Control: 916 mg.day^{-1} Intervention: 1723 mg.day^{-1}	DXA - BMD Radius – meta & diaph, LS, FN, trochanter, femoral diaphysis	Radius - Metaphysis: 5.4% v 3.0% - Diaphysis: 5.4% v 3.0% LS: 3.7% v 4.0% FN: 3.5% v 2.1% F Troch: 4.9% v 3.0% F Diaph: 6.4% v 5.3% Difference in % gain from baseline	p < 0.08 p < 0.02 NS NS p < 0.05 p < 0.01

DXA – Dual energy x-ray absorptiometry
SPA – Single photon absorptiometry
LS – Lumbar spine
FN – Femoral neck
GT – Greater trochanter
TB – Total body
BW – Bone width

Nowson et al. (1997) investigated the effect of 18 months of calcium supplementation on bone mineral accrual in young female twins. An effervescent calcium tablet (containing calcium carbonate and calcium lactate gluconate) was used to enhance baseline calcium levels to greater than 1600 $mg.day^{-1}$. Forty-two pairs of pre- and post-menarcheal female mono- and di-zygotic twins (mean age 14.0 ± 2.6 years) were involved. One twin from each pair was randomly assigned to receive the supplement. No differences were reported for anthropometrics, maturity status, physical activity levels, and calcium intake between the supplement and placebo groups. Greater gains with supplementation were reported for lumbar spine BMD (1.53%, $p < 0.01$) and total hip BMD (1.27%, $p < 0.05$), but not for femoral neck BMD within the first six months of intervention. At 12 months the difference in gain was maintained at the lumbar spine (1.5%, $p < 0.01$), but not at the total hip. A difference between the groups however, was reported at the femoral neck (2.0%, $p < 0.01$). By 18 months no significant differences in gains in BMD at the three sites were reported between the supplemented and placebo twins. No difference in bone area was reported.

Nowson et al. (1997) postulated that the initial greater increase in BMD reported in the supplemented group may have been due to the slowing of bone turnover, which had been previously observed in children supplemented with calcium [Johnston et al., 1992]. In their calcium supplementation study described earlier Johnston et al. (1992) reported reduced serum osteocalcin levels (as a measure of bone turnover) in calcium supplemented male and female twins when compared to twins receiving the placebo (48.5 ± 17.3 versus 54.0 ± 21.1 $\mu.litre^{-1}$, $p = 0.008$).

While all the aforementioned studies have supplemented calcium intake using tablet forms of calcium salts, Bonjour et al. (1997) conducted a 12-month intervention study involving 108 pre-pubertal girls (mean age 7.9 ± 1 years), using foods enriched with calcium from a milk extract. Participants were randomly allocated to consume either two calcium-enriched foods per day (850 $mg.day^{-1}$ additional calcium) or foods of equal composition and caloric value, but without added calcium.

The mean gain in BMD at the six measured sites was greater in the supplemented group when compared with the placebo group (4.7% ± 0.4% versus 3.4% ± 0.5%, $p < 0.001$). The gains however were not uniform at all sites. Greater gains in BMD were reported at the radius diaphysis (5.8% versus 3.1%, $p < 0.05$), trochanter (4.8% versus 3.0%, $p < 0.05$) and femur diaphysis (6.6% versus 5.2%, $p < 0.01$) in the supplemented group when compared to the unsupplemented group. No differences between the groups for gains in BMD were reported for the radius metaphysis, femoral neck and lumbar spine.

Methodological differences between studies make it difficult to compare the results. Girls are included in all the studies however, three of the seven studies contained both sexes [Johnston et al., 1992; Lee et al., 1994; Lee et al., 1995]. No studies have investigated the effects of calcium supplementation on males exclusively. Age and maturity status of participants also differed between studies. Three studies included pre-pubertal children only [Bonjour et al., 1997; Lee et al., 1994; Lee et al., 1995], while the remaining four studies involved children of mixed maturity. Furthermore, the proportion of children in each maturity group, or the number of children who moved into higher maturity groups during the course of the intervention was not uniform between the studies.

Baseline calcium and total calcium load with supplementation varied between the studies. Dietary intakes ranged from 269 $mg.day^{-1}$ [Lee et al., 1994] to 916 $mg.day^{-1}$ [Bonjour et al., 1997], with baseline calcium levels augmented by between 300 $mg.day^{-1}$ [Lee et al., 1994; Lee et al., 1995] to 1000 $mg.day^{-1}$ [Nowson et al., 1997] with supplementation. Four different forms of calcium supplementation were used, which may have affected calcium absorption efficiency [Levenson & Bockman, 1994]. Three studies used calcium citrate malate [Johnston et al., 1992; Lloyd et al., 1993; Lloyd et al., 1996], two used calcium carbonate [Lee et al., 1994; Lee et al., 1995], one used an effervescent tablet containing calcium carbonate and calcium lactate gluconate [Nowson et al., 1997] and the final study used calcium enriched foods, supplemented with calcium from a milk extract [Bonjour et al., 1997]. Intervention periods also varied between studies, ranging from 12 months to three years. Furthermore, differences were also evident in the methods used to determine bone measures, and the sites measured. Although results are positive but modest, site-specific comparisons of results from different studies may appear inconsistent. The potential effect of maturity and baseline calcium intake on the response of bone to calcium supplementation is also likely to affect the results.

2.17.1 The effect of calcium supplementation on bone mineral accrual at different stages of maturity

The initial calcium supplementation study conducted by Johnston et al. (1992), indicated that pre-pubertal children (n = 22 pairs) may be more responsive to supplementation than peri- (n = 4 pairs) and post-pubertal (n = 19 pairs) children. The pre-pubescent children who received supplementation reported significantly greater gains in BMD at the distal radius (mean = 3.8%, range 1.4% to 6.2%), midshaft radius (mean = 5.1%, range 1.5% to 8.7%), and lumbar spine (mean = 2.8%, range 1.1% to 4.5%) when compared with the non-supplemented children. No differences in gains in BMD were reported for the three femoral sites in the pre-pubertal children. No difference in gains in BMD were reported between

the supplemented peri- and post-pubertal twins and their non-supplemented counterpart for the six measured sites (mean = 0.3%, range: -1.6% to 2.2%) or at any one individual site. It was not stated if the baseline values differed between the two maturity groups (pre- versus peri- / post-pubertal).

In contrast to the maturity related findings by Johnston et al. (1992), Lloyd et al. (1996) reported a greater response to supplementation in more mature girls compared to the less mature girls. The 94 girls involved in the study were grouped into those of above median maturity and those of below median maturity based on their score for Tanner staging. For the girls of below median maturity, no difference in the yearly rate of bone mineral accrual (total body BMD) was reported between the supplemented and placebo group. The more mature girls receiving the calcium supplemented however, reported greater yearly gains in total body BMD when compared to their maturity matched peers who received the placebo (approximately 0.05 $g.cm^{-1}$ versus 0.045 $g.cm^{-1}$, respectively, $p < 0.006$).

Nowson et al. (1997) also reported a greater response to supplementation in more mature girls when compared to less mature girls. The girls were divided into those who were pre-menarcheal on entry into the study, and those who reached menarche either before or during the course of the study. There were no significant differences in the gains in BMD between the pre-menarcheal girls who received calcium supplementation and those who received the placebo. A positive effect was reported in post-menarcheal girls who received supplemented with greater gains in BMD being reported at the lumbar spine ($1.48 \pm 0.54\%$, $p < 0.01$) and hip ($1.37 \pm 0.56\%$, $p< 0.02$). The number of pre-menarchial girls in this study, however was small ($n = 11$ pairs). Therefore the lack of effect of supplementation in the younger girls may have been due to insufficient numbers to provide adequate power, rather than to a maturity-related difference.

The effect of calcium supplementation on bone mineral accrual at different stages of maturity appears inconclusive. Johnston et al. (1992), who reported no effect of supplementation on bone mineral accrual in mature children, postulated that the lack of effect of calcium supplementation on more mature children is possibly due to hormonal stimulation during puberty already maximising bone mineral accrual. The osteogenic effect of puberty may have masked the smaller effect due to calcium supplementation. Alternatively, Lloyd et al. (1996) suggests that the hormonal changes taking place during puberty enhance the effect of calcium supplementation. The effect of calcium supplementation on bone mineral accrual at different stages of maturity warrants further investigation.

2.17.2 Does daily dietary calcium intake influence the osteogenic response of bone to calcium supplementation?

Baseline calcium intake may influence the effect of calcium supplementation on bone mineral accrual. Children with lower calcium intakes have demonstrated greater responses to calcium supplementation when compared with those with higher calcium intakes [Bonjour et al., 1997; Lee et al., 1994; Lee et al., 1995]. Lee et al. (1994 & 1995) performed two identical calcium supplementation intervention studies involving seven-year-old children. Mean habitual calcium intake of the children in these two studies differed by approximately 300 mg. day^{-1} (280 $mg.day^{-1}$ v 567 $mg.day^{-1}$).

No differences were reported for gains in distal radius BMC, bone width (BW) or BMC/BW between the supplemented and non-supplemented children with higher calcium intakes. In comparison, significantly greater gains in radius BMC and BMC/BW were reported in the supplemented children when compared with the non-supplemented children in the lower calcium intake study. A regression analysis showed that baseline calcium intake was a significant predictor of net gains in lumbar spine BMC ($r = -0.29$, $p = 0.009$) [Lee et al., 1995], supporting the notion that lower baseline calcium intakes result in greater gains in lumbar spine BMC.

Bonjour et al. (1997) divided the female participants ($n = 108$, mean age 7.9 ± 0.1 years) involved in the intervention trial into those with above median calcium intake (> 880 $mg.day^{-1}$) and those with below median calcium intake (< 880 $mg.day^{-1}$) at baseline. While no difference in baseline BMD values were reported between the above median and below median calcium groups, a greater response to supplementation was reported in the low calcium group when compared with the high calcium group. Supplemented girls in the low calcium group gained approximately 2% more BMD at the six measured sites ($p < 0.01$) than the non-supplemented girls in the same group. The mean difference in gain in BMD between the supplemented and non-supplemented girls in the high calcium group was not significant. The results from the above studies indicate that greater responses to calcium supplementation may be made in children with lower calcium intakes compared to children with higher calcium intakes.

2.17.3 The Effect of Calcium Supplementation Withdrawal on Bone Mineral Accrual: The Long Term Benefits

Calcium supplementation is of most benefit to bone mass if the gains made are maintained beyond the supplementation period. The long-term benefits of calcium supplementation are equivocal.

The 22 pre-pubertal twins involved in the previously reported study by Johnston et al. (1992) we measured 12 months after the cessation of supplementation [Slemenda et al., 1993]. During the study period, the supplemented twin gained 5.1% more BMD at the radius compared to the twin receiving the placebo. The difference was reduced to 0.8% 12 months after the cessation of supplementation. Furthermore, during the follow up period greater rates of accrual were reported at the lumbar spine (1.3%, $p = 0.03$) in the twin who received the placebo compared to the supplemented twin. Slemenda et al. (1993) suggested that as osteocalcin was reduced in the supplemented twin the effect of supplementation was due to suppression of bone remodelling.

Slemenda et al. (1997) continued to track the same group of twins for an additional two years. Data from two years post- and three years post-supplementation were available for 41 of the original 45 sets of twins who participated in the calcium supplementation study. By the third year post-supplementation, no difference in gains in BMD between the previously supplemented pre-pubescent children and those who received the placebo were reported. Serum osteocalcin levels in the pre-pubescent children, which had been reduced during the calcium supplementation period (-15.1%, $p < 0.05$), no longer differed from the placebo group two years and three years after the cessation of calcium supplementation. Baseline calcium intake during the follow up period (920 $mg.day^{-1}$) did not to differ from that reported at baseline for the supplementation period (930 $mg.day^{-1}$).

Lee et al. (1996) conducted an 18-month follow up study of children previously supplemented with 300 mg of calcium from calcium carbonate for 18 months (Lee et al., 1995). At follow up, no significant difference in gains in height, weight, and bone measures were reported between those children previously supplemented and those who received the placebo. The significant difference in lumbar spine BMC and lumbar spine bone area reported between the groups at the end of the supplementation period was no longer evident at the completion of the 18-month follow up period.

Lee et al. (1997) conducted an 12-month follow up study of their previous work (Lee et al., 1994) in which children accustomed to low calcium intakes were supplemented with 300mg of calcium from

calcium carbonate. At the end of the 18-month supplementation period significant differences were reported for gains in radius BMC and BMC/BW between the supplemented and non-supplemented children. By the end of the 12-month follow up period, no differences were apparent in bone measures at the radius between the previously supplemented children, and those who received the placebo.

In contrast to results from the previous follow-up studies, Bonjour et al. (1997) reported residual benefits of calcium supplementation on bone mass and area one year after the cessation of supplementation in 100 of the 108 girls originally tested. Mean gains in BMD from the six measured sites remained significant (58 ± 3 versus 53 ± 3 mg.cm^{-2}, $p < 0.05$), at the end of the follow up portion of the study. Mean gains in BMC however, did not remain significant. The greatest residual effect of supplementation in this study was in the girls with below median calcium intakes ($n = 49$). The greater gains made in femoral shaft width ($p < 0.05$), BMC ($p < 0.02$), and lumbar spine height ($p < 0.05$) at the end of the supplementation period was still evident by the end of the 12 month follow up period.

The major difference between the study conducted by Bonjour et al. (1997) and those described earlier, is that the earlier studies used calcium salts as the means of supplementation, while Bonjour et al. (1997) used calcium from a milk extract, which was incorporated into food products. This may indicate that factors inherent to the extract, or the foods it was incorporated into may have contributed to the sustained benefits reported. Further research in this area is needed to confirm the apparent differences reported in the calcium supplementation follow up studies.

From the literature presented, it appears that calcium supplementation may have a positive effect on bone mass accrual in children. Greater gains in bone mass as a result of calcium supplementation have been modest, but evident [Bonjour et al., 1997; Lee et al., 1994; Lee et al., 1995]. Maturity related differences in response to calcium supplementation are inconclusive and require further research. Children with lower habitual dietary calcium intakes appear to respond more favourably to calcium supplementation, and reported greater gains in bone mass when compared to children with higher calcium intakes [Bonjour et al., 1997; Lee et al., 1994; Lee et al., 1995]. The long-term benefits of calcium supplementation on bone mineral accrual appear inconclusive. In all studies using calcium salts as the form of supplementation [Johnston et al., 1992; Lee et al., 1994; Lee et al., 1995] the greater gains in bone mass accrual were no longer evident following the cessation of supplementation. In contrast residual benefits to bone mass and area were detected in children supplemented with food-based calcium supplements, following supplementation withdrawal [Bonjour et al., 1997]. The following section will focus on the effect of physical activity on bone mass.

2.18 Introduction to the effects of physical activity on bone mass

Physical activity is reported to enhance bone mass accrual in children [Bailey, 1995; Blimkie et al., 1996], maintain bone mass in adults [Snow, 1996], and minimise bone loss in later life [Ernst, 1998]. Early evidence to support these claims came from cross sectional studies comparing active or athletic populations, with their inactive counterparts. Greater bone mass has been reported in both adult [Dook et al., 1997] and younger athletes [Bass et al., 1998] compared to non-athletic controls. Similarly, greater bone mass was reported in active adults [Halioua & Anderson, 1989] and children [Ruiz et al., 1995] compared to their sedentary counterparts.

The osteogenic effects of exercise appear to be specific to the bone or area of bone to which the load is applied. For example, greater bone mass has been reported in the arms of gymnasts, when compared to age-matched controls [Bass et al., 1998]. The arms are commonly loaded in gymnastic movements. Alternatively, figure skaters have been found to have similar bone mineral density in the arms and ribs, when compared to age-matched controls, but greater bone mineral density in the pelvis and legs [Slemenda & Johnston, 1993].

Exercise induces an osteogenic response that is dependant on the magnitude of the load [Frost, 1992]. Loads that are greater than usually experienced stimulate bone mass accrual during growth (via modelling) and conserve bone mass during adulthood (via remodelling). If loading to bone is less than regular levels, remodelling is accelerated and bone loss occurs [Zerwekh et al., 1998]. Exercise interventions studies involving adults have resulted in no effect or minor gains in BMD. Although few exercise intervention studies have been conducted involving children, enhanced bone mass accrual was reported with exercise [Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997].

Results from bilateral studies also indicate a potential for a greater osteogenic effect during growth when compared to adulthood. Haaspasalo et al. (1994) investigated the side-to-side difference in BMD and BMC between the playing and non-playing arms of elite female squash players, and reported mean differences of between 15.0% – 17.8%. Girls who commenced playing before menarche had a greater side-to-side difference (mean differences: 20.6% – 24.0%), compared to those who commenced after menarche (mean differences: 7.7% – 11.1%, $p < 0.01$ – $p < 0.05$). Similar findings have also been reported by Kannus et al. (1995). The authors noted that the difference between humeral BMC of the playing and non-playing arm of female squash and tennis players were greater for those who had commenced playing during the growth period (17%-24%), when compared to women who commenced playing in adulthood (8%-14%).

The optimal age or stage of development when the greatest osteogenic response occurs remains unknown. The earlier study by Haaspasalo et al. (1994) indicated that a greater osteogenic response occurred before menarche than after. More recently the author [Haaspasalo et al., 1998] reported that the side-to-side difference did not become apparent until players were Tanner stages 3 to 5. The authors suggest, therefore that the adolescent growth spurt may, in fact be the most opportune time to maximise the osteogenic effect of exercise.

2.19 The mechanisms of the osteogenic response to loading

The adaptation of bone to mechanical loading has been recognised as far back as the nineteenth century. In his earlier work, Darwin (1872) noted that the leg bones of domestic ducks were larger and heavier than the leg bones of wild ducks, despite similar ancestry. Darwin attributed the difference to the greater use of the legs by domestic ducks, as more walking and less flight had become part of their normal movement patterns. Although adaptation to loading is apparent, the mechanisms that bring about an osteogenic response remain unclear.

Frost (1992) proposed a model, to describe the relationship between the magnitude of loads and the adaptation by bone. Frost's 'mechanostat' theory introduces the concept of different strain thresholds at which modelling and remodelling of bone are initiated. These strain thresholds he termed mechanical usage (MU) set points. A MU set point of 50 microstrains (a measure of load on bone) is considered the minimal effective strain for controlling remodelling units (MES_r). Strains below this magnitude are associated with increased bone remodelling and decreased bone modelling resulting in a net loss of bone mass. The normal strain magnitudes required to maintain bone (remodelling = modelling) lie between 50 – 1500 microstrains.

Strains exceeding 1500 microstrains are considered to be the minimal effective strain for controlling modelling drifts (MES_m). Strains of this magnitude promote bone modelling during growth and the conservation of bone during adulthood. During growth therefore, bone will continue to be accrued until sufficient bone is added to accommodate the load. The addition of bone in response to mechanical usage will result in the load being distributed over a wider area, hence reducing the magnitude of the load per unit area. When the strain magnitude fall below the MES_m (returning within the normal range) bone modelling is again 'switched off', and a new steady state between modelling and remodelling is reached. The exact mechanism by which the mechanical load is 'sensed' and the appropriate adaptation initiated, remains unclear. Numerous theories have been proposed which include a greater blood flow to the bone providing more nutrients and growth related hormones, or a streaming potential being generated as a result of the bending of the bone in response to mechanical stress [Lanyon, 1992; Chilibeck et al., 1995].

Further evidence from animal studies indicate a favourable osteogenic response by bone may also be initiated by strains that are of a high rate, are abnormally distributed, and are dynamic in nature [Lanyon, 1996]. Greater bone formation has been reported with submaximal strains, if unevenly distributed, when compared with repetitive cycles of normal strain distribution [Lanyon, 1992]. Furthermore, Lanyon, (1992) reported that a static load applied continuously to a bone resulted in an accelerated rate of bone remodelling that was similar to the rates of bone remodelling associated with immobilisation. In contrast, a dynamic load applied for a short duration resulted in bone formation proportional to the magnitude of the strain.

The translation of findings from animal studies to human responses to mechanical loading is not definitive. The changes to bone architecture and dimensions in response to loading are complex processes influenced by the characteristics of the strain applied and the loading history of the bone. Although results from animal studies indicate the most appropriate loading is dynamic, of high strain rate and magnitude and unevenly distributed, results from human studies are inconclusive.

2.20 The osteogenic response of adult bone to exercise

Exercise in adults in promoted, to reduce the risk of osteoporosis by slowing the rate of bone loss. These recommendations were initially based on studies comparing physically active adults and athletes, and their sedentary counterparts. While greater bone mass has been reported in more active [Haliou & Anderson, 1989] and athletic adults [Dook et al., 1997], it is unclear to what extent the greater bone mass can be attributed to the exercise itself. Furthermore results from intervention studies that ensued have resulted in no effect or small gains in BMD. To quantify the effect of exercise on bone loss in adults a number of meta-analytical assessments [Kelley, 1998a; Kelley 1998b] and reviews [Ernts, 1998] have been conducted.

Kelley (1998a) reviewed 11 randomised trials investigating the effects of exercise on regional BMD. The mean change in BMD from the 11 trials was 0.27% (95% CI, 0.16 – 0.37%). On closer examination four studies included participants undertaking hormone replacement therapy (HRT), with an additional study not specifying HRT use. Furthermore, one study included calcium supplementation in the control group. Of the remaining six studies, three reported bone loss in both the control and exercise group. Two of these studies however measured BMD at the radius although this site was not loaded in the exercise program. One study reported gains in the femoral region, but not the lumbar spine for their calcium/exercise group, and bone loss at the lumbar spine and two femoral sites in the exercise only group. Only two studies reported gains in bone mass. One study used high intensity walking resulting in gains at the lumbar spine, while the second used high

intensity strength training, and reported gains at the lumbar spine and femoral neck. The gains were approximately 1%, which were within the coefficient of variation of the instrumentation used to measure the changes.

Kelley (1998b) also conducted a meta-analysis, investigating the effect of aerobic-based activity on lumbar spine BMD in post-menopausal women. Six of the 10 studies included in the meta-analysis were non-randomised and the remaining four randomised. Two studies reported HRT use in some participants, while a further four studies reported use of calcium supplements. Eight studies reported gains in BMD, one reported both gains and losses, and a final study reported a loss at the lumbar spine. Gains were reported with both weight bearing exercise (eg. walking, step-ups, jogging), and non-weight bearing exercise (eg. stationary cycling, water aerobics). Following analysis the mean difference in lumbar spine BMD between the exercise and control groups for the ten studies was 2.83% ± 0.77%. The mean gain at the lumbar spine however, was only 0.32% ± 4.46%. The difference between the two groups was predominantly due to bone loss in the control group (mean loss = -2.51% ± 2.69%).

Most exercise intervention studies involving pre-menopausal women have reported modest or no increases in BMD, while one study reported a loss at the lumbar spine [Rockwell et al., 1990]. Gleeson et al. (1990) conducted a 12-month non-randomised exercise intervention trial involving pre-menopausal women to determine the effects of weight training on lumbar spine and os calcis BMD. Thirty-four women in the exercise group and 38 controls completed the study. All participants received 500 mg of calcium daily. Following the intervention period a non-significant gain in lumbar spine BMD of 0.8% (± 3.4%) was reported in the exercise group. A non-significant loss of 0.5% (± 3.5%) was reported in the control group. The difference in percent change between the two groups at the end of the intervention period was significant. No change in os calcis BMD was reported for either the exercise or control group. The reported mean changes in BMD were within the error limits of the measuring instrumentation. Selection bias may also have accounted for the findings as participants self selected the study groups in which they were involved. Furthermore, although both groups were matched for all other baseline variables, they varied in their baseline physical activity levels.

Rockwell et al. (1990) conducted a non-randomised 9-month weight training intervention program involving 10 pre-menopausal women, (matched to seven controls) to determine the effect of weight training on bone mass at the lumbar spine and femoral neck. Participants were supplemented with 500 mg of calcium daily. Following the 9-month period a loss of approximately 4% in lumbar spine BMD ($p = 0.01$) was reported in the exercise group, compared to no change reported for the control group. No changes in BMD were reported at the femoral neck for either group. Similar to Gleeson et

al. (1990), participants self selected the group they were involved in, with some participants in the exercise group already involved in strenuous exercise. In addition the participants that remained in the exercise group were lighter than the controls by almost seven kilograms. This difference was not accounted for when group comparisons were made.

Lohman et al. (1995) conducted an 18-month randomised weight training intervention study to determine the effects of resistance training on total body and regional BMD in pre-menopausal women. Twenty-two women partook in the weight training program 3 times a week. Thirty-four women acted as controls. All participants were given a 500mg calcium supplement daily. At the end of the intervention period, gains in BMD in the exercise group were reported at the trochanter (1.5%, $p < 0.05$), while no change was reported at the lumbar spine, radius, Ward's triangle, femoral neck, or for total body BMD. No changes to BMD were reported in the control group. The study however, reported a large drop out rate for the exercise group with 22 participants remaining from an initial 59 women. The change in trochanter BMD reported was within the coefficient of variation (1% – 4%) of the measuring instrumentation.

The results from the meta-analysis and intervention trials indicate that both aerobic-based and strength-based programs, and activities of either a weight bearing or non-weight bearing nature may contribute to small gains in bone mass at the loaded sites in mature and older women. The data is somewhat more contradictory at sites not directly loaded. Furthermore, of the reported changes many were within the coefficient of variation of the measuring instruments. From the available data it appears that a variety of exercises may result in a small osteogenic effect, but the ability to produce large gains in bone mass in mature and older females appears limited. Exercise may play a role in slowing the rate of bone loss. However, the benefit may be lost when exercise is ceased. It may be warranted therefore to turn attention to the immature skeleton to investigate the osteogenic response of growing bone to mechanical loading.

2.21 The osteogenic response of immature bone to exercise

Animal studies were amongst some of the first research to investigate the osteogenic response of the growing skeleton to mechanical loading. Forwood and Parker (1987) noted that the growing skeleton may be more responsive to mechanical loading than a mature skeleton. Subsequent cross-sectional comparisons between active [Ruiz et al., 1995] and athletic children [Conroy et al., 1993; Dyson et al., 1997; Slemenda & Johnston, 1993] and their less active counterparts supported the findings from these animal studies. Greater measures of bone mass were reported in more active and athletic children when compared to inactive controls. More convincing support of childhood activity conferring a greater osteogenic effect than similar activities undertaken in adulthood arose from

unilateral studies investigating the side-to-side difference in bone mass in the arms of elite squash and tennis players [Haaspasalo et al., 1994; Haaspasalo et al., 1996; Haaspasalo et al., 1998; Kannus et al., 1995], and intervention studies involving children [Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997].

2.21.1 The effect of exercise on measures of bone mass in children

Ruiz et al. (1995) investigated the effects of normal calcium intake and physical activity levels on femoral neck and lumbar spine BMD in 151 healthy children aged between 7 – 15.3 years. Physical activity ranged from 1-3 hours a week of sporting activity to more intense participation in organised sports (3 - 12 hours.week^{-1}). Sixty-four percent of the children in the later group participated in weight bearing activities. Hours spent per week in sporting activity had a significant influence on lumbar spine and femoral neck BMD ($p < 0.001$ and $p = 0.01$, respectively) for the entire group of children. When the sexes were separated however, the effect was evident for girls ($p = 0.004$ and $p = 0.03$, respectively), but not for boys. The authors however, did not specify how physical activity was assessed.

Greater measures of bone mass have been reported in athletic children when compared to matched controls. Dyson et al. (1997) investigated the effects of gymnastics training on bone mass by comparing bone mass in pre-adolescent female gymnasts ($n = 16$, mean age 9.82 ± 0.89 years) to non-athletic controls ($n = 16$, mean age 9.82 ± 0.89 years). Whole body, femoral neck, trochanter and lumbar spine BMD and BMAD (to adjust for differences in bone size) were assessed using DXA. Radius BMD and cortical:trabelcular bone ratios were determined using peripheral quantitative computed tomography (pQCT). The gymnasts were shorter and leaner, and spent more time in activity when compared to the controls, however the two groups did not differ for weight, maturity status and dietary intake. Gymnasts reported greater whole body (7.4%), femoral neck (19.5%) and lumbar spine (8.1%) BMAD when compared to controls ($p < 0.05$). Bone mineral density at the femoral neck (7.7%, $p < 0.05$), trochanter (16.2%, $p < 0.01$) and radius (19.6%, $p < 0.01$) were also greater for the gymnasts when compared to the controls. No differences in whole body, or lumbar spine BMD were reported between the groups.

Conroy et al. (1993) reported greater BMD at all measured sites in 25 elite male junior weight lifters (mean age 17.4 ± 1.4 years) when compared to 11 matched controls (16.9 ± 1.1 years). BMD at the lumbar spine, femoral neck, trochanter and Ward's triangle was 33%, 24%, 18% and 27% greater for the weight lifters when compared to controls, respectively ($p < 0.05$). The number of controls however, was small ($n = 11$). Bone mineral density at the lumbar spine and femoral neck for the

weight lifters was also significantly greater than adult male reference values. Strength accounted for between 30-56% of the variance in BMD for the weight lifters. Strength measures were not assessed in the control group. Although the two groups were matched for age, height and weight, no assessment was made of lean mass in spite of lean mass being correlated with measures of bone mass [Seeman et al., 1996].

Slemenda and Johnston (1993) compared the upper and lower body BMD between 22 figure skaters aged 11-23 years and 22 controls aged 10-23 years. At baseline, skaters were leaner (body fat: 18.7 ± 5.4% v 24.3 ± 6.0%, $p = 0.0004$), and reported greater menstrual irregularities ($p = 0.01$). Skaters reported greater BMD for the total body (5.6%, $p = 0.02$), pelvis (14.1%, $p = 0.001$), legs (10.0%, $p = 0.005$) and trunk (8.0%, $p = 0.005$) when compared to the controls. No difference was reported between the groups for BMD at the arms, ribs and spine. Even when adjusted for weight and age, the difference in BMD at the legs (5.5%, $p = 0.04$) and pelvis (11.1%, $p = 0.001$) remained significant. Bone mineral density however, negatively correlated with hours of training ($r = -0.40 - 0.45$), suggesting lower BMD values in girls that trained longer. It was not reported however, if the skaters who trained more intensely also reported more menstrual irregularities, were leaner or reported a greater incidence of inadequate nutrient intake, which may contribute to lower BMD [Young et al., 1994].

Grimston et al. (1993) investigated the osteogenic effect of active loading versus impact loading in children. The authors compared femoral neck and lumbar spine BMD in 17 children (mean age 12.6 ± 0.4 years) predominantly involved in swimming with 17 children (mean age 13.2 ± 0.4 years) involved in sports where loads of at least three times body weight are experienced. Activities in the impact load group included running, tumbling, dance and gymnastics. No differences were reported between the groups for age, weight, height, hours of training, years of training and nutritional intake. The impact load group however, spent more time in weight bearing activities than the active loading group ($p < 0.05$). The impact group reported approximately 8% greater BMD at the femoral neck when compared to the active group. No difference between the groups was found for lumbar spine BMD. The authors reported no correlation between average hours spent engaged in weight bearing activity and femoral neck BMD. It is unclear however, if the hours spent weight bearing were distributed evenly throughout all the activities in the groups (running, gymnastics, dance) or were characteristic of a specific type of activity eg. running. The effect of exercise per se on bone mass is also unclear, as sedentary controls were not included in the study.

Nichols et al. (1995) compared lumbar spine and hip BMD, in 10 peri-pubescent gymnasts (mean age 11.2 ± 0.4 years) to 10 age-matched swimmers and 10 age-matched non-athletic controls. Gymnasts were significantly shorter, lighter and had a lower percentage body fat when compared to swimmers

and controls. Gymnasts had a tendency towards greater BMD at Ward's triangle (7.5%, $p = 0.10$) and at the trochanter (7.2%, $p = 0.06$) when compared with the swimmers, but did not differ from the controls. No differences were found between the groups for lumbar spine, femoral neck and whole body BMD. No non-weight bearing sites were measured. The magnitude of the effect of impact loads however is difficult to distinguish. Firstly the gymnasts had commenced training earlier than swimmers (4.7 ± 1.0 years v 7.5 ± 3.4 years) and trained for longer (6.7 ± 1.2 years v 3.8 ± 3.4 years) although it was not stated if the differences were significant. Additionally, despite gymnasts being smaller and lighter than both the swimmers and the controls, no adjustments were made to BMD values to account for these differences.

Bass et al. (1998) compared the regional and total body BMD of 45 active female pre-pubescent gymnasts to 35 bone-age matched controls. Greater BMD was reported for total body (3.4%, $p < 0.01$), and for the spine (6.5%, $p < 0.01$), legs (4.9%, $p < 0.05$) and arms (11.5%, $p < 0.001$), but not for the skull in the gymnasts when compared to controls. Greater bone mass (g) and volumetric BMD ($g.cm^{-3}$) at the lumbar spine (24% and 14.5%, $p < 0.001$) and femoral midshaft (16% and 12%, $p < 0.01$) were reported for the gymnasts when compared to the controls. No difference was reported between the two groups for height however, gymnasts has less fat mass ($p < 0.001$) and more lean mass ($p < 0.05$) compared to controls.

The results from the studies presented support the site specificity of the ostoegenic response to mechanical loading. For example, Slemenda and Johnston (1993) reported greater BMD in the legs (5%) and pelvis (11%) of skaters, but no difference in the arms and ribs when compared to controls. The nature of skating tends to load the lower body, and not the upper body. Alternatively, Bass et al. (1998) reported approximately 11% greater BMD in the upper body (radius and arms) and 5% greater BMD in the lower body (femoral neck and trochanter) of gymnasts compared to the controls. Gymnastics training characteristically loads both the upper and lower body.

Athletic studies provide promising evidence that physical activity confers an osteogenic benefit to growing bone. However, the results from cross sectional comparisons may be influenced by selection bias that may contribute to the apparent differences in bone mass between athletic and non-athletic children. Studies examining unilateral loading of the skeleton and randomised exercise intervention trials may reduce the potential for selection bias.

2.21.2 The effect of unilateral loading on the growing skeleton

Unilateral loading studies provide the unique opportunity to investigate the effects of differential mechanical loading on bone, while controlling for other influential factors such as genetic disposition,

hormonal status and nutrient intake. Evidence to support the potential for a greater osteogenic response to mechanical loading in the growing skeleton when compared with mature bone arose from studies comparing the playing and non-playing arms of squash and tennis players.

Kannus et al. (1995) investigated the effect of starting age of playing on bone mass in the dominant and non-dominant arm of 105 elite squash and tennis players (mean age 27.7 years, range 16 – 50 years). The side-to-side difference in the BMC of the dominant versus non-dominant arm was greater for the players (8.5% – 16.2%, $p < 0.05 – 0.01$) than for the fifty matched controls (3.2% – 4.6%). Players where then divided into 6 groups, depending on the number of years before or after menarche they commenced playing. Following adjustment for height and age, the side-to-side difference at the proximal humerus and humeral shaft, but not the radius, remained significant for all groups. The differences were greatest in those who commenced playing the earliest (> 5 years before menarche, mean difference 20.1% – 22.6%), compared to those who commenced the latest (> 15 years after menarche, mean difference 8.6% – 9.5%). The side-to-side difference for each of the six groups is depicted in Figure 2.6. The side-to-side difference in general decreased with increasing chronological age players commenced playing.

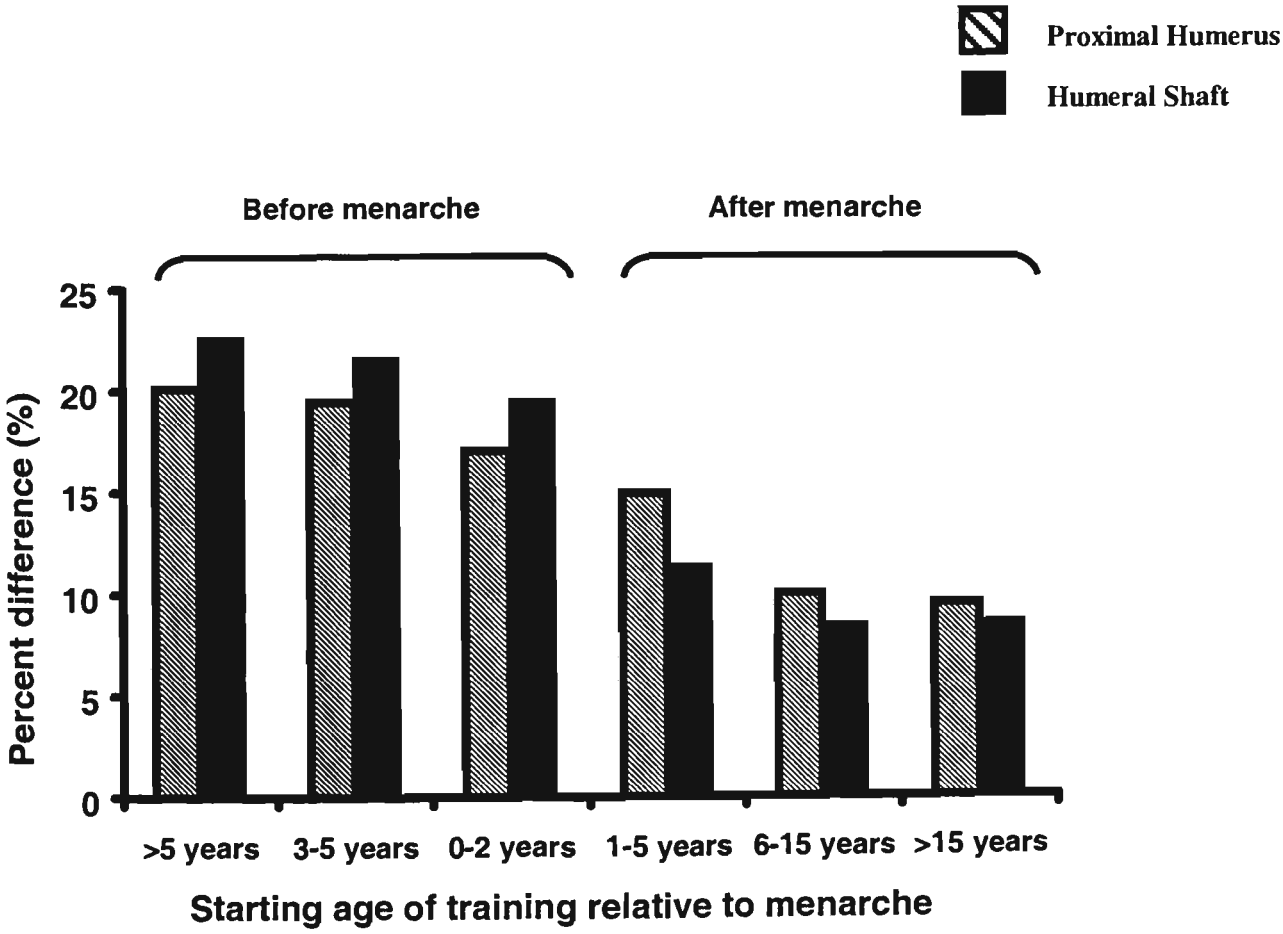

Figure 2.6: Side to side difference in BMC of the upper arm of players relative to the age they commenced training [Kannus et al., 1995].

The limitations to quantifying the magnitude of the effect of mechanical load as a result of starting age of playing were that the training volume and intensity of each of the subsets based on starting age were not disclosed. In addition, changes to training for each individual over their entire training life were not reported. Players assessed ranged in age from 16 – 50 years; therefore some players may be post-menopausal, while others may still be accruing bone. The range in playing years was also not reported. The division of players into the subsets based on age they commenced playing was not equal, therefore it is difficult to determine with precision at what age before or after menarche the greatest osteogenic effect may occur.

Haaspasalo et al. (1998) investigated the effect of maturity status on the side-to-side difference between the playing and non-playing arms of 91 female tennis players aged 7 – 17 years. Bone mineral density was measured at three sites in the arms (proximal humerus, humeral shaft and distal radius) and at the lumbar spine. The side-to-side differences in the players were compared to that of 58 female controls. The mean side-to-side difference in the controls ranged from –0.2% to +4.6%, while the mean side-to-side difference in the players ranged from 1.6% to 15.7%. When the side-to-side differences in players were compared to controls of a similar maturity status, no difference was found between the groups for girls in Tanner stage I. For girls in Tanner stage II, a difference was only found at the humeral shaft. Differences in the three measured sites were reported between the players and controls for girls in Tanner stages III to V. Greater BMD at the lumbar spine was reported for the players when compared to controls for girls in Tanner stages IV and V only. No difference was found between the two groups for BMD in the non-dominant radius from Tanner stages II to V. The distal radius was not assessed in girls in Tanner stage I. Within the player's group, the number of sessions per week, the number of training years and the total number of training hours increased with Tanner stages. The correlations between the relative side-to-side differences of the players, and the training variables for girls within each maturity group are depicted in Table. 2.5.

Table 2.5. Correlation between the relative side-to-side differences in BMD and training variables for players of different maturity status. (Adapted from Haspassalo et al., 1998)

Site	Training variable	Tanner I	Tanner II	Tanner III	Tanner IV	Tanner V
Proximal humerus	Total training hours	NS	NS	0.50	NS	NS
	Sessions per week	NS	NS	0.55	NS	0.80
Humeral Shaft	Total training hours	NS	NS	0.63	0.70	0.52
	Session per week	NS	NS	0.77	NS	0.61
Distal radius	Total training hours	-	NS	0.61	NS	0.61
	Session per week	-	NS	0.66	NS	0.67

As reported in Table 2.5, total training hours and sessions per week did not correlate with the relative side-to-side difference in BMD at the three measured sites for girls in Tanner stages I and II ($r = -0.23$ to 0.44). Both variables correlated with the BMD differences at all three measured sites for girls in Tanner stage III ($r = 0.50$ to 0.77, $p < 0.05$). Only total training hours correlated with the side-to-side difference in humeral shaft BMD for girls in Tanner stage IV ($r = 0.70$, $p < 0.01$). For players at Tanner stage V both variables correlated with BMD differences at all three sites ($r = 0.52$ to 0.80, $p < 0.05$), except for total training hours which did not correlate with the BMD difference at the proximal humerus ($r = 0.43$).

Although the authors concluded from their data that the osteogenic effect of exercise may not become apparent until Tanner stage III, and that strenuous exercise during Tanner stage I does not result in gains in BMD, the data is not conclusive. The intensity of training was not assessed and training loads were greater in the more mature girls. In addition the two indicators of training load did not correlate uniformly with the side-to-side difference in BMD for girls from Tanner stages III to V. Correlations were significant during Tanner stage III and for the majority of sites in Tanner stage V. The correlation however, only existed for total training hours and humeral shaft BMD for girls in Tanner stage IV. Although the data indicates that the peri-pubertal period may be more opportune to maximise the effect of exercise on bone than the pre-pubertal period, more data is needed to support this claim. The results from unilateral loading studies however, have reported a greater side-to-side difference in players who started playing while young compared to those who started playing after maturity.

2.21.3 The effect of exercise intervention on the growing skeleton

Few exercise intervention studies involving children have been conducted, however from the data that is available, the effects have been positive. To date, three studies have been published which have conducted exercise intervention trials involving young children [Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997]. The former study involved pre-pubescent males, the second pre and peri-pubescent females and the later both males and females.

Morris et al. (1997) conducted a non-randomised 10-month exercise intervention program involving 71 pre-menarchial girls (Tanners stages 1 – 3) aged 9-10 years to determine the effects of exercise on total body, appendicular and axial bone mass. The program consisted of three weekly weight bearing physical education classes, in addition to their normal physical education curriculum. The sessions involved dance, running and jumping based games, and weight training. Thirty-eight girls received the additional exercise sessions, while the remaining 33 girls acted as controls. No difference was reported between the groups for baseline characteristics.

Following the intervention period greater gains in total body and regional BMD were reported in the exercise group (3.3% - 12.0%, $p < 0.05$) when compared to the control group (1.2% - 2.8%). Greater gains were also reported for total body (12.0% v 6.5%, $p = 0.001$), proximal femur (11.9% v 3.6%, $p = 0.01$) and femoral neck BMC (10.4% v 5.9%, $p = 0.001$) in the exercise group when compared to the control group. The greater gains in lumbar spine BMC in the exercise group compared to the control group neared significance (7.0% v 1.5%, $p = 0.05$). No differences were reported between the groups for changes in bone mineral apparent density (to adjust for bone size) at the lumbar spine and femoral neck. No differences were reported between the groups for bone area, except for the femoral neck, where gains were greater in the exercise group compared to the control group (4.7% v 0.6%, $p = 0.01$) following the intervention period. Greater gains in lean mass were also reported in the exercise group compared to the control group (2.2 % v 1.4%, $p = 0.01$). Lean mass was an independent predictor of total body, lumbar spine, and femoral neck BMD, and total body BMC, accounting for 10-58% of the variance in bone accrual. The control group reported greater gains in fat mass over the study period (1.0% v 0.5%, $p = 0.04$). Total body fat content was positively associated with lumbar spine BMC accrual.

The study consisted of girls who were both pre- (Tanner stage 1) and peri-pubertal (Tanner stages 2-3). Failure to match the girls for pubertal stage makes it difficult to establish if the changes to bone mass reported in the exercise group were due to the exercise, or to skeletal growth due to maturity. The measurement of BMD or BMC is a function of the size of the bone and the amount of bone within the bone (volumetric density). No change to FN BMAD was reported with exercise, therefore the increase in FN BMD was likely to result from an increase in size (increase in FN area reported in exercise group, $p < 0.01$) than to an increase in the amount of bone within the bone (volumetric density).

Bradney et al. (1998) conducted an 8-month school-based exercise intervention trial involving 20 pre-pubescent boys (mean age 10.4 ± 0.2 years, Tanner stage 1), and compared changes in bone mass to 20 age-, height-, sitting height-, weight- and baseline BMD-matched controls. The program consisted of 30 minutes of additional weight bearing activities three times a week. Activities such as ball games, weight training, gymnastics and dance were included in the program. Volumetric density, true density, cortical width, medullary and periosteal diameters and bone strength measures were calculated from the DXA results. No differences in bone and anthropometric measures were reported between the groups at baseline.

Both the control and exercise groups increased BMD and BMC following the eight-month period. The gains in BMD over the total period calculated from the monthly gains reported were greater for

the exercise group when compared to the control group. Changes of 2.6% v 1.7% for total body BMD, 4.3% v 2.1% for lumbar spine BMD and 6.2% v 2.6% for BMD of the legs were reported for the exercise and control groups, respectively ($p < 0.05$ - < 0.01). Greater BMC accrual was reported at the femoral midshaft in the exercise group compared to the control group (9.3% v 3.4%, $p < 0.01$), but not at the lumbar spine. No differences were reported between the groups for volumetric densities ($g.cm^{-3}$) at the lumbar spine or femoral midshaft.

More recently, McKay et al. (2000) conducted an 8-month school-based exercise intervention program involving 144 pre- and early-pubescent (Tanner stages 1 – 2) males and females of Asian and Caucasian descent. The ten participating schools were randomly assigned to the exercise group (n = 63 children) or acted as controls (n = 81 children). The exercise group participated in three additional exercise session per week, involving between 10 –30 minutes of weight bearing activity. The minimal requirement per session was 10 tuck jumps.

At baseline no differences were reported between the groups for bone and anthropometric measures. Children at the control school however, were more active and performed better on a vertical jump test than children at the exercise school. Following the intervention period, both the control and exercise groups increased BMD and anthropometric measures. The control group accrued more height than the exercise group (3.5 $cm.yr^{-1}$ v 1.5 $cm.yr^{-1}$, respectively), which was due to a greater increase in sitting height. No differences were reported between the groups for gains in bone measures except for the trochanter, where the exercise group reported a greater gain in trochanteric BMD compared to the control group (4.4%.yr^{-1} v 3.2%.yr^{-1}, respectively, $p = 0.03$). Using a regression model to account for confounding factors (including changes in height, but not maturity status), group selection (exercise) remained a significant predictor of trochanter BMD, accounting for 3% of the variance. Groups were not matched for pubertal stage. Therefore it may be suggested that the failure to detected enhanced bone mass accrual at other weight bearing sites with exercise could be due to greater bone mass accrual in the control group due to advanced maturity. The greater gain in height (and sitting height) in the control group supports this suggestion.

Although the data indicates that moderate weight bearing exercise results in enhanced bone accrual in young children, the exact amount of exercise and the most appropriate type of exercise to maximise the benefit to bone is still not clear. The additional exercise provided in the three intervention programs however, was sufficient to enhance bone mineral accrual, and was a realistic and achievable amount of exercise for young children. In addition, the changes in BMD reported in the three intervention studies (approximately 1% - 10%) were greater than those reported with mature and older women (< 2%). Although other factors such as participants' motivation and movement efficiency

need to be considered, the results indicate that the growing skeleton may be more responsive to mechanical loading, than more mature bone.

2.22 The long-term benefits of childhood physical activity on bone mass.

Enhanced bone mass accrued during childhood as a result of physical activity is only of assistance in preventing osteoporosis if the benefits are maintained into adulthood. Greater BMD has been reported in athletic children compared to age-matched controls. In order to determine if these greater benefits are maintained, studies have compared retired athletes to matched controls. Greater or equal measures of bone mass have been reported in retired athletes when compared to controls [Bass et al., 1998; Duppe et al., 1996; Etherington et al., 1996; Karlsson et al., 1996; Karlsson et al., 2000; Khan et al., 1996; Kirchner et al., 1996].

Higher values for BMD in athletes have been reported in some but not all studies. Kirchner et al. (1996) compared total and regional BMD in 18 female former college gymnasts (mean age 36.3 ± 1.0 years) to 15 age-, height- and weight-matched controls. Gymnasts had commenced training, on average at age 11.9 ± 0.5 years, and competed for, on average 7.3 ± 0.8 years. Groups were matched for age, height and weight at baseline however, gymnasts were involved in more strenuous activity, expended more energy, had lower percent body fat and exercised more over the past 10 years compared to the controls ($p < 0.003 - p < 0.05$). Following adjustment for past and current physical activity levels, greater BMD was reported for the gymnasts at the lumbar spine (16%), femoral neck (18%), Ward's triangle (22%) and for the total body (9%) compared to controls ($p < 0.01$).

Duppe et al. (1996) compared the total and regional BMD of 25 retired soccer players (age range 34 – 48 years) to 57 age-matched controls. Players had retired for 9.7 years (range 5 – 20 years). Players did not differ from controls for height and weight however, they did report greater lean mass ($p < 0.05$). Following adjustment for age, height and BMI, greater BMD was reported in the players for total body (3.5%, $p < 0.05$), femoral neck (7.3%, $p < 0.05$), trochanter (11.1%, $p < 0.01$) and Ward's triangle (8.9%, $p < 0.05$), but not for lumbar spine, when compared to controls. No training history of past players was provided however, controls and players did not differ in current physical activity levels. No dietary assessments were conducted. Furthermore, adjustments were not made for lean mass despite greater lean mass reported in the ex-players compared to the control.

Khan et al. (1996) investigated the effects of osteoporosis risk factors in elite female ballet dancers on BMD in later life. One hundred and one retired dancers (mean age 51.1 ± 1.4 years) were compared to 101 age-, height-, weight-, and menopausal status-matched controls. Dancers were lighter, drank

more alcohol, smoked more, had later menarche, reported more incidences of menstrual disturbances and drank less milk during adolescence, compared to controls ($p < 0.05$). Despite elite female ballet dancers exhibiting many osteoporosis risk factors during their years of dancing, no differences were reported between the dancers and controls for total body, total hip, femoral neck, trochanter, intertrochanter and lumbar spine BMD. Lower BMD at the radius however, was reported for the dancers compared to the controls ($p < 0.05$).

Etherington et al. (1996) compared lumbar spine, femoral neck and forearm BMD of 83 retired elite female athletes (mean age 52.4 years) to 585 age-matched controls. The athlete group consisted of 67 runners (mean time since retirement 15.3 years), and 16 tennis players (mean time since retirement 19.8 years). Athletes were taller, lighter and smoked less than controls (significance not reported). Following adjustment for age, height, weight and smoking, greater BMD was reported for the athletes at the femoral neck (12.1 %, $p < 0.001$) and lumbar spine (8.7%, $p < 0.001$) compared to the controls. Forearm BMD was not reported. No dietary intakes, training histories or menstrual details were provided. Furthermore, 40 of the 67 runners and 15 of the 16 tennis players were still active in their sport.

Bass et al. (1998) reported greater BMD at the arms, legs, femoral neck, lumbar spine and total body (range: 6% – 16%, $p < 0.05 – 0.001$) of 36 retired gymnasts (mean age 25 ± 0.9 years, range 18 – 35 years) when compared to 15 age-, height- and weight-matched controls. No difference between the groups was reported for BMD at the skull. Gymnasts had commenced training at age 7.5 ± 0.4 years (range 3 – 12 years) and retired at age 16.7 ± 0.4 years (range 12 – 24 years). The gymnasts had less fat mass ($p < 0.05$), a later menarche ($p < 0.05$) and tended towards greater lean mass ($p < 0.06$) when compared to the controls. The greatest difference in BMD between gymnasts and controls was reported at the arms (16%), which is a site uniquely loaded during gymnastics training. The magnitude of the difference between the two groups (for this and other sites) did not diminish with increased years since retirement. Furthermore, the magnitude of the difference in BMD at the arms of the retired gymnasts (16%) was similar to active pre-pubertal gymnasts compared to bone-age matched control (11%). These data indicate that the residual benefits to bone mass conferred by exercise, may be prolonged as the magnitude of the difference in BMD between the gymnasts (active and retired) and matched controls were similar even after retirement.

Few studies have investigated the prolonged benefit of exercise on bone mass well into old age. Karlsson et al. (1996) investigated the residual and prolonged benefits of weight lifting on bone mass by comparing 64 retired male weightlifters (age range 35 – 79 years) to 133 male controls. Weight lifters had retired for 25 ± 13 years. Greater total body (7.4%, $p < 0.01$) and femoral neck BMD (10.6,

$p < 0.01$) was reported for the retired lifters aged 35 – 49 years (n = 16) when compared to the controls. The greater BMD in the lifters remained significant at the total body (5.8%, $p < 0.01$), but not at the femoral neck, for those aged 50 – 64 years (n = 24), compared to controls. Lower BMD at the skull (-8.6%, $p < 0.01$) was reported in the lifter in this age group, when compared to the controls. No differences in total body, femoral neck or skull BMD were reported between the lifters aged 65 – 79 years (n = 24) and the controls.

Results from Bass et al. (1998) suggest that the residual benefits of childhood activity is maintained during adulthood as the difference in BMD between controls and ex-gymnasts did not decrease with years from retirement. Karlsson et al. (1996) however, reported greater BMD at the femoral neck and total body for retired lifters ages 35-49 years when compared to controls, but no significant difference between controls and retired weight lifters aged 65-79 years. These findings may be interpreted as the residual benefit decreasing with increasing years since activity however, the controls were not age matched. The lifters may, in fact have had greater BMD than similarly aged controls, as lifters in the older age group (65-79 years) would be expected to have commenced normal age-related bone loss [Seeman, 1997].

More recently, Karlsson et al. (2000) compared BMD of the arms and legs of former soccer players aged 48 – 94 years (n = 128) to age matched controls (n = 138). The authors reported a diminishing in the difference in leg BMD (but not arm) between the former players and controls with increasing years since retirement. By 35 years after retirement, no difference in leg BMD was reported between the former players and controls. The inference that exercise during growth does not confer prolonged benefit to bone mass generated considerable debate [Sievanen et al., 2000; Lorentzon et al., 2000]. The controls were age-matched but not body mass matched, which may influence BMD. Also when data for the 70+ year old retired players was corrected for current activity levels and body composition, they reported 6.5% higher BMD compared to the controls. The training history of the former players varied considerably in relation to volume of training (4-20 hours per week) and years of training (1-35 years). In response to the debate raised the authors presented three rebuttals. Firstly, that residual benefits were not reported at the sites most prone to fractures (hip and lumbar spine). Secondly, data may be skewed by systematic bias in that healthier people who are more suited to exercise, do so, than the exercise leading to their improvement in health. And thirdly, the residual benefits may be due to continued exercise, even if at a reduced rate.

A further difficulty in determining if benefits to bone mass from exercise are maintained, it that initial BMD values are unknown. Khan et al. (1996) reported no difference in BMD values between retired dancers and controls. It is unclear however, if the dancers commenced with greater BMD at weight bearing sites than the controls (hence the rate of loss was greater), or lesser BMD than controls. Bass

et al. (1998) attempted to answer this question by comparing the BMD of active gymnasts to that of the retired gymnasts. While BMD values were less in the retired gymnasts compared to the active gymnasts, the authors acknowledged the limitation that training loads may have differed between the two groups.

An additional limitation in determining the residual benefit of elite training in childhood on adult BMD, is that many of the retired athletes are still active. Etherington et al. (1996) reported that 60% of the runners and 94% of the tennis players participating in their study were still active. Kirchner et al. (1996) also reported that the gymnasts involved in their study were more active in general and performed more strenuous exercise compared to the controls. It may be that some of the residual benefits result from active children maintaining an active lifestyle as adults. Whether the residual benefit is due to the exercise during childhood per se, or due to an active lifestyle being maintained remains unknown. Both outcomes however, may impart a positive influence on adult bone mass and reduce subsequent fracture risk in later life.

2.23 The combined effect of exercise and calcium on bone mass

The literature presented thus far indicates that individually, moderate exercise or calcium supplementation may enhance bone mass accrual in children [Bradney et al., 1998; Bonjour et al., 1997; Johnston et al., 1992; Morris et al., 1997]. There is however, limited data available on the combined effect of exercise and calcium. There are few calcium/exercise intervention studies involving adults [Nelson et al., 1991; Prince et al., 1995], and none, to date, involving children. Furthermore, cross sectional studies investigating the association of calcium intake and physical activity levels with bone mass in adults and children are inconclusive. Table 2.6 presents a summary of previous studies investigating the associations between current (and past) calcium intake, exercise and bone mass in adults and children.

Halioua and Anderson (1989) investigated the effect of lifetime calcium intake and physical activity on distal and mid radius bone mass in 181 pre-menopausal women aged 20 – 50 years. Calcium intake was associated with distal radius BMC ($p = 0.004$), and BMD ($p = 0.0001$), and mid radius BMC ($p = 0.0008$) and BMD ($p = 0.0004$). Physical activity was associated with distal radius BMC ($p = 0.03$) and mid radius BMC ($p = 0.007$). When calcium intake was categorised into low (< 500 $mg.day^{-1}$), intermediate (500-800 $mg.day^{-1}$) and high (> 800 $mg.day^{-1}$), there was a significant difference in bone values at intermediate and high calcium intakes, compared to the low intake group ($p < 0.003$). Furthermore, when physical activity was combined with calcium intake bone mass values for the intermediate calcium / high activity and high calcium / high activity were greater than for the low calcium / low activity group ($p < 0.05$).

Uusi-Rasi et al. (1998) investigated the association of weight bearing exercise and current calcium intake with total body, femoral neck and distal radial bone mass in 422 women in three age groups (25-30, 40-45, 60-65 years). The highly active women had greater total body BMC (1.8%) and femoral neck BMD (5.0%) when compared with the low activity group. The high calcium group also had greater total body BMC (4.6%) compared to the low calcium group. No association was reported between femoral neck BMD or for radius BMD and either physical activity or calcium intake. Furthermore, no significant interaction was reported between calcium intake and physical activity relative to any of the bone variables.

Tylasvsky et al. (1992) evaluated the relationship between adolescent physical activity levels and calcium intake and radial bone mass in 705 Caucasian college women aged 18-22 years. Calcium intake was based on milk and cheese consumption only. Participants were divided into high (> 4 hours per week), moderate (1-4 hours per week) or low (<1 hour per week) activity groups. The calcium intakes were rank ordered with the lower 25^{th} percentile denoted as the low consumers, the

25^{th}-75^{th} percentiles the moderate consumers and the upper 25^{th} percentile the high consumers. The high calcium consumers had greater distal radius BMC (1.8%) and BMD (2.7%) when compared with the low consumers. No difference was reported for bone variables between the moderate and low calcium groups. The high and moderate activity groups had greater distal radius BMC (12.3% and 6.4%, respectively) and BMD (9.1% and 5.3%, respectively) compared to the low activity group. An interaction between calcium and physical activity was reported. The high calcium / high activity and moderate calcium / moderate activity groups had greater distal radius BMC (16.5% and 9.5%, respectively) and BMD (12.3% and 8.9%, respectively) when compared with the low calcium / low activity group.

Mazess and Barden (1991) investigated the effects of age and lifestyle variables (including calcium intake and physical activity) on bone mass over a two-year period in 200-300 pre-menopausal women aged 20-39 years. Lumbar spine, femoral and humeral bone mass (n = 300, n = 218 and n = 215, respectively) was measured using DPA. Radial shaft and distal radial were assessed using SPA. No association was reported between physical activity or calcium intake, and bone mass, or changes to bone mass over the two-year period. Furthermore, no interactive effects of calcium and physical activity on bone mass, or changes to bone mass were reported.

Of the four studies presented in adults only the distal radius is a site common to all. Two of the studies reported an association between calcium, exercise and bone mass at this site [Halioua & Anderson, 1989; Tylavsky et al., 1991]. An interactive effect of calcium and exercise on bone mass was also reported for these two studies. The remaining two studies reported no association between calcium, exercise and bone mass, and no interactive effect between the two lifestyle variables [Mazzess & Barden, 1991; Uusi-Rusi et al., 1998]. The effects of mechanical loading are site specific [Lanyon, 1992] therefore an interaction would be more likely to occur at a weight bearing site (loaded) than a non-weight bearing site (not loaded).

Table 2.6: Summary of previous studies investigating the relationship between calcium, exercise and bone mass in adults and children.

Reference	Study group	Measurement techniques			Bone sites measured	Assoc. Calcium	Assoc. Activity	Interaction
		Bone mass	Calcium Intake	Physical activity				
Adult studies								
Halioua & Anderson, 1989	181 pre-meno women (20-50 yrs)	SPA	Food frequency Questionnaire	Questionnaire	Distal radius BMC BMD Mid radius BMC BMC	 Yes Yes Yes Yes	 Yes No Yes No	Intermed. Ca/ High PA & High Ca/ High PA > Low Ca/ Low PA
Uusi-Rasi et al., 1998	422 women (25-30, 40-45, 60-65 yrs)	DXA	4-day food record	Pedometer (3 days) Diary (3 days) Questionnaire	Total body BMC Femoral neck BMD Distal radius BMD	 Yes No No	 Yes Yes No	No interaction reported
Tylasvsky et al., 1992	705 pre-meno women (18-22 yrs)	SPA	Food frequency questionnaire	Questionnaire	Distal radius BMC BMD Mid radius BMC BMD	 Yes Yes Not reported	 Yes Yes Not reported	High Ca/ High PA & Mod Ca / Mod PA > Low Ca / Low PA
Mazzess and Barden, 1991	200-300 pre-meno women (20-39 yrs)	DPA SPA	24-hour food record	Pedometer (2-days) Accelerometer (2 days)	Lumbar spine BMD Femoral neck BMD Humerus BMD Radius shaft BMD Distal radius BMD	 No No No No No	 No No No No No	No interaction reported

Children & Adolescents								
Welten et al., 1994	84 males and 98 females aged 27 years	DXA	Diet history	Interview	LS BMD	No	Yes – males No - females	No
Ruiz et al., 1995	70 males and 81 females aged 7-15 years	DXA	Semi-quantitative food frequency questionnaire	Questionnaire	LS BMD	Yes-boys No-girls	No-boys Yes-girls	Not reported
					FN BMD	Yes-boys No-girls	No boys Yes-girls	
Boot et al., 1997	205 males and 295 females aged 4-20 years	DXA	Food frequency questionnaire	Questionnaire	LS BMD	No – boys No – girls	Yes-boys No-girls	Not reported
					TB BMD	Yes– boys No-girls	Yes-boys No-girls	

The association between calcium intake and physical activity and bone mass in younger populations is also inconclusive. Welten et al. (1994) investigated if weight bearing activity and dietary calcium intake during adolescent and young adulthood was related to lumbar spine BMD (LS BMD) in 84 males and 98 females aged 27 years. Activity levels and dietary calcium intake was assessed four times between the age of 13-17 years, once at 21 years of age and again at 27 years of age. Weight bearing exercise during adolescence and young adulthood was a significant predictor of LS BMD in males (accounting for 15-17% of the variance), but not in females. Dietary calcium intake during adolescence and young adulthood was not a predictor of LS BMD in either sex. No interaction between weight bearing exercise and dietary calcium intake was detected. Dietary calcium intakes in this group of participants were relative high. Mean values for each of the age ranges were between 1100 $mg.day^{-1}$ - 1435 $mg.day^{-1}$ for males and 941$mg.day^{-1}$ – 1204$mg.day^{-1}$ for females.

Boot et al. (1997) investigated the relationship between dietary calcium intake and physical activity and total body (TB) and LS BMD in 205 boys and 295 girls aged 4-20 years. Calcium intake (mean 1180 $mg.day^{-1}$) was positively associated with TB BMD in males ($p < 0.009$). Physical activity expressed as $hours.week^{-1}$ was associated with TB and LS BMD in males ($p < 0.04$). No associations between physical activity or calcium intake and BMD were reported for females. An interaction between calcium and exercise was not reported.

Ruiz et al. (1995) investigated the association between dietary calcium intake and physical activity and LS and FN BMD in 81 girls and 70 boys aged 7-15 years. A positive association was reported between dietary calcium intake and LS and FN BMD in boys but not in girls. Physical activity was positively associated with TB and LS BMD in girls but not in boys. No interaction between calcium intake and activity levels and BMD was reported.

Interpretation of the data is difficult. Inconsistencies occur in relation to the association of BMD with dietary calcium or physical activity. For example, the lack of association between calcium intake and BMD may be compounded by the fact that calcium intakes are often uniformly high and do not vary between the sexes or correlate with age [Boot et al., 1997]. Overall physical activity levels do not necessarily give an indication of loads experienced. For example activities may include non-weight bearing sports such as swimming, which may be performed at high intensity, but may not necessarily involve mechanical loads sufficient to stimulate additional bone mass accrual. Furthermore, an interaction between exercise and calcium would most likely be present at a site where loads are applied. An interaction may be expected at weight bearing sites. This implies that it may be the quality of the activity (in relation to loads applied) not the quantity of the activity that may allow for an interaction. Few studies have categorised the activities in relation to the their loading qualities, rather than their energy value (METS) or volume ($hr.week^{-1}$). There is a need to quantify activities

from the perspective of loads applied to bone. Intervention studies where activity levels and calcium intakes can be quantified may provide a clearer insight into the effects of exercise and calcium on bone mass.

2.23.1 Calcium and exercise intervention studies

Results from calcium / exercise intervention studies involving adults are also limited. Nelson et al. (1991) conducted a year-long walking program, and calcium supplementation intervention study involving 36 post-menopausal women aged 60.2 ± 6.5 years. Participants were recruited into the exercise groups based on preference, but were randomly assigned to receive either a high calcium milk drink (831 mg calcium) or a low calcium placebo (41 mg calcium). Lumbar spine and femoral neck BMD was assessed using DPA. Lumbar spine trabecular BMD was measured using QCT and radius BMD assessed using SPA. Total body calcium was determined using neutron activation. A difference in trabecular BMD at the lumbar spine was reported between the exercise groups, with the sedentary group reporting a 7.0% reduction in BMD, and the exercise group a 0.5% increase ($p = 0.028$). No effect of calcium was reported at this site. A positive effect of calcium was reported for femoral neck BMD with the high calcium group reporting a 2.0% increase in BMD, while the placebo group reported a 1.1% decrease. No effect of exercise was reported at this site. No effect of either calcium or exercise was reported for radius BMD or total body calcium. Although the two by two design was employed, results were not analysed accordingly, therefore the potential for interactions were not determined.

Prince et al. (1995) conducted a two-year, randomised calcium/exercise intervention trial involving 168 women aged 50-70 years who were at least 10 years post-menopausal. The women were randomly assigned to receive either one gram of calcium from skim milk powder, one gram of calcium from calcium lactate gluconate, a placebo, or receive calcium lactate gluconate and undertake 4 hours of additional exercise each week (two of which was supervised). Bone mineral density at the lumbar spine, at three hip sites and two tibial sites were measured every six months. Changes in BMD at the trochanter, intertrochanter, ultradistal ankle, and total hip, for the calcium and calcium/exercise groups, were greater than those reported for the placebo group ($p < 0.05$). A difference in annual change in BMD between the calcium/exercise group and the calcium only group was only reported at the femoral neck ($0.23 \pm 0.34\%$ v $-0.18 \pm 0.20\%$, $p < 0.05$). No differences were reported between the groups for lumbar spine and mid tibial BMD.

While the results appear to indicate that an interaction between calcium and exercise may have occurred at the femoral neck, results need to be further examined. For example, compliance for the

exercise group was poor reporting only a 10% greater activity level (measured in METS) compared to the non-exercise group. No interaction (or exercise effect) was reported at the other weight-bearing site of the ankle. Furthermore, no anthropometric data was provided for the groups. To determine the potential for an additive effect, the study would need to include an exercise only group. A two by two design may provide the necessary comparisons to detect an interaction.

It has been suggested that an adequate calcium intake is necessary to enable physical activity to be effectual [Bailey & Martin, 1994]. The minimal amount of calcium required however, is not definitive. Specker (1996), who performed an analysis of calcium intake and bone mineral density changes in response to exercise attempted to determine if the effect of exercise on bone mass is augmented at a specific level of calcium intake. Seventeen exercise intervention trials involving adults were included in the analysis. Changes to lumbar spine BMD in response to exercise were plotted against calcium intake and compared to the changes relative to the controls. A significant difference existed between the regression lines for exercise versus controls ($p < 0.05$). No differences were reported for changes in lumbar spine BMD relative to calcium intake with the controls. The gains in lumbar spine BMD in response to exercise increased with increasing calcium intake. This analysis indicates that the effect of the exercise on lumbar spine BMD was augmented by an increased calcium intake. No effect of calcium intake on the changes to radius BMD in response to exercise was apparent.

From the literature reviewed it appears that the ability for calcium or exercise to make clinically significant changes to bone mass in adults may be limited. If in adults, the only effect of calcium is to reduce the rate of bone turnover (thus reduce the size of the remodelling space), then the reported changes are small and reversible once the calcium supplementation ceases. In addition, if exercise in the adult skeleton is only able to reduce the rate of bone loss, then bone mass can only be maintained, not added to in any appreciable manner. The potential for a combined effect of exercise and calcium however, cannot be dismissed. If the rate of bone loss can be slowed and the combined effects of exercise and calcium are synergistic or additive, then the progression towards bone fragility may be delayed.

Alternatively, the singular effects of exercise and calcium interventions on the growing skeleton are larger than those reported for adults. If exercise is able to stimulate modelling during the growth phase, and sufficient calcium is available, then the mechanisms and the materials are available to provide for maximal bone mass accrual. An intervention study involving both exercise and calcium may provide insight into this potential synergistic effect.

Chapter Three

Methodology

Sub-headings

3.1 Methods

The methodology described in this chapter was used in the 8.5 month calcium supplementation and exercise intervention study. The results from the intervention study are reported in Chapter 6. Methodologies that are common to all studies are also reported in this chapter. Methodology employed exclusively for the studies reported in Chapters 4 and 5 are described within the respective chapters.

Data for the intervention study was collected at baseline, 4.25 months (mid) and 8.5 months (post). The timing of data collection is described in Table 3.1.

Table 3.1: Data collection time lines for the calcium/exercise intervention program

Measurement	Baseline	Mid	Post
Body composition / bone mass	✔		✔
Pubertal staging	✔		✔
Dietary assessment	✔	✔	✔
Physical activity assessment	✔	✔	✔
Anthropometry	✔		✔

3.2 Recruitment

Participants were recruited from Milgate primary school, situated in Doncaster east, an outer middle class suburb of Melbourne, Australia. This school was selected as a result of the school having a sufficient number of potential participants, and the willingness of the school principal to incorporate the program into the school's physical education curriculum. Two parent information sessions describing the program and its requirements were conducted. Parents of girls eligible for inclusion (female students in years 2-5, n = 154) were invited to attend. Following the information sessions, plain language statements and consent forms were mailed to all eligible participants. Written parental consent was obtained for 88 girls.

3.3 Allocation to study groups

Subjects were stratified on the basis of age, ethnicity and total body scan mode, then randomly assigned to one of four study groups using a randomisation table. Participants were not stratified on the basis of pubertal staging as this assessment was not completed prior to the allocation to the study groups. The study groups were: high impact exercise + calcium supplementation (HI ex + Ca), high impact exercise + placebo (HI ex + Pla), low impact exercise + calcium supplementation (LI ex + Ca) and low impact exercise + placebo (LI ex + Pla).

3.4 Pubertal staging

Pubertal staging was determined using a parental-assisted self-report, based on Tanner staging for pubic hair and breast development [Tanner, 1978]. Participants were mailed illustrations of pubic hair and breast development stages, and instructions on completing the self-assessment sheet. Pre-pubertal participants were defined as those with Tanner stages 1 for pubic hair and breast development. Participants reporting Tanner stages 2-4 were classified as peri-pubertal. Early puberty was defined as participants in Tanner stage 2. Participants who had achieved menarche were classified as post-pubertal. The accuracy of this method of self-reported pubertal status has been described previously [Duke et al., 1980]

3.5 Body composition and bone mineral content

Body composition and bone mineral content and density were assessed using dual energy x-ray absorptiometry (DXA) using pencil beam mode (Lunar Radiation Corporation, Madison, WI, DPX-L). Total body, anterior-posterior lumbar spine (L1-L4) and femoral neck scans were conducted at the beginning and end of the intervention period. Pediatric or adult software (version 4.6d) was used depending on the participant's trunk thickness, which was determined immediately prior to the scan using a Harpenden anthropometer. Scan modes were selected on the basis of body mass of the participant and were based on the manufacturer's criteria. All metal objects eg. jewellery were removed prior to each scan. Subjects were required to wear light clothing such as bathers, T-shirt and shorts, or a gown provided. All scans were performed and analysed by the same operator. The coefficient of variation was 1.3% ± 0.4% for TB BMC as determined on five repeated measures taken on five adults over a two week period [Walker et al., 1996]. Adults were used to determine the CV as repeated scans are not permissible on children.

3.5.1 Regional analysis of the total body scans

Proximal and distal segments of the arms (humerus & ulna-radius, respectively) and the legs (femur & tibia-fibula, respectively) were determined using the 'region of interest' option from the total body scans. All scans were analysed by the same investigator. The coefficient of variation for regional determinations was 1.68 ± 0.4 %.

3.5.2 Total body scans

Subjects were required to lay supine and straight on the bed, with hands placed prone on either side of the body. The legs were straight and approximately 10 cm apart, with the feet relaxed and rotated in. Scan time was from 10 min to 15 min depending on the size of the participant.

3.5.3 Femoral neck

Subjects were required to lay supine and straight on the bed. The scanned leg (left) was gently rocked, and the greater trochanter located. The scan start position is 5 cm inferior of the greater trochanter and in the middle of the femoral shaft. The leg was gently lifted and rotated inwards and strapped to a plastic positioner. Scan time was from 2 min to 4 min depending on the size of the participant.

3.5.4 Lumbar spine

The participant was required to lie supine and straight on the bed. A support block was placed under their lower legs so the knees were bent and the femur was at 45° from horizontal. The iliac crest was located, and the scan position located 2 cm below an imaginary line between the iliac crests. The scanner moves from inferior (L5) to superior (T12). Scan time was from 1 min to 3 min depending on the size of the participant.

3.6 Anthropometrics

Standing height was measured using a Holtain wall stadiometer. Subjects were required to stand barefooted, with hands by side and heels touching the wall. The head was tilted forward so the eyes were in line with the ears. Subjects were required to breath in, and the measurement taken. At least two measures are taken, and the average recorded. Sitting height was measured using the same standiometer while sitting on a stool of known height, with knees bent at 90° and hands placed on their lap. Body mass while wearing light-weight clothing was measured using a SECA electronic scale. Limb lengths (humerus, ulna, tibia) and body widths (bi-acromial, bi-trochanter, bi-iliac) were measured using a Harpenden anthropometer, accurate to one millimetre. Femur length was determined from the total body scans, and was defined as the distance from the inferior border of the lateral epicondyle to the superior border of the greater trochanter. Humerus length was defined as the distance between and including the head of the humerus and the most distal portion of the trochlea. Ulna length was the distance between and including the olecranon process and the styloid process. Tibia length was the distance between and including the medial condyle and medial malleolus. Appendicular measurements were taken on the left-hand side of the body. Body width measurements were bi-acromial (distance between the acromion processes), bi-iliac (distance between the two iliac crests) and bi-trochanter (distance between the greater trochanters of the femur). All land marks were clearly identified and repeated measure performed. The same investigator performed all measurements. The coefficient of variation was 0.1 ± 0.1 %.

3.7 Physical activity

Physical activity was assessed using a parental-assisted physical activity questionnaire. Questionnaires and instructions were mailed to participants. Completed questionnaires were returned

in a sealed envelope. Total hours of activity, and hours of weight bearing and / or non-weight bearing exercise were determined. Participants were classified into either the high activity group (> 6 hours of activity per week) or low activity group (< 6 hours of activity per week). Classification was validated using school-yard video surveillance of recess activity on a sub-set of 24 participants randomly selected from the high and low activity groups. Video surveillances were performed twice during the intervention period. The participant was videoed undertaking their normal recess time activity using a National video camera with zoom lens. The researcher was as inconspicuous as possible and minimised their interference of normal recess time activity. Where possible, videoing was performed from a distance using the zoom lens. The questionnaire was validated by correlating participants' rank score for the activity questionnaire (high – med – low) with their ranking from the video surveillance (high – med – low).

3.8 Dietary intake

Dietary intake was assessed using a three-day diet diary. Participants were provided with a set of weighing scales, household cups and spoons, a diet diary and a set of instructions. All foods and drinks consumed were recorded for two weekdays and one weekend day. To assist with the analysis of diet entries, participants were encouraged to include food wrappers and packaging. Diet diaries were analysed by the same qualified nutritionist. If discrepancies were noted in the entries, a phone interview was conducted with a parent of the participant to clarify the entries. Total energy and nutrient intake were analysed using FoodWorks Version 2 (Xyris Software Pty Ltd, Australia). Weighed food records were used to assess dietary intake for this age group of girls as this method of dietary assessment is reported to be more accurate than 24-hour recalls and food frequency questionnaires in this age group [Crawford et al., 1994].

3.9 Calcium supplementation

Calcium supplementation was provided in the form of calcium-enriched food products. The recipes for the food products were devised in conjunction with the Head Dietitian of the Royal Children's Hospital, Melbourne, Australia. The food products consisted of nine varieties of cookies, seven varieties of muesli slices, and seven varieties of muffins. All food products were similar in their energy, fat, carbohydrate, protein and dietary fibre content. Each food product was fortified with 2 grams of milk minerals, which provided 400mg of calcium. The milk minerals were provided in 15kg bags directly from the supplier (Murray Goulburn Co-operative Ltd, Australia). A sample analysis was performed on each batch of milk mineral provided, using standardised methods [Australian Standards Methods].

Ten food products were given to the participants each week, providing a total of 4000mg of calcium per week. The food products were produced weekly, and delivered directly to the participants in sealed and labelled bags. The combination of food products provided to each participant was based on personal preference, which was determined from weekly feedback sheets and food product order forms. The same procedure was performed for the control group however, the food products did not contain additional calcium. During term breaks, a delivery of two weeks of food products was made to participants on the last day of school. (See appendix 3.1 for food recipes). Participants were randomly assigned to either the calcium supplement group, or the placebo group. Food acceptability was monitored via weekly feedback sheets. As foods were not commercially produced, but made in weekly individual batches, it was difficult to maintain consistency on quality. Due to production problems during the intervention period which may have negatively affected consumption mid way through the intervention period, participants were given the option of consuming the milk minerals (or a placebo equivalent – Poly Joule, Sharp Lab, Australia) in powdered form. Participants were provided with instructions on how to include the powder into their regular foods and drinks. Furthermore, in the final month of the intervention period, Ca-enriched milk (or regular milk) was offered to participants. One satchel of the milk mineral powder or a 300ml carton of the Ca-enriched milk was equivalent to two food products. Murray Goulburn Co-operative Ltd, Australia provided the regular and Ca-enriched milks. The calcium supplementation sequence is demonstrated in Figure 3.1. Supplementation was performed in a double-blind manner.

To assess compliance, participants were asked to return their labelled bag and any uneaten food products each week. Uneaten food products were counted, and recorded for each participant. The number of returned bag and the mean weekly calcium intake from supplementation was determined. The mean daily calcium intake from supplementation ($mg.day^{-1}$) was calculated as the number of foods consumed x 400mg.7 $days^{-1}$.

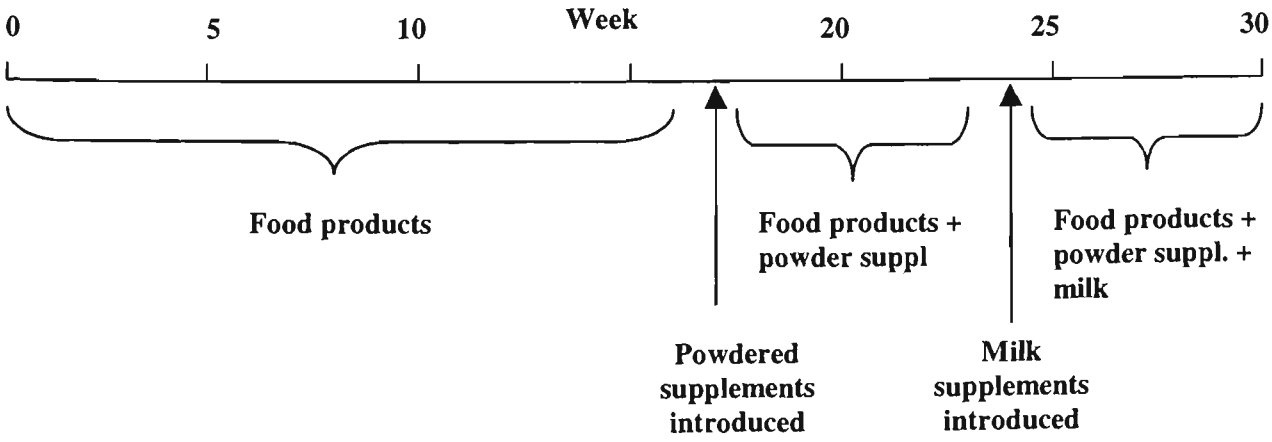

Figure 3.1. Total calcium supplementation intervention period demonstrating the timing of the introduction of the three calcium supplementation methods

3.10 Exercise intervention

The exercise intervention program consisted of three, 20 minute warm up sessions as part of physical education or sport classes. The warm up sessions were either high impact consisting of hopping jumping, skipping based games and activities, or low impact consisting of skill, stretching and dance based movements. A qualified pediatric exercise scientist developed the exercise program curriculum. A qualified physical education teacher conducted the exercise sessions. The movements incorporated into the sessions were developmentally appropriate for the age and level of development of the participants. The high impact sessions were progressive in nature, incorporating impacts of greater frequency and magnitude during the course of the intervention period. To assess the number of impacts, warm up sessions were filmed every three weeks for the duration of the intervention period. The number and type of movements per session were recorded. The magnitude of the impacts was determined using a pedar mobile system on a sub-set of 12 participants. The pedar sensored insole was worn in the participant's shoe and receiver worn in a fitted backpack as the participant performed the movements involved in the sessions. Recorded information was downloaded onto a Toshiba laptop computer and the average and peak ground reaction forces recorded for each of the respective movements. Impacts were classified as low (0.8 – 1.2 x body weight), moderate (1.3 – 1.7 x body weight), high (1.8 – 2.2 x body weight) or very high (> 2.2 x body weight) based on ground reaction forces experienced while performing the respective movements. Movements classified as low impact include walking, steps and shuffles. Moderate impact movements include running and heel raises. High impact movements were hops, skips, jumps and leaps. Very high impact movements were leaps over raised objects eg. a small hurdle, and jumps from heights less than 30 cm eg. off a bench. The reliability of the Pedar Mobile was determined from a subset of 12 girls randomly selected from each of the four study groups. The CV for the Pedar which was determined by repeated measures for each girl performing four different movements (walking, running, skipping and hurdling) was 6 ± 2%. The attending teacher recorded attendance at each exercise session.

3.11 Ethical approval

Ethical approval was sought and obtained from the Ethics departments of the Directorate of School Education, and the two participating Universities, Deakin University, Burwood Australia and Victoria University, Footscray, Australia.

3.12 Power calculations

Based on the findings reported by Bonjour et al (1997), 28 girls were required in the supplementation group to have 90% power to detect a difference (two tailed).

3.13 Statistical analysis

Data are presented in absolute terms, percentage terms or as adjusted values relative to control groups. All data were checked for normality. Comparisons between groups at baseline were made using ANOVA. Gains in bone mass and growth parameters over the intervention period were determined using repeated measures ANOVA. Changes to dietary intake pre- mid- and post-intervention period were determined using repeated measures ANOVA. Group differences in gains in bone mass were determined using ANCOVA adjusting for baseline BMC and growth in limb length. Variance accounted for, for each variable was determined from the ETA squared values. A 2 x 2 design was used to test for the main effects for calcium and exercise, and for an interaction. All data are expressed as mean ± SE. A significance level of $p < 0.05$ was used for all comparisons. Data were analysed using the statistical package SPSS for Windows, Version 9.0 (SPSS Inc, Chicago, Ill).

Chapter Four

Study One

Level, Sources and Seasonality of Dietary Calcium Intake in Children and Adolescents Enrolled in the University of Saskatchewan Pediatric Bone Mineral Accrual Study

4.1 Abstract

An adequate calcium intake during the growth period may be critical in maximising peak bone mass, a potential determinant of future risk for developing osteoporosis. Decreases in dietary calcium intakes have been attributed to decreases in dairy food consumption so it is important to know the amount and sources of dietary calcium. To determine the intake and sources of calcium of subjects in the University of Saskatchewan Pediatric Bone Mineral Accrual Study, individual food records were analysed for calcium content using records over six years of the study in 226 subjects aged 8 to 19 years who each provided up to four recalls per year. Recalls with very low or very high reported energy intakes were omitted, leaving 3113 records. Foods were grouped according to Canada's Food Guide, and subdivided to examine specific food types. Subjects were considered younger if they were in elementary school, and older if post-elementary (9th grade or beyond). The major food source of calcium in this group of children was from the Milk Products group, contributing 57%-63% to total dietary calcium intake. Fluid milk was the single greatest contributor to dietary calcium (39%-50%). Older girls consumed significantly less dietary calcium compared to younger girls (951 $mg.day^{-1}$ v 1021 $mg.day^{-1}$, $p < 0.01$), however, older boys had significantly greater dietary calcium intakes compared to younger boys (1386 $mg.day^{-1}$ v 1179 $mg.day^{-1}$, $p < 0.001$). Older girls demonstrated a marked decrease in dietary calcium from Milk Products, especially from fluid milk, compared to younger girls. No significant differences were reported between seasons for either younger or older boys or older girls for calcium intake or food sources of calcium. Younger girls consumed significantly less total dietary calcium (1001 $mg.day^{-1}$ v 1076 $mg.day^{-1}$, $p < 0.05$) and obtained less calcium from fluid milk (501 $mg.day^{-1}$ v 568 $mg.day^{-1}$, $p < 0.05$) during winter compared to summer. Concerns regarding the adequacy of calcium intakes in this group of North American children should be focused on girls, and in particular older girls, whose declining fluid milk intake may potentially compromise bone mass accrual, and younger girls during winter when calcium metabolism may be limited.

4.2 Introduction

Recent evidence suggesting an increase in the incidence and prevalence of osteoporosis [Wark, 1996] has re-newed interest in dietary factors, in particular dietary calcium, as potential contributors to the prevention of this chronic disease. Osteoporosis, though a disease of the elderly, may have its etiology during youth. The development of peak bone mass during the growth years is considered an important determinant of future risk of osteoporosis in later life [Heaney, 1991; Matkovic et al., 1994; Bailey & McCulloch, 1992]. An adequate calcium intake during the growth period, therefore may be critical in maximising bone growth potential. The importance of calcium for skeletal growth has led to the recent increase in the recommended North American adequate intake (AI) level of calcium for children aged between 9-18 years, to 1300 $mg.day^{-1}$ [Inst. of Med., 1997].

Concerns have been raised over the adequacy of dietary calcium intakes, especially of children and adolescents. A downward trend in dietary calcium intake over the past 10-20 years is apparent in both adults [National Inst. of Nutrition, 1996] and children [Albertson et al., 1997] in North America. Mean dietary calcium intakes of North American children appear to fall short of the current recommendations [Albertson et al., 1997; Donovan & Gibson, 1996; Evers & Hooper, 1995; Fleming & Heimbach, 1994; Ghadirian et al., 1995; Shatenstein & Ghadirian, 1996] with similar findings reported in other westernised countries [Bergstrom et al., 1993; Crawley, 1997; Moynihan et al., 1996; Strain et al., 1994]. An equally alarming trend is the decrease in dietary calcium intake of girls with maturity. Lower calcium intakes have been reported for older (adolescent) girls compared to younger girls [Bergstrom et al., 1993; English, 1989; Fleming & Heimbach, 1994; Roma-Giannikou et al., 1997; Shatenstein & Ghadirian, 1996; Strain et al., 1994;].

Peak bone mineral accrual follows peak height velocity by approximately one year and occurs at approximately 13.0 years of age in Canadian girls [Bailey, 1997; Martin et al., 1997]. During this stage of development, girls appear to be decreasing their calcium intake rather than increasing it to meet their higher calcium needs. Potential explanations for this apparent decrease in calcium intake in older girls include a decrease in fluid milk consumption [Fleming & Heimbach, 1994] and an increase in energy-restricted dieting [Chapman, 1994]. In contrast, the opposite has been reported in boys, with calcium intakes reported to increase with maturity [Bergstrom et al., 1993; English, 1989; Shatenstein & Ghadirian, 1996; Strain et al., 1994] at around the time of peak bone mineral content accrual which occurs at approximately 14.5 years of age in boys [Martin et al., 1997].

Dairy products are the major source of dietary calcium for children and adolescents, contributing from 42%-71% of total calcium intake [Bergstrom et al., 1993; English, 1989; Donovan & Gibson, 1996; Fleming & Heimbach, 1994; Moynihan et al., 1996]. However, variations in dietary sources of calcium are apparent between countries [Adamson et al., 1992; Bergstrom et al., 1993; English, 1989; Donovan & Gibson, 1996; Fleming & Heimbach, 1994; Moynihan et al., 1996], which may reflect social and cultural differences. For example, children in Australia acquire over 70% of their dietary calcium from dairy products [English, 1989], while for British children dairy products provide 42% of total dietary calcium [Moynihan et al., 1996].

To our knowledge, seasonal variations in calcium intake have not been reported, though seasonal differences in the intake of calcium rich foods may have implications for bone metabolism. Seasonal variation in the linear growth of Canadian children has been reported in this group of children [Mirwald & Bailey, 1997]. Seasonal variations in bone loss have been reported in post-menopausal women living in Boston, who demonstrated greater bone loss during the winter months compared to the summer period [Dawson-Hughes et al., 1991]. Reductions in the intake of calcium rich foods during the winter months, may further compromise bone metabolism during this period. This factor is particularly pertinent in countries of high and low latitude where vitamin D synthesis is impaired during the winter period [Rosen et al., 1994].

To optimize calcium intake during childhood and adolescence it is important to understand the extent to which calcium inadequacies may be occurring, when these inadequacies may be occurring, and to determine the changes in eating patterns or food choices which are resulting in the apparent reduction in dietary calcium intakes, especially in girls. The aim of this study was to determine and compare total dietary calcium intake and sources of dietary calcium of younger and older boys and girls and to determine differences that may be apparent between sexes, maturational groups and seasons.

4.3 Methods

4.3.1 Recruitment

Dietary intake data were derived from the Saskatchewan Pediatric Bone Mineral Accrual Study. The study was initiated in 1991 to investigate bone mineral accrual in growing children and to examine lifestyle factors related to nutrition and physical activity which may influence bone mineral accrual. Three hundred and seventy five students aged 8-14 years, who were attending two elementary schools in the city

of Saskatoon, were eligible for inclusion in the study. Written parental consent was obtained for 228 students (113 males and 115 females).

Additional participants were included in the study with, 21 new participants included in 1992, three in 1993 and one in 1994. The number of participants per year, and by sex, is described in Table 4.1. This on-going study involved collection of dietary and physical activity information, bone scans and anthropometric measurements every year. The data presented in this study used recalls by subjects collected over six years, i.e., 1991-1996. Over 3,500 recalls were collected, of which 3,113 were eligible for analysis (see below for exclusion criteria). During this time, bone scans were conducted annually and physical activity levels and anthropometric measurements assessed every six months. The dietary intake assessment schedule varied over the study period and is outlined in Table 4.1. All tests were performed by trained and qualified personnel, with some of the testing procedures described in Chapter 3.

Table 4.1. Data Collection Times and Subjects in the University of Saskatchewan Pediatric Bone Mineral Accrual Study.

Year	Subjects (M/F)	Recalls (M/F)	Timing of Dietary Assessment[1]
1991	226 (107/107)	699 (366/333)	Apr, Jun, Sept, Nov
1992	236 (110/122)	713 (364/349)	Jan, Mar#, May, Nov
1993	232 (106/118)	603 (297/306)	Feb#, May, Nov
1994	197 (102/107)	482 (232/250)	Feb#, May, Nov
1995	191 (87/97)	334 (159/175)	May, Nov
1996	179 (72/88)	282 (130/151)	May, Nov
TOTAL		3113 (1548/1565)	

[1] Post-elementary school (i.e. older) subjects were not assessed at the mid-winter times indicated as #

4.3.2 Dietary Intake

Dietary intake over the six year collection period was assessed via serial 24-hour recalls conducted both at the participating schools and in the hospital setting where the bone scans were performed. All days of the week, except Friday and Saturday were recalled. Initially, all participants received a 20 minute training session on food portion sizes, which were reviewed prior to each school based testing session. Display boards of life-size, two dimensional pictures (National Dairy Council, 1990, Rosemont II) were present at

the time of recalls to assist with portion sizing. School-based dietary assessments were supervised by graduate and under-graduate nutrition students who assisted the children, and checked forms for completeness. Dietary assessments performed at the hospital were conducted on a one-on-one basis with graduate nutrition and physical education students or a qualified nutritionist. Nutrient supplement use was determined and analysed separately. Data used in this report represents intake from food sources only, as supplemental calcium use was minimal.

4.3.3 Exclusion criteria

A single 24-hour recall record was excluded for a subject if the energy intake was below 1000 kcal per day, or greater than 5000 kcal per day. This criteria was used to avoid extremes that represent actual, but not typical, intakes or as a result of under- or over-reporting of intakes [Mertz et al., 1991]. Approximately 10% of recalls were excluded so that of the 3,504 recalls obtained over the six years, 3,113 were analysed for calcium intake and food source of dietary calcium.

4.3.4 Nutrient Analysis

Food intake from the 24-hour recalls were analysed using the Nutritional Assessment System (NUTS) program, version 3.7 (Quilchena Consulting Limited, Victoria, Canada), which uses the 1988 Canadian Nutrient File and provides imputed values for nutrients that are missing in the Canadian Nutrient Files. When a food item was missing from the database, a similar food was coded. All data were generated as ASCII Files and transferred to an interfacing data base for further analysis. Nutrient intake per food item was determined for three nutrients, energy, calcium and Vitamin D. Percent nutrient from each of the 12 food groups was determined by dividing total nutrient intake for that dietary recall by the nutrient contained in each of the 12 food groups. eg. (calcium from milk/total calcium intake) x 100

4.3.5 Food Grouping

All foods included in the NUTS database were divided into one of 12 groups (Figure 4.1). Five main groups were devised based on those of Canada's Food Guide to Healthy Eating [Health Canada, 1992]. These groups were further subdivided as follows. The group *Milk Products* was further divided into: *fluid milk*, which included full cream, low fat, skim and flavoured milks; *cheese*; *yogurt*; and *dairy-others* which included ice-cream, thick shakes and milk shakes. The *Vegetables and Fruit* group was divided into: *fruits*, including fruit juices; and *vegetables*, including all legumes, herbs, spices, coffee, tea and

vegetable-only soups and vegetable juices. The *Grain Products* group was kept as an entire group and include foods such as crackers, buns and muffins. The *Meat and Alternatives* group was separated into: *meat and eggs*; and *fish*. Foods not included in the basic four food groups were categorised into *Other Foods*. This last group was divided into: *fats and oils*, which included nuts and seeds, peanut butter, mayonnaise and salad dressings; *sweets and snacks*, which included foods such as soft drinks, candies, cookies, cakes, sweet pastries and alcoholic beverages; and *combined dishes* which were those foods not readily itemised into one of the above groups. Included in the *combined dishes* group were composite foods such as pizza, macaroni and cheese, lasagna, burgers and mixed soups, or individual items made with a number of ingredients such as pancakes.

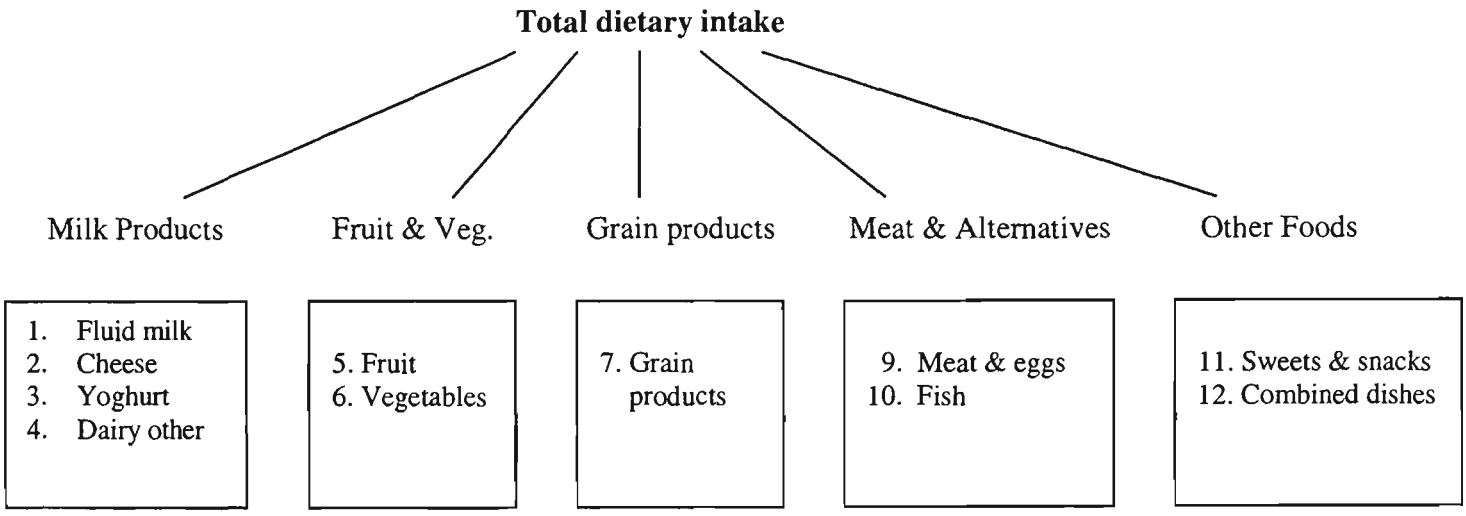

Figure 4.1 Categorisation of foods from the Canadian Food Composition Table into 12 groups

4.3.6 Seasonal Categories

The year was divided into summer and winter seasons based on average monthly temperatures, and the official definitions for the seasons in the study catchment area [Masterson et al., 1976]. Winter is defined as commencing on November 1 and concluding March 20, while summer is defined as commencing on May 1 (including the spring shoulder) and concluding September 20. The average daily temperature over the winter period is minus 11.7 degrees Celsius compared with an average daily temperature of plus 14.9 degrees Celsius for the summer period [Environment Canada, 1993]. For dietary assessments performed in April, those conducted up to and including April 15 were included in the winter category, and those conducted after April 15 were categorised as summer. For assessments conducted in October, those that occurred up to and including October 15 were denoted as summer, and those performed after October 15 were denoted as winter

4.3.7 Maturational Categories

As biological maturity is variable with respect to age of onset, maturity was defined as entering high school, i.e. post-elementary school. Based on the public school system in the province of Saskatchewan, elementary school is from kindergarten through to year 8, with high school from years 9 to 12. The age range of entry into high school is 13-15 years, while the mean age of biological maturity for this group as defined by the age of peak height velocity (PHV) is 11.6 years in girls and 13.5 in boys [Bailey, 1997]. The age of PHV however, can vary considerably. Therefore, entry into high school or beyond (post-elementary) was used as the maturational bench mark as changes to the adolescent diet have been associated with numerous lifestyle factors including changes to the school curriculum [Clavien et al., 1996]. The age range of children in elementary and post-elementary schools were 8-12 and 13-18, respectively.

4.3.8 Statistical Analysis

Values were reported as mean ± standard error. Data analyses were conducted using SPSS (version 6.5.1). Data were not normally distributed, and were not correctable with data transformation. Means were tested, therefore using a non-parametric equivalent to the unpaired T-test (Mann-Whitney test), to determine differences between the groups based on sex, maturity and seasons.

4.4 Results

4.4.1 Total dietary calcium intake

Total dietary calcium from foods, and calcium intake by food groups are presented in Table 4.2. Males consumed more total calcium, and derived more calcium from *fluid milk*, *meat/eggs*, *fish* and *vegetables* compared to females ($p < 0.05 - p < 0.001$). Maturity related differences were also reported. The older girls consumed significantly less dietary calcium, compared to the younger girls ($p < 0.01$). The opposite was found for boys, with the older boys having a significantly greater dietary calcium intake compared to the younger boys ($p < 0.01$). Mean dietary calcium intakes for both the younger and older girls and for the younger boys were below the current recommended level of 1300 $mg.day^{-1}$ [Food and Nutrition Board, 1997]. On average 78% of the dietary recalls for girls and 60% of the recalls for boys had dietary calcium intakes of less than 1300 $mg.day^{-1}$. A greater percentage of dietary recalls from the older girls (81.2%) were below the recommendations for calcium, compared to the younger girls (74.9%) and both the younger and older boys (65.9% and 53.1%, respectively).

Older boys gained more dietary calcium from *fluid milk*, *cheese*, *vegetables*, *meats/eggs*, *grains*, *fats and oils* and *sweets/snacks* compared to the younger boys. The exceptions were *yogurt*, *dairy 'others'* and *fruit*. Older girls demonstrated a marked decrease in dietary calcium from milk products, in particular *fluid milk*, and to a lesser extent from grain products, compared to their younger counterparts. Older girls however, obtained significantly more calcium from *cheese* ($p < 0.001$) compared to the younger girls. When total dietary calcium was expressed relative to energy intake, the older girls demonstrated the lowest nutrient density (531 $mg.1000\ kcal.day^{-1}$), compared to the younger girls (575 $mg.1000\ kcal^{-1}$) and the younger and older boys (578 $mg.1000\ kcal^{-1}$ and 551 $mg.1000\ kcal^{-1}$, respectively). Nutrient density only differed significantly between the older girls and the younger boys ($p = 0.02$).

4.4.2 Calcium Sources

The percent contribution of food groups to total dietary calcium intake is presented in Table 4.3. All of the food groups comprising *Milk Products* were the major source of dietary calcium for younger and older boys and girls, contributing between 57.2% to 63.3% to total dietary calcium. The second largest source

of dietary calcium was from the *Other Foods* contributing from 18.7% to 24.6%. Included in this category are foods that contain dairy products as an ingredient, for example pizza, and macaroni and cheese. The

contribution from *Grain Products* ranged from 8.5% to 9.0% and for *Vegetables and Fruit* the range was 6.0% to 7.6%. The smallest contribution to dietary calcium was from *the Meat and Alternatives group*, which ranged from 2% to 3%. The largest contribution to total dietary calcium from a single food group was *fluid milk*, which provided between 39.5% to 49.6% of total dietary calcium. Males gained a greater percentage of calcium from *fluid milk*, and *fish* compared to females. However, females gained a greater percentage of calcium from *fruit* and *sweets/snacks* than males.

4.4.3 Calcium sources and seasonal variations

Seasonal variations in calcium intake for males and females are presented in Tables 4.4 and 4.5, respectively. No significant differences were reported between seasons for both younger and older boys (Table 4.4), and for older girls for total dietary calcium intake, sources of dietary calcium and percentage contribution to total dietary calcium by food groups. Seasonal variations were apparent however, for younger girls (Table 4.5). The younger girls consumed significantly less total dietary calcium (1001 ± 22 v 1076 ± 22, $p < 0.05$), and obtained significantly less calcium from *fluid milk* (501 ± 16 v 568 ± 17, $p < 0.05$) and *dairy 'other'* during the winter months compared to the summer months. Younger girls gained more calcium from *fruit* and *Combined dishes* in winter compared to summer. Additionally, the younger girls gained a significantly smaller percentage of dietary calcium from *fluid milk* and *dairy-other* food groups, but significantly more from *fruit* and *Combined dishes* during the winter months compared to the summer months.

4.5 Discussion

The mean daily dietary calcium intake of each of the four sex and maturity groups were compatible with [Bergstrom et al., 1993; English, 1989], and higher than [Albertson et al., 1997; Crawley, 1997; Fleming & Heimbach, 1994; Moynihan et al., 1996; Roma-Giannikou et al., 1997; Strain et al., 1994] intakes reported for similar age and sex groups in other westernised countries. It appears, however, that the general concerns over the adequacy of dietary calcium intakes in children and adolescents may be applicable to some, but not all maturity and sex groups. The mean dietary calcium intake of the older boys met the current North American recommendation for calcium of 1300 $mg.day^{-1}$ [Inst. of Med.,

1997]. In contrast the older girls had the lowest mean dietary intake (951 mg.day^{-1}) of the four groups, and the greatest proportion of dietary recalls (81.2%) with calcium intakes below the current recommendation for calcium. The issue of calcium inadequacy appears to be more applicable to girls, in particular older girls, than it is to boys. Calcium inadequacy during growth may have implications for skeletal integrity later in life by not optimising peak bone mass [Bailey & M^{c}Culloch, 1992; Heaney, 1991; Matkovic et al., 1994].

It appears that older boys consumed sufficient calcium to meet skeletal needs. The recommendation for calcium [Inst. of Med., 1997] is based on the measured amount of calcium retained in bone during peak bone mineral content (BMC) accrual, assuming a standard calcium absorption efficiency of 20.3%. The age of peak BMC accrual for boys occurs at 14.5 years, with a peak BMC accrual of 320 g.year^{-1} [Martin et al., 1997]. The age of entry into high school for this group of boys ranges from 13-15 years, therefore at around the time of peak BMC accrual, when calcium needs are greatest, older boys are more likely to meet these needs with their current dietary calcium intake. Although the dietary calcium intake reported by the younger boys did not meet the current recommendations for calcium, their intake of 1179 mg.day^{-1} may have been sufficient to meet their needs at their stage of skeletal development. The age range of the younger boys in this group is 9-12 years. The rate of bone accrual in this younger age group is likely to be less than that estimated for the older boys.

For girls, age of peak BMC accrual occurs at approximately 13 years of age [Martin et al., 1997], which coincides with the minimal age of entry into high school. Based on a peak BMC accrual of 240 g.year^{-1} reported for girls [Martin et al., 1997] there is some doubt that their reported dietary calcium intake of 1042 mg.day^{-1} for the younger girls and 951 mg.day^{-1} for the older girls would have met skeletal needs based on current assumptions [Inst. of Med., 1997; Martin et al., 1997]. Calcium adequacy appears to be an issue with girls, in particular older girls. While the older boys increased dietary calcium intake to meet heightened skeletal needs, the opposite occurred with older girls. Dietary calcium intake decreased in this group, when skeletal needs were greatest. Sub-optimal calcium intakes reported in the girls, in particular the older girls, may have resulted from an insufficient energy intake, under-reporting of nutrient intake or a low nutrient density diet as a result of inappropriate food choices. Relative to total energy intake, the older boys consumed significantly more total energy and more energy from each of the food groups when compared with younger boys. In contrast, the older girls reported similar total energy intakes as the younger girls in spite of the older girls being taller and heavier. Cross sectional data utilising the same group of children reported the mean height and weight of 10-12 year old girls as 151 ± 8.9 cm and 42.7

± 12.0 kg, respectively compared with a mean height and weight of 162.1 ± 6.4 cm and 55.6 ± 13.0 kg, respectively for 13-15 year old girls [Whiting et al., 1995]. The finding of lower energy intakes in older girls compared to younger girls has also been observed by others [Bergstrom et al., 1993].

The likelihood of under-reporting of dietary intakes by the older females potentially skewing the data was acknowledged. The incidence of under-reporting in adolescents has been previously reported by Bandini et al. (1997) and Carter & Whiting, (1997). The problem of under reporting in this group of children was addressed and may have been partly rectified with the elimination of all dietary recalls with intakes below 1000 kilocalories. Even with the low intakes removed, there are indications that food choices of the older girls appeared to influence calcium adequacy. The older girls exhibited diets with the lowest calcium density when compared to the other groups. The older girls obtained a greater portion of their dietary calcium from *cheese* and *vegetables* compared to the younger girls. They also consumed less *fluid milk*, which resulted in significantly less calcium being derived from *fluid milk*. On the other hand, the younger boys exhibited diets with the highest mean nutrient densities compared to the other groups. The younger boys derived more calcium from *Milk Products* (63.3%) and in particular *fluid milk* (49.6%) compared to the other groups. This suggests than in relation to calcium, the younger boys, or their parents on their behalf, were making appropriate food choices, by consuming sufficient milk products.

Decreases in dietary calcium intakes have been attributed to decreases in dairy consumption [Fleming & Heimbach, 1994; Kennedy & Goldberg, 1995]. Our data suggest the decrease in calcium intake in the older girls is due more to a decrease in *fluid milk* consumption, than total milk products. The energy derived from *Milk Products* were similar for the older girls (18.4%) when compared with the younger girls (18.1%), however, the contribution of *Milk Products* to total dietary calcium intake differed. The amount of dietary calcium provided by *Milk Products* was 620 $mg.day^{-1}$ and 696 $mg.day^{-1}$ for the older and younger girls, respectively. The decrease in *fluid milk* consumption by the older girls had a direct bearing on their calcium intake. The increase in cheese consumption made up some, but not all, of the deficit created by the decreased consumption of *fluid milk*.

The major food source of calcium in this group of children was *Milk Products*, contributing over 60% of dietary calcium. *Fluid milk* was the single greatest contributor to dietary calcium intake contributing over 45% of dietary calcium. The contribution *of Milk Products* reported here was less than that reported for 10 to 15 year old children in Australia at approximately 69% [English, 1989] and 14 and 17 year old adolescents in Sweden at approximately 65% [Bergstrom et al., 1993].

Table 4.2. Total dietary calcium intake from food in mg per day (SE) and contribution of food groups to total calcium intake by sex and social-maturational[1] groups[2]

	BOYS			GIRLS		
	Elementary School[1] n = 1115[3]	Post-Elementary[1] n = 433	Total n = 1548	Elementary School[1] n = 1198	Post-Elementary[1] n = 367	Total n = 1565
Milk Products	808	952	848	696	620	679
- Fluid milk	626 (15)[b]	726 (28)[b]	654 (14)[i]	537 (12)[f]	438 (22)[f]	514 (11)[i]
- Cheese	139 (9)[b]	190 (16)[b]	153 (8)	116 (7)[f]	151(15)[f]	125 (6)
- Yogurt	12 (2)	9 (3)	11 (2)	11 (2)	7 (2)	10 (1)
- Other	31 (3)	27 (4)	30 (2)	32 (3)	24 (4)	30 (2)
Vegetables & Fruit	56	61	58	53	54	53
- Fruit	25 (1)	24 (2)	25 (1)	24 (1)[f]	21 (2)[f]	24 (1)
- Vegetables	31 (1)	37 (2)	33 (1)[g]	29 (1)[e]	33 (2)[e]	29 (1)[g]
Meat & Alternatives	22	29	24	15	15	15
- Meat/Eggs	18 (1)[c]	24 (2)[c]	20 (1)[i]	13 (1)	14 (1)	13 (1)[i]
- Fish	4 (1)	5 (2)	4 (1)[h]	2 (1)	1 (1)	2 (1)[h]
Grain Products	80 (2)[a]	90 (4)[a]	83 (2)[i]	73 (2)[f]	60 (2)[f]	70 (1)[i]
Other Foods	213	254	224	205	202	204
- Fats & Oils	7 (1)[c]	10 (1)[c]	8 (1)	7 (1)	10 (1)	8 (1)
- Sweets/snacks	61 (3)[b]	69 (4)[b]	63 (2)	57 (2)	63 (4)	58 (2)
- Combined dishes	145 (7)	175 (15)	153 (7)	141 (6)	129 (10)	138 (6)
Total (5 Food Groups)	1179 (20)[c]	1386 (37)[c]	1237 (18)[i]	1042 (16)[e]	951 (27)[e]	1021 (13)[i]

[1] Social-maturation according to attendance in elementary school (grades 1-8) or post-elementary school (grades 9-12) and beyond.

[2] Pairs of values sharing same superscript (elementary versus post-elementary) are significantly different for boys ($a = p < 0.05$; $b = p < 0.01$; $c = p < 0.001$), for girls ($d = p < 0.05$; $e = p < 0.01$; $f = p < 0.001$) and between the sexes ($g = < 0.05$; $h = p < 0.01$; $i = p < 0.001$).

[3] n represents the number of recalls.

Table 4.3. Percentage contribution of food groups (SE) to total dietary calcium intake by sex and social-maturational[1] groups[2].

	BOYS			GIRLS		
	Elementary School[1] n = 1115	Post-Elementary[1] n = 433	Total n = 1548	Elementary School[1] n = 1198	Post-Elementary[1] n = 367	Total n = 1565
Milk Products	63.3	62.3	63.0	62.1	57.2	61.0
- Fluid milk	49.6 (0.8)	48.2 (1.3)	49.2 (0.7)[h]	48.4 (0.7)[f]	39.5 (1.5)[f]	46.3 (0.7)[h]
- Cheese	10.0 (0.5)[a]	11.7 (0.8)[a]	10.5 (0.4)	9.5 (0.5)[f]	14.6 (1.1)[f]	10.7 (0.5)
- Yogurt	0.9 (0.2)	0.4 (0.2)	0.7 (0.1)	0.9 (0.2)	0.7 (0.2)	0.9 (0.1)
- Other	2.8 (0.2)	2.0 (0.3)	2.6 (0.2)	3.3 (0.3)	2.4 (0.4)	3.1 (0.2)
Vegetables & Fruit	6.5	6.0	6.4	6.7	7.6	6.9
- Fruit	2.9 (0.1)[b]	2.2 (0.2)[b]	2.7 (0.1)[h]	3.1 (0.1)[e]	2.8 (0.2)[e]	3.0 (0.1)[h]
- Vegetables	3.6 (0.2)	3.8 (0.3)	3.7 (0.1)	3.6 (0.2)[f]	4.8 (0.4)[f]	3.9 (0.2)
Meat & Alternatives	2.5	3.0	2.6	2.0	2.1	2.0
- Meat/Eggs	2.1 (0.1)[b]	2.5 (0.2)[b]	2.2 (0.1)	1.7 (0.1)	1.9 (0.2)	1.8 (0.1)
- Fish	0.4 (0.1)	0.5 (0.2)	0.4 (0.1)[h]	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)[h]
Grain Products	9.0 (0.3)	8.6 (0.4)	8.9 (0.2)	8.9 (0.2)[d]	8.5 (0.2)[d]	8.8 (0.2)
Other Foods	18.7	20.1	19.1	20.3	24.6	21.3
- Fats & Oils	0.8 (.01)[b]	1.1 (0.1)[b]	0.9 (0.1)	0.9 (0.1)	1.5 (0.2)	1.0 (0.1)
- Sweets/snacks	6.2 (0.3)	7.0 (0.5)	6.4 (0.2)[h]	6.7 (0.3)	8.4 (0.6)	7.1 (0.2)[h]
- Combined dishes	11.7 (0.6)	12.0 (0.9)	11.8 (0.5)	12.7 (0.5)	14.7 (1.1)	13.2 (0.5)

[1] Social-maturation according to attendance in elementary school (grades 1-8) or post-elementary school (grades 9-12) and beyond.

[2] Pairs of values sharing same superscript (elementary versus post-elementary) are significantly different for boys (a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$), for girls (d = $p < 0.05$; e = $p < 0.01$; f = $p < 0.001$) and between the sexes (g = < 0.05; h = $p < 0.01$; i = $p < 0.001$).

[3] n represents the number of recalls

Table 4.4. Seasonal variations in total dietary calcium intake from food in mg per day (SE) and contribution of food groups to total calcium intake in boys by social-maturational[1] groups[2]

	Boys			
	Elementary		Post-Elementary	
	Summer n = 617[3]	Winter n = 498	Summer n = 180	Winter n = 253
Milk Products	824	787	925	970
- Fluid milk	641 (22)	607 (21)	711 (43)	736 (37)
- Cheese	136 (14)	142 (13)	176 (25)	200 (21)
- Yogurt	15 (4)	9 (2)	9 (5)	8 (5)
- Other	32 (4)	29 (4)	29 (8)	26 (4)
Vegetables & Fruit	56	57	52	50
- Fruit	25 (1)	25 (1)	25 (3)	23 (2)
- Vegetables	31 (2)	32 (2)	37 (4)	37 (3)
Meat & Alternatives	25	20	28	31
- Meat/Eggs	20 (2)	17 (1)	21 (2)	27 (2)
- Fish	5 (1)	3 (1)	7 (3)	4 (2)
Grain Products	81 (2)	79 (3)	96 (5)	87 (5)
Other Foods	221	202	268	244
- Fats & Oils	7 (1)	6 (1)	9 (1)	11 (2)
- Sweets/snacks	61 (4)	62 (4)	64 (6)	73 (5)
- Combined dishes	153 (10)	134 (11)	195 (29)	160 (15)
Total (5 Food Groups)	1206 (28)	1146 (27)	1379 (61)	1391 (47)

[1] Social-maturation according to attendance in elementary school (grades 1-8) or post-elementary school (grades 9-12) and beyond.

[2] Pairs of values sharing same superscript (elementary versus post-elementary) are significantly different for boys ($a = p < 0.05$; $b = p < 0.01$; $c = p < 0.001$)

[3] n represents the number of recalls

Table 4.5. Seasonal variations in total dietary calcium intake from food in mg per day (SE) and contribution of food groups to total calcium intake in girls by social-maturational[1] groups[2]

	Girls			
	Elementary		Post-Elementary	
	Summer n = 653[3]	Winter n = 545	Summer n = 142	Winter n = 225
Milk Products				
- Fluid milk	568 (17)[d]	501 (16)	400 (33)	461 (28)
- Cheese	127 (10)	103 (9)	151 (24)	151 (19)
- Yogurt	12 (2)	9 (3)	4 (3)	8 (3)
- Other	36 (4)	27 (4)	24 (5)	24 (5)
Vegetables & Fruit				
- Fruit	23 (1)	26 (1)	21 (3)	21 (2)
- Vegetables	28 (2)	29 (2)	34 (4)	32 (2)
Meat & Alternatives				
- Meat/Eggs	13 (1)	13 (1)	12 (2)	15 (2)
- Fish	2 (1)	2 (1)	3 (2)	1 (1)
Grain Products	74 (2)	72 (2)	59 (4)	60 (3)
Other Foods				
- Fats & Oils	7 (1)	8 (1)	14 (3)	8 (1)
- Sweets/snacks	54 (3)	61 (3)	60 (7)	65 (5)
- Combined dishes	132 (9)	152 (10)	130 (17)	128 (13)
Total (5 Food Groups)	1076 (22)[d]	1001 (22)	913 (39)	974 (37)

[1] Social-maturation according to attendance in elementary school (grades 1-8) or post-elementary school (grades 9-12) and beyond.

[2] Pairs of values sharing same superscript (elementary versus post-elementary) are significantly different for girls ($d = p < 0.05$; $e = p < 0.01$; $f = p < 0.001$)

[3] n represents the number of recalls

However, it was greater than 12 to 19 year old girls in the United States at approximately 56% [Albertson et al., 1997], 14 to19 year old Canadian girls at approximately 45% to 53% [Donovan & Gibson, 1996], Scottish youth at 49%, which included eggs [Crawley, 1997], and 12 year old English children at approximately 46% including eggs [Adamson et al., 1992]. *Fluid milk* has been reported as the single most significant contributor of dietary calcium in others nations, ranging from 25% for 12 year old English children [Moynihan et al., 1996], to up to 56% in Australian children [English, 1989]. A reduction in *fluid milk* consumption will, therefore have a different impact on total dietary calcium intake, depending on the importance placed on *fluid milk* as a calcium source. In the case of this sample of children, the reduction in *fluid milk* consumption in the older girls had a significant impact, resulting in intakes well below the current recommendations, and potentially not providing sufficient calcium to meet skeletal needs.

Seasonal variation in linear growth has been reported in children [Mirwald & Bailey, 1997], however to our knowledge, seasonal variations in nutrient intake in children have not been reported. Seasonal variations in intake were not observed in the boys, however differences were apparent in the young girls, where less *fluid milk* and less *dairy-others* were consumed in winter compared to summer. This resulted in significantly less dietary calcium being consumed during the winter months. This may have implications on bone metabolism, which may already be compromised in winter [Dawson-Hughes et al., 1991]. Inhabitants of areas at very high and very low latitudes must rely on dietary sources of Vitamin D during the winter months, as they are unable to synthesis Vitamin D during this period. Food products fortified with Vitamin D, such as milk, therefore become a primary source of this vitamin. A reduction in *fluid milk* intake during this period has two implications for bone health. The first being a reduction in calcium intake, and the second being a reduction in Vitamin D intake, which is needed for the absorption of calcium. For the younger girls both Vitamin D (Appendix 4.1) and calcium intake were significantly lower during winter when compared to summer, as a result of less *fluid milk* being consumed.

The concerns regarding the adequacy of calcium intake in North American children does not appear to be universal, but rather should be focused on girls, and in particular older girls. The reduced calcium intake in the older girls appears to be due to less *fluid milk* being consumed. In addition, the younger girls appear to be consuming less *fluid milk* during winter, which may have implications on bone metabolism during this period. These findings may have implications to the education and promotional campaigns designed at increasing dietary calcium intakes in children. Such campaign may be best targeted at girls, with an emphasis on promoting an increase in *fluid milk* consumption, especially during the winter period.

Chapter Five

Study Two

The Timing and Magnitude of Peak Height Velocity and Peak Tissue Velocities for Early, Average and Late Maturing Boys and Girls

5.1 Abstract

The process of maturation follows a relatively consistent pattern, with peak height velocity (PHV) preceding other peak tissue velocities. There are however, large variations in the timing and magnitude of peak height and tissue velocities. These variations occur between the sexes and for individuals within a sex group. To investigate these variations, height, body mass and tissue accrual was determined in a group of 60 male and 53 female adolescents measured annually over a six-year period using standard measuring procedures and dual-energy X-ray absorptiometry (DXA). Annual velocity values were derived and the age and magnitude of peak height and peak tissue velocities determined using a cubic spline fit of individual data. Individuals were rank ordered on the basis of sex and age at peak height velocity (PHV) then divided into quartiles to divide participants into early (lowest quartile), average (middle two quartiles) and late (highest quartile) maturers. Sex- and maturity-related comparisons in the ages and magnitudes of peak height and peak tissue velocities were then made. Males reached peak velocities significantly later than females for all tissues (mean difference 1.5 years, $p < 0.001$) and demonstrated significantly greater magnitudes at peak ($p < 0.001$). The age of PHV was negatively correlated with the magnitude of PHV in both sexes ($r = -0.4$, $p < 0.05$). At a similar maturity point (age of PHV) no differences were apparent in body mass and fat mass between the maturity groups for both sexes. Late maturing males however, accrued more bone mineral (1779 g v 1493 g, $p < 0.05$) and lean mass (41.8 kg v 36.6 kg, $p < 0.05$), and were taller (168.1 cm v 161.6 cm, $p < 0.05$) by the age of PHV in comparison to early maturers. It was concluded that maturational status (early, average or late maturity) as indicated by age of PHV was inversely related to the magnitude of PHV in both sexes. At a similar maturational point there were no differences between early and late maturers for body mass and fat mass in boys and girls.

5.2 Introduction

The process of maturation follows a relatively consistent pattern, with peak height velocity (PHV) preceding other peak tissue velocities [Hagg & Taranger, 1992; Lindgren, 1978; Tanner et al., 1966]. There are however, large variations in the timing and magnitude of peak height and tissue velocities, and the rate at which individuals pass through each stage of maturity [Tanner, 1989]. These variations occur between the sexes and for individuals within a sex group. For example, females generally mature approximately two years earlier than males [Hagg & Taranger, 1991]. Boys however, tend to demonstrate greater magnitudes in peak height and tissue velocities compared to girls [Bailey, 1997].

Longitudinal data suggest that differences may be apparent in body composition and magnitude of peak tissue velocities in early versus late maturing children. Reports indicate that both male and female early maturers are fatter with greater sums of skinfolds both during the growth period [van Lenthe et al., 1996] and in adulthood [Garn et al., 1986; Post & Kemper, 1993] compared to late maturers. In addition, early maturers appear to be heavier and have a greater body mass index (BMI) compared to late maturers [Beunen et al., 1994; van Lenthe et al., 1996]. Furthermore, some studies suggest that early maturers are taller at maturity and demonstrate a greater magnitude of PHV compared to late maturers [Hagg & Taranger, 1992; Kemper et al., 1985; Lopez-Blanco et al., 1995]. These differences, however, may not necessarily carry through to adulthood. While some authors have reported differences in adult height between children who mature at different ages [Garn et al., 1986; Hagg and Taranger, 1991; Kemper et al., 1985], others have reported no significant difference in adult height between maturational groups [Beunen et al., 1994].

To date, there is a paucity of longitudinal data describing the timing and magnitude of peak tissue velocities as they relate to the timing of maturation. Recent longitudinal data derived from the Saskatchewan Pediatric Bone Mineral Accrual Study [Bailey, 1997] have provided measures of body composition and tissue changes during maturity, as measured by dual energy x-ray absorptiometry (DXA). The purpose of this study was to describe and compare the timing and magnitude of peak height and tissue velocities in early, average and late maturing boys and girls.

5.3 Methods

5.3.1 Recruitment

Longitudinal data were derived from the Saskatchewan Pediatric Bone Mineral Accrual Study. The study was initiated in 1991 to investigate bone mineral accrual in growing children and to look at lifestyle factors related to nutrition and physical activity which may influence bone mineral accrual.

Three hundred and seventy five students aged 8-14 years, who were attending two elementary schools in the city of Saskatoon, were eligible for inclusion in the study. Written parental consent was obtained for 228 students (113 males and 115 females). Data has been collected annually since 1991. By the end of the sixth year, complete data were collected on 68 males and 72 females.

5.3.2 Body composition and bone mineral content

Total body bone mineral content, fat mass and lean mass were assessed annually in the months of October and November, using dual energy x-ray absorptiometry (Hologic 2000 QDR, software version 7.10) in array mode. Total body scans were analysed using software version 5.67A. All scans were analysed by the same qualified person. Details of scan procedures are described in Chapter 3.

5.3.3 Anthropometry

Anthropometric dimensions were taken every six months. Height, without shoes, was recorded to the nearest 0.1cm using a manual wall stadiometer. Body mass was measured to the nearest 0.05 kg using a calibrated electronic scale (SECA). Details of the procedures are described in Chapter 3. Age of menarche in females was determined retrospectively via an annual interview performed by a qualified female researcher immediately prior to the DXA scan.

Annual gains in height were determined using a rolling whole year velocity. This method was used to reduce the effect of seasonal fluctuations in longitudinal growth that has been reported in this group of children [Mirwald & Bailey, 1997]. Height and tissue velocities were calculated as the difference between the absolute values for height, bone mineral content (BMC), bone-free lean mass (LM), fat mass (FM) and body mass (BM). Velocity values (corrected for the time differences between measures) were expressed as cm per year for height, grams per year for BMC, and kilograms per years for LM, FM and BM. Data for each individual were analysed separately. Peak height velocities for each individual were determined by fitting a cubic spline curve to the velocity data for each individual. A cubic spline curve fitting procedure was used as the polynomial curve fitting procedures used to examine the data tended to fit the data to the pre-existing curve, resulting in a blunting of the peak value (Figure 5.1). From the individual velocity curves that were derived from the velocity data, the age and magnitude of PHV for each individual was determined. The procedure was repeated for the velocity data for LM, FM, BM, and BMC accrual.

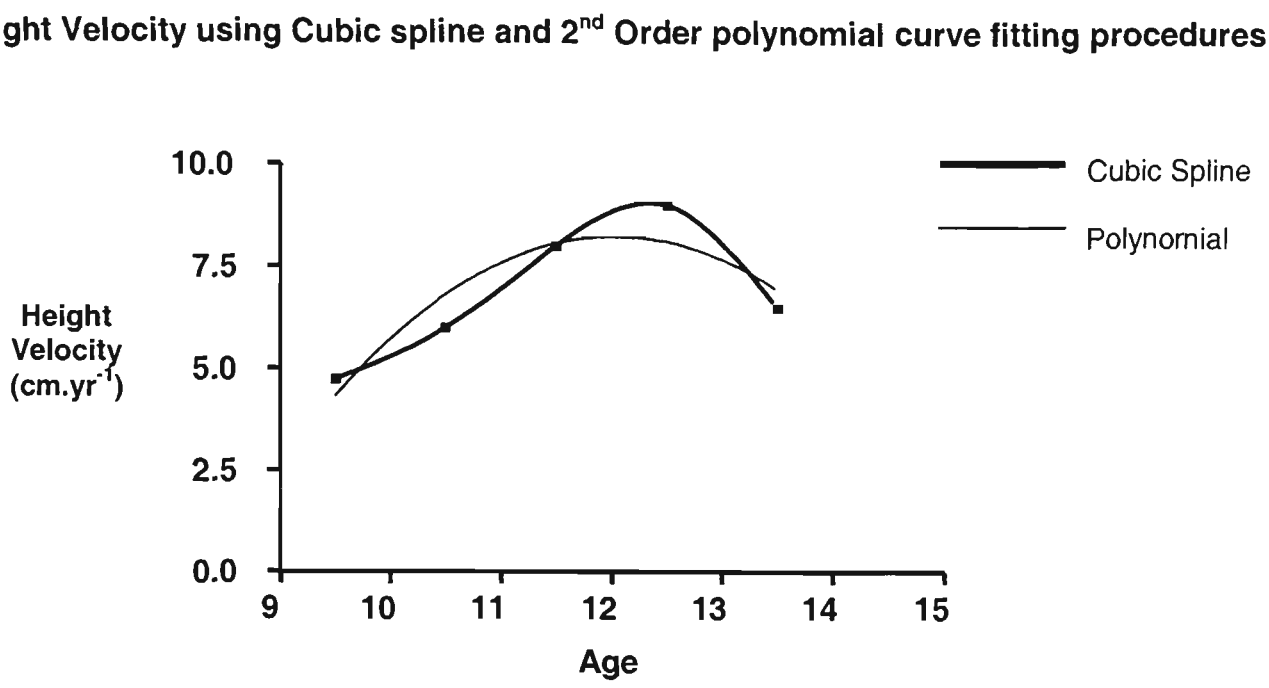

Figure 5.1 Comparison of PHV values using two different curve fitting procedures.

5.3.4 Inclusion criteria

Of the data available, only children who had at least attained peak height, peak BMC and peak lean mass velocities were included in the analysis. Sixty boys and 53 girls were eligible for inclusion. Of the remaining tissue velocities, three males and four females had not reached peak fat mass velocity, and three males and one female had not reached peak body mass velocity. All subjects were of Caucasian decent.

5.3.5 Maturity categorisation

To establish maturity groups, males and females were separated and rank ordered on the basis of age at PHV. The ranked lists were divided into quartiles. Early maturers were defined as individuals in the lowest quartile of the ranking, average maturers as those within the middle two quartiles, and late maturers as those in the highest quartile. The number of participants in each category is depicted in figure 5.2.

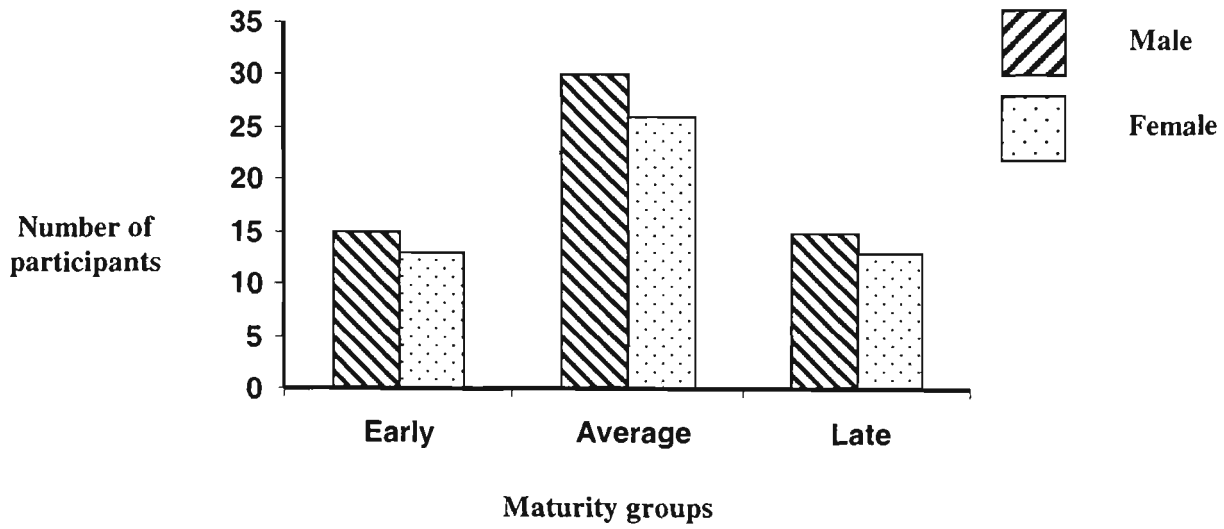

Figure 5.2. Breakdown of male and female participants into early-, average- and late-maturity groups based on the age of PHV.

5.3.6 Statistical analysis

Statistical differences between maturity groups were tested with ANOVA (SPSS version 6.5.1). If a difference was reported ($p < 0.05$), a post hoc analysis (Scheffe) was conducted. Sex differences in age and magnitude of peak tissue velocities were determined using t-tests for independent means. Pearson correlations were used to determine relationships between age and magnitude of PHV, and the age and magnitude of the remaining peak tissue velocities. Data are expressed as means (SD).

5.4 Results

5.4.1 Comparisons of Males and Females

The timing of peak height and tissue velocities for boys and girls are presented in Table 5.1. The mean age of PHV and age of all peak tissue velocities occurred significantly later in boys compared to girls ($p < 0.001$). Boys were later in reaching peak height, lean mass, body mass, BMC and fat mass velocities by 1.6 years, 1.6 years, 1.5 years, 1.5 years and 1.4 years respectively, compared to girls.

The magnitude of PHV, and peak tissue velocities are presented in Table 5.1. The magnitude of all velocities were significantly greater for males than females ($p < 0.001$). Males, on average gained 1.8 $cm.yr^{-1}$ more height, 82 $g.yr^{-1}$ more bone mineral at peak, 3.6 $kg.yr^{-1}$ more lean mass, and 1.6 $kg.yr^{-1}$ more body mass at peak. For fat mass there was a negative velocity in both sexes, with boys losing 1.9 $kg.yr^{-1}$ and girls losing 0.4 $kg.yr^{-1}$ at peak. The variance for fat mass velocity at peak was greater than for the other tissues in males and females.

The sequence of attaining peak height and tissue velocities was similar in both sexes (Figure 5.3). PHV preceded peak lean and peak body mass velocities in that order. Peak fat and peak BMC velocities occurred almost simultaneously in both sexes, and were the latest peak velocities.

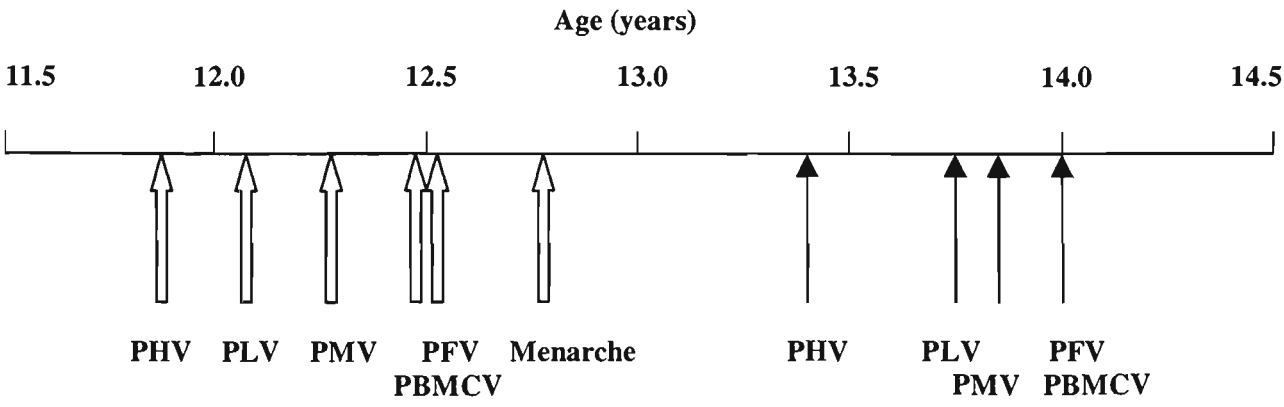

Figure 5. 3. Sequence of PHV and peak tissue velocities for males (solid arrow) and females (open arrows)

PHV = peak height velocity
PLV = peak lean mass velocity
PFV = peak fat mass velocity
PMV = peak body mass velocity
PBMCV = peak bone mineral content velocity

Table 5.1. Sex comparison of mean values (SD) for the timing and magnitude of peak height and tissue velocities.

	Males	Females	Significance
Age PHV (years)	13.4 (1.0)	11.8 (0.9)	$p < 0.001$
Age PLV (years)	13.7 (0.9)	12.1 (1.0)	$p < 0.001$
Age PFV (years)	14.0 (1.3)	12.6 (2.0)	$p < 0.001$
Age PMV (years)	13.8 (1.1)	12.3 (1.2)	$p < 0.001$
Age PBMCV (years)	14.0 (1.0)	12.5 (0.9)	$p < 0.001$
Age of Menarche (years)	-	12.7 (1.0)	-
PHV ($cm.y^{-1}$)	10.4 (1.2)	8.6 (1.1)	$p < 0.001$
PLV ($kg.y^{-1}$)	8.8 (1.6)	5.2 (1.2)	$p < 0.001$
PFV ($kg.y^{-1}$)	-1.9 (2.2)	-0.4 (1.8)	$p < 0.001$
PMV ($kg.y^{-1}$)	10.3 (1.9)	8.7 (1.4)	$p < 0.001$
PBMCV ($g.y^{-1}$)	407 (93)	325 (67)	$p < 0.001$

PHV = peak height velocity
PLV = peak lean mass velocity
PFV = peak fat mass velocity
PMV = peak body mass velocity
PBMCV = peak bone mineral content velocity

Absolute values at the age of PHV are presented in Figures 5.4 a)-e). At the time of PHV, boys were significantly taller and heavier and had greater lean mass and bone mineral content than girls. There was no difference however, between boys and girls for fat mass at the age of PHV.

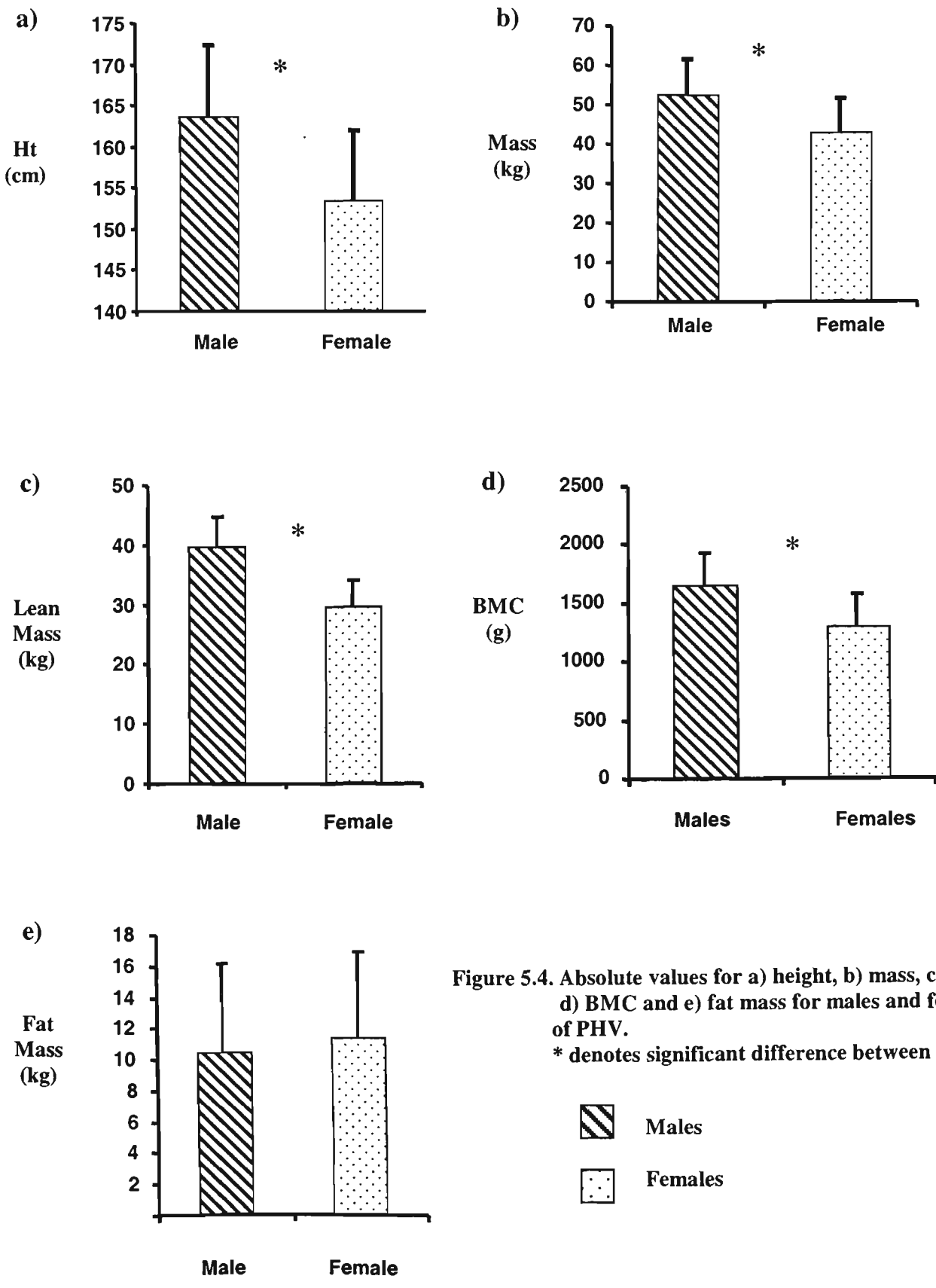

Figure 5.4. Absolute values for a) height, b) mass, c) lean mass, d) BMC and e) fat mass for males and females at the age of PHV.
*** denotes significant difference between the sexes ($p < 0.001$)**

5.4.2 Maturity comparisons – Males

Correlations between age of PHV and peak tissue velocities and their respective magnitudes in males are presented in Table 5.2. There was a significant negative relationship between age of PHV and magnitude of PHV. None of the other relationships between age and magnitude of the remaining peak tissue velocities were significant for males.

Table 5.2. Correlations between age and magnitude of peak height and peak tissue velocities for males
*** = p < 0.05**

	PHV	PLV	PMV	PFV	PBMCV
Age PHV	- 0.40*				
Age PLV		-0.24			
Age PMV			0.03		
Age PFV				0.21	
Age PBMCV					0.01

The timing of peak height and tissue velocities for the three maturity groups of male and females are presented in Tables 5.3. As expected, a significant difference existed for age of PHV among maturity groups ($p < 0.001$). There was a similar trend for all of the peak tissue velocities ($p < 0.05$ - $p < 0.01$).

Table 5.3. Timing of peak height and tissue velocities for early, average and late maturing males and females. a = significant difference between all maturity groups ($p < 0.05$)
b = significant difference between early and late maturers ($p < 0.05$)

	Male			Females		
	Early	**Average**	**Late**	**Early**	**Average**	**Late**
Age PHV (years)	12.2[a] (0.5)	13.4[a] (0.3)	14.7[a] (0.5)	10.5[a] (0.4)	11.8[a] (0.4)	13.0[a] (0.4)
Age PLV (years)	12.7[a] (0.6)	13.6[a] (0.5)	14.8[a] (0.6)	11.0[a] (0.9)	12.1[a] (0.6)	13.1[a] (0.6)
Age PFV (years)	12.9[a] (1.1)	14.0[a] (0.9)	15.0[a] (1.1)	11.6[b] (2.0)	12.7 (1.7)	13.5[b] (2.0)
Age PMV (years)	12.9[a] (1.0)	13.8[a] (0.9)	14.9[a] (0.7)	11.2[a] (0.9)	12.3[a] (0.9)	13.4[a] (0.9)
Age PBMCV (years)	13.2[a] (0.7)	13.9[a] (0.7)	15.1[a] (0.6)	11.7[a] (0.6)	12.5[a] (0.6)	13.5[a] (0.6)
Age of Menarche (years)	-	-	-	11.7[a] (0.6)	12.7[a] (0.6)	13.9[a] (0.7)

The magnitudes of peak height and peak tissue velocities are presented in Table 5.4. The magnitude of PHV was significantly greater for early maturing boys compared with late maturing boys with a

difference of 1.1 $cm.y^{-1}$ reported between the two maturity groups. There was no difference between early and average maturers for magnitude of PHV. There also was no difference in the magnitude of peak tissue velocities among maturity groups of boys.

Table 5.4. Magnitude (SD) of peak height and tissue velocities for early, average and late maturing males and females.
b = significant difference between early and late maturers ($p < 0.05$)
c = significant difference between average and late maturers ($p < 0.05$)

	Male			Females		
	Early	**Average**	**Late**	**Early**	**Average**	**Late**
PHV ($cm.y^{-1}$)	10.8[b] (1.6)	10.6 (1.1)	9.7[b] (0.8)	9.2[b] (1.2)	8.6 (0.9)	7.9[b] (0.9)
PLV ($kg.y^{-1}$)	9.0 (1.5)	9.0 (1.7)	8.2 (1.6)	5.2 (1.3)	5.6[c] (1.1)	4.2[c] (0.9)
PFV ($kg.y^{-1}$)	-1.7 (1.4)	-2.4 (2.7)	-1.2 (1.3)	0.2 (1.0)	-0.4 (1.5)	-0.8 (2.2)
PMV ($kg.y^{-1}$)	10.7 (1.7)	9.9 (1.9)	10.7 (2.0)	8.4 (1.6)	9.1 (1.3)	8.1 (1.5)
PBMCV ($g.y^{-1}$)	426 (68)	406 (98)	389 (104)	335 (71)	342[c] (63)	278[c] (51)

Absolute values for lean mass, fat mass, bone mineral content (BMC), height and body mass at the age of PHV for the three maturity groups of males are presented in Table 5.5. There were no differences for fat mass and body mass at the time of PHV among maturity groups. Early maturers, however, were shorter (161.6 cm v 168.1 cm, $p < 0.05$) and had significantly less lean mass (36.6 kg v 41.8 kg, $p = 0.013$) and bone mineral content (1493 g v 1779 g, $p = 0.016$) compared to late maturers.

Table 5.5. Tissue values at time of PHV, for early, average and late maturing males and females.
b = significant difference between early and late maturers ($p < 0.05$)

	Male			Females		
	Early	**Average**	**Late**	**Early**	**Average**	**Late**
Height at PHV (cm)	161.6[b] (7.4)	163.8 (5.3)	168.1[b] (8.9)	149.9 (7.8)	154.8 (7.4)	154.1 (5.0)
Lean mass at PHV (kg)	36.6[b] (4.6)	40.2 (3.5)	41.8[b] (6.5)	27.7 (4.6)	30.4 (4.3)	29.6 (4.5)
Fat mass at PHV (kg)	10.7 (6.3)	10.2 (4.6)	10.6 (7.6)	9.00 (5.38)	12.6 (6.3)	11.3 (2.4)
Mass at PHV (kg)	49.6 (9.6)	53.0 (6.7)	55.1 (11.2)	38.7 (9.0)	45.2 (8.9)	43.1 (5.9)
BMC at PHV (grams)	1493[b] (241)	1665 (212)	1779[b] (363)	1157 (266)	1362 (278)	1330 (171)

5.4.3 Maturity comparisons – females

Correlations between age of PHV and peak tissue velocities and their respective magnitudes in females are presented in Table 5.6. There was a significant negative relationship between age of PHV and magnitude of PHV. Similarly, there was a significant negative relationship between the ages and magnitudes of peak lean mass velocity (PLV) and peak bone mineral content velocity (PBMCV). The relationships between age and magnitude of peak fat mass velocity (PFV) and peak body mass velocity (PMV), however, were not significant for females.

Table 5.6. Correlations between age and magnitude of peak height and peak tissue velocities for females
* = $p < 0.05$

	PHV	PLV	PMV	PFV	PBMCV
Age PHV	- 0.40*				
Age PLV		-.034*			
Age PMV			-0.14		
Age PFV				0.25	
Age PBMCV					-.033*

The timing of PHV and peak tissue velocities for the three maturity groups of females are presented in Table 5.3. There was a significant difference among maturity groups for age of PHV ($p < 0.001$). There was a similar trend for the ages of peak velocities for BMC ($p < 0.001$), lean mass ($p < 0.001$) and body mass ($p < 0.01$), with early maturers reaching peak velocities before average maturers, who reached peak velocities before late maturers. Age of PFV differed significantly between early and late maturers ($p < 0.05$), but not between early and average matures, or average and late maturers. Age at menarche was significantly different among maturity groups ($p < 0.001$).

The magnitude of PHV, and peak tissue velocities for the three maturity groups of females are presented in Table 5.4. There was a difference in magnitude of PHV between early and late maturing girls (9.2 $cm.yr^{-1}$ v 7.9 $cm.yr^{-1}$, $p < 0.05$), but not between early and average maturers, or between average and late maturing girls. The magnitude of peak fat mass and body mass velocities among maturity groups did not differ. The magnitude of PBMCV was similar for early and average maturing girls. Average maturing girls, however, demonstrated a greater magnitude of PBMCV compared to late maturers (342 $g.yr^{-1}$ v 278 $g.yr^{-1}$, $p = 0.015$). There was a similar trend for the magnitude of PLV, with a greater magnitude at peak for average maturers compared to late maturers (5.6 $kg.yr^{-1}$ v 4.23 $kg.yr^{-1}$, $p = 0.002$).

Absolute values for tissues at the time of PHV for the three maturity groups of girls are presented in Table 5.5. At the age of PHV, there were no differences among the maturity groups for height, body mass, and body tissue variables.

5.5 Discussion

5.5.1 Comparisons of peak height and peak tissue velocities between males and females

Peak height velocity was achieved, on average, 1.7 years earlier in females than in males. These findings are consistent with others [Tanner, 1989; Malina & Bouchard, 1991] however, the sex difference in timing of PHV was less than previously reported. Tanner et al. (1966) reported a two year difference between mean ages of PHV of females and males (12.1 v 14.1 years) while Lindgren (1978) reported a difference of 2.2 years between females and males (11.9 v 14.1 years). Data from Hagg and Taranger (1991, 1992) also indicate a sex difference of approximately 2 years between mean ages at PHV. More recently, Lopez-Blanco et al. (1995) and Bailey (1997) report a mean sex difference of 1.8 years and 1.9 years, respectively, for age at PHV. The small differences among studies may reflect sampling variation, methodological strategies in fitting longitudinal data, and perhaps secular variations. The present values were based on longitudinal analysis, whereas the earlier data for the Saskatoon sample were analysed cross sectionally [Bailey, 1997]. In addition to PHV, these sources of variation may also apply to other tissue velocities.

Although the timing and magnitude of peak velocity values differed between the sexes, the pattern of growth as reflected by the sequence of peak velocities of the different tissues is consistent between the sexes and among maturity groups. In both males and females, PHV is attained first followed by peak lean velocity and subsequently and concurrently, peak fat and BMC velocities. Since body mass represents the composite of the lean, fat and BMC velocities, peak body mass velocity occurs between lean and the later fat and BMC peak velocities. Only limited, partial data is available to compare some of the peak tissue velocities. Earlier work by others, reported in Malina and Bouchard (1991) suggested that peak body mass, fat free mass and fat mass velocities occur almost simultaneously with PHV. Lindgren (1978) however, reported that PHV preceded PMV by 0.3 and 0.6 years for male and females, respectively. Beunen and Malina (1988) summarized the mean ages for peak height and peak mass velocities from six studies, with the difference in timing ranging from 0.3 – 0.9 years for females and 0.2 – 0.4 years for males. Bailey (1997) reported that PHV preceded PBMCV by 1.1 years in both males and females. In relation to the timing of PMV and PBMCV, and based on cross sectional analysis, PMV precedes PBMCV. These results are comparable to the current study. The results from this study however, are unique as the body composition data was derived longitudinally using DXA, and a complete sequence of peak velocity values was obtained for each individual.

Males demonstrated a greater magnitude in height gain at peak when compared to females. Gains of 10.4 $cm.yr^{-1}$ and 8.5 $cm.yr^{-1}$ were reported for males and females, respectively. The height gains at peak were similar to those reported by others. Lindgren (1978) reported peak height velocities of 9.8 $cm.yr^{-1}$ and 8.3 $cm.yr^{-1}$ for males and females, respectively. Beunen and Malina (1988) summarised the mean PHV values from 20 studies. PHV values ranged from 8.2 – 10.3 $cm.yr^{-1}$ for males and 7.0 – 9.1 $cm.yr^{-1}$ for females. Lopez-Blanco et al. (1995) reported peak height velocities of 9.6 $cm.yr^{-1}$ and 8.6 $cm.yr^{-1}$, respectively for males and females. In the present study males also demonstrated greater magnitude in peak tissue velocities when compared to females. Limited comparative data from other studies are available describing the magnitude of peak tissue velocities. Lindgren (1978) reported peak body mass velocities of 9.1 $kg.yr^{-1}$ and 7.3 $kg.yr^{-1}$, respectively for males and females. Beunen and Malina (1988) summarised the mean PMV values from three other studies. Values ranged from 8.7 – 9.8 $kg.yr^{-1}$ for males and 6.8 – 8.8 $kg.yr^{-1}$ for females. Peak mass velocities for males and females in our group of adolescents were 10.3 $kg.yr^{-1}$ and 8.7 $kg.yr^{-1}$, respectively. The PHV values, especially those of males, and PMV values reported in this group tended to be greater than values previously reported. This tendency may be, in part due to the curve fitting method used in this study, which is less likely to modify the timing and magnitude of the peak values [Bailey et al., 1999].

At peak, a negative velocity was reported for fat mass in both males and females. Tanner (1989) in reporting some of his earlier work noted that subcutaneous fat growth in the limbs of males and females during adolescents as assessed by x-rays, decreased at around the time of PHV. The decrease in fat mass for the males in this earlier work fell below zero indicating a negative velocity, while the velocity for the females slowed, but remained positive. Similarly, Malina et al. (1999) demonstrated the relationship between subcutaneous adipose tissue distribution, trunk and extremity skinfolds and associated ratios with the timing of PHV. If subcutaneous fat mass of the limbs and trunk are indicative of total body fat mass, then our findings are in general agreement with Tanner (1989) and Malina et al. (1999).

When males and females are compared at the common maturational benchmark of PHV, there are significant sex differences for height, lean mass, body mass and BMC. Fat mass showed no significant difference between males and females. A possible explanation for this latter result is the higher variability in fat mass compared to the other body tissues.

5.5.2 Maturity comparisons

A significant difference was reported for age of PHV among the maturity groups for both sexes. Previous comparisons between maturity groups have used alternative methods such as an arbitrary cut

off in age of PHV which produced a narrow categorization range [van Lenthe et al., 1996] or menarche [McKay et al., 1998] to define maturity groups. The division of this data into rank ordered quartiles and assigning early and late maturers to the lowest and highest quartiles appears to be an adequate means of defining maturity groups. It resulted in a mean difference between adjacent maturity groups of over one year, and two years between early and late maturation groups in both males and females.

When males and females are grouped into maturity categories, it is noteworthy that the sequencing of peak tissue velocities is comparable in each maturity group with PHV occurring first, followed by peak lean velocity and then peak fat and BMC velocities. Peak body mass velocity occurred between peak lean and peak fat and BMC velocities. All ages of peak velocity are statistically significant between maturity groups in males and females with the exception of the age of peak fat velocity. Only the early and late maturity groups in females were significantly different.

A negative relationship of r = -0.40 was found between the age and magnitude of PHV for both boys and girls. These findings are in agreement with Malina and Bouchard (1991) who noted that the age of PHV was moderately, but negatively correlated (r = -0.3 to -0.5) with the magnitude of PHV. McKay et al. (1998) also found that the magnitude of PHV was negatively related to maturity in girls when menarche was used as the maturational benchmark. In the present study a greater magnitude of PHV was reported in the early maturers, when compared to the late maturers for both sexes. Lindgren (1978) and Hagg and Taranger (1992) also reported greater magnitudes of PHV in early maturers compared to late maturers.

When the correlation between the age and magnitude of peak velocity are applied to the other tissues, the negative relationship does not persist for all tissues. For males, none of the other correlations between age and magnitude of peak tissue velocities were significant. For females significant negative relationships were found for age and magnitude of PBMCV and PLV. Results for PBMCV are in agreement with McKay et al. (1998) who used menarche at the maturational benchmark to distinguish early, average and late maturers. Comparative data for peak lean mass velocities are not available. For PMV, no differences were reported among maturity groups of both sexes. These findings are in agreement with Lindgren (1978) who reported no difference in PMV among maturity groups for both sexes.

No difference in height (and body mass) at the time of PHV was reported between the maturity groups for females. For males however, while no difference was reported between maturity groups for body mass, late maturers were taller than early maturers at the time of PHV. Lindgren (1978) found that both late maturing boys and girls were taller than their earlier maturing counterparts at the age of

PHV. Mean height at age of PHV were 148.9 cm, 151.4 cm and 156.5 cm for early, average and late maturing girls, respectively ($p < 0.001$), and 159.8 cm, 164.0 cm and 167.0 cm for the three male maturity groups, respectively ($p < 0.001$). Beunen et al. (1994) reported that early maturing boys were consistently taller (and heavier) between the age 13-16 years. If the average distance curves for height (and body mass) for the different maturity groups were aligned on age of PHV however, little difference was apparent between the height (and body mass) at the age of PHV between the maturity groups.

Hagg and Taranger (1991) also reported that early maturing boys and girls were taller than their late maturing counterparts from the ages of 9 months to 16 years, with the differences being statistically significant between the ages of 5 – 14 years for girls and 12 – 15 years for boys. Similar to the aforementioned study by Beunen et al. (1994) if distance values were aligned on the age of PHV, minor differences were also apparent between the maturity groups for height at PHV. When data is expressed in chronological years, it stands to reason that for a given chronological age during adolescence an early maturer is more likely to be taller than a late maturer as they have already experienced their adolescent growth spurt. Aligning children on age of PHV to make comparisons appears to be more appropriate in determining differences that may be present in height (and body mass) at the age of PHV among maturity groups. The difference in height at age of PHV for males may be due to a longer period of childhood growth eg. an extra two years, before the adolescent growth spurt occurs.

The effect of height at maturity on adult stature remains inconclusive. Garn et al. (1986) reported that early maturing girls, based on their age of menarche, were shorter as adults by between 1.0 – 1.7 cm when compared to late maturing girls. Wellens et al. (1992) also reported greater adult height in late maturing girls (menarche > 13.99 years) when compared to earlier maturing girls (menarche 12.00 – 12.99 years). Differences in height at the time of maturity remains unknown however, as menarche was assessed retrospectively. Beunen et al. (1994) found the greater height demonstrated by early maturing boys during the adolescent years (13 – 16 years) was reduced by ages 17 – 18 years, and eliminated by age 30. Hagg and Taranger (1991) reported similar results to Beunen et al. (1994), but for females only. No difference in height was evident between early, average and late maturing girls between the ages of 15 – 25 in spite of the early and average maturers being taller than late maturers between the ages of 5 – 14 years and 12 – 14 years, respectively. In contrast to previous results, late maturing boys who were shorter than early maturing boys up to the age of 16 years, surpassed both the average and early maturers by 4.2 cm ($p < 0.05$) and 6.5 cm ($p < 0.01$), respectively by age 18 years [Hagg & Taranger, 1991]. Although maturity related difference in height at PHV were not evident in the females involved in this study, the continual tracking of participants into adulthood may reveal differences in final adult height.

The data presented demonstrates that maturity related differences do occur in relation to PHV for both sexes. In addition maturity related differences in the magnitudes of peak lean and bone mineral content velocities were evident for girls. The impact of these differences remains unknown. In males, peak lean, body mass and fat velocities appear not to differ between maturity groups, suggesting that these peak tissue velocities simply occurred later, but with no less magnitude in later maturers when compared to early maturers. Peak mass and fat velocities did not differ between maturity groups for females however, average maturing girls accrued more bone mineral and lean mass at peak when compared to late maturers. The implication of this disparity on adult size, and bone mineral content remains unknown. At maturity, early maturing females were no taller, heavier or demonstrated a greater fat mass when compared to late maturers. Early maturing males were similar in body mass and fat mass, however were shorter and had less bone mineral content and lean mass than their late maturing counterparts.

This study was the first of its kind to report PHV in relation to other peak tissue velocities. The sequence and timing of these maturity related events however, are relative to this group of children, until other data becomes available for comparison.

Chapter Six

Study Three

The Effect of Weight Bearing Exercise and Calcium Supplementation on Bone Mass Accrual in Pre- and Early-Pubertal Girls

6.1 Abstract

Optimising the attainment of peak bone mass plays an important role in osteoporosis prevention strategies. Dietary calcium and exercise are modifiable environmental factors that may increase bone mass during growth and if maintained will subsequently lead to a higher peak bone mass. The aim of this 2 x 2 (calcium v placebo and high impact v low impact exercise) randomised controlled trial was to determine if there were site specific osteogenic effects of calcium (Ca) and exercise (ex), and if exercise combined with additional calcium resulted in a greater osteogenic effect than exercise or calcium alone.

Sixty-six girls aged 8.8 ± 0.1 yrs (Tanner stages 1 & 2) were randomly assigned to one of four groups: high impact exercise (HI ex) + Ca, HI ex + placebo (Pla), low impact exercise (LI ex) + Ca or LI ex + Pla. The 8.5-month school-based program consisted of 20 minutes of HI or LI ex, three times a week. Participants also received either Ca-fortified foods or an equivalent food without additional Ca. Body composition, BMC (DXA), anthropometry and maturity were assessed at baseline and post-intervention. Normal dietary calcium intake and physical activity levels were assessed at baseline, mid- and post-intervention periods. Main effects for Ca and ex, and interaction between the two were determined using ANCOVA controlling for baseline BMC and growth in limb lengths.

At baseline, no differences were reported between groups for anthropometric and BMC values, dietary calcium intake or hours of weight bearing activities. All anthropometric and BMC variables increased over the 8.5-month period in all groups ($p < 0.05$). The Ca group consumed an additional 434 ± 19 mg Ca per day. Following adjustment, the Ca group accrued 3.3 grams (3.4%) more BMC at the arms compared to the Pla group. The HI ex group accrued 9.8 grams (2.5%) more BMC at the legs and 18.6 grams (1.7%) more BMC for the total body compared to the LI ex group. An interaction between exercise and calcium was detected at the femur ($p < 0.05$). There was no effect of either Ca or ex detected for BMC accrual at the lumbar spine.

In summary the effects of exercise and calcium were site specific and evident at the appendicular skeleton. The exercise was most effective at enhancing bone mass accrual at the legs, and the calcium effective at the arms. In conclusion exercise combined with increased dietary calcium may be an effective way to increase bone mass accrual in young girls, with the potential for a combined effect at sites that experience mechanical loading.

6.2 Introduction

Low bone mineral density (BMD) in the elderly may result from low peak BMD, age-related bone loss or both. Of these two factors, low peak BMD is considered a more important contributor to low BMD in the elderly than age-related bone loss [Bonjour et al., 1994]. Calcium and exercise are two environmental factors considered important for the acquisition and maintenance of bone mass. High calcium intakes during childhood and adolescence have been associated with greater BMD in adulthood [Murphy et al., 1999; New et al., 1997,]. Calcium supplementation has also shown to be effective in enhancing bone mass accrual in children [Johnston et al., 1992; Lee et al., 1994; Lee et al., 1995]. Long term benefits to bone mass from calcium supplementation however, have not been reported following the cessation of supplementation [Lee et al., 1996; Lee et al., 1997; Slemenda et al., 1997]. In contrast one study reported greater gains in bone mass in children with calcium supplementation, with the benefits still detectable one year after the cessation of supplementation [Bonjour et al., 1997]. The uniqueness of this study was that supplementation was in the form of food products enriched with calcium from a milk extract, while the previous studies used calcium salts.

High intensity weight bearing exercise in childhood has been associated with higher BMD, with residual benefits maintained into adulthood [Bass et al., 1998]. Short-term (less than 1 year) school-based weight-bearing exercise interventions have also resulted in modest gains in BMD in normally active children [Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997]. All three school-based exercise intervention studies supplemented the existing physical education curriculum with additional exercises, or additional physical education classes. There are no studies that have examined the effects of high- versus low-impact exercise programs on bone mass accrual in children. Furthermore, the combined effect of calcium and exercise on bone mass accrual in children is yet to be determined.

The aim of this study was to determine if increased calcium intake from calcium-enriched foods and a school-based exercise intervention will result in greater gains in bone mass in pre- and early-pubescent girls. It was also the aim of this study to examine if the effect of calcium when combined with exercise is greater than the singular effects of exercise or calcium alone. It is hypothesised that an exercise effect may be detected at the legs and a combined effect may be detected at loaded sites.

6.3 Methods

Full details of the methodology used in this chapter are presented in Chapter 3.

6.3.1 Statistical analysis

Data are presented in absolute terms, percentage terms or as adjusted values relative to control groups. All data were checked for normality. Comparisons between groups at baseline were made using ANOVA. Gains in bone mass and growth parameters over the intervention period were determined using repeated measures ANOVA. Changes to dietary intake pre- mid- and post-intervention period were determined using repeated measures ANOVA. Group differences in gains in bone mass were determined using ANCOVA adjusting for baseline BMC and growth in limb length. Variance accounted for each variable was determined from the ETA squared values. A 2 x 2 design was used to test for the main effects for calcium and exercise, and for an interaction. All data are expressed as mean ± SE. A significance level of $p < 0.05$ was used for all comparisons. Data were analysed using the statistical package SPSS for Windows, Version 9.0 (SPSS Inc, Chicago, Ill).

6.4 Results

6.4.1 Group characteristics and Compliance

Eighty-eight girls provided written consent to be included in the study and were randomly assigned to the study groups base on the methods reported in Chapter 3. Following randomisation, two girls left the school within the first month of the intervention. Of the remaining 86 girls, 66 were included in the study cohort. Participants were excluded if they presented with the following conditions: menarche (n = 3), Tanner stage 3 or greater (n = 10), obesity (body mass > 4SD above the mean, n = 2), involvement in high intensity weight bearing exercise (> 10 hours of WB ex per week, n = 1), and non-compliance with the foods (n = 3 in the calcium group, n = 1 in the placebo group). Non compliance was defined as refusal to consume the food products within the first 6 weeks of the study. Fifteen percent of the remaining participants (n = 10) were of Asian decent. Group characteristics are presented in Table 6.1. The mean age of all participants was 8.8 ± 0.1 years (range 6.9 to 11.0 years). No differences were reported between the groups for age at baseline. Similarly, when groups were combined to determine the main effects for calcium and exercise, no differences were reported between groups for age.

6.4.2 Maturation

Maturational differences between the groups are presented in Table 6.1. Of the girl's who commenced the intervention at Tanner stage 1 (n = 53), five from the HI ex + Ca group, three from the HI ex + Pla

Table 6.1. Mean group characteristics (± SE) for pre- and early-pubescent girls involved in 10 months of calcium supplementation (or placebo) and either high impact or low impact exercise. Maturity data was collected pre- and post-intervention period. Dietary and exercise data was collected pre-, mid- and post-intervention period.

	HI ex + Calcium (n = 16) 8.7 ± 0.3 yrs*	HI ex + Placebo (n = 18) 9.0 ± 0.2 yrs*	LI ex + Calcium (n = 14) 8.8 ± 0.3 yrs*	LI ex + Placebo (n = 18) 8.9 ± 0.3 yrs*
Tanner stage 1 - 1	10	8	12	8
Tanner stage 1 - 2	5	3	0	7
Tanner stage 2 - 2	1	7	2	3
Weight bearing exercise$^{\nabla}$ ($hr.wk^{-1}$)	7.5 ± 0.9	6.0 ± 0.6	6.6 ± 0.5	7.4 ± 0.8
Calcium ($mg.day^{-1}$)	664 ± 42	644 ± 48	684 ± 60	705 ± 47
Supplemented calcium ($mg.day^{-1}$)	426 ± 30	-	442 ± 24	-
Total calcium^ ($mg.day^{-1}$)	958 ± 56	644 ± 48	1002 ± 55	705 ± 47
Total energy ($kj.day^{-1}$)	6965 ± 365	6259 ± 275	6671 ± 383	6848 ± 290
Carbohydrate ($g.day^{-1}$)	224 ± 14^{c}	190 ± 10^{c}	223 ± 13	219 ± 9
Protein ($g.day^{-1}$)	60 ± 3	56 ± 3	57 ± 4	60 ± 3
Fat ($g.day^{-1}$)	60 ± 4	58 ± 3	54 ± 5	59 ± 3

* Value at baseline

^{c} Like letters denote significant difference between groups ($p < 0.05$)

^ = dietary calcium + supplemented calcium

$^{\nabla}$ Exercise additional to the intervention program

group and seven from the LI ex + Pla group progressed to Tanner stage 2 by the end of the 8.5-month period. Maturational differences between the groups when determining the main effects for calcium and exercise are presented on Tables 6.4 and 6.6, respectively. When determining the main effect of calcium, five pre-pubescent girls from the calcium group, and 10 from the placebo group progressed to Tanner stage 2. For the main effect of exercise, eight girls from the HI ex group and seven girls from the LI ex group advanced from Tanner stage 1 to Tanner stage 2.

6.4.3 Exercise intervention

Both the low impact and high impact sessions were progressed throughout the intervention period. The complexity of movements performed during the LI ex sessions was increased ie. more intricate dance routines, without change to the magnitude of impacts. Ninety-seven percent of impacts in the low impact classes were less than 1.2 x body mass. The HI ex sessions were progressed by increasing the number of impacts, but more specifically the number of very high impact movements (>2.2 x body mass). Initial HI ex sessions included an average of four very high impact movements. This number was increased to almost 50 by the completion of the intervention period. The mean number and types of impacts during the intervention period are reported in Table 6.2. Mean attendance for the exercise sessions was 93% (range 44% - 100%).

Table 6.2. Sequential comparison of the number and types of impacts for the HI ex and LI ex groups. Superscripts $^{(1\text{-}4)}$ indicate the magnitude of the impacts.

	High Impact			Low Impact		
	Baseline	**Mid**	**Post**	**Baseline**	**Mid**	**Post**
Walk [1]	0	3	0	33	147	0
Step [1]	0	3	11	269	343	311
Shuffle [1]	0	0	0	30	31	6
Stationary action [1]	0	0	0	139	22	265
Squat [1]	0	0	0	0	0	5
Run [2]	167	125	227	0	0	0
Skip [2]	88	127	62	0	0	0
Hop [2]	27	0	31	0	0	0
Leap [3]	26	0	1	0	0	0
Leap (raised object) [4]	4	0	24	0	0	0
Jump (low) [2]	0	0	4	23	0	8
Jump (normal) [3]	0	43	47	0	0	0
Jump (from ht) [4]	0	17	23	0	0	0
Total leg movements	312	317	419	359	521	326
Low impact [1]	0%	2%	2%	94%	100%	98%
Moderate impact [2]	54%	39%	54%	6%	0%	2%
High impact [3]	45%	54%	33%	0%	0%	0%
Very high impact [4]	1%	5%	11%	0%	0%	0%

Table 6.3. Body composition, anthropometry, and bone mass values (± SE) for pre- and early-pubescent girls before and after 10 months of calcium supplementation (or placebo) and either high impact or low impact exercise[†].

	Calcium / HI ex (n = 16)			Calcium / Low ex (n = 14)			Placebo / HI ex (n = 18)			Placebo / Low ex (n = 18)		
	Baseline	Changes		Baseline	Changes		Baseline	Changes		Baseline	Changes	
		Absolute	Percent		Absolute	Percent		Absolute	Percent		Absolute	Percent
					Body composition (kg)							
Weight	28.3 ± 1.7	2.9 ± 0.5	10.0 ± 1.4	29.5 ± 1.4	2.5 ± 0.2	8.7 ± 1.0	30.5 ± 1.5	2.6 ± 0.4	8.6 ± 1.2	29.9 ± 1.7	2.8 ± 0.3	9.7 ± 1.1
Lean mass	20.1 ± 0.7	1.6 ± 0.2	7.9 ± 0.6	21.0 ± 0.7	1.6 ± 0.1	7.8 ± 0.5	20.2 ± 0.5	1.5 ± 0.1	7.7 ± 0.7	20.1 ± 0.7	1.7 ± 0.2	8.4 ± 0.8
Fat mass	6.5 ± 1.0	1.3 ± 0.4	20.7 ± 3.9	6.7 ± 0.8	0.8 ± 0.2	14.0 ± 3.6	8.5 ± 1.1	1.1 ± 0.3	14.9 ± 3.7	8.0 ± 1.1	1.1 ± 0.3	17.4 ± 4.7
Percent fat	22.1 ± 1.9	1.8 ± 0.6		22.7 ± 1.7	0.9 ± 0.6		26.9 ± 2.2	1.0 ± 0.6		25.8 ± 2.1	1.2 ± 0.7	
					Anthropometry(cm)							
Height	130.2 ± 2.3	3.9 ± 0.2	3.0 ± 0.2	133.8 ± 2.2	4.3 ± 0.3	3.2 ± 0.3	132.2 ± 1.6	3.8 ± 0.2	2.9 ± 0.2	131.9 ± 1.7	4.0 ± 0.2	3.0 ± 0.2
Sitting height	68.9 ± 1.1	1.9 ± 0.3	2.8 ± 0.4	70.2 ± 0.9	1.9 ± 0.2	2.7 ± 0.3	69.7 ± 0.7	1.7 ± 0.1	2.5 ± 0.2	69.4 ± 0.8	1.5 ± 0.2	2.1 ± 0.3
Bone lengths												
Leg	61.3 ± 1.3	2.2 ± 0.2	3.6 ± 0.3	63.6 ± 1.3	2.4 ± 0.2	3.8 ± 0.4	62.4 ± 1.0	2.1 ± 0.2	3.4 ± 0.3	62.5 ± 1.3	2.5 ± 0.2	4.1 ± 0.3
Femur	31.5 ± 0.7	1.4 ± 0.1	4.6 ± 0.4	32.7 ± 0.6	1.5 ± 0.1	4.8 ± 0.4	32.3 ± 0.6	1.5 ± 0.2	4.7 ± 0.7	32.7 ± 0.6	1.5 ± 0.1	4.6 ± 0.4
Tibia -fibula	28.9 ± 0.7	1.2 ± 0.1	4.1 ± 0.3	30.3 ± 0.6	1.2 ± 0.1	4.1 ± 0.4	29.6 ± 0.5	1.1 ± 0.1	3.6 ± 0.3	29.8 ± 0.5	1.2 ± 0.1	4.0 ± 0.3
Arm	46.3 ± 0.9	1.3 ± 0.2	2.8 ± 0.4	47.5 ± 0.8	1.2 ± 0.2	2.6 ± 0.4	47.0 ± 0.8	1.3 ± 0.1	2.7 ± 0.3	46.6 ± 0.8	1.2 ± 0.1	2.6 ± 0.3
Humerus	26.3 ± 0.6	0.7 ± 0.1	2.5 ± 0.5	26.9 ± 0.5	0.6 ± 0.1	2.2 ± 0.6	26.7 ± 0.5	0.6 ± 0.1	2.2 ± 0.5	26.5 ± 4.1	0.5 ± 0.1	2.0 ± 0.4
Ulna -radius	20.0 ± 0.4	0.6 ± 0.1	3.2 ± 0.3	20.6 ± 0.4	0.6 ± 0.2	3.1 ± 0.8	20.3 ± 0.4	0.7 ± 0.1	3.4 ± .04	20.1 ± 0.4	0.7 ± 0.1	3.5 ± 0.4
					Bone mass(g)							
Total body	1042 ± 61	106 ± 13	10.3 ± 1.1	1115 ± 46	101 ± 6[d]	9.3 ± 0.7[d]	1038 ± 42	118 ± 10[d]	11.4 ± 0.8[d]	1043 ± 52	103 ± 10	9.8 ± 0.7
Lumbar spine	16.0 ± 1.0	2.0 ± 0.4	12.1 ± 1.7	17.4 ± 0.8	1.9 ± 0.3	11.0 ± 1.4	16.7 ± 0.8	1.8 ± 0.2	11.1 ± 1.5	15.8 ± 0.8	2.2 ± 0.3	14.5 ± 2.4
Leg	333 ± 27	59 ± 7[b]	17.5 ± 1.1[ab]	379 ± 23	50 ± 3[b]	14.0 ± 1.5[b]	346 ± 22	54 ± 4	16.1 ± 1.0	362 ± 29	53 ± 5	14.7 ± 0.8[a]
Femur	156 ± 12	30 ± 4[bc]	18.7 ± 1.5[b]	175 ± 11	24 ± 2[b]	14.7 ± 1.9[b]	163 ± 10	25 ± 2[c]	15.8 ± 1.1	168 ± 13	27 ± 3	15.7 ± 1.0
Tibia-fibula	131 ± 10	19 ± 2	15.0 ± 1.1	148 ± 9	17 ± 1[d]	12.2 ± 1.2[d]	134 ± 8	21 ± 2[df]	16.2 ± 1.3[df]	141 ± 11	18 ± 2[f]	13.0 ± 1.1[f]
Arm	104 ± 7	16 ± 2[ac]	15.1 ± 1.8[ac]	112 ± 6	13 ± 1	11.9 ± 1.0	103 ± 5	10 ± 1[c]	10.0 ± 1.1[c]	103 ± 6	11 ± 1[a]	10.8 ± 1.4[a]
Humerus	56 ± 4	7 ± 1[c]	13.1 ± 1.3	61 ± 3	6 ± 1	10.5 ± 1.3	55 ± 3	5 ± 0.5[c]	9.7 ± 0.9	56 ± 3	5.5 ± 0.7	10.0 ± 1.4
Ulna-radius	37 ± 3	4 ± 0.5[ac]	12.1 ± 1.3[ac]	40 ± 2	5 ± 0.4[de]	12.5 ± 1.7[de]	37 ± 2	3 ± 0.4[cd]	8.5 ± 1.0[cd]	37 ± 2	3.3 ± 0.4[ae]	9.2 ± 1.2[ae]

[†] Unadjusted means (± SE).

[a-f] Like letters denote significant difference between adjusted values for groups (ANCOVA), $p < 0.05$

Table 6.4. Group characteristics for pre- and early-pubescent girls before and after 10 months of calcium supplementation or a placebo equivalent[†].

	Calcium (n = 30) Baseline ± SE	Placebo (n = 36) Baseline ± SE
Age (years)	8.8 ± 0.2	8.9 ± 0.2
High Impact exercise	16	18
Low Impact exercise	14	18
Tanner stage 1	27	26
Tanner stage 2	3	10
Asian decent	4	6
Weight Bearing Exercise[▽] ($h.week^{-1}$)	7.2 ± 0.5	6.7 ± 0.5
Dietary Calcium ($mg.day^{-1}$)	685 ± 33	676 ± 34
Supplemented Calcium ($mg.day^{-1}$)	434 ± 19	-
Total Calcium ($mg.day^{-1}$)	946 ± 40	676 ± 34

[▽] Exercise additional to the intervention program

Table 6.5. Anthropometry, bone mass and body composition values for pre- and early-pubescent girls before and after 10 months of calcium supplementation or a placebo equivalent[†].

	Calcium (n = 30)			Placebo (n = 36)		
	Baseline ± SE	Changes ± SE		Baseline ± SE	Changes ± SE	
		Absolute	Percent		Absolute	Percent
			Anthropometry(cm)			
Height	131.9 ± 1.6	4.1 ± 0.2	3.1 ± 0.2	132.0 ± 1.1	3.9 ± 0.2	2.9 ± 0.1
Sitting height	69.5 ± 0.7	1.9 ± 0.2	2.8 ± 0.3	69.6 ± 0.5	1.6 ± 0.1	2.3 ± 0.2
Leg length	62.4 ± 0.9	2.3 ± 0.1	3.7 ± 0.2	62.5 ± 0.8	2.3 ± 0.1	3.7 ± 0.2
Femur length	32.1± 0.5	1.5 ± 0.1	4.7 ± 0.3	32.5 ± 0.4	1.5 ± 0.1	4.6 ± 0.4
Tibia length	29.5 ± 0.5	1.2 ± 0.1	4.1 ± 0.2	29.7 ± 0.4	1.1 ± 0.1	3.8 ± 0.2
Arm length	46.9 ± 0.6	1.3 ± 0.1	2.7 ± 0.3	46.8 ± 0.6	1.2 ± 0.1	2.7 ± 0.2
Humerus length	26.6 ± 3.7	0.6 ± 0.1	2.4 ± 0.4	26.6 ± 0.3	0.5 ± 0.1	2.1 ± 0.3
Ulna Length	20.3 ± 0.3	0.6 ± 0.1	3.1 ± 0.4	20.2 ± 0.3	0.7 ± 0.1	3.5 ± 0.3
			Bone mass (g)			
Total Body	1076 ± 39	104 ± 7	9.8 ± 0.7	1041 ± 33	111 ± 7	10.6 ± 0.5
Lumbar spine	16.7 ± 0.7	2.0 ± .02	11.6 ± 1.1	16.2 ± 0.6	2.0 ± 0.2	12.8 ± 1.4
Leg	355 ± 18	55 ± 4	15.9 ± 1.0	354 ± 18	54 ± 3	15.4 ± 0.7
Femur	165 ± 8	27 ± 2	16.7 ± 1.2	165 ± 8	26 ± 2	15.7 ± 0.7
Tibia-fibula	139 ± 7	18 ± 1	13.7 ± 0.9	137 ± 7	20 ± 1	14.6 ± 0.9
Arm	108 ± 4	14 ± 1[b]	13.6 ± 1.1[b]	103 ± 4	11 ± 1	10.4 ± 0.9
Humerus	58 ± 2	6.7 ± 0.6[c]	11.9 ± 0.9[c]	55 ± 2	5.3 ± 0.4	9.9 ± 0.8
Ulna-radius	38.6 ± 1.7	4.5 ± 0.3[a]	12.3 ± 1.0[a]	36.6 ± 1.4	3.2 ± 0.3	8.8 ± 0.8
			Body composition (kg)			
Weight	28.9 ± 1.1	2.7 ± 0.3	9.4 ± 0.9	30.2 ± 1.1	2.7 ± 0.2	9.1 ± 0.8
Lean mass	20.5 ± 0.5	1.6 ± 0.1	7.9 ± 0.4	20.1 ± 0.4	1.6 ± 0.1	8.0 ± 0.5
Fat mass	6.6 ± 0.6	1.1 ± 0.2	17.6 ± 2.7	8.2 ± 0.7	1.1 ± 0.2	16.1 ± 2.9
Percent fat (%)	22.4 ± 1.3	1.4 ± 0.4		26.3 ± 1.5	1.1 ± 0.4	

[†] Unadjusted means (± SE).
[a] Significant when adjusted for baseline BMC and growth in length ([a] = $p < 0.01$, [b] = $p < 0.05$, [c] = $p < 0.1$).

Table 6.6. Group characteristics for pre- and early-pubescent girls before and after 10 months of either high impact or low impact exercise[†].

	High impact (n = 34) Baseline ± SE	Low impact (n = 32) Baseline ± SE
Age (years)	8.8 ± 0.2	8.8 ± 0.2
Calcium group	16	14
Placebo group	18	18
Tanner stage 1	26	27
Tanner stage 2	8	5
Asian decent	6	4
Weight Bearing Exercise[∇] (hr.week^{-1})	6.7 ± 0.5	7.0 ± 0.5
Dietary Calcium (mg.day^{-1})	654 ± 32	696 ± 37
Supplemented Calcium (mg.day^{-1})	442 ± 24	427 ± 30
Total Calcium (mg.day^{-1})	730 ± 51	748 ± 50

[∇] Exercise additional to the intervention program

Table 6.7. Anthropometry, bone mass, and body composition values for pre- and early-pubescent girls before and after 10 months of either high impact or low impact exercise[†].

	High impact (n = 34)			Low impact (n = 32)		
	Baseline ± SE	Changes ± SE		Baseline ± SE	Changes ± SE	
		Absolute	Percent		Absolute	Percent
			Anthropometry(cm)			
Height	131.2 ± 1.4	3.8 ± 0.2	2.9 ± 0.1	132.7 ± 1.3	4.1 ± 0.2	3.1 ± 0.2
Sitting height	69.3 ± 0.6	1.8 ± 0.1	2.6 ± 0.2	69.8 ± 0.6	1.6 ± 0.2	2.4 ± 0.2
Leg length	61.9 ± 0.8	2.1 ± 0.1	3.5 ± 0.2	63.0 ± 0.9	2.4 ± 0.1	3.9 ± 0.2
Femur length	31.9 ± 0.5	1.5 ± 0.1	4.7 ± 0.4	32.7 ± 0.4	1.5 ± 0.1	4.7 ± 0.3
Tibia Length	29.3 ± 0.4	1.1 ± 0.1	3.8 ± 0.2	30.0 ± 0.4	1.2 ± 0.1	4.1 ± 0.2
Arm length	46.7 ± 0.6	1.3 ± 0.1	2.8 ± 0.2	47.0 ± 0.6	1.2 ± 0.1	2.6 ± 0.2
Humerus length	26.5 ± 0.4	0.6 ± 0.1	2.3 ± 0.3	26.7 ± 0.3	0.5 ± 0.1	2.1 ± 0.3
Ulna length	20.2 ± 0.3	0.7 ± 0.1	3.3 ± 0.3	20.3 ± 0.3	0.7 ± 0.1	3.3 ± 0.4
			Bone mass (g)			
Total Body	1040 ± 36	112 ± 8	10.8 ± 0.7	1075 ± 35	102 ± 6	9.6 ± 0.5
Lumbar spine	16.4 ± 0.6	1.9 ± 0.2	11.6 ± 1.1	16.5 ± 0.6	2.1 ± 0.2	13.0 ± 1.5
Leg	340 ± 17	56 ± 4[b]	16.7 ± 0.7[b]	369 ± 19	52 ± 3[b]	14.4 ± 0.8[b]
Femur	159 ± 8	27 ± 2	17.2 ± 0.9	171 ± 8	26 ± 2	15.3 ± 1.0
Tibia-fibula	132 ± 6	20 ± 1[a]	15.6 ± 0.9[a]	144 ± 7	18 ± 1[a]	12.6 ± 0.8[a]
Arm	103 ± 4	13 ± 1	12.4 ± 1.1	107 ± 4	12 ± 1	11.3 ± 0.9
Humerus	55 ± 2	6 ± 1	11.3 ± 0.8	58 ± 2	6 ± 0.5	10.3 ± 1.0
Ulna-radius	37 ± 2	4 ± 0.3	10.2 ± 0.9	38 ± 2	4 ± 0.3	10.6 ± 1.0
			Body composition (kg)			
Weight	29.5 ± 1.1	2.7 ± 0.3	9.2 ± 0.9	29.7 ± 1.1	2.7 ± 0.2	9.2 ± 0.8
Lean mass	20.2 ± 0.4	1.6 ± 0.1	7.8 ± 0.4	20.5 ± 0.5	1.6 ± 0.1	8.2 ± 0.5
Fat mass	7.5 ± 0.7	1.2 ± 0.2	17.6 ± 2.7	7.4 ± 0.7	1.0 ± 0.1	15.9 ± 3.0
Percent fat (%)	24.7 ± 1.5	1.4 ± 0.4		24.4 ± 1.4	1.1 ± 0.4	

[†] Unadjusted means (± SE) are presented.
[a] Significant when adjusted for baseline BMC and growth in length ([a] = $p < 0.01$, [b] = $p < 0.05$, [c] = $p < 0.1$).

6.4.4 Anthropometry and body composition

Anthropometric and body composition data are shown in Tables 6.3, 6.5 and 6.7. No differences were reported between the four groups for all baseline anthropometric measures and body composition (Table 6.3). Similarly, no differences were evident for baseline anthropometric or body composition measures when groups were combined to report the main effects for calcium (Table 6.5) or exercise (Table 6.7). There was however, a non-significant trend towards greater percent body fat in the placebo group when compared to the calcium group ($p = 0.055$). There were no differences between Asians and Caucasians for anthropometric and body composition measures at baseline (Appendix 6.1). All subsequent analyses were conducted with the two ethnic groups combined.

All groups reported significant gains in anthropometric and body composition measures over the 8.5-month period. No differences were reported between groups for changes in anthropometric measures and body composition (Table 6.3). When groups were combined to determine the main effect for calcium there were no differences in the gains in anthropometric and body composition measures (Table 6.5). A similar result was found when comparing exercise groups except for leg length gains (Table 6.7). There was a non-significant tendency for the LI ex group to gain more leg length than the HI ex group ($p < 0.1$). There were no differences between Asians and Caucasian for changes to anthropometric and body composition measures, when adjusted for maturity and baseline values (Appendix 6.1 reports unadjusted values).

6.4.5 Dietary intake

Dietary intake data are presented in Table 6.1 and Table 6.8. Dietary intake data was obtained from 63 girls at baseline, 61 girls mid-intervention, and 58 girls post-intervention. No differences were reported between groups for intakes of calcium, total energy, and macronutrients, at baseline, mid- or post-intervention (Table 6.8). Mean reported dietary intake of calcium, total energy, protein and fat did not differ between the group (Table 6.1). Mean carbohydrate intake differed between the HI ex-Ca group and the HI ex-Pla group (Table 6.1). The mean reported calcium intake for the entire group ($n = 66$) was 674 ± 24 mg.day^{-1} (range from all diet entries 111 mg.day^{-1} to 1362 mg.day^{-1}), which is below the lower limit of the recommended intake levels for children of this age. Following calcium supplementation, the mean calcium intake for the calcium group exceeded the lower limits of the recommended intake by approximately 40%.

When the four study groups were combined a time effect was reported for calcium ($p < 0.01$) and carbohydrate intake ($p < 0.05$). Mean calcium and carbohydrate intakes were lower at the mid-point

when compared to baseline. The calcium by time effect is depicted in Figure 6.1. No time effect was reported for protein, fat and total energy intakes. No time by study group effect was reported (Table 6.8).

Dietary intake data when reporting the main effects of calcium and exercise are reported in Tables 6.9 and 6.10, respectively. When determining the main effects of calcium no differences were reported between the calcium and placebo groups for all nutrient intakes (Table 6.9). Similarly, no differences were reported between the HI ex group when compared to the LI ex group for all nutrients except for post- carbohydrate intake (Table 6.10). The LI ex group consumed approximately 29g more carbohydrate than the HI ex group ($p = 0.034$).

Asians girls on average consumed less calcium compared to Caucasian girls (536 ± 70 $mg.day^{-1}$ v 700 ± 24 $mg.day^{-1}$, $p < 0.05$). There were no differences between Caucasian girls and Asian girls for total energy, carbohydrate, protein and fat intakes. Baseline values are reported in Appendix 6.1.

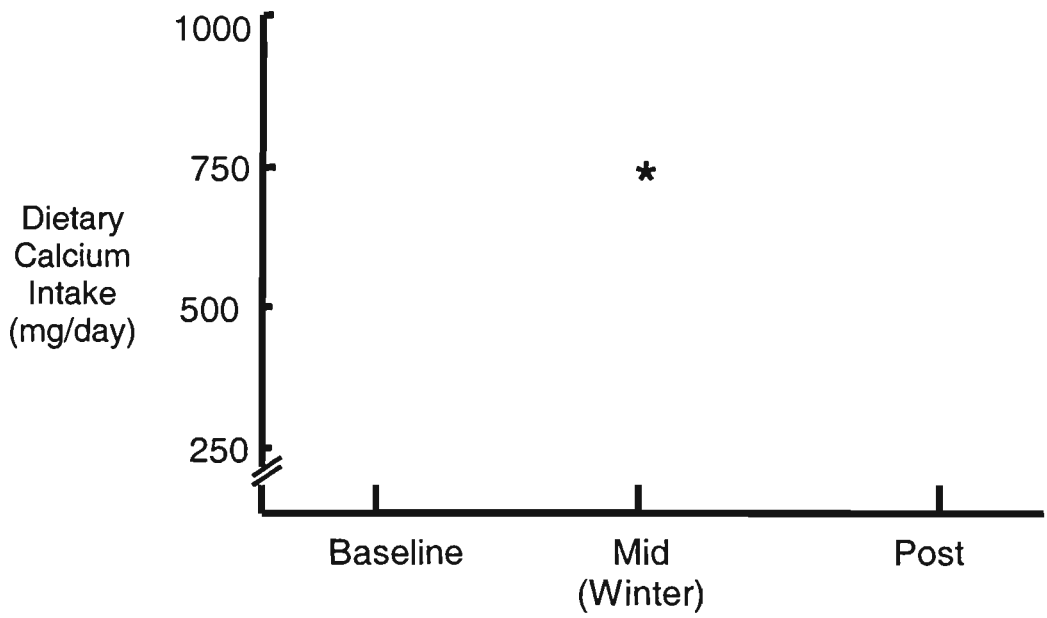

Figure 6.1 Seasonal change in dietary calcium intake in 63 pre- and early-pubescent girls based on 3 x 3-day weighed food records.
*** $p < 0.05$, different to baseline**

6.4.6 Calcium supplementation and food compliance

The augmentation of dietary calcium intake with supplementation is presented in Tables 6.3. The mean calcium intake from supplementation was 434 ± 19 $mg.day^{-1}$. Following supplementation the total calcium intake (dietary + supplement) was greater for the HI ex + Ca, and LI ex + Ca groups

than the non-supplemented groups. No difference was reported for supplemented calcium intake or total calcium intake when groups were combined to report the main effect for exercise (Table 6.6). No difference was reported between the calcium and placebo group for the mean number of foods consumed each week. Both groups consumed an equivalent of 7.5 of the required 10 food products each week. Food consumption on a week by week basis did not differ between the calcium and placebo groups. The mean number of food items (or equivalents) consumed each week ranged from six to nine.

6.4.7 Baseline bone mass

Baseline bone measures are presented in Table 6.2. Groups were matched for all bone measures. Similarly, when groups were combined to report the main effects for calcium (Table 6.5) or exercise (Table 6.7), groups were matched for baseline bone measures. No differences between Asian girls and Caucasian girls were reported for baseline bone measures. There was however, a tendency for Asian girls to have lower bone mineral content at the arms compared to Caucasian girls (93g v 107g, $p < 0.07$).

6.4.8 The effect of 8.5 months of high- or low-impact exercise on bone mass accrual

The main effect for exercise on changes to bone mass is presented in Table 6.7 and Figure 6.2a. HI ex had a positive effect on total body BMC accrual when expressed in absolute terms and as a percentage. Following adjustment for baseline total body BMC and height gain, girls in the HI ex group accrued approximately 18.6 grams (1.7%) more BMC for the total body compared to the LI ex participants ($p < 0.05$). Group membership (HI ex or LI ex) accounted for 8% of the variance in total body BMC accrual.

HI ex had a positive effect on BMC accrual at the legs when expressed in absolute terms and as a percentage. Following adjustment for baseline leg BMC and growth in leg length, girls in the HI ex group accrued approximately 9.8 grams (2.5%) more BMC at the legs compared to the LI ex participants ($p < 0.05$). Group membership (HI ex or LI ex) accounted for 9% - 10% of the variance in BMC accrual at the legs.

Table 6.8. Dietary intake values for pre- and early-pubescent girls before, during and after 10 months of calcium supplementation and either high impact or low impact exercise.

	Calcium / High impact (n = 16)			Calcium / Low impact (n = 14)			Placebo / High impact (n = 18)			Placebo / Low impact (n = 18)		
	Baseline	Mid	Post	Baseline	Mid	Post	Baseline	Mid	Post	Baseline	Mid	Post
Calcium (mg)	699 ± 66	615 ± 59	674 ± 44	732 ± 71	611 ± 56	718 ± 75	725 ± 67	589 ± 48	642 ± 62	776 ± 69	676 ± 55	688 ± 49
Ca Suppl. (mg)	427 ± 30	427 ± 30	427 ± 30	442 ± 24	442 ± 24	442 ± 24	-	-	-	-	-	-
Total energy	7192 ± 548	6628 ± 386	6791 ± 388	6752 ± 450	6478 ± 394	6800 ± 533	6463 ± 291	6145 ± 340	6264 ± 361	6991 ± 395	6882 ± 341	6894 ± 335
Carbohydrate	248 ± 29	209 ± 13	206 ± 10	229 ± 18	213 ± 13	226 ± 22	203 ± 12	181 ± 12	187 ± 12	224 ± 15	212 ± 10	226 ± 11
Protein	60 ± 4	57 ± 4	62 ± 4	59 ± 5	53 ± 4	60 ± 5	56 ± 4	56 ± 4	57 ± 4	60 ± 4	62 ± 4	62 ± 4
Fat	56 ± 4	58 ± 5	63 ± 6	53 ± 4	55 ± 6	55 ± 5	58 ± 3	59 ± 4	59 ± 4	61 ± 4	63 ± 4	56 ± 3

Table 6.9. Dietary intake values for pre- and early-pubescent girls before, during and after 10 months of calcium supplementation or a placebo equivalent.

	Calcium (n = 30)			Placebo (n = 36)		
	Baseline	Mid	Post	Baseline	Mid	Post
Calcium (mg)	714 ± 47	613 ± 40	694 ± 41	751 ± 48	634 ± 37	666 ± 39
Ca Suppl. (mg)	434 ± 19	434 ± 19	434 ± 19	-	-	-
Total energy (kj)	6988 ± 356	6558 ± 272	6795 ± 312	6735 ± 248	6525 ± 246	6589 ± 248
Carbohydrates (g)	239 ± 17	211 ± 9	215 ± 11	214 ± 10	197 ± 8	208 ± 9
Protein (g)	60 ± 3	55 ± 3	61 ± 3	58 ± 3	59 ± 3	60 ± 3
Fat (g)	54 ± 3	57 ± 4	59 ± 4	60 ± 3	61 ± 3	57 ± 3

Table 6.10. Dietary intake values for pre- and early-pubescent girls before, during and after 10 months of either high impact or low impact exercise

	High impact (n = 34)			Low impact (n = 32)		
	Baseline	Mid	Post	Baseline	Mid	Post
Calcium (mg)	713 ± 46	602 ± 37	658 ± 37	756 ± 49	647 ± 39	701 ± 42
Ca Suppl. (mg)	442 ± 24	442 ± 24	442 ± 24	426 ± 30	426 ± 30	426 ± 30
Total energy (kj)	6805 ± 302	6379 ± 256	6528 ± 265	6891 ± 293	6707 ± 256	6854 ± 292
Carbohydrates (g)	224 ± 15	194 ± 9	197 ± 8*	226 ± 11	212 ± 8	226 ± 11*
Protein (g)	58 ± 3	57 ± 3	59 ± 3	60 ± 3	58 ± 3	61 ± 3
Fat (g)	57 ± 3	59 ± 3	61 ± 4	58 ± 3	60 ± 4	56 ± 3

* difference between groups ($p < 0.05$)

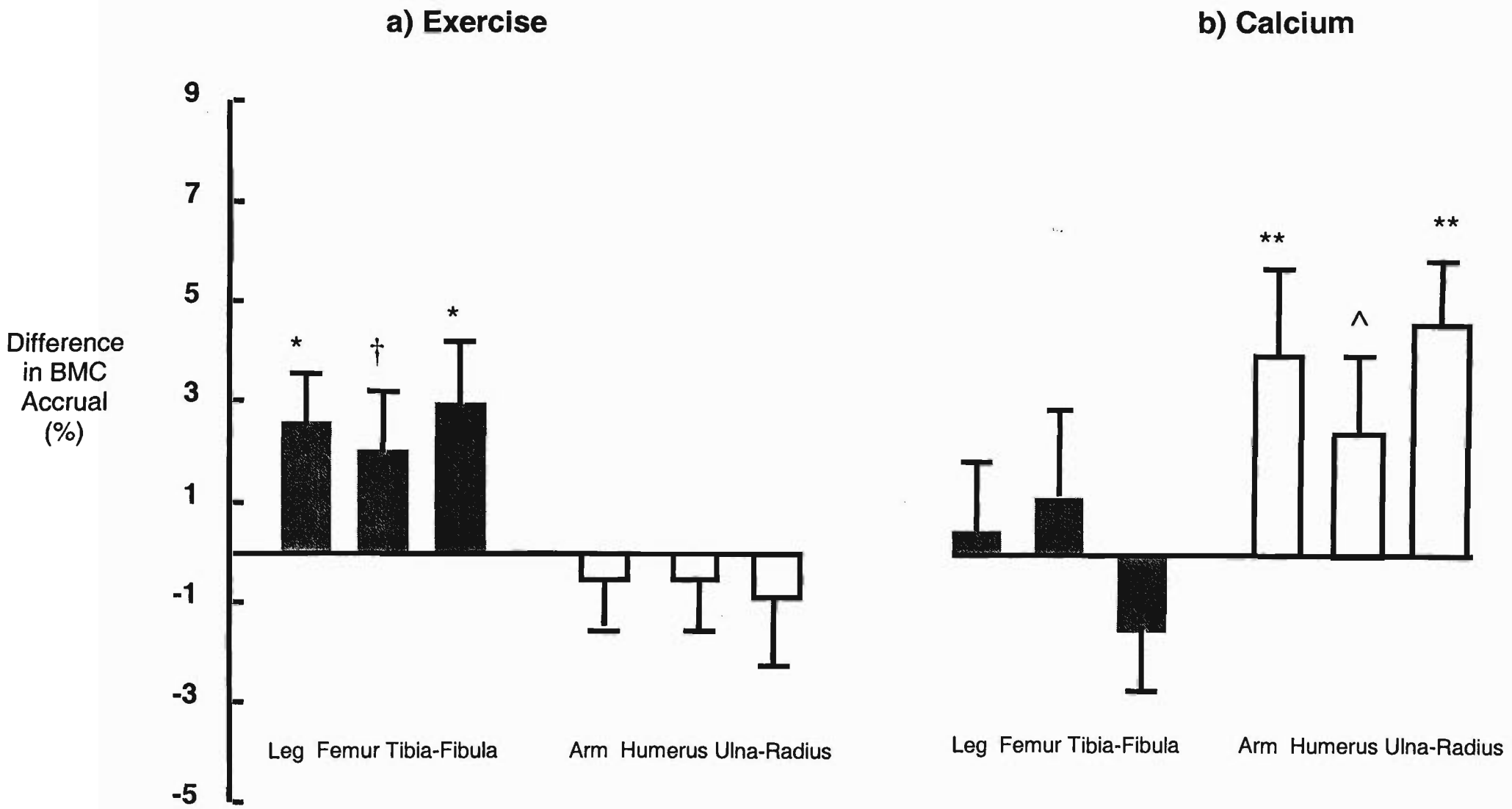

Figure 6.2: The main effect of exercise and calcium over 10 months in pre- and early-pubertal girls after adjusting for baseline bone mass and change in bone length: bone mass accrual was higher at the weight-bearing sites in the HI ex group relative to the LI ex group (zero), and higher at the non-weight bearing sites in the Ca group relative to the Pla group (zero) ** $p < 0.01$ * $p < 0.05$ † $p < 0.06$ ^ $p < 0.1$

The difference in BMC accrual between the HI ex and LI ex groups remained at the tibia-fibula when total leg was divided into proximal (femur) and distal (tibia-fibula) segments (Figure 6.2a). Following adjustment for baseline tibia-fibula BMC and growth in tibia length, the HI ex group accrued approximately 4.3 grams (3.1%) more BMC at the tibia-fibula than the LI ex group. Group membership (HI ex or LI ex) accounted for 11%-12% of the variance in tibia-fibula BMC accrual. There was a trend towards greater BMC accrual at the femur for the HI ex groups compared to the LI ex group ($p < 0.1$). The HI ex group accrued approximately 3.8 grams (2.1%) more BMC at the femur than the placebo group. No effect of exercise was detected for BMC accrual at the arms or lumbar spine.

6.4.9 The effect of 8.5 months of calcium supplementation on bone mass accrual

The main effect for calcium on changes to bone mass is presented in Table 6.5 and Figure 6.2b. Calcium had a positive effect on arm BMC accrual expressed in absolute terms and as a percentage. Following adjustment for baseline arm BMC and growth in arm length the calcium group accrued approximately 3.3 grams (3.4%) more BMC at the arms than the placebo group ($p < 0.05$). Group membership (Ca or Pla) accounted for 9%-10% of the variance in arm BMC accrual.

The difference in BMC accrual between the Ca and placebo group remained at the ulna-radius when the arm was divided into proximal (humerus) and distal (ulna-radius) segments. Following adjustment for baseline ulna-radius BMC and growth in ulna length the calcium group accrued approximately 1.8 grams (4.1%) more BMC at the ulna-radius than the placebo group (Figure 6.2b). Group membership (Ca or Pla) accounted for 15%-16% of the variance in ulna-radius BMC accrual ($p < 0.01$). The calcium group accrued approximately 1.2 grams (2.1%) more BMC at the humerus than the placebo group. No effect of calcium supplementation was detected for BMC accrual at the legs, lumbar spine or for the total body.

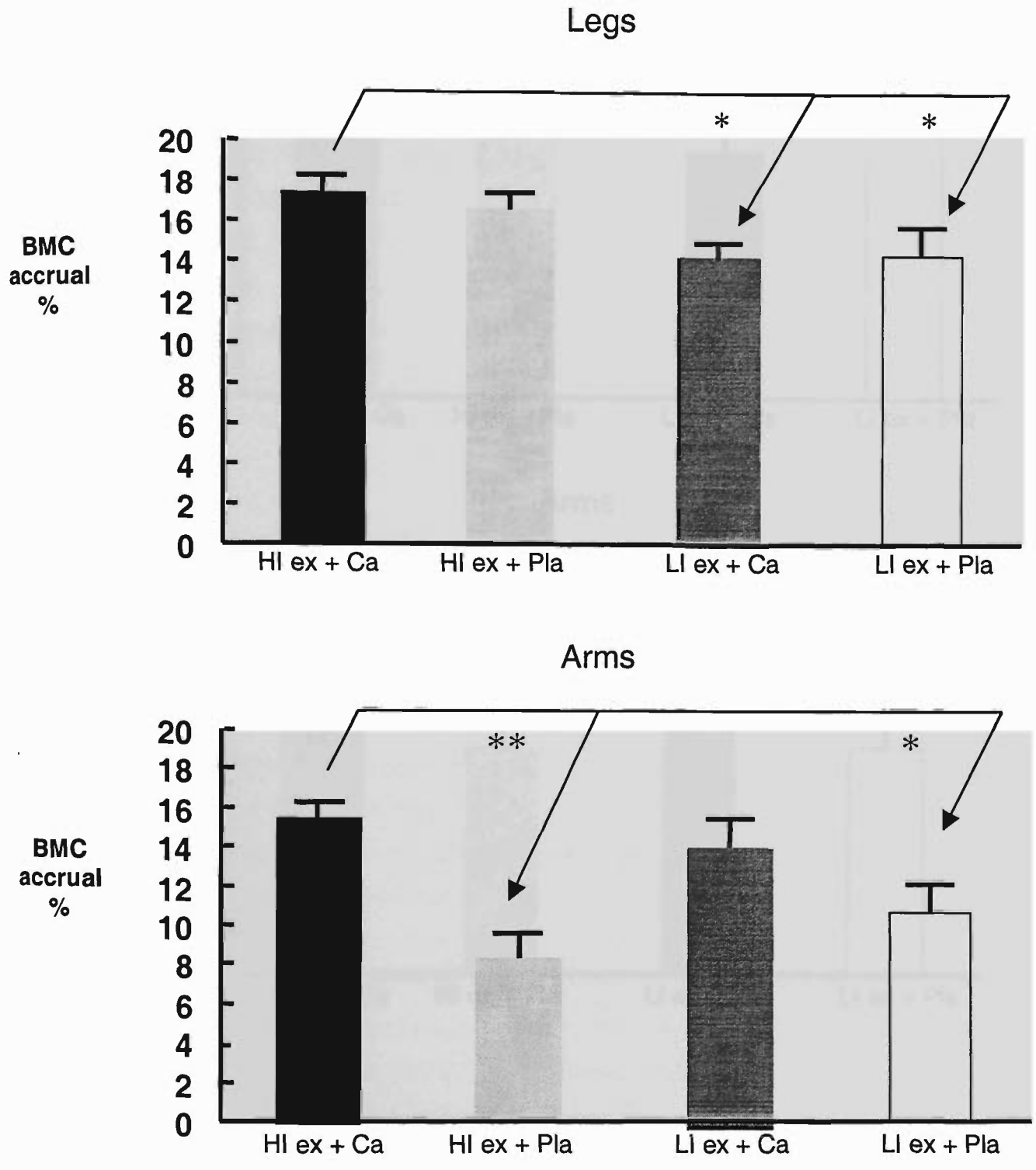

Figure 6.3 Group comparisons in percentage bone mass accrual in pre- and early-pubescent girls following 10 months of calcium and exercise intervention after adjusting for baseline bone mass values and change in limb lengths.
A connecting arrow denotes a difference between the groups ** $p < 0.01$ * $p < 0.05$

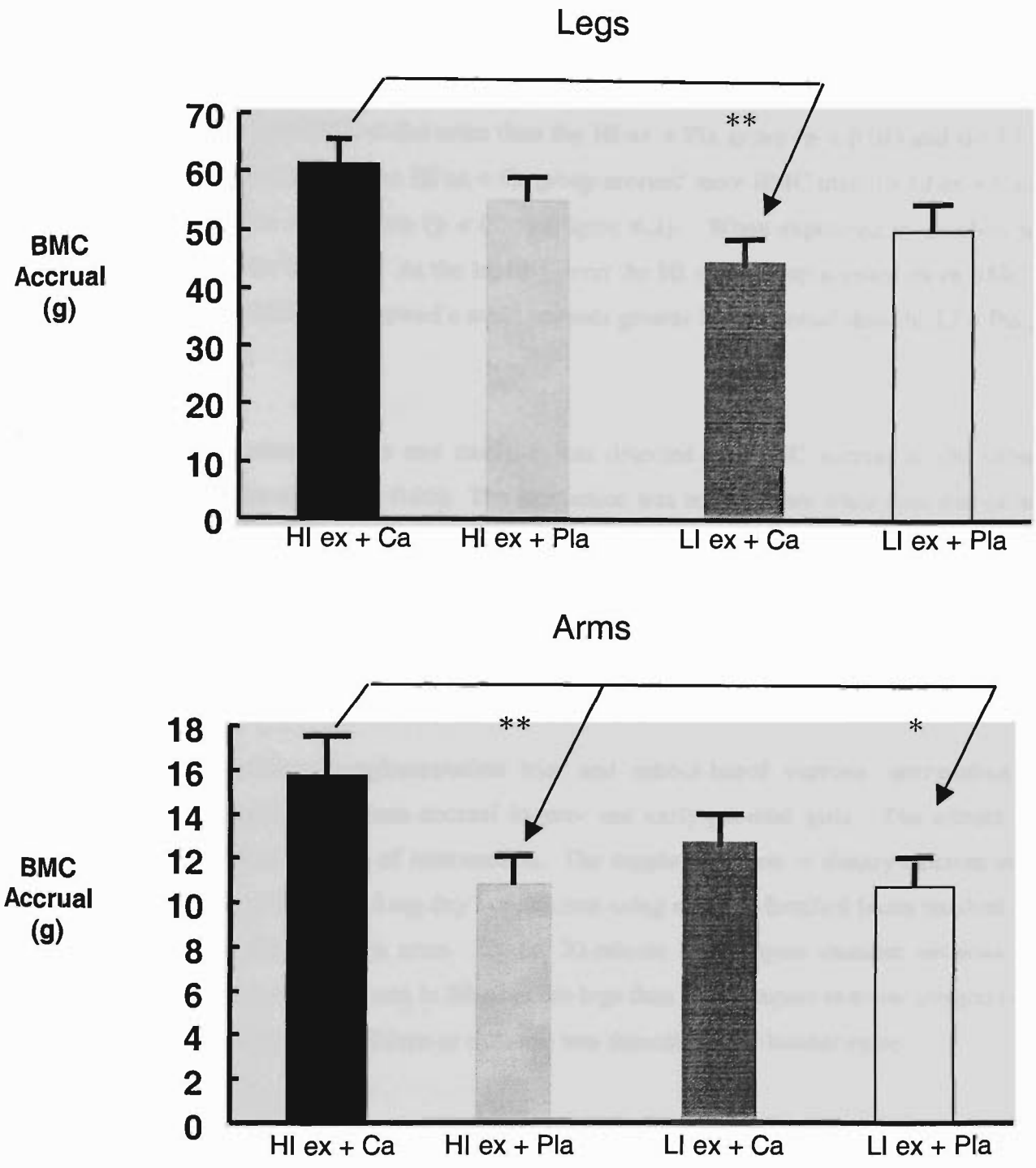

Figure 6.4 Group comparisons in bone mass accrual in pre- and early-pubescent girls following 10 months of calcium and exercise intervention after adjusting for baseline bone mass values and change in limb lengths.
A connecting arrow denotes a difference between the groups ** $p < 0.01$ * $p < 0.05$

6.4.10 The combined effect of calcium and exercise on bone mass accrual

Group comparisons are reported in Figures 6.3 and 6.4. When expressed as a percentage the HI ex + Ca group accrued more BMC at the arms than the HI ex + Pla group ($p < 0.01$) and the LI ex + Pla group ($p < 0.05$). At the legs, the HI ex + Ca group accrued more BMC than the LI ex + Ca group ($p < 0.01$) and the LI ex + Pla group ($p < 0.05$) (Figure 6.3). When expressed in absolute terms the trend was repeated for the arms. At the legs however the HI + Ca group accrued more BMC than the LI + Ca group ($p < 0.05$), and showed a trend towards greater BMC accrual than the LI + Pla group ($p < 0.06$) (Figure 6.4).

An interaction between calcium and exercise was detected for BMC accrual at the femur, when expressed in absolute terms ($p < 0.05$). The interaction was not apparent when data was expressed as a percentage. No interaction between calcium and exercise was detected for bone mass accrual at the other measured sites.

6.5 Discussion

This food-based calcium supplementation trial and school-based exercise intervention program resulted in augmented bone mass accrual in pre- and early-pubertal girls. The effects were site specific and related to the type of intervention. The supplementation of dietary calcium intake (674 $mg.day^{-1}$) with an additional 434 $mg.day^{-1}$ of calcium using calcium-fortified foods resulted in a 2-4% greater increase in BMC at the arms. Three, 20-minute high impact exercise sessions per week resulted in a 2–3% greater increase in BMC at the legs than a low impact exercise program of similar duration. No effect of either calcium or exercise was detected at the lumbar spine.

An effect of calcium supplementation on bone mass accrual was detected at the arms. The magnitude of the effect at this non-weight bearing site was comparable to those reported from other calcium supplementation trials involving pre-pubertal children. Johnston et al. (1992) reported a 5.1% difference in BMD at the midshaft radius between supplemented and non-supplemented twins after 3 years of supplementation with $1000mg.day^{-1}$ of calcium. Lee et al. (1993) reported a 2.5% greater increase in radius BMC in children supplemented with $300mg.day^{-1}$ of calcium for 18 months. Bonjour et al. (1997) reported 2.4% greater BMD at the radius in girls after 12 months of supplementation with $850mg.day^{-1}$ of calcium from fortified foods.

Despite the duration of this 8.5 month intervention study being less than other reported studies (12 – 36 months), the magnitude of the effect of calcium on bone mass accrual was similar (2-4%). The magnitude of the response to calcium supplementation may have been influenced by the baseline

dietary calcium intakes in this group of girls being relatively low (mean dietary calcium intake < 700mg.day^{-1}). Bonjour et al. (1997) reported a significantly greater change in BMD with calcium supplementation for girls with low calcium intakes (mean intake of 675 mg.day^{-1}) compared to those with high calcium intakes (mean intake of 1185 mg.day^{-1}). The enhanced response in BMD to calcium supplementation in low calcium consumers has also been reported in randomised trials involving adults [Dawson-Hughes et al., 1990]. The recommended daily intake (RDI) for children ranges from 700 – 1200 mg.day^{-1}, depending on their age. The mean reported calcium intake in this group of girls was below the RDI of 700 mg.day^{-1}. None of the girls had a mean dietary calcium intake above the RDI of 1200 mg.day^{-1}. The supplementation level of 434 mg.day^{-1} of calcium was sufficient to produce a detectable effect at the arms.

The effect of calcium supplementation was detected at the distal arm (ulna-radius) with only a trend towards an effect of calcium detected at the proximal arm (humerus). This observation may be potentially explained in two ways. Firstly, the temporal pattern of growth of the appendicular skeleton generally progresses from the distal segment to the proximal segment [Cameron et al., 1982]. This is supported by a greater percentage change in ulna length compared to humerus length (3.3 ± 0.2% v 2.2 ± 0.2%, $p < 0.01$), in this group of girls. More relative growth (and relative bone mineral accrual) was likely to be occurring in the distal portion of the arm compared to the proximal portion. The effect of calcium therefore was potentially influencing proportionally more bone at the distal segment of the arm, due to more growth occurring at this site, compared to the proximal segment. Secondly, the ulna and radius being two bones would have a greater surface area to volume ratio than the humerus. The process of bone remodelling occurs on the surface of bone. Therefore if calcium supplementation results in a slowing of the remodelling process, [Johnston et al., 1992] then per unit volume of bone, more surface remodelling may have been slowed (more remodelling spaces filled) on the ulna and radius compared to the humerus.

No effect of calcium supplementation was detected at the legs. Similarly, Bonjour et al. (1997), Johnston et al. (1992) and Lee et al. (1995) reported no difference in gains in femoral neck BMD between calcium supplemented pre-pubertal children and controls. Bonjour et al. (1997) however, reported greater gains in femoral trochanter (1.9%, $p < 0.01$) and femoral diaphysis (1.4%, $p < 0.05$) BMD in supplemented pre-pubertal girls receiving 850 mg.day^{-1} of calcium compared to controls. The lack of effect at the legs in this study may have resulted from the supplemented calcium not being sufficient for an effect to be detected at weight bearing sites. In this group of girls, dietary calcium intake was supplemented with an additional 434 mg.day^{-1} of calcium. Even with supplementation, 41% of the girls in the supplementation group still had calcium intakes below the RDI of 1200 mg.day^{-1}. It may be that the calcium dose in this study was insufficient to result in a detectable effect

at weight bearing sites. The supplementation dose in this study was limited to less than 450 $mg.day^{-1}$ as with larger doses the colour, texture and taste of the foods became unacceptable. Bonjour et al (1997) utilised alternative foods to add the milk extract to, therefore appeared able to increase the calcium dose of the foods, without compromising their palatability.

An effect of exercise on bone mass accrual was detected at the legs. The magnitude of the effect at this weight-bearing site (2-3%) was compatible to those reported for other school-based exercise intervention trials involving children. Morris et al. (1997) reported a 4% greater increase in leg BMD in pre-menarcheal girls involved in an exercise program compared to controls. Bradney et al. (1998) reported a 3.6% greater increase in leg BMD in exercising pre-pubertal boys compared to controls. McKay et al. (2000) reported a 1.2% greater gain in BMD at the trochanter in exercising pre- and early-pubertal boys and girls compared to controls. The greater gains in bone mass with exercise are less than those reported for young gymnasts (3-11%) [Bass et al., 1998] however, the loads involved with gymnastics training are substantially higher than loads involved in this program [Daly, et al., 1994].

Similar to the effect of calcium at the arms, an exercise effect was detected at the distal portion of the leg (tibia-fibula), with only a trend towards an exercise effect in the proximal portion of the leg (femur). This observation may have resulted from the magnitude of the impact on the lower leg being greater than the upper leg. This may be due to a few reasons. Firstly, the femur is further from the point of impact with the ground, so ground reaction forces may be attenuated through the bones and muscles of the lower leg before reaching the upper leg, which may result from improved landing efficiency. Secondly, the femur is a much larger bone than the tibia and fibula, therefore may have been better able to accommodate the ground reaction forces associated with the exercise program. Thus larger forces may be required to induce a detectable difference in bone mass accrual at the femur. As expected no effect of exercise was detected at the arms, as they were not loaded in this exercise program.

A combined effect of exercise and calcium was detected for bone mass accrual at the femur. A combined effect was not detected at other weight bearing sites. As no other calcium / exercise intervention trials involving children have been reported comparable data are not available. However, Prince et al (1995) reported greater gains in femoral neck BMD in post-menopausal women with calcium and exercise, compared to the calcium only group, with no combined effect reported at other weight bearing and non-weight bearing sites. A possible explanation for the combined effect being detected only at the femur in this study is that there was insufficient power to detect an interaction at other sites.

No effect of either calcium supplementation or exercise was detected at the lumbar spine. Morris et al. (1997) reported 6.5% greater BMC accrual at the lumbar spine with exercise in pre-menarcheal girls. This effect was relatively large however, the girls were more mature. The girls in this study were pre- and early-pubertal (Tanner stage 2) based on Tanner rating of breast development. Gains in leg length were greater than gains in sitting height ($p < 0.001$), which is the growth pattern consistent with a low dose of sex-steroids. It may be postulated therefore that the effect of either calcium supplementation or exercise may be more readily detected at the lumbar spine in more mature girls. An alternative explanation for the lack of effect detected at the lumbar spine was that neither the magnitude of the impacts, or the amount of additional calcium were sufficient to result in augmented BMC accrual at this site. The ground reaction forces (GRF's) experienced in the high impact exercise sessions were up to 2.4 times body mass. These GRF's were only sightly higher than those reported for adults while running (1.6 – 2.3 x body mass) [Munro et al., 1987] and much less than GRF's reported during basketball lay-ups (13.2 – 14.6 x body mass) [McCray et al., 1994]. Relative to other sporting activities, the loads in this school-based program were relatively conservative. However, the program was designed on the physical, maturational and developmental levels of the participants so that is could be incorporated into the existing physical education curriculum.

Due to ethical limitations involved with this study it was elected not to take blood samples. Therefore, we were not able to determine if calcium and / or exercise influenced the modelling process, the remodelling process or both. Johnston et al. (1992) reported a reduction in serum osteocalcin levels in calcium supplemented (pre-pubescent) children when compared to non-supplemented children, suggesting suppression of the remodelling process with calcium supplementation. Serum osteocalcin levels (and the rate of remodelling, and BMD) however, reverted to non-supplemented levels once supplementation ceased [Slemenda et al., 1997]. This observation raises the question of the permanency of the effect of calcium supplementation on bone mass. Bonjour et al. (1997) reported residual benefits of calcium supplementation on femoral shaft BMC and width in 100 pre-pubescent girls one year after the cessation of supplementation. The uniqueness of this study compared to previous calcium supplementation intervention trial was the calcium was derived from a milk extract and incorporated into foods. Factors inherent to the milk minerals or its incorporation into foods may have contributed to the greater permanency of its effect on bone mass. Milk minerals were also utilised as the means of calcium supplementation in this study. Following up participants of this study a year or more after the cessation of supplementation may indicate whether the gains made at the arms are maintained. The follow up may also indicate if residual benefits result from the high impact exercise. Greater bone mass relative to controls has been reported in retired athletes who participated in long term high impact exercise training [Bass et al., 1998]. The residual benefits of short-term (less than one year) high impact exercise is yet to be determined.

6.6 Conclusion

The results demonstrate that this school-based exercise and calcium intervention program can result in augmented bone mass accrual in the appendicular skeleton in pre- and early-pubescent girls. The effects of calcium or exercise were site specific which stresses the need for both an adequate calcium intake and appropriate exercise to develop bone mass in both the upper and lower extremities. The results of this study are promising as the amount of additional high impact exercise in this program was easily incorporated into the existing school curriculum and enhanced bone mass accrual. The direct assessment of the loads involved in this intervention study has added to the knowledge of the type and amount of mechanical loading that will have an osteogenic effect. The amount of additional calcium was also effective in augmenting bone mass accrual. The incorporation of the milk minerals into foods such as muffins and muesli bars improved the acceptability of the calcium supplementation while still maintaining the concept of healthy eating. The stage of maturity at which a potential effect of either calcium or exercise may be detected at the axial skeleton warrants further investigation. If the reported benefits to bone mass accrual are maintained it further emphasises the importance of growth as a critical period to positively influence bone development with the long-term aim of reducing the risk of osteoporosis in later life.

Chapter Seven

Conclusion and Recommendations

7.1 Conclusion

The growth period is an important time in the development of bone. Lifestyle habits undertaken during childhood and adolescents may have implications to bone status both during the growth phase itself, and in later life. The bone mass established during this period may determine the amount of bone that is available to cope with age- and hormone-related bone loss in later life. Two important lifestyle habits that have a positive effect on bone mass are calcium intake and physical activity.

Calcium is an important mineral component of bone, therefore is an essential nutrient during the growth of the skeleton. An adequate reserve of calcium prior to the establishment of the adult skeleton is particularly pertinent for females who experience proportionally more bone loss than males in later life. The first study asked the question does calcium intake decrease with age in girls and does calcium intake change with the seasons? Results from study one demonstrated that the dietary calcium intakes of young girls, and adolescent females are frequently not meeting recommended levels. Furthermore, as skeletal needs for calcium increase around the time of maturity, females are decreasing rather than increasing calcium intakes. These findings reinforce findings by others who reported a reduction in calcium with age or maturity in girls. This is the first study of its kind to report seasonal changes in calcium intake in children. The reduction in calcium intake in young girls during the winter months raises the issue of calcium adequacy during a time when calcium metabolism may be compromised as a result of reduced Vitamin D availability. The reductions in calcium intake reported in girls were predominantly due to a decrease in fluid milk intake. The effect of the reductions in calcium intake on peak bone mass is yet to be determined. The trend however, may have repercussions for resistance to bone fragility in later life. The trend was not apparent in males who appeared to be consuming sufficient calcium, therefore are more likely to be meeting their skeletal calcium needs during childhood and adolescence. From these findings and findings by others it may be recommended that educational and marketing strategies to increase calcium intake may be more effective if specifically targeted at females. It may be worthwhile considering specific marketing of milk in winter (targeting young females) and examining the feasibility of food fortification to enhance calcium intake. The efficacy of such strategies would require evaluation.

Study two asked the question do late maturers have lower peak height and tissue velocities than early maturers? Results from study two demonstrated that the magnitude of PHV is greater in early maturers compared to late maturers based on longitudinal analysis. Average maturing females accrued more bone mineral at peak than late maturing females. Calcium needs therefore would vary depending on the age of maturity. Calcium intake recommendations have been determined from bone mass accrual data and are based on peak rates of bone mineral accrual. As a minimal requirement,

calcium intake should meet these recommended levels during this period of rapid skeletal growth. An adequate calcium intake may be most beneficial if it extends from the time of PHV (detectable through observation) through to the age of PBMCV (not detectable through observation) which occurs approximately six months later.

Physical activity is also an important stimulus for bone development. The effect of mechanical loading is specific to the site to which the load is applied. The results from study three demonstrated augmented bone mass accrual at the legs (weight bearing site), but not the arms (non-weight bearing site) in response to weight bearing exercise. Calcium supplementation however, enhanced bone mass accrual in the arms. It is not clear why there was a differentiation on the location of the effects of exercise and calcium. This observation has stimulated questions that are worthy of further investigation. However, the overall school-based exercise and calcium intervention program resulted in augmented bone mass accrual in the appendicular skeleton in pre- and early-pubescent girls. The effects of the calcium or the exercise appear site specific which stresses the need for both an adequate calcium intake and appropriate exercise to develop bone mass in both the upper and lower extremities. The site specificity of the exercise effect may indicate that a combined effect of exercise and calcium requires at least an adequate calcium intake and mechanical loading to the site at which the benefit is being sought. A combination of the two appears better than the individual effects of each therefore it is recommended that both be considered equally when lifestyle factors are emphasised to promote bone health.

Despite the design of this exercise program being relatively conservative in relation to its duration and magnitude, it did result in positive gains in bone mass at the loaded sites. The accurate recording of the loads involved in this program has provided valuable information about the type and amount of exercise that has an osteogenic effect. This information can assist in the appropriate prescription of exercise for the pediatric population. The incorporation of a 'bone specific' fitness component into the school curriculum to promote bone mass accrual is a feasible option. The calcium supplementation was effective in enhancing bone mass accrual in the arms. The incorporation of milk mineral into foods was a novel approach to enhancing calcium intake, and was well accepted by the participants in the study. Our knowledge of the mechanisms that result in the osteogenic effect of milk minerals is limited, and requires further research. The feasibility of calcium enrichment of foods to enhance bone mass accrual would require further scrutiny. The stage of maturity at which a potential effect of either calcium or exercise may occur at the axial skeleton also warrants further investigation. Results from such a study would assist in enhancing the understanding of the effects of calcium and exercise over the entire growth period.

The next step in our understanding of the effects of exercise it to further investigate the mechanisms of how bone responds to mechanical loading, and to follow up the participants to determine of the reported benefits are maintained after the intervention period. If the reported benefits of calcium and exercise to bone mass accrual are maintained into adulthood, it further emphasises the importance of growth as a critical period to positively influence bone development, with the long-term aim of preventing bone fragility in later life.

7.2 Summary of recommendations

1. An adequate calcium intake should be encouraged in young females via educational and marketing strategies. An important aspect of this strategy is to determine why intakes in girls are decreasing during adolescence.

2. The continual tracking of children through puberty and into adulthood would provide valuable information about the effect of age of maturity on adult size and bone mass.

3. Conducting similar intervention programs with participants of various levels of maturity would expand our understanding of the effects of this program on more mature boys and girls.

4. The inclusion of a school-based exercise program to enhance bone mass accrual is a feasible option. Such a program would add another dimension to the overall health benefits of physical activity.

5. More research is required to investigate the mechanisms, which result in the osteogenic effect of the milk minerals.

6. Wide-scale fortification of foods with calcium to augment calcium intake would require further investigation.

7. Following up participants in the intervention program to determine if the benefits of the calcium or exercise are maintained.

8. Further work to determine the threshold of mechanical loading that has an osteogenic effect.

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Appendix

Appendix 3.1 Recipes for foods used in the calcium / exercise interventions study

Calcium Study Recipes

CHOCOLATE CRACKLES

QUANTITY	200	250
RICE BUBBLES	825G	1035G
ICING SUGAR	1370G	1715G
COCONUT	455G	570G
COPHA (MELTED)	1425G	1785G
COCOA	170G	215G

ANZAC COOKIES

QUANTITY	***60***	***120***	***150***
OATS	350G	700G	875G
PLAIN FLOUR	300G	600G	750G
SUGAR	550G	1.1KG	1.375KG
COCONUT	175G	350G	440G
GOLDEN SYRUP	190G	375G	470G
MARGARINE	350G	700G	875G
BICARB	10G	20G	25G
BOILING WATER	75ML	150ML	190ML

CHOCOLATE CHIP COOKIES

QUANTITY	***100***	***250***	***300***	***350***
BUTTER 2.34KG	680G	1.68KG	2.0KG	
BROWN SUGAR 5.2KG	800G	3.2KG	4.8KG	
SELF RAISING FLOUR 4.2KG	1.2KG	3.0KG	3.6KG	
EGGS	6	14	16	19
CHOC BITS 2.95KG	540G	1.34KG	2.68KG	

PEANUT BUTTER COOKIES

QUANTITY	***100***	***200***	***250***	***300***
SELF RAISING FLOUR 1.8KG	600G	1.2KG	1.5KG	
SALT 1.5TSP	0.5TSP	1TSP	1.25TSP	
BROWN SUGAR 1.5KG	500G	1.0KG	1.25KG	
WHITE SUGAR 1.5KG	500G	1.0KG	1.25KG	
BUTTER 1.2KG	400G	800G	1.0KG	
EGGS	6	12	15	18
PEANUT BUTTER 1.2KG	400G	800G	1.0KG	
VANILLA 12TSP	4TSP	8TSP	10TSP	

FACE COOKIES

QUANTITY	*100*	*200*	*250*	*300*
PLAIN FLOUR 1.5KG	600G	900G	1.05KG	
SELF RAISING FLOUR 1.5KG	600G	900G	1.05KG	
BUTTER/MARGARINE 1.25KG	500G	750G	825G	
CASTER SUGAR 1.25KG	500G	750G	825G	
EGGS	4	6	7	10
MILK 400ML	160ML	240ML	280ML	

BREAM BUTTER & SUGAR ADD EGG, MIX WELL. ADD FLOURS AND MIX TO FIRM DOUGH. ROLL OUT AND CUT INTO SHAPES.

APPLE & CIMNNAMON MUFFINS

QUANTITY	*20*	*50*	*100*	*200*
SELF RAISING FLOUR 3KG	300G	750G	1.5KG	
PLAIN FLOUR 1.5KG	150G	375G	750G	
CINNAMON 15TSP	1.5TSP	3.75TSP	7.5TSP	
BICARBONATE SODA 5TSP	0.5TSP	1.25TSP	2.5TSP	
BROWN SUGAR 2.0KG	200G	500G	1.0KG	
EGGS	1	2.5	5	10
MILK 3.30LT	330ML	825ML	1.65LT	
VEGETABLE OIL 800ML	80ML	200ML	400ML	
TINNED APPLE 2.0KG	200G	500G	1.0KG	

BANANA CHOC CHIP MUFFINS

QUANTITY	*100*	*200*
MILK	1LT	2LT
EGGS	10	20
VANILLA	2.5TBS	5TBS
MELTED MARGARI NE	500G	1.0KG
SELF RASINING FLOUR	2.0KG	4.0KG
WHITE SUGAR	500G	1.0KG
BANANAS	2.5KG	5.0KG
CINNAMON	12G	25G
NUTMEG	6G	12G

CHOC CHIP SLICE

QUANTITY	*150*	*200*
ROLLED OATS	60G	800G
PLAIN FLOUR	950G	1.27KG
CORNFLAKES	100G	135G
COCONUT	150G	200G

CHOC CHIPS	900G	1.2KG
BUTTER	775G	1035G
HONEY	1.0KG	1.335KG
SUGAR	200G	270G

MELT BUTTER & HONEY ADD TO DRY INGREDIENTS, MIX WELL. PRESS INTO PAN, BAKE

ANZAC SLICE WITH SULTANAS

QUANTITY	***120***
ROLED OATS	700G
PLAIN FLOUR	600G
SUGAR	350G
COCONUT	350G
SALT	1TSP
GOLDEN SYRUP	375G
BOCARBONATE SODA	20G
BOILING WATER	150G

RICE BUBBLE SLICE

QUANTITY	***150***	***200***
MARGARINE	450G	600G
MARSHMALLOWS	1.5KG	1.98KG
SOFT CARAMELS	625G	830G
RICE BUBBLES	30 CUPS	40 CUPS

MELT MARGARINE IN LARGE SUACEPAN OVER LOW HEAT. ADD CARAMELS & MARSHMALLOWS. STIR UNTIL COMPLETLEY MELTED, REMOVE FROM HEAT. ADD RICEBUBBLES STIR UNTIL WELL COATED. PRESS INTO PAN. ALLOW TO COOL. CUT INTO SQUARES OR FINGERS.

Appendix 4.1. Seasonal variations in Vitamin D intake from food in iu per day (SEM) and contribution of food groups to Vitamin D intake in elementary school females

	Vitamin D (iu.day^{-1})	
	Summer n = 653[2]	Winter n = 545
Milk Products		
- Fluid milk	194 (6)[e]	172 (6)[e]
- Cheese	5 (1)	4 (1)
- Yogurt	0	0
- Other	2 (1)	3 (1)
Vegetables & Fruit		
- Fruit	0	0
- Vegetables	2 (1)	4 (2)
Meat & Alternatives		
- Meat/Eggs	8 (1)	8 (1)
- Fish	7 (2)	7 (2)
Grain Products	9 (1)	7 (1)
Other Foods		
- Fats & Oils	5 (1)	6 (1)
- Sweets/snacks	5 (1)	5 (1)
- Combined dishes	15 (1)	18 (1)
Total Vitamin D (5 Food Groups)	253 (6)[d]	234 (6)[d]

[1] Pairs of values sharing same superscript (summer versus winter) are significantly different (d = $p < 0.05$; e = $p < 0.01$; f = $p < 0.001$)

[2] n represents the number of recalls

Appendix 6.1. Group characteristics, anthropometry, bone mass, body composition and dietary intake values for Asian and Caucasian pre- and early-pubescent girls at baseline and after 10 months of calcium supplementation (or placebo) and either high impact or low impact exercise.

	Asian (n = 10)			Caucasian (n = 56)		
	Baseline ± SE	Changes ± SE		Baseline ± SE	Changes ± SE	
		Absolute	Percent		Absolute	Percent
			Group Characteristics			
Age (yrs)	8.8 ± 0.4			8.8 ± 0.1		
Hi ex + Ca	3			13		
Hi ex + Pla	3			15		
Low ex + Ca	1			13		
Low ex + Pla	3			15		
Tanner stage 1-1	5			33		
Tanner stage 1-2	3			12		
Tanner stage 2-2	2			11		
WB exer (hr.day^{-1})	5.8 ± 1.0			7.1 ± 0.4		
			Anthropometry(cm)			
Height	131.1 ± 1.3	4.1 ± 0.4	3.1 ± 0.3	132.1 ± 1.1	3.9 ± 0.1	3.0 ± 0.1
Sitting height	70.1 ± 0.5	2.1 ± 0.4	3.0 ± 0.7	69.4 ± 0.5	1.7 ± 0.1	2.4 ± 0.1
Leg length	61.0 ± 1.0	2.3 ± 0.3	3.9 ± 0.4	62.7 ± 0.7	2.3 ± 0.1	3.7 ± 0.2
Femur length	31.5 ± 0.5	14.0 ± 1.4	4.4 ± 0.4	324.4 ± 3.6	14.9 ± 0.8	4.7 ± 0.3
Tibia Length	28.6 ± 0.4	1.2 ± 0.2	4.2 ± 0.6	29.8 ± 0.3	1.2 ± 0.1	3.9 ± 0.2
Arm length	46.2 ± 0.8	1.2 ± 0.1	2.5 ± 0.3	46.9 ± 0.5	1.3 ± 0.1	2.7 ± 0.2
Humerus length	26.3 ± 0.1	0.6 ± 0.1	2.1 ± 0.5	26.6 ± 0.3	0.6 ± 0.1	2.2 ± 0.3
Ulna length	20.0 ± 0.3	0.6 ± 0.1	3.1 ± 0.4	20.3 ± 0.2	0.7 ± 0.1	3.4 ± 0.3
			Bone mass (g)			
Total Body	992 ± 39	108 ± 11	11.0 ± 1.1	1068 ± 28	107 ± 6	10.1 ± 0.5
Lumbar spine	15.9 ± 0.7	2.0 ± 0.4	12.9 ± 2.9	16.5 ± 0.5	2.0 ± 0.2	12.1 ± 1.0
Leg	321 ± 21	54 ± 5	17.0 ± 1.3	360 ± 14	54 ± 3	15.4 ± 0.6
Femur	150 ± 9	26 ± 3	17.3 ± 1.2	168 ± 7	27 ± 2	16.1 ± 0.8
Tibia-fibula	125 ± 8	20 ± 2	16.4 ± 1.5	140 ± 6	19 ± 1	13.8 ± 0.7
Arm	93 ± 6	14 ± 2	16.0 ± 2.5	107 ± 3	12 ± 1	11.1 ± 0.7
Humerus	51 ± 3	7 ± 1	13.7 ± 2.0	58 ± 2	6 ± 0	10.3 ± 0.6
Ulna-radius	34 ± 2	5 ± 1	13.6 ± 1.8	38 ± 1	4 ± 0	9.8 ± 0.7
			Body composition (kg)			
Weight	27.6 ± 1.3	3.1 ± 0.4	11.0 ± 1.2	30.0 ± 0.9	2.6 ± 0.2	8.9 ± 0.7
Lean mass	19.8 ± 0.4	1.9 ± 0.2	9.5 ± 0.9	20.4 ± 0.4	1.6 ± 0.1	7.7 ± 0.4
Fat mass	6.1 ± 1.1	1.1 ± 0.3	17.6 ± 3.6	7.7 ± 0.6	1.1 ± 0.2	16.7 ± 2.3
Percent fat (%)	21.8 ± 2.8	1.1 ± 0.6	-	25.1 ± 1.1	1.3 ± 0.3	-
			Dietary intake			
Diet Ca (mg.day^{-1})	517 ± 90	-	-	776 ± 33	-	-
Protein (g.day^{-1})	57 ± 7	-	-	59 ± 2	-	-
Fat (g.day^{-1})	52 ± 7	-	-	58 ± 2	-	-
CHO (g.day^{-1})	204 ± 15	-	-	229 ± 10	-	-
Energy (kj.day^{-1})	6271 ± 524	-	-	6956 ± 221	-	-

† Unadjusted means (± SE) are presented.