The Impact of Diet and Lifestyle on Bone Health in the Elderly

A thesis presented in partial fulfillment of the
requirements for the degree of

Master of Science

In

Nutritional Science

At Massey University, Turitea,
Palmerston North
New Zealand.

Caroline Morva Stanley

2001

i

ABSTRACT

Osteoporosis is a multi-factorial disorder in which nutrition and lifestyle play an important role. There were two main reasons for doing this study. The purpose of the first part of the study was to determine the prevalence of nutritional and lifestyle factors related to bone health in a group of senior citizens from the Manawatu who were over the age of 70 years. This was followed by an intervention trial in which the purpose was to assess the impact of a single serve of a high-calcium milk on bone resorption using two ingestion strategies.

Calcium, magnesium and zinc intakes were below currently recommended levels in many of the study participants. Some of the participants and particularly those in rest homes received very little sunlight exposure and low dietary vitamin D. Participants spent an average of around 3.5 hours/day in physical activity. Weight-bearing activities such as walking, gardening and certain sports were common in this group. Institutionalised women were most compromised by nutrition and lifestyle. Dietary supplementation may therefore benefit many in this group.

Of the 52 participants in the diet and lifestyle study, a group of 28 women and 14 men volunteered to take part in an intervention trial, which was approved by the Massey University Human Ethics Committee. The mean calcium intake of trial participants was only 70% of the current US recommended adequate intake (AI). Each person consumed a supplementary serve of 250mls of high calcium milk (640mg Ca), every evening for two weeks. Half consumed a whole dose one hour before bedtime (single serve group), whilst the rest consumed the milk in three divided doses of 80mls every hour before bedtime (divided dose group). Free deoxypyridinoline (Dpd), a biochemical marker of bone resorption, was measured in urine that was collected overnight on two consecutive days before and after two weeks of milk intervention. In the single serve group Dpd was 4.15 ± 1.99 at the start and 3.94 ± 2.15 mmol/mmol creatinine after two weeks (NS). In the divided dose group Dpd was 4.25 ± 2.21 at the start and 4.79 ± 2.27 mmol/mmol creatinine after two weeks (NS). In conclusion, a supplementary serve of milk in this group of elderly people did not produce significant changes in urinary Dpd, whether the milk was consumed as the whole amount or in divided doses.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADI	Average daily intake
AI	Adequate intake
AM	Morning
B-ALP	Bone Alkaline Phosphatase
BMD	Bone mineral density
B-SP	Bone sialoprotein
Ca^{2+} or Ca	Calcium
CTx	C-telopeptide
d	Day
Dpd	Deoxypyridinoline
EAR	Estimated average requirement
FFQ	Food frequency questionnaire
Hr	Hour
HRT	Hormone Replacement Therapy
HYP	Hydroxyproline
IU	International units
M	Men
MoH	Ministry of Health
MP	Menopausal
mths	Months
NIH	National Institute of Health
NM	Not mentioned
NTx	N-telopeptide
Ocn	Osteocalcin
PM	Evening
PostM	Postmenopausal
PreM	Premenopausal
Pyd	Pyridinoline
R	Range
RDA	Recommended daily allowance

s.d.	Standard deviation
TI	Total intake
UK	United Kingdom
US	United States
VitD	Vitamin D
W	Women
Wks	Weeks
Y	Young
yrs	Years

1. LITERATURE REVIEW

1.1 OSTEOPOROSIS

1.1.1 Normal Bone Remodelling

The word skeleton comes from the Greek word '*skeletos*' meaning 'dried up'. However bone is a dynamic and living tissue that is continually being broken down and rebuilt in a process called remodelling (Shown in figure 1.1). Bone resorption occurs via special cells called osteoclasts, which create cavities, or lacunae in the bone. Cells called osteoblasts are then recruited to fill in the resorption cavity with bone matrix, which subsequently becomes mineralised. Figure 1.1 demonstrates the process of bone resorption within a single resorption lacunae, however overall bone remodelling represents a sum of many such units. The whole process of bone resorption and formation occurs over a period of several months and is vital for the normal growth and repair of bone. The relative amount of resorption and formation is known as the remodelling balance. During growth, remodelling balance is positive with formation exceeding resorption. In adulthood the balance is close to zero and in older age the balance becomes negative with resorption effectively outpacing formation.

Figure 1.1: The Bone Remodelling Cycle
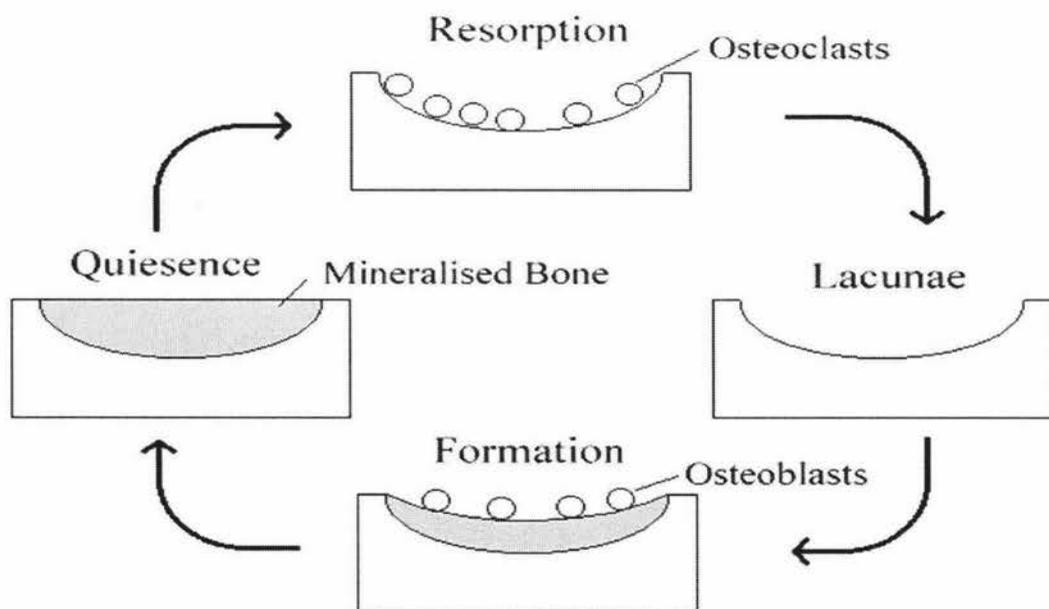
1.1.2 Definition and Pathophysiology of Osteoporosis

Osteoporosis was defined in the 1993 Consensus Development Conference statement as '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' (Consensus Development Conference, 1993). The World Health Organisation developed the working definition of osteoporosis as a bone mineral density 2.5 standard deviations below the mean peak value in young adults (Kanis *et al.* 1994, cited in Eastell, 1998). This criterion provides a useful tool for determining the prevalence of osteoporosis in populations. However proper diagnosis of the disease in individuals requires thorough examination of diet, medical, family and fracture history in addition to the presence of low bone mass.

The porosity of osteoporotic bone generally results from an imbalance in bone remodelling (Aloia, 1994). In osteoporosis the rate of bone resorption exceeds the rate of bone formation. Architectural integrity is compromised with every remodelling cycle as normal resorption cavities are made deeper or are incompletely filled by osteoblasts. In trabecular bones there is a reduction in the number of trabeculae struts and remaining struts become perforated and thinned causing the bones to become weak and break more easily (Twomey *et al.* 1983). Scanning electron micrographs reveal that broad connective trabecular plates within normal trabecular bone appear as thin rods in osteoporotic bone (Marcus, 1996). Demineralisation, accumulation of cement lines (produced by bone remodelling) and increased cortical porosity may also contribute to the weakening of aging bones (Marcus, 1996).

Fractures typically occur at the hip, spine and wrist although any bones can be affected (National Osteoporosis Foundation USA, 2000). The incidence of fractures increases after menopause, steadily at first and then exponentially in the elderly (Avioli, 1987, cited in Aloia, 1994). Fractures often have serious complications. Hospitalisation is commonly necessary after the occurrence of fractures particularly those of the hip or spine. Serious disabilities and even death are among some of the complications commonly associated with osteoporotic fractures of the hip or spine. In addition spinal fractures often lead to loss of height, chronic back pain and deformity.

The decreased bone mass associated with osteoporosis makes the elderly more susceptible to bone fracture. Osteoporosis and consequently bone fractures are especially prevalent in women as the dramatic decline in oestrogen levels, which follows menopause accelerates the age-related bone loss.

1.1.3 Epidemiology and Economic Burden

Osteoporosis is a prevalent condition in today's society and is an increasing health concern as the world's aging population expands. Most at risk are post menopausal women with over half of women experiencing some osteoporotic fracture in their lifetime (Reid, 1992). Elderly men also suffer from this condition, up to one-third of all men suffer from osteoporotic fractures (Cummings *et al.* 1989 & Jones *et al.* 1994 both cited in Center & Eisman, 1997). It follows then that there must exist a large economical burden on society due to the cost of hospital care for those suffering osteoporotic fractures.

1995 estimates of the annual cost to New Zealand to treat osteoporosis were $NZ 3.4 million (Lane, 1996). Direct costs related to osteoporotic hip fractures have been estimated at over $NZ 66 million (Lane, 1996). The incidence of fractures worldwide is large. Estimations of worldwide hip fracture rates in 1990 were 1.7 million with women sustaining the majority (1.2 million) (Center & Eisman, 1997). As the number of elderly worldwide is rapidly increasing the cost of osteoporosis to societies is expected to increase dramatically.

1.1.4 Aetiology

1.1.4.1 Age, Sex and Menopausal Status

Bone loss and subsequently fracture rate increases with age in both men and women (Twomey *et al.* 1983; Jones *et al.*, 1994). However the type of bone and years since menopause in women affect the pattern of bone deterioration. The exciting discovery of oestrogen receptors in bone cells suggested that oestrogen might have direct effects on bone. Mice lacking this oestrogen receptor were also found to have bone mineral densities 20 to 25% lower than their normal counterparts. Plasma

oestradiol and oestrone (derivatives of oestrogen), correlate well with rate of bone loss in both perimenopausal (Johnston *et al.* 1985a), and postmenopausal women (Cauley *et al.* 1986). It is commonly accepted that the oestrogen deficiency experienced by women following menopause is the major factor leading to the development of type I or postmenopausal osteoporosis.

In women bone loss is thought to have two components, one due to the hormonal changes experienced after menopause and the other to age. Oestrogen deficiency is largely responsible for the rapid bone loss experienced by women in the first 5-10 years following menopause while the exponential bone loss experienced by both men and late postmenopausal women is thought to be age-related. Each of these two factors act differently on different bones within the body. Trabecular bone is more sensitive to oestrogen deficiency than cortical bone thus fractures of bones with high trabecular to cortical ratios such as the vertebrae predominate in women during the first 20 years following menopause (Nordin *et al.* 1992; Center & Eisman, 1997). An exponential rise in hip fractures in those over the age of 65 years is thought to be the result of age-related bone loss which effects both trabecular and cortical bone (Nordin *et al.* 1990; Center & Eisman, 1997).

Whilst menopausal bone loss is largely the result of oestrogen withdrawal, several factors, including reduced physical activity, bone fatigue, accumulation of microfractures, poor diet and malabsorption of nutrients, may contribute to age-related bone loss. Serum parathyroid hormone, which correlates well with increases in bone turnover, also increases with age (Khosla *et al.* 1997). Long-term oestrogen therapy can eliminate the age-related increase in serum parathyroid hormone, indicating that it remains an important factor in bone loss even in older age.

1.1.4.2 Ethnicity/ Genetics

There are clear racial differences in peak bone mass, rate of bone loss and in fracture rate. In the US, African-Americans generally have higher bone mineral mass than those of Asian, Hispanic or Caucasian origins (Liel *et al.*1988; Luckey *et al.*1989; Oritz *et al.*1992; Bachrach *et al.* 1999). Caucasian women have greater incidence of hip fractures than either Asian or African-American women (Farmer et al. 1984). Japanese women have lower incidence of fracture than Caucasians despite lower body mass,

calcium intake and surprisingly lower bone mineral densities (Fujita & Fukase, 1992; Fujita, 1994). Women in other Asian countries such as Singapore, Hong Kong and China also experience this phenomenon (Lau & Woo, 1994). In a study by Kung *et al.* Chinese women were shown to absorb calcium more efficiently than Caucasian Americans (Kung *et al.* 1998). Racial differences such as this may help explain differences in osteoporosis epidemiology. In New Zealand, Maori have bone mineral densities 20% higher and fracture rates only half that of New Zealanders of Caucasian decent (Melton & Riggs, 1983, cited in Pollitzer & Anderson, 1989; Reid *et al.*1986, cited in Pollitzer & Anderson, 1989; Maggi *et al.*1991, cited in Lau & Woo, 1994). One of the few ethnicities with lower bone mineral content than Caucasians are the North Alaskan Inuit who have also been shown to lose bone at a faster rate than Caucasians in later life (Mazess & Mather, 1974)

Up to 60-80 % of population variability in peak bone mass is thought to be due to genetics (Pollitzer & Anderson, 1989). Twin and family studies have provided evidence for a genetic contribution. Similar peak bone size, mass and bone density has been demonstrated in mother-daughter pairs (Lutz, 1986; Lutz & Tesar, 1990; Matkovic *et al.* 1990). A paternal influence on these parameters has also been demonstrated in at least one study (Matkovic *et al.* 1990). In one large prospective study, a maternal history of hip fracture doubled the risk of hip fracture in women (Cummings *et al.* 1995). Markers of bone turnover have also been shown to correlate well in twin studies (Kelly *et al.* 1991; Tokita *et al,* 1994). Familial studies must be treated with caution as confounding factors such as shared environmental, nutritional and lifestyle factors may be present in such groups.

Whether or not genetics has an influence on bone loss is less clear. Lutz and Tesar (1990) found that the correlation between the bone mineral densities in mother daughter pairs was higher for premenopausal mothers and their daughters than for postmenopausal mothers and their daughters (Lutz & Tesar, 1990). This may indicate the existence of separate components for inheritance of peak bone mass and bone loss in later life. Alternatively it suggests that while there is a strong genetic influence on peak bone mass, postmenopausal bone loss may not be genetically determined.

Several gene loci involved in the determination of bone mass have been identified. The most promising gene loci so far has been one that codes for the vitamin D receptor (VDR). Specific forms of this gene have been associated with lower bone mineral density and increased risk of osteoporosis in populations (Morrison *et al.* 1994; Yamagata *et al.*1994; Spector *et al.* 1995; Ferrari *et al.* 1995; Matsuyama *et al.* 1995; Gennari *et al.* 1998). Not all studies have found significant associations between the BB genotype and bone mineral density however the small sample sizes used in these studies does not lend credit to their findings (Looney *et al.*1995; Lim *et al.* 1995). It is hoped that VDR genotypes may prove to be a useful tool in the prediction of osteoporosis, so that preventative therapy can be focused on those most at risk of developing the disease.

Some claim that the VDR gene may account for up to 75% of the genetic component for bone mineral density (Morrison *et al.*1994). However a plethora of other loci, such as oestrogen receptor genes and the collagen type I? 1 locus have also been shown to impact on bone mass. It is thought highly likely that several genes may interact in some way both with each other and with the environment to determine an individual's bone mass.

1.1.4.3 Physical Activity

It is generally well accepted that physical activity has a positive influence on bone mass. Cross-sectional studies have shown a positive relationship between historical levels of physical activity and bone area (Kriska *et al.*1988), bone density (Halioua & Anderson, 1989; Zhang *et al.*1992; Välimäki *et al.* 1994) and risk of hip fracture (Jaglal *et al.* 1993). Some case-control studies have also shown positive benefits of high impact exercise on bone mineral density and other selected factors associated with osteoporosis (Nelson *et al.* 1994; Heinonen *et al.* 1996).

Bone responds to the loading effect of weight-bearing exercise by increasing bone formation and bone mineral density according to the magnitude and frequency of mechanical stresses (Channay *et al.* 1972; Smith *et al.*1994). In the absence of such stresses reductions in bone mass are seen. Bed-rested patients and astronauts experiencing weightlessness in space both go into negative calcium balance and experience reductions in bone mass (Krølner *et al.*1983; Smith *et al.* 1998). Mechanical

loading of immobilised limbs in rats has been shown to prevent the decline in bone mass associated with disuse (Inman *et al.* 1999).

Because the level of strain determines changes in bone mineral density the type of exercise undertaken becomes important. Varied high-impact aerobic exercise produces the greatest effects on bone (Center & Eisman, 1997). Sports that involve jumping such as gymnastics are favourable while swimming is less beneficial due to the absence of loading forces (Snow, 1996 cited in Center & Eisman, 1997)

The effect of physical activity in reducing fracture risk is two-fold. The favourable architectural changes produced by loading the bones are accompanied by improvements in muscle mass and fitness. Increased muscle mass and fitness assist in fall prevention by improving balance, strength, concentration and coordination (Nelson *et al.* 1994; Province *et al.*1995; Heinonen *et al.*1996).

The benefits of physical activity on bone depend to some extent on the calcium intake of an individual. There exists a synergism between the effects of calcium and physical activity on bone whereby the additive effects of the two are greater when applied in combination. Cross-sectional retrospective studies have found that the greatest bone mineral densities occur in individuals who have adhered to an active lifestyle whilst maintaining an adequate calcium intake (Halioua & Anderson, 1989; Suleiman *et al.* 1997). Caution should be taken in assuming this from the study of Halioua & Anderson (1989) as they failed to produce groups where one factor was high while the other was low, thus creating difficulties in dissecting individual effects.

1.1.4.4 Body Weight

Greater body weight is associated with higher bone mass particularly at the weight-bearing sites of the skeleton (Bauer *et al.* 1993; Greenspan *et al.* 1994). Dietary and lifestyle interventions that result in weight loss are commonly associated with reduced bone mineral density (Compston *et al.*1992; Svensden *et al.* 1993;Ramsdale *et al.*1994; Salamone *et al.* 1999). Weight gain incurred over a lifetime confers considerable protection against fractures. The Study of Osteoporotic Fractures found that, in a large group of Caucasian women with mean age 72 years, a 20% increase in

weight since the age of 25 years translated into a 40% reduction in fracture risk (Cummings *et al.* 1995).

The relationship between body weight and bone mineral density is probably largely due to the direct loading effect of body weight on the skeleton. However the level of circulating estrogens are also likely to contribute. In addition to being secreted from the ovaries, oestrogen is also produced peripherally in the adipose tissue where it is formed via the aromatisation of androgens (Schindler *et al.* 1972; Bleau *et al.* 1974). Peripheral metabolism in adipose tissue becomes of increased importance after the menopause when oestrogen secretion from the ovaries is down regulated. Therefore compared with women of lower adiposity, women with greater adipose contents (fat mass) will have higher circulating levels of oestrogen, which is associated with a reduced loss of bone mass (Johnston *et al.* 1985a; Cauley *et al.* 1986).

1.1.4.5 Dietary Factors

1.1.4.5.1 Calcium Intake

There is strong evidence for the role of calcium in bone health and in the prevention of osteoporosis (See section 1.2.3). Hence nutritional factors that reduce calcium absorption or increase calcium excretion (see section 1.2.5) may also have adverse effects on the skeletal system. In this regard, via positive or negative influences on calcium status, vitamin D, protein, sodium, oxalates and oligosaccharides also have possible role in bone health

1.1.4.5.2 Vitamin D Status

Most of our body's vitamin D is formed when our skin is exposed to sunlight. Food is a relatively poor source of vitamin D and often contributes very little to total serum vitamin D levels. However food sources of vitamin D become of more significance when exposure to sun is limited. Thus it is especially important that vitamin D intakes from food are adequate in the wintertime, in extreme northern and southern latitudes and in the institutionalised and bedridden.

Severe vitamin D deficiency over a prolonged period leads to the development of osteomalacia, a condition characterised by bone pain, muscle weakness, fractures and

Looser zones. Osteomalacia is usually considered a rare condition restricted to specific risk groups such as those with malabsorption syndromes. It is now also recognised that the elderly and especially those who are bedridden or institutionalised, are also at risk from occult vitamin D deficiency leading to osteomalacia (Aaron *et al.* 1974; Johnston *et al.*1985b; Leboff *et al.* 1999). Sub-optimal levels of vitamin D or hypovitaminosis D, occur even more frequently and may also lead to compromised bone health and osteoporosis. A recent study found that hypovitaminosis D occurred in as many as 57% of medical inpatients (Thomous *et al.* 1998).

The metabolites of vitamin D are directly required for adequate calcium and phosphate absorption and as such are vital to maintain bone health (DeLuca *et al.*1988). When vitamin D is low the absorption of calcium will be reduced. Hypovitaminosis D also leads to the development of secondary hyperparathyroidism and increased bone resorption, which is likely to contribute to bone weakening and risk of fracture (Parfitt *et al.* 1982).

Bone mineral density has been positively correlated with serum 25-OHD (a metabolite of vitamin D) with differences in bone density between the highest and lowest tertile of vitamin D status being 5-10% (Villareal *et al.* 1991; Khaw *et al.*1992). Baker *et al.* (1979) observed that patients with femoral neck fractures had lower intakes of vitamin D than control subjects. Vitamin D supplementation has been shown to reduce bone loss from the femoral neck in postmenopausal women (Ooms *et al.* 1995; Dawson-Hughes *et al.* 1995).

Controlled trials of the effects of vitamin D metabolites on fracture rate have yielded conflicting results. Many vitamin D supplementation trials have suffered from small sample numbers, short duration and lack of proper controls, blinding and randomisation. Vitamin D supplementation has been shown to effectively reduce fracture rate in the elderly when administered alongside calcium [see section 1.2.3 for further discussion of these studies] (Chapuy *et al.* 1994; Dawson-Hughes *et al.* 1997). Another large randomised controlled study failed to find any effect of vitamin D supplementation (400 IU) alone on the rate of hip fracture in an elderly Dutch population (Lips *et al.* 1996). However baseline calcium intakes were much higher in this study and as such passive calcium absorption (occurs independently of vitamin D)

may have predominated. A quasi-randomised study where annual intramuscular vitamin D injections were given to an elderly Finnish population also failed to show a reduction in the number of hip fractures but found a reduction in the overall fracture rate (Heikinheimo *et al*. 1992, cited in Compston, 1998).

1.1.2.5.3 Magnesium and Zinc

As well as its presence in bone as part of the crystalline mineral, magnesium has an important role in bone metabolism and may be involved in bone mineral mobilisation via it's actions on parathyroid hormone, vitamin D and calcium (Linder, 1991). The role of the trace mineral zinc in bone health is less well defined. Zinc has been shown to stimulate bone growth and calcification in weaning rats (Yamaguchi & Yamaguchi, 1986 cited in Herzberg, 1990). Zinc is a co-factor for several enzymes involved in bone formation and remodelling including alkaline phosphatase (Johnstone *et al*. 1978 and Yamaguchi & Yamaguchi, 1986 cited in Herzberg, 1990). Zinc has been implicated in the pathogenesis of osteoporosis and fracture risk. Herzberg et al. found that urinary zinc excretion was elevated in subjects with osteoporosis compared with those with no osteoporosis (Herzberg *et al*. 1990). More recently low zinc intake was significantly associated with higher risk of fracture in men aged 46-68 years (Elmståhl *et al*. 1998).

Of some concern also is the interaction between calcium and zinc intakes. Calcium intakes of between 1200 and 1500 mg/day are recommended to reduce bone loss in old age (NIH Consensus Development Panel, 1994; Yates et al. 1998). However calcium intakes at this level have been shown to reduce zinc absorption efficiency in a group of postmenopausal women by around 20% (Wood & Zheng, 1997). Zinc supplementation was able to offset the reduction in zinc absorption and therefore it is important that zinc intakes are adequate when calcium intakes meet the recommended levels.

1.1.4.5.4 Retinol

A subject of considerable confusion for several years has been the unusually high incidence of osteoporosis in Northern Europe despite the high intakes of calcium in this region (Hegsted, 1986; Abelow *et al*. 1992 cited in Whiting, 1999). The confounding factor in this puzzle was recently identified as retinol [vitamin A] (Melhus *et al*. 1998). Animal studies investigating hypervitaminosis A demonstrate accelerated

bone loss, increased bone fragility and spontaneous fractures (Hathcock *et al.*1980). In addition vitamin A poisoning causes bone abnormalities such as impaired bone remodelling (Hathcock *et al.* 1980; Bendich *et al.* 1989). Scandinavian countries have vitamin A intakes up to six times higher than their Southern Europe neighbours (Melhus *et al.* 1998). A significant contributor to this high intake of vitamin A comes from dairy products, which are fortified with retinol in Northern European Countries. (Melhus *et al.*1998). As dairy products are also a major source of calcium it is clear to see where the confusion stems from. The inclusion of vitamin A in bone density analyses revealed a significant negative association between retinol intake and bone mineral density at all sites and incidence of hip fracture (Melhus *et al.* 1998).

1.1.4.5.5 General Dietary Patterns

There is a school of thought that broadening the research focus to include whole foods rather than just single nutrients may uncover new and interesting patterns. In whole foods combinations of nutrients may act synergistically and as nutrients are usually consumed in whole food forms this approach may provide answers, which can be more directly related to diet.

A positive association has recently been discovered between the intake of fruit and vegetables and bone health (Tucker *et al.* 1999; New *et al.*2000). Fruit and vegetables are naturally high in many vitamins and minerals. Positive associations linking the intake of zinc, magnesium, potassium, fibre and vitamin C with higher bone mass all provide plausible reasons for the beneficial effect of vegetables and fruit on bone health (Tucker *et al.*1999; New *et al.*2000). However the minerals thus far receiving the most attention are magnesium and potassium. This is due to the belief that these calcium salts function as an alkaline buffer for the acid load provided by components of a usual diet. The ingestion of alkaline-forming minerals and foods lowers the skeletal buffering load and therefore reduces the loss of bone mineral.

Another whole food that is also naturally high in magnesium and potassium is milk. Milk has frequently been associated with good bone health. In one recent intervention trial the consumption of three eight ounce servings of milk in addition to usual dietary intake reduced levels of circulating parathyroid hormone and N-telopeptide excretion, which are both indicative of reduced bone resorption (Heaney,

1999). The presence of magnesium and potassium may act in synergism with the high levels of calcium also naturally present in milk. Vitamin D is also often added to milk further enhancing its effect on bone. Milk may therefore represent a naturally occurring dietary preventative for bone loss and osteoporosis.

1.1.4.6 Cigarette Smoking, Alcohol Abuse and Caffeine Intake

In some, but not all, studies cigarette smoking, alcohol abuse and caffeine intake have been associated with negative effects on bone (Hernanda-Avila *et al.* 1991; Bauer *et al.* 1993; Burger *et al.*1998). It has been suggested that smoking exerts its effect on bone due to its bodyweight lowering properties, however a recent study has shown that smoking disturbs vitamin D status and calcium metabolism indicating a more direct effect (Brot *et al.* 1999). It is not known how caffeine affects bone and indeed it is thought that caffeine may just be a marker for other factors such as an unhealthy lifestyle. Alcohol may have direct toxic effects on osteoblasts or act indirectly via hypogonadism, however its mode of action is far from proven.

1.1.4.7 Summary

Osteoporosis is a crippling disorder, which imposes a growing economic and human cost on today's society. The aetiology of this disease is very complex and is still not completely understood. Genetics certainly plays the most significant part in determining peak bone mass and bone loss in later life. Bone loss increases with age, and more rapidly in women around the time of menopause, leaving the elderly and especially elderly women at risk of low bone mass and fractures. Excesses and deficits of certain nutrients may play an important role in obtaining and maintaining the maximal bone mass possible. Weight-bearing physical activity and a good nutritional status can lead to a greater peak bone mass and a reduced rate of bone loss in later years. Assessing the nutritional and lifestyle inadequacies in populations and using intervention strategies to improve them is one way that osteoporosis levels may be reduced. Those at greatest risk of low bone mass and osteoporotic fractures should be targeted for dietary, pharmaceutical and lifestyle intervention measures to minimise the impact of this disease in our society.

1.2 CALCIUM

1.2.1 Calcium's Role in Bone Physiology

Calcium is a major structural component of bone. Bone matrix comprises collagen and crystallized minerals of which tricalcium phosphate or hydroxyapatite is the greatest contributor (Tortora and Grabowski, 1996). Calcium salts are deposited in the bone between spaces in the collagen framework. They are subsequently crystallized, hardening the bone, in a process known as calcification or mineralisation. Consequently calcium has a strong influence on the hardness and density of bone.

1.2.2 The Calcium Requirement

An adequate intake of calcium throughout life is essential for the development and maintenance of a healthy skeleton. Dietary calcium is deposited in the bones and contributes significantly to the overall bone mass. It is vital that sufficient calcium be ingested during the first 2-3 decades of life in order to achieve the maximal peak bone mass possible. Bone calcium reaches a maximum between the ages of 35-45 years after which it begins to decline (Bronner & Pansu, 1999). It is thought that the decline in skeletal calcium and subsequent bone loss experienced during the aging process may result, at least in part, from inadequate calcium intakes and impaired absorption of calcium. Increasing dietary calcium levels throughout life may offset bone loss due to aging.

Bone contains around 99% of the body's calcium stores and has been referred to as the body's calcium bank. Concentrations of calcium outside of the skeleton are small and are kept at a relatively constant level via homeostatic mechanisms. When insufficient calcium is supplied by the diet bone resorption is increased to free the stores of calcium present in the bone. Long-term insufficiency of dietary calcium may therefore result in partially demineralised and subsequently weakened bones. Therefore it is vital that dietary calcium intake is sufficient to maintain blood homeostasis hence avoiding the need for bone resorption and subsequent loss of bone mass.

Because blood calcium is so tightly regulated, and is maintained even in the face of marked calcium deprivation, it cannot be used as a measure of calcium deficiency. Estimates of calcium requirement are therefore mainly derived from balance studies. In

addition, estimates of calcium requirement are often derived from research where changes or differences in bone density and fracture incidence are related to varying amounts of calcium intake or supplementation.

Research suggests that the optimal amount of calcium required to maintain bone calcium and slow bone loss in later life is considerably greater than the 1989 Australian recommended dietary intake (RDI) of 800mg/day for men and 1000mg/day for women aged 54 years and older. However calcium exhibits threshold behaviour (Matkovic & Heaney, 1992); that is, once a certain threshold intake is reached further increases in calcium intake will have no additional benefit. The calcium requirement should not therefore be set higher than this threshold. The exact threshold level is unknown but is probably around 1200-1500mg/day. Evidence from a balance study completed in the late 1970's indicated that zero calcium balance is achieved at a daily intake of around 1200 for middle aged women and at around 1500mg/day for older postmenopausal women (Heaney *et al.* 1977).

Most calcium supplementation trials have suggested that calcium intakes up to 1200-1500mg/day have beneficial effects on bone density and fracture risk in the elderly (Reid *et al*, 1993; 1995; Dawson-Hughes *et al*. 1997). However, Dawson-Hughes *et al*, 1993 found that increasing total calcium intake to levels over 900mg/day had no added benefit. In a recent study extrapolation of data found that in women more than 10 years menopausal the daily calcium intake required to halt bone loss at the hip was around 1700mg (Devine *et al*. 1995). This figure would have been lower if the women in the trial had sodium intakes closer to the current RDA (recommended daily allowance). As calcium excretion is increased by increases in sodium intake, women with lower sodium intakes would have achieved calcium balance is achieved at lower calcium intakes. With this in mind calcium requirement is likely to vary among individuals. As such Nordin proposed a 'sliding scale' model for calcium recommendations where the requirement is modified depending on other dietary components such as protein and sodium, which increase excretion of calcium (Nordin, 2000).

Calcium's role in the prevalence of osteoporosis in today's society may have an evolutionary basis. It is thought likely that primitive human diets contained far greater

amounts of calcium than present contemporary intakes (Heaney *et al.*1977; Eaton & Nelson, 1991). Stone age diets were abundant in calcium rich foods including roots, tubers, greens as well as insects (Heaney *et al.*1977; Eaton & Nelson, 1991). Consequently human physiology may well have adapted to a high dietary calcium intake unlike those seen today. This may explain why the calcium needs of today's society are often not being met by diet alone.

Recent changes in calcium recommendations reflect the findings of supplementation trials, balance studies and other evidence of a higher calcium requirement. In 1994 the National Institute of Health Conference on Optimal Calcium Intake recommended that all adults over the age of 65 should be consuming 1500mg of calcium per day (NIH Consensus Development Panel, 1994). The current USFDA recommendation for daily calcium intake in those over 51 years of age is 1200mg (Yates, *et al.* 1998). In New Zealand and Australia the RDA remains at 800mg/day for men and 1000mg/day for women over the age of 54 years. Whether or not the RDI should be higher than this is still a matter of some debate among scientists and nutritionists (Heaney, 2000, Specker, 2000).

1.2.3 Calcium Supplementation and BMD/ Fracture Incidence

Large cohort studies have found that an adequate calcium intake throughout life is associated with considerable protection against osteoporotic fractures (Matkovic *et al.* 1979; Holbrook *et al.*1988; Suleiman *et al.* 1997). In a cross-sectional study of 124 women calcium intakes greater than 700mg/day were associated with higher bone mineral density (Suleiman, *et al.* 1997). In another study carried out by Holbrook *et al.* examined the occurrence of hip fractures in 957 men and women aged 50-79 in relation to dietary information obtained by 24 hour recall 13 years earlier (Holbrook *et al.* 1988). An adequate calcium intake was associated with considerably reduced incidence of hip fractures in both men and women.

However cohort studies such as these are dogged by confounding factors and can only provide evidence of correlations not cause and effect. The most reliable information is obtained from randomised controlled clinical trials in which confounding variables are considered and the control groups are well matched. Clinical trials have shown that calcium supplementation, with and without concurrent vitamin D

supplementation, reduces bone loss and the incidence of fractures in postmenopausal women and men (Chapuy *et al.* 1992, 1994; Reid *et al.* 1993, 1995; Dawson-Hughes, *et al.* 1990, 1997). However not all studies have found significant improvements. The results of several studies investigating the effectiveness of calcium supplementation in the elderly are summarised in table 1.1.

Two factors which can have an impact on the outcome of calcium supplement trials are the baseline calcium intakes of the participants and, in women, the number of years since menopause. The pathogenesis of bone loss in postmenopausal and age-related osteoporosis may not be the same. Different processes or a combination of different processes may be acting in an individual depending on their age, postmenopausal status and sex. As a result early postmenopausal women might respond differently to treatment than men or older women. It has been suggested that during the first 3-5 years following menopause bone loss is due primarily to estrogen deficiency and consequently calcium supplements should be largely ineffective (O'Brien, 1998; Heaney, 2000).

Nilas *et al.* found that supplementation with 500mg of calcium had no effect on a group of early postmenopausal women with baseline calcium intakes between 550 and 1150 mg/day (Nilas et al, 1984, cited in Heaney, 1999). Dawson-Hughes *et al.* found that in the first 5 years after menopause women responded poorly to calcium supplementation of 500mg/day, while those 6 years or more postmenopausal showed significant improvements in bone health (Dawson-Hughes *et al.* 1990). However other studies using considerably higher levels of calcium supplementation (1000-1700mg/day) have found that calcium supplementation can be significantly effective in early postmenopausal women (Polley *et al.* 1987; Smith *et al.* 1989; Aloia *et al.* 1994). One study found larger improvements in women within 10 years of their menopause than in older women (Polley *et al.* 1987).

The lack of success of some calcium trials in early postmenopausal women does not necessarily indicate that calcium is ineffective in this group. The apparent failure of calcium supplementation in this group may have more to do with the overwhelming effect of oestrogen withdrawal on bone resorption. The comparatively tiny changes produced by calcium supplementation, and especially low dose calcium

supplementation, may be masked by the huge increases in bone resorption seen in early postmenopausal women. In men and in older women, malabsorption of nutrients, poor diet, sedentary lifestyle and limited sunlight exposure become more important in the pathology of bone loss. Improvements produced by calcium supplementation therefore become more significant and recognisable.

The baseline habitual calcium intake of participants has also been related to the success of calcium supplementation. In a trial conducted by Dawson-Hughes *et al.* only those participants who had a baseline calcium intake of less than 400mg showed significant improvements in bone density (Dawson-Hughes *et al.* 1990). Calcium supplementation was also found to have no effect in a group of men who already had intakes close to 1200mg/day. (Orwell *et al.* 1990 cited in Heaney, 2000). However other trials have found significant effects in groups of subjects with mean daily intakes of around 700mg (Dawson-Hughes *et al.* 1997; Reid *et al.* 1993). These findings are not surprising considering the nature of calcium absorption. The threshold behaviour that calcium exhibits (refer section 1.2.2) means that increasing calcium intake over a certain level will not produce additional benefit.

Table 1.1: Calcium Supplementation and BMD/Fracture Incidence

Reference	Subjects	Baseline Calcium Intake	Supplement dose intake	Duration	Results
Polley *et al.* 1987	PostM Women.		Dairy product to bring TI=1250mg with or w/out restricted sodium or 1000mg Ca-TI=1700mg	3 mths	? rate forearm bone loss in those <10 yrs PostM with either calcium or dairy products plus salt restriction but not with dairy products alone.
Dawson-Hughes *et al.* 1990	PostM Women	Half<400mg/d & Half>400mg/d	500mg /d	2 yrs	? bone loss in older Post M women with ADI of <400mg/d.
Reid *et al.* 1993	PostM Women (>3yrs).	750mg/d	1000mg/d	2yrs	? loss of total body & femoral BMD loss eliminated in trunk.
Reid *et al.* 1995	Same women in previous trial	750/mg/d	1000mg/d	Another 2 yrs. Total time=4yrs	Loss of total body BMD sustained. Benefit <in first yr Also ? Fracture rate.

Abbreviations:
PostM	postmenopausal	mths	months	yrs	years
TI	Total intake	ADI	average daily intake	BMD	Bone mineral density

Table 1.2: Supplementation Trials using Calcium and Vitamin D

Reference	Subjects	Baseline Calcium Intake	Supplement dose	Duration	Results
Chapuy et al. 1992	Older PostM women, 69-106 yrs old	511 ? 172 mg/d	1200mg/d Ca^{2+} +800 IU/d VitD.	18 mths	? femoral BMD (2.7%) ? hip fractures (43%) ? nonvertebral fractures (32%).
Chapuy et al. 1994	Same as previous study	Same as previous study	Extension of 1992 trial	Another 18 mths	Sustatined ? in hip and nonvertebral fractures
Dawson-Hughes et al. 1997	Men & Women 65 yrs +	730mg/d	500mg/d Ca^{2+} 700IU/d Vit D	3 yrs	? Total-body bone loss in both men and women ? osteoporotic fracture in women.

Abbreviations:
PostM postmenopausal   VitD Vitamin D   d day   mths months Ca^{2+} Calcium

The combination of calcium therapy with vitamin D supplements has yielded impressive results. In a large randomised-controlled study of older postmenopausal women Chapuy *et al*. demonstrated a 43% reduction in hip fractures and a 32% decline in total nonvertebral fractures (Chapuy *et al*. 1992). Dawson-Hughes *et al*. also demonstrated the effectiveness of this combination therapy, albeit less convincingly, in older women and men (Dawson-Hughes *et al*. 1997). However because the nutrients were only given in combination it is impossible to separate the beneficial effects of the two nutrients and to conclude whether the results are due to calcium or vitamin D alone or a combination effect.

1.2.4 Milk and Bone Health

In 1979 Matkovic *et al*. examined a Yugoslav population where the participants came from either a dairying or non-dairying region (Matkovic *et al*.1979). Those in the dairying region had significantly higher metacarpal bone cortical area and reduced incidence of femur fracture. Matkovic *et al*. claimed that the superior bone health in the dairying region was due to the population in this area having calcium intakes twice as high as in the non-dairying region. A similar effect was seen in dairy-consuming areas

of China (Hu *et al.* 1993) and in women with a history of high milk consumption (Murphy *et al*, 1994) although not all studies have shown positive relationships between historical dairy consumption and bone health (Feskanich *et al.* 1997).

Milk and many other dairy products are arguably the best dietary source of calcium. Not only do milk and dairy products contain high levels of calcium, they also contain other nutritional components that enhance its bioavailability such as lactose and sometimes added vitamin D (see sections 1.2.4.5.5 & 1.2.5.2.1). Furthermore milk and milk products are also good sources of magnesium, potassium and zinc intakes all of which have been shown to have positive effects on bone (see section 1.1.4.5.5).

Very few studies have examined the effect of milk and dairy products on parameters of bone health in controlled clinical trials. However a milk supplement of 831mg/day for one year was found to retard femoral bone loss in post-menopausal women. Another study found that milk supplementation (equivalent of 729mg/day) decreased urinary excretion of N-telopeptide, a marker of bone resorption, in 204 men and women aged between 55 and 85 years (Heaney *et al*, 1999). Therefore although further studies are required to produce convincing proof of a substantial benefit to bone health and fracture risk, it seems that favourable changes in skeletal metabolism are produced by increases in milk intake.

1.2.5. Calcium Absorption

1.2.5.1. Physiology of Calcium Absorption and the Role of Vitamin D

In the intestine, calcium absorption occurs both transcellularly via an active mechanism and paracellularly by passive means. The transcellular active process occurs in the duodenum and is up regulated when calcium intakes are low (Bronner, 1997). Low levels of calcium in the blood stimulate secretion of parathyroid hormone, a hormone synthesised by the parathyroid glands (Tortora and Grabowski, 1996). Parathyroid hormone acts to increases bone resorption causing the release of calcium into the blood. A second action of parathyroid hormone is to promote the formation of calcitriol, or 1, 25-dihydroxy vitamin D, which is the active hormone form of the vitamin. Active transport of calcium depends on the presence of calcitriol (Pansu *et al.*

1983). This biologically active form of vitamin is required for the biosynthesis of a calcium-binding protein, calbindin D_{9k} (Bronner, 1997). By binding calcium calbindin D_{9k} facilitates the movement of calcium across the brush border of the intestine (Bronner *et al.* 1986). As vitamin D is th precursor for active calcitriol, adequate vitamin D status is therefore a vitally important factor in calcium absorption especially when calcium intakes are low.

1.2.5.2 Dividing the Supplementary Dose

It is now generally accepted that the calcium is better absorbed when given in smaller doses. The inverse relationship between calcium absorption efficiency and calcium intake has been demonstrated both in animals (Cashman & Flynn, 1996) and in humans (Heaney *et al.* 1975). Several studies have demonstrated this relationship using various methods and formulated equations to describe the nature of the effect (De Grazia *et al.* 1965; Ireland & Fordtran, 1973; Heaney *et al.* 1975; Bronner *et al.* 1982). However the calcium doses used in these studies have been from non-food sources and were often not within levels ingested as part of a normal diet (Heaney *et al.* 1990). A precise definition of the relationship between calcium intake and fractional absorption in humans, using a natural nutrient source and calcium intakes typical of naturally ingested meals, had yet to be described.

In 1990 Heaney *et al.* investigated the effect of calcium load on its absorption in humans using milk with increasing calcium contents (Heaney *et al.* 1990). Fractional calcium absorption was measured by tracing a radioactively labelled milk calcium load, a method, which produces a reasonably accurate measure of absorption. Fractional calcium absorption was demonstrated to be higher when the doses of calcium consumed are smaller [see figure 1.2] (Heaney *et al.* 1990). Using the equations derived by Heaney *et al.* it can be shown that a single 500mg dose of calcium is absorbed from milk with 29% efficiency. When the same amount is given as three divided doses the absorption efficiency rises to approximately 40% (Heaney *et al.* 1990). This represents an additional 60mg of calcium absorbed.

Figure 1.2: The Effect of Calcium Load on Absorption Fraction (Adapted from Heaney et al. 1990).
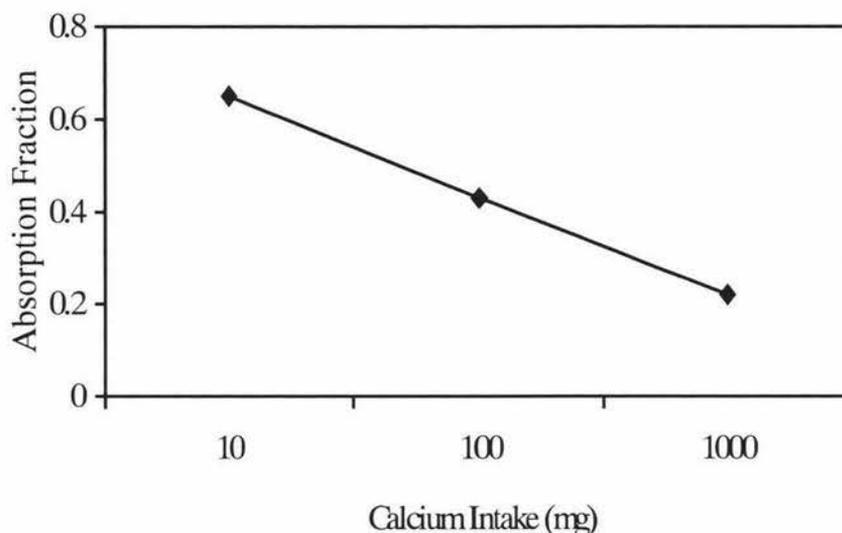
Although 60mg of calcium is not a large saving, when the effect of dividing the dose over a total day's calcium intake it may mean that less calcium need be ingested making it considerably easier to achieve the recommended daily amounts of calcium.

1.2.5.3 Evening Supplementation

Bone turnover exhibits a circadian pattern with levels of bone resorption peaking during the overnight fast (Eastell *et al.* 1992). The increase in bone resorption seen at night is even higher in osteoporotic women and this may contribute to the accelerated bone loss experienced by these women (Eastell *et al.* 1992). Oral calcium supplementation was shown to be more effective at reducing the overnight peak in bone resorption when given at bedtime rather than in the morning (Blumsohn *et al.* 1994). During the overnight fast serum calcium homeostasis is maintained by increased bone resorption and reduced urinary calcium excretion. Taking calcium supplements in the evening presumably reduces the need for calcium to be resorbed from bone stores. This effect is likely to be of even more value for osteoporotic subjects as they experience far smaller adjustments in calcium excretion and rely almost entirely on bone resorption to maintain their serum calcium level at night (Eastell *et al.* 1992).

1.2.5.4 Nutritional Factors Which Affect Calcium Absorption and Excretion

1.2.5.4.1 Milk Oligosaccharides

Lactose, a component of milk has been demonstrated to increase calcium absorption (Schutte *et al.* 1989). The increased bioavailability of calcium, and also related nutrients such as magnesium has been shown to be due to the certain soluble oligosaccharides, such as the hydrolysis products of lactose (Ohta *et al.*1995; van den Heuvel *et al.* 1999; Takahara *et al.* 2000). The effect of these oligosaccharides on gut viscosity may well be the mechanism by which they increase mineral absorption. By slowing the procession of gut contents, absorption time is increased.

1.2.5.4.2 Sodium Intake

High intakes of sodium chloride promote bone loss in animals (Goulding *et al.* 1983) and are considered a risk factor for osteoporosis in humans (Goulding *et al.*1981; Parfitt *et al.*1983). Calcium excretion is driven by, and occurs in proportion to, sodium excretion (Nordin *et al.* 1993; Devine *et al.* 1995). Therefore when sodium intake, and consequently sodium excretion, is high, calcium excretion will also be high. In a longitudinal study of 124 postmenopausal women dietary sodium was found to be a significant determinant of bone loss at both the hip and the ankle (Devine *et al.* 1995). A high calcium intake was able to counteract the negative effects of sodium on bone, however a reduction in sodium intake considerably lowered the amount of calcium required to achieve calcium balance and slow bone loss (Devine *et al.*1995).

1.2.5.4.3 Protein Intake

Studies examining the relationship between protein intake and bone loss have produced conflicting results. Increases in protein intake produce parallel increases in calcium excretion (Kim *et al.* 1979; Schutte *et al.*1980; Pannemans *et al.*1997). For every 50g increase in protein intake a further 60mg of calcium is excreted (Kerstetter & Allen, 1994 cited in Kerstetter *et al.* 1998). A high protein intake may therefore cause increased bone resorption and weaken bones. However protein intakes in New Zealand are typically between 60 and 80mg/day and an increase of 50g protein would represent a huge increase in intake that is extremely unlikely to occur. In the unlikely event that protein intake were to increase substantially a loss of 60mg of calcium does not

represent a hugely significant amount when average intakes are around 700-800mg.day in New Zealand elderly. Indeed restricting protein intake may be just as detrimental to bone health as low protein intakes have also been associated with hyperparathyroidism and reduced calcium absorption. (Kerstetter *et al*.1998).

1.2.6 Summary

Calcium is an important nutrient for bone health. Research suggests that the amount of calcium required to maintain bone mass and minimise bone loss in the elderly is around 1200mg/day. Supplementation trials have demonstrated the effectiveness of calcium in slowing bone loss, and reducing the incidence of fractures. Milk is an excellent dietary source of calcium both quantitatively and qualitatively and may offer an excellent supplement for dietary intervention. The efficiency of calcium absorption from such a supplement is improved when supplements are taken in divided amount and in the evening. These are important strategies to consider when trying to optimise calcium treatment. The absorption of calcium can be altered by several nutritional factors. Some of the oligonucleotides in milk such as lactose enhance the absorption of calcium while protein and sodium have a negative impact by increasing calcium excretion. However being a threshold nutrient, calcium is only beneficial to those who do not already receive enough of it in their regular diets.

1.3 DIETARY INTAKE OF THE ELDERLY

1.3.1 Special Dietary Needs of the Elderly Concerning Bone Health

1.3.1.1 Energy Intake

As people age there is a tendency towards a more sedentary lifestyle in Western countries. Reduced physical activity lead to the decline in muscle mass and increment in fat tissue often seen in the elderly (Evans & Campbell, 1993). Less muscle mass also means a decline in metabolic rate and subsequently a smaller appetite and reduced energy requirement. Although the energy needs of the elderly are lower than in young adults, micronutrient requirements mostly remain unchanged or are elevated (Ministry of Health, 1996). As a result the elderly may find it increasingly difficult to achieve the recommended daily intakes of micronutrients. Therefore the nutrient density of the older persons diet becomes of great importance.

1.3.1.2 Calcium

An adequate calcium intake is important to minimise bone loss in later life. The calcium requirement of the elderly may be greater due to defective calcium absorption. A fall in calcium absorption is seen around the age of 55-60 in women and 65-70 in men (Bullamore *et al.* 1970; Bouillion *et al.*1997). Declining gastrointestinal function may be responsible for the reduced calcium absorption with age however some suspect that inadequate levels of vitamin D may play a role. The ability to adapt to a low calcium diet may also be impaired in the elderly (Bouillon *et al.*1997).

1.3.1.3 Vitamin D

As people age it becomes increasingly difficult to maintain adequate vitamin D status. As mobility declines exposure to sunlight becomes limited especially in those who are housebound or institutionalised. Excessive use of sunscreens, which are recommended for the prevention of skin cancer, can further reduce exposure to the ultraviolet light required for vitamin D synthesis in the skin (Matsuoka *et al.* 1987). In a recent study of a group of elderly New Zealanders living in an Auckland aged care-facility, limited sun exposure and poor mobility were the strongest predictors of poor

vitamin D status (Ley *et al.*1999). The main reasons given for limiting sun exposure included concerns about skin cancer, the risk of colds or pneumonia, fear of falling and many excursions occurring mainly undercover (Ley *et al.* 1999).

The skin of older persons has an impaired ability to synthesise the vitamin D precursor cholecalciferol (Holick *et al.*1989). Holick *et al.* exposed both elderly and young people to the same amount of simulated sunlight and measured the level of serum vitamin D produced. Although the sample number was very small (only 6 elderly subjects) the difference in vitamin D production was marked. The young volunteers (aged 22-30) were able to produce a maximum serum vitamin D concentration of 78nmol/L while the elderly group (aged 62-80) could only achieve a maximum of around 21nmol/L (Holick *et al.* 1989).

Further exacerbating the problem, kidney responsiveness to parathyroid hormone for the synthesis of active vitamin D is reduced with advancing age (Dandona *et al.*1986). Moreover at latitudes of about 40 degrees north or south and higher, which includes Auckland, New Zealand, very little vitamin D is produced by the skin in winter (Utiger, 1998). It is therefore not suprising that hypovitaminosis D is a common problem in the elderly and causes concern as to the ability of elderly to absorb calcium efficiently in order to maintain bone health.

1.3.1.4 Other Factors which affect Nutrient Intake in the elderly

There are several other factors related to advancing age that may affect nutrient intake. Olfactory (smell) and gustatory (taste) sensations gradually decline with age. Reductions in the senses of taste and smell may result in food seeming dull and bland. As a consequence the elderly may eat less or eat foods that are high in sugar and salt but have little nutritional value. In a review by Horwath poor dentition and oral health, poverty, social isolation, loneliness and depression were listed as other factors commonly responsible for poor dietary intakes in the elderly (Horwath, 1989).

1.3.2 Habitual Dietary Intake of Elderly New Zealanders

1.3.2.1 Habitual Intake of Calcium

Dietary survey studies performed in New Zealand have indicated that many New Zealand elderly, both men and women have calcium intakes which are well below the current United States (US) recommended adequate intake of 1200mg (see table 1.3.1). Over the last ten years estimates of calcium intake have ranged from 500-855mg in women and from 600-890mg in men. Follow-up of a population registered with the Mosgiel Health Centre in Dunedin, New Zealand indicated a trend for increasing calcium intakes over the last ten years (Fernyhough *et al.* 1999). Data from National Nutrition Surveys also supports a trend towards an increased calcium intake in those over the age of 65 years (Hillary Commission, 1991; MoH, 1999).

Table 1.3: Studies Measuring the Calcium Intake of Elderly New Zealanders in the Last 10 years

Author/ Year/ Location	Age Group	Sample No.	Questionnaire type	Mean Ca Intake (mg/d)		Current RDI & % Below RDI
				Women	Men	
Horwarth, 1990, Dunedin, Otago	W=54-84; M=60-86 ? age =70yrs	W=29 M=24	2-d diet records ? 5	**771;** R= 462-1580	**888;** R= 407-1720	800mg; , W=17%, M=14%[1]
Hillary Commission, 1989/1990/ National survey	65+		24-Hr Diet Recall	**500**	**600**	
Sharpe et al. 1994,	PostM W 45-71yrs	140	4-d diet records ? 3	**700**		86% below 1000mg
Upritchard & Ball, 1996, Otago	50-65	247	FFQ	**754**		
Fernyhough et al. 1999, Dunedin, Otago	70+	W=167 M=81	FFQ	'88/'89= **788;** '95/'96= **855**	'88/'89= **825;** '95/'96= **890**	
National Nutrition Survey, New Zealand Food: New Zealand People, 1997	65+		24-Hr Recall	**636**	**751**	

Abbreviations:
W = women   yrs = years   R = range   PostM = postmenopausal   Ca Calcium
M = men   d = day   Hr = hour   FFQ = food frequency questionnaire

The large variation in estimates provided by the different surveys are probably due to a number of factors. Firstly, the populations being surveyed vary and may not all be representative of New Zealand's elderly population. Secondly, dietary assessment methods cannot be assumed to be equal. Each different method of dietary assessment is associated with its own strengths and weaknesses and the results must be interpreted with these in mind. The largest and perhaps most representative studies of the intake of elderly New Zealanders' are the National Nutrition Surveys. These large surveys have employed the 24-hour recall as their method of assessing dietary intake. The 24-hour recall often encounters the problem known as the 'flat slope syndrome' in which the interviewee 'talks' a good diet so that those foods which they perceive as 'good' are overestimated and those they perceive as 'bad' are underestimated (Thompson & Byers,

---

[1] Percentage below 2/3 of the RDA

1994). As they are usually conducted the day after the foods are consumed they are also susceptible to the under-reporting of foods due to memory failure, a problem especially pertinent in elderly groups.

Several of the other studies have employed a food frequency questionnaire. Food frequency questionnaires consist of a list of foods/ food groups. The participant is asked to give the amount of each listed item that they have consumed during each day. The accuracy of food frequency questionnaires largely depends on their level of detail, that is, the number of foods/ food groups described in the survey. While short lists tend to underestimate nutrient intakes, long lists may result in overestimation (Thompson & Byers, 1994). For these reasons food frequency questionnaires are best used to determine approximate nutrient intakes and are better employed as a tool for ranking individuals than for estimating intake levels.

Multiple diet records are considered the 'gold standard' of dietary assessment although these require greater participant motivation and can lead to sample bias as participants must be highly motivated and literate to perform them. In addition as diet records are performed the day of food consumption there is a temptation for participants to alter their diet to produce a more 'favourable' outcome. The group studied by Horwarth (1990) is likely to be a biased group. The participants were all members of a group aiding the local Medical Research Foundation as volunteers for a study on aging. As such it is likely that the participants would share other common interests and lifestyles and may not be representative of the New Zealand population as a whole. The comparatively small sample used in this study is also disadvantageous. Providing the sample is randomly selected, the larger a group is the more representative and accurate the data will be as a whole.

1.3.2.2. Habitual Intake of Other Nutrients Important in Bone Health

In the Auckland-based study of people aged 70 and older, dietary contributions of vitamin D was well below the recommended 15?g [mean intakes of around 3?g in men and 2.5?g in women] (Fernyhough *et al.* 1999). Ordinarily low dietary intakes of vitamin D are more than compensated for by that produced during sunlight exposure. However as this age group have reduced ability produce vitamin D via this route, and

may also spend little time outdoors (see section 1.3.1.3), dietary sources of the vitamin are of greater relevance than they are for younger people. A recent study of thirty-nine elderly New Zealanders living in an Auckland aged-care facility the presence of frank hypovitaminosis was found to be 49% in midwinter and 33% in midsummer (Ley *et al.* 1999). This finding mimics findings overseas, where hypovitaminosis in the very old has been a common finding (Thomous *et al.* 1998; Warden, 1998). Although the mean age of Ley's group was 80 years, most of the participants were able to go outdoors unassisted (Ley *et al.* 1999). The author of the study suggests that hypovitaminosis D may be normalised by 15-20 minutes of daily sun exposure. For those unable to venture outdoors without assistance, vitamin D supplementation may be required to maintain normal levels.

Other important nutrients commonly inadequate in the diet of elderly are magnesium and zinc (Fernyhough *et al.* 1999; Horwath, 1990; Ministry of Health, 1999). Zinc deficiency has been reported to be as high as 24% in a population of elderly women from Dunedin (Horwath, 1990). The percentage of men with deficient intakes was reported to be 33% although the author makes reference to Australian estimated requirement of 15mg and not the lower recommended dietary allowance (RDA) of 12 mg/day (Horwath, 1990). Fernyhough *et al.* 1999 found that the mean zinc intake was below the Australian RDA (12mg) in both men (9.2) and women (8.3) in their Auckland population, but the percentage of inadequate intake was not reported. In the most recent National Dietary Survey the greatest level of zinc inadequacy was in the 65+ age group. (Ministry of Health, 1999) This was quoted as being only 4.8% but the value used to determine adequacy of nutrient intake was the Estimated Average Requirement (EAR) taken from the UK Dietary Reference Values (1991) (Ministry of Health, 1999). The EAR used, 7.3mg for males and 5.5mg for females is much lower than the current Australian RDA of 12mg for both men and women. They conclude that only a very small proportion of New Zealanders are at risk of zinc inadequacy. However zinc inadequacy may have been more predominant had they used the RDA as a cutoff not the EAR.

1.3.3 Summary

The elderly have special requirements for some nutrients including calcium and vitamin D. The reduced energy intake that commonly accompanies aging however means that it becomes increasingly difficult to meet dietary needs. Few large studies have examined nutrient intakes in New Zealand elderly. Average intakes of calcium found in New Zealand studies are frequently well below the current US recommended adequate intake (AI) for calcium. Magnesium and zinc are among other nutrients often found to be inadequately supplied in the diets of New Zealand elderly. Hypovitaminosis D may be a common problem among our elderly with the institutionalised and bed ridden at increased risk due to sufficient exposure to sunlight. Reduced levels of physical activity also lead to lower energy requirements, reducing appetite further and making it increasingly important the diet of elderly is as nutrient dense as possible.

1.4 PHYSICAL ACTIVITY IN THE ELDERLY

1.4.1 Benefits of Physical Activity in the Elderly

Sarcopenia or muscle wasting is a common condition associated with aging. Reduced muscle mass leads to declines in strength, which contributes significantly to disability in the elderly (Frontera *et al.* 1991; Mazzeo *et al.* 1998). A sedentary lifestyle contributes to muscle wasting and bone loss, while increasing physical activity levels increases bone mass and reduces the risk of falling through promoting muscle development and physical fitness (see section 1.1.4.3). Increasing strength and endurance exercises can reduce or prevent the decline in muscle mass seen in old age and may even help rebuild to lost muscle mass (Buchner *et al.*1992 cited in Ministry of Health (MoH), 1996; Fiatarone *et al.*1995). Even the very frail and older elderly may obtain such benefits (Fiatarone *et al.*1995).

Greater energy expenditure and an increased metabolic rate achieved through enhanced physical activity may also improve appetite. Improved appetite may lead to greater energy intakes. Larger intakes of food will increase the ability of the elderly to achieve recommended daily intakes of nutrients thus improving nutritional status, further enhancing health. Moreover many of the symptoms of diseases commonly seen in the elderly such as cardiovascular problems, diabetes and obesity may benefit from increased physical activity.

1.4.2 Habitual Physical Activity of New Zealand Elderly

Information concerning the physical activity habits of elderly New Zealanders' is very limited. Data from a large Auckland-based study indicates that inactivity is common among the elderly with around 40% of men and women over the age of 60 years not participating in any leisure-time physical activity and approximately 6% not participating in any physical activity (Galgali *et al.*1998). Physical activity was shown to decline with age, and women were less likely to be physically active than men were. Hours spent on feet per day ranged from around two hours per day to seven hours per day. The mean number of hours spent on feet per day was less for those living in

institutions (2.41 hours/day) and declined with age, from age 60 onwards (2.49 hours/day for those over 90 years).

In the 1996/1997 New Zealand Health Survey an active individual was defined as one who had taken part in 2.5 hours or more of leisure-time physical activity in the seven days before the interview (MoH, 1999). Physical activity was lowest in the oldest age group (75+), however the 65-74 age group had the highest percentage of physically active people. Although the level of physically active people in the 75+ age group was reported as being over half the group (53%), 47% were regarded as being sedentary, indicating that inactivity is very common amongst this age group.

The observation that the 65-74 years age- group had the highest percentage of physically active individuals and were comparably and even more physically active than the youngest group (aged 15-24 years) was interesting to note (MoH, 1999). The majority of this group would be retired and as such the amount of time they have available for leisure activities would be larger.

The steep decline in activity seen between the 65-74 year old group and the 75+ age group may reflect the prevalence of disease conditions and disability in the very old that can lead to the cessation of previously undertaken leisure activities. For example, level of physical activity was strongly associated with a person's self-rated health status (MoH, 1999). In addition those who were sedentary were more likely to have been admitted to hospital in the last year than those in the active category. Whether the lack of exercise was a direct result of, or the cause of, hospitalisation could not be determined from the data collected although it was presumed likely that both may have been contributing factors (MoH, 1999). In the Auckland-based study it was found that the feeling of being 'physically unable to exercise' became more prevalent with increasing age (Galgali *et al.* 1998). In addition the 1991 LINZ (Life in New Zealand) survey conducted by the Hillary Commission found that the desire to be more active and the perceived benefits of activity decline in the elderly (Hillary Commission for Recreation and Sport, 1991).

There were also gender differences in physical activity. Older women were found to be less active than older men (Galgali *et al.* 1998). In the 75+ age group

women were also seen to be less active than men, however in both the 45-64 and 65-74 age groups women were more active than their male counterparts. As on average women live longer than men do there are probably more women in the 85+ bracket of the 75+ group. As the 75+ group is not sub-divided any further however it is impossible to tell if this is the case. In addition the increased prevalence of osteoporosis in women may contribute to the lowered activity of women in this age group. This inconsistency may be due to the higher proportion of all men in full-time employment. Assuming that retirement or unemployment enables an individual more time for leisure, the sudden rise in activity in later life for men as opposed to women may be indicative of the proportionally larger impact of retirement in this gender.

1.4.3 Summary

An adequate level of physical activity is instrumental in obtaining and maintaining good health even in old age. Benefits include increases in the mass and strength of both muscle and bone, greater energy expenditure, and reduced risk of several diseases. Despite the apparent advantages of an active lifestyle it appears that many New Zealand elderly lead sedentary lifestyles. However it is not clear how greater part illness and the inability to exercise plays in these statistics. The number of New Zealand studies which define physical activity in this age group are limited and further, more detailed studies are required in order for the reasons and possible solutions to inactivity can be ascertained.

1.5 BIOCHEMICAL MARKERS IN THE ASSESSMENT OF BONE TURNOVER

1.5.1 Introduction to Biochemical Markers of Bone Resorption

The assessment of bone disease previously relied solely on techniques such as radiography, bone scans, and bone biopsies. Whilst measurements of bone density achieved through radiography and bone scans are vital in the diagnosis of bone disease, they offer no information on the precise balance of bone metabolism. Bone biopsies are an invasive procedure and as such cannot be routinely utilised in human studies. Bone density measurements are still commonly used in the assessment of clinical or dietary treatments in clinical trials. Measurements of bone density and fracture risk still have an important role in the assessment of therapies as they are probably the most convincing evidence of the effectiveness of a treatment. However the 2-3 years required to observe significant changes in bone density slows the monitoring of pharmaceutical therapies and other interventions. The more recent introduction of bone turnover markers however provides a faster way of obtaining answers. A few biochemical assays for markers such as urinary and serum calcium, serum alkaline phosphatase and urinary hydroxyproline were available, and although these are still used, their lack of specificity for bone makes changes in bone turnover difficult to assess accurately.

The discovery of new, highly specific biochemical markers of bone metabolism is relatively recent and has opened new doors for bone research. These 'bone markers' are enzymes, molecules or hormones that are closely associated in some way with either the formation or resorption of bone. Biochemical bone markers can indicate changes occurring over much shorter periods than measurements of bone density. Because calcium and anti-resorptive treatments such as oestrogen therapy act on the start of remodelling sites i.e. the resorption process, changes in markers of bone resorption show earlier changes than markers of bone formation. Markers of bone resorption can detect changes within hours and bone formation markers after 6-9 months, thus representing a much quicker method of obtaining information about bone losses (Christenson, 1997; Rubinacci *et al.* 1996). This means that clinical trials that screen

for potential treatments can be completed in much shorter time periods, thus not only speeding up scientific advancement but making the research of diseases such as osteoporosis more economical and accessible.

Biochemical markers of bone turnover are classified based on the phase of the bone remodelling cycle (see section 1.1.1), to which they relate. Bone formation involves osteoblast cells building collagen fibres from procollagen molecules. The terminal ends of each procollagen molecule are enzymatically 'clipped' just before being incorporated into the collagen molecule. These terminal ends more properly known as procollagen I extension peptides and termed the procollagen I carboxyterminal propeptide (PICP) and the procollagen I aminoterminal propeptide (PIAP), according to which end of the molecule they were clipped from, are released into circulation during bone formation (Christenson, 1997). Levels of these extension peptides in the circulation can therefore be measured to be used as indicators (or markers) of the level of bone formation. As part of the formation process active osteoblasts also secrete the enzyme bone-specific-alkaline phosphatase (B-ALP) and the peptide osteocalcin. The levels of these in the circulation can also be measured and used as markers of bone formation.

In the same way products of collagen breakdown, which are released into the circulation during bone resorption, and enzymes involved in the process, can be used as markers for bone resorption. Markers of bone resorption include urinary calcium, the enzyme acid phosphatase, hydroxyproline, N- and C-telopeptide fragments of type I collagen (NTx and CTx), and pyridinium cross-links. (Christenson, 1997). Again the specificity and sensitivity of these markers vary, especially when the molecule exists in tissues other than bone collagen [hydroxyproline] or in food [calcium and hydroxyproline] (Christenson, 1997; Garnero & Delmas, 1997). The more recent bone markers such as osteocalcin, procollagen extension peptides and pyridinium cross-links are more bone specific thereby representing a more precise instrument, which gives better credibility to studies of bone turnover.

1.5.2 Pyridinium Cross-links

1.5.2.1 Biochemical Background of Pyridinium Cross-links

Figure 1.3: Diagram of Collagen showing the Pyridinium Cross-links Between Adjacent Collagen Fibres.
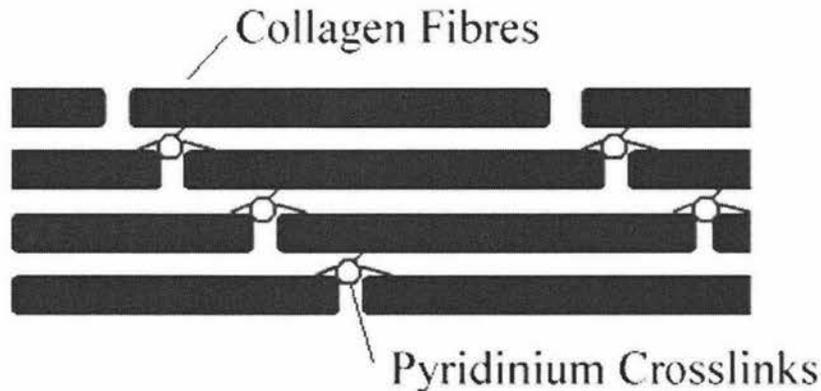
The 3-hydroxypyridinium derived amino acids, pyridinoline and deoxy-pyridinoline, (also called hydroxylyslpyridinoline and lyslpyridinoline respectively) form non-reducible cross-links in the mature form of collagen [see figure 1.3] (Delmas, 1990). Pyridinoline and deoxypyridinoline have similar structures that differ only by the presence of a single hydroxyl group. The covalent links form between adjacent collagen chains via a reaction catalysed by the enzyme lysl oxidase, which results in aldehyde formation from the lysine and hydroxylysine side-chains (Eyre, 1984b). These inter-chain bonds, which occur at the terminal telopeptide regions of the collagen chain, stabilize the whole collagen molecule within the extracellular matrix (Garnero & Delmas, 1997; Delmas, 1990).

Pyridinium cross-links are unique to collagen and elastin and the two forms are present in varying amounts in different types of collagen (Delmas, 1990; Black *et al*, 1988). Pyridinoline cross-links occur in several types of collagen, including tendon, cartilage, dentin, aorta and bone (Delmas, 1990; Black *et al*, 1988). However deoxypyridinoline is less widespread and occurs in significant amounts only in the type I collagen of bone (Delmas, 1990). Therefore bone collagen has an unusually high deoxypyridinoline: pyridinoline ratio (Eyre *et al*, 1984).

36

1.5.2.2 Pyridinium Cross-links as Bone Markers

As pyridinium cross-links are non-reducible they cannot be recycled during collagen turnover and are released into the bloodstream after collagen proteolysis (Garnero & Delmas, 1997). As a consequence the cross-links are excreted in the urine where they are present both as free amino acids (40%), and bound to peptides (60%) (Garnero & Delmas, 1997). Although collagen turnover is very slow in other forms of collagen it is continuously remodeled in bone collagen and consequently has a high turnover in this tissue (Erikson et al, 1996; Eyre, 1992). Consequently it can be assumed that the majority of pyridinium cross-links present in urine are bone-derived with only a very small percentage coming from other sources (Eyre, 1992; Erickson et al, 1996). Deoxypyridinoline is considered even more bone specific as it is virtually absent in all other collagen types (Erikson et al, 1996).

Pyridinium cross-links offer many advantages over hydroxyproline, which until recently was the principal marker of bone resorption. Firstly pyridinium cross-links, and in particular deoxypyridinoline, are more specific for bone as hydroxyproline is present in all types of collagen (Garnero & Delmas, 1997; Bettica et al, 1996). Also unlike hydroxyproline, pyridinium cross-links are not absorbed in the gut and consequently they are not affected by diet (Bettica et al, 1996; Garnero & Delmas, 1997). This removes the need for food restriction prior to sampling.

Hydroxyproline is highly metabolised within the body. (Garnero & Delmas, 1997). This means that the hydroxyproline measured is not a direct measure of hydroxyproline turnover. It is commonly believed that owing to their molecular structure, pyridinium cross-links are not metabolised within the body which means that urine pyridinium cross-link content is a direct measure of the mass of collagen resorbed (Bettica et al, 1996; Eyre et al, 1988). Furthermore pyridinium cross-links exist only in the mature form of collagen. As a result newly synthesized collagen molecules, which have not been integrated into the extracellular collagen fibrils, cannot contribute to the marker measurement (Eyre et al, 1988). In addition the more recent development of an enzyme-linked-immunoassay (ELISA) specific for pyridinium cross-links provides a more sensitive and convenient means of measurement than the more expensive and labour-intensive HPLC method (Seyedin et al, 1993; Robins et al, 1994). Therefore pyridinium cross-links are chosen as bone resorption markers because they represent a

far superior method for analysing bone turnover then previously used markers such as hydroxyproline.

1.5.2.3 Relationship of Pyridinium Cross-links to Age, Gender, Menopausal Status and in Osteoporosis

Several studies have demonstrated that markers of bone resorption are more sensitive than bone formation markers for distinguishing between normal and osteoporotic patients (Kushida *et al*, 1995; Garnero *et al*, 1984b). This is likely to be due to the increased ratio of bone resorption; bone formation seen in this condition. Of all the resorption markers total urinary pyridinium cross-links have been cited to be the superior marker in the clinical assessment of osteoporosis (Garnero & Delmas, 1997). However with the development of new markers this may now not be true. Pyridinoline and deoxypyridinoline are significantly increased in osteoporotic patients (Bettica *et al*, 1996; Garnero *et al*, 1994). Pyridinium cross-links and especially levels of free pyridinoline measured by ELISA have been shown to have high t- and z- scores (measures of statistical significance), when used to compare normal and osteoporotic patients (Kushida *et al*, 1995; Garnero *et al*, 1984b). In addition pyridinium cross-links have been shown to be much more sensitive than hydroxyproline, previously the only available marker of bone resorption (as discussed in section 1.5.2.2).

Men have higher excretion rates of bone resorption markers than women (Green, 2001). It is thought that the rate of bone resorption may be a function of skeletal size as well as rate of bone turnover and, as men generally have larger skeletal masses than women, this may account for their higher levels of bone resorption.

Pyridinoline cross-links have also been used to measure the changes occurring with age and the onset of menopause. Kushida *et al*, 1995 showed that pyridinoline and deoxypyridinoline cross-links were already significantly increased in premenopausal women in their fifties compared to those in their thirties and forties. The onset of menopause however causes a marked and rapid increase in the excretion of pyridinium cross-links. Uebelhart *et al*. found that the levels of pyridinoline and deoxypyridinoline rose by 62% and 82%, respectively, following the onset of menopause (Uebelhart *et al*. 1991).

Pyridinium cross-links are elevated in several disease conditions with bone involvement, including hyperparathyroidism, cancer and Cushing's syndrome (Seibel *et al.* 1992; Chiodini *et al.* 1998; Lipton *et al.* 1993). Consequently measurements of pyridinium cross-links can be used to monitor the success of therapy for such diseases. For example levels of cross-links in patients with hyperparathyroidism are reduced to levels seen in normal healthy controls following parathyroidectomy surgery (Seibel *et al.* 1992).

1.5.3 Biochemical Markers in Calcium Supplementation Trials

The advent of biochemical markers of bone formation and resorption have provided a more accessible means of determining the effectiveness of treatments on bone health. Several studies have investigated the effectiveness of calcium supplementation via these markers (See table 1.4). Most have demonstrated the efficacy of calcium supplementation in reducing bone turnover in postmenopausal women (Woo *et al.* 1991; Blumsohn *et al.* 1994; Rubinacci *et al.* 1996; McKane *et al.* 1996; Fardellone *et al.* 1998; Kamel *et al.* 1998; Prestwood *et al.* 1999). Changes have been produced in as little as 2-4 hours (Rubinacci *et al.* 1996; McKane *et al.* 1996), however different markers produce conclusions after longer amounts of time and of varying significance.

Table 1.4 Calcium Supplementation and Biochemical Markers

Reference	Subjects	Average Baseline Ca intake	Ca Dose +Time of Supplementation	Duration	Results
Woo et al. 1991	20 PostM W	7.2 mmol/d (288mg/d)	10mmol/d (400mg/d) PM	12 hrs	? HYP
Blumsohn et al. 1994	18 PreM W	AM=847? 98mg/d; PM=1129? 126mg/d	1000mg/d Either AM or PM	2 wks	? in nocturnal Dpd and NTx by evening but not morning supplementation
Rubinacci et al. 1996	7 Y W and 11 PostM W (53-78 yrs old).	NM	1000mg + 200mls milk (200mg Ca). AM	3 hrs	? Pyd & Dpd ? PostM W >Y W
McKane et al. 1996	28 PostM W & 12Y W	Post M 815mg/d Y W 918mg/d	1600mg/d With 3 main meals and at bedtime	3 yrs	? Pyd 17% ? Dpd 25% PostM W Pyd 18% > Y W PostM W Dpd 30% > Y W Supplemented PostW Pyd and Dpd = Y W Pyd and Dpd.
Rosen et al. 1997	227 MP W.	R = 400 – 800mg/d	500mg/d or HRT NM	1 yr	No change in NTx or Dpd with Ca but ? B-ALP ? Ocn, B-ALP, NTx & Dpd with HRT
Fardellone et al. 1998.	PostM W >5 years PostM	576 ? 142 mg/d	1200mg/d as 2 doses AM & PM	2 mths	? B-ALP, HYP, Pyd & Dpd. ? greater when ADI<576mg/d
Kamel et al. 1998	PostM W <75 yrs old but >5 yrs PostM	32 <800mg/d, ?=503 ? 159 mg/d & Control Group 21 1130 ? 352 mg/d	1200mg/d as 2 doses AM and PM	2 mths	? HYP, Total Pyd, Total Dpd, NTx & CTx No change in Free Dpd No changes in control group
Prestwood et al. 1999	31 PostM W >70 yrs old + placebo 11 PostM W >70 yrs old .	1. 865 ? 117 mg/d 2. 897 ? 78 mg/d 3. 838 ? 116 mg/d	0.5 mg/d 17?-estradiol (low dose) or 1500 mg/d Ca + 800 IU/d Vit D; then combined. NM	12 wks single treatment, 12 wks both	? CTx, NTx, FDpd & B-SP (bone-sialoprotein) Only in Ca & Vit D group ? B-ALP, Ocn, and PP (type I procollagen peptide)

Abbreviations:							
		AM	Morning	PM	Evening	NM	Not mentioned
PostM	Postmenopausal	Ca	Calcium	W	Women	d	Day
HYP	Hydroxyproline	PreM	Premenopausal	wks	Weeks	Dpd	Deoxypyridinoline
NTx	N-telopeptide	Pyd	Pyridinoline	Ocn	Osteocalcin	MP	Menopausal
HRT	Hormone Replacement Therapy	CTx	C-telopeptide	Vit D	Vitamn D	hr	Hour
ADI	Average Daily Intake	B-SP	Bone sialoprotein	Yr	Yr	Y	Young
B-ALP	Bone Alkaline Phosphatase	mths	Months	AM	Morning	PM	Evening

Fardellone *et al.* 1998 demonstrated that supplementation of 1200mg/day, given as two doses of 600mg taken in the morning and evening, calcium/day reduced excretion of the bone formation marker B-ALP as well as decreasing levels of resorption markers, hydroxyproline (HYP), pyridinoline (Pyd) and deoxypyridinoline (Dpd). The changes were observed within 1-2 months of supplementation and were greatest in subjects with baseline calcium intakes below the mean intake of 576mg/day (Fardellone *et al.* 1998). However an earlier study 3 months of supplementation failed to produce changes in resorption markers, even in those with lower baseline intakes, and achieved an increase in B-ALP measurements (Rosen *et al.* 1997). It is possible that the considerably lower amount of calcium (500mg/day) used in this study may not have been enough to produce significant changes in resorption although Woo *et al.* 1991 achieved a drop in HYP with a single 400mg dose of calcium. The Chinese women in this study did however have exceptionally low calcium intakes (mean intake=288mg) and were of different ethnicity than the study population in Rosen *et al.* 1997. It is difficult to make direct comparisons between ethnic groups. The RDA for Chinese has not been formally estimated and may well be lower than for Caucasians due to differences in body size, calcium absorption or vitamin D production (see section 1.1.4.2). In yet another study evening supplementation of 1000mg/day for 2 weeks was effective in reducing the nocturnal rise in urinary Dpd, where morning supplementation of the same dose failed to produce changes (Blumsohn *et al.* 1994). Hence timing of supplementation may also be an important factor to consider when analyzing or comparing separate studies.

Kamel *et al.*1998 demonstrated the ability of 1200mg/day, given as two doses of 600mg taken one in the morning and one in the evening, to reduce levels of urinary HYP, total Pyd and Dpd, NTx and CTx, but not those of free Dpd. The authors quote several other studies in which free deoxypyridinoline has failed to produce significant responses where other markers have succeeded (Blumsohn *et al.* 1995; Ebeling *et al.* 1996; Kamel *et al.* 1996).   It has been suggested that free Dpd may lack the specificity for the osteoclastic resorption process that the conjugated form has or that renal clearance of the free and conjugated forms may differ (Eyre *et al.* 1995; Cowell *et al.* 1996 both cited in Kamel *et al.* 1998. Despite the apparent inadequacies of the free form, conjugated Dpd is often quoted as producing the largest and most significant changes of all the resorption markers (McKane *et al.* 1996; Rubinacci *et al.* 1996).

However the intra-individual CV (within-person biological variability) for total Dpd was the highest amongst a group of other resorption markers in a recent study, where NTx was shown to be the most reproducible (Kamel *et al*. 1998).

1.5.3 Summary

Biochemical markers represent an effective medium for the study of bone metabolism. Being able to formulate conclusions within short periods of time (week to months as opposed to years when dealing with bone mineral density as an endpoint) is a huge plus in terms of such trials and will enhance the accessibility and efficiency of intervention studies. However measurements of bone mineral density and fracture risk remain the most conclusive evidence of changes in bone health and will therefore remain crucial in addition to marker studies. Pyridinium cross-links are among the most respected and widely used of all the bone markers and have been used successfully in the study of osteoporosis and its treatment. Bone markers have shown the efficacy of calcium supplementation in reducing bone turnover. However as in trials using bone density or fracture rate as end-points, variables such as mean baseline calcium intake, time of supplementation and sample collection as well as other confounding factors can all alter the results obtained.

2. STUDY TO EVALUATE DIETARY AND LIFESTYLE FACTORS IN A GROUP OF ELDERLY

2.1 INTRODUCTION

Diet and lifestyle can have a large impact on bone health. Some nutrients can have physiological effects, either positive or negative, on bone. Nutrients that have important roles in bone health in include calcium, phosphorus, potassium, magnesium zinc and vitamin D (see sections 1.1 and 1.2). Several nutrients such as sodium, protein and retinol can also have deleterious effects on bone when consumed in high amounts. In addition lifestyle factors such as exposure to sunlight, heavy alcohol consumption, smoking and weight-bearing physical activity are also recognised to impact on bone health (see section 1.1). Nutrition and lifestyle represent factors that can be changed to produce beneficial effects. Thus recognising dietary and lifestyle inadequacies and problems provides a tool whereby bone health can be improved. The main purpose of this study was to identify and measure dietary and lifestyle factors related to bone health in a group of elderly, some of which were recruited to take part in an intervention trial. This information was used to identify dietary and lifestyle inadequacies in order to establish where improvements may be made. In those volunteers who went on to participate in the intervention trial, dietary and lifestyle information collected was used in the following chapter to consider any impact they may have had on the results found.

2.2 METHODS

2.2.1 Recruitment/ Anthropometric Measurements

2.2.1.1 Participant Recruitment

Participants were recruited from the regions of Palmerston North and Fielding. A small descriptive paragraph, detailing the study and participants required was placed in the 'Interests' section of the local community newspaper *The Guardian*. An information sheet with contact telephone numbers was placed in local supermarkets, the Manawatu Golf club and the Palmerston North Public Library. A small informatory news item describing the study and participant requirements aired on several radio

stations through the Radio Network Central Limited. Information sheets were also placed in several retirement villages and rest homes. Jillian Richards contributed to the recruitment process, answering calls, explaining study details to volunteers and arranging meetings with them. Jillian also assisted in dietary and physical activity evaluation of participants.

2.2.1.2 Introduction to /Explanation of Study

Once a volunteer expressed interest in participating further details of the study were explained and discussed with them in their own homes by either Caroline Stanley or Jillian Richards. Once consent was obtained medical conditions and potentially complicating medications were taken into consideration and those unsuitable for the trial were eliminated. All measures and interviews were carried out in the participant's home by either Caroline Stanley or Jillian Richards.

2.2.1.3 Anthropometric Measures

?? *Body weight* was measured in kilograms (Kg) using conventional weighing scales (PM-150, Taiwan). Measurements were made to the nearest 0.01kg. Shoes were removed.

?? *Standing height* was measured in metres using a portable stadiometer specially designed for our purposes by the technical laboratory at Massey University. Measurements were made to the nearest millimeter (mm). Shoes were removed.

?? *Maximal handgrip* on both sides was measured in duplicate using a standard handgrip monitor (Lafayette model 78010, Indiana). Measurements were taken to the nearest 0.5kg.

Weight, height and handgrip were all taken as the mean of duplicate values. Body mass index (BMI) was calculated from the following equation:

$$BMI = Weight / Height^2$$

Basal metabolic rate (BMR) was calculated from Schofield's equation for men and women over 60 years of age (Schofield, 1985):

> *Men*   BMR = (0.038 x weight) + (4.068 x height) − 3.491
>
> *Women* BMR = (0.033 x weight) + (1.917 x height) − 0.074

2.2.2 Dietary Assessment Methods

Dietary assessment to estimate the mean nutrient intake of the participant group was by 24-hour dietary recall. During the interview participants were asked to recall their food intake from the previous 24 hours. This method was performed the day following the 24-hour period being recalled, in a one-on-one interview. The interviewees were given visual aids to help estimation of portion sizes. Visual aids used included metric and non-metric cups and glasses, standard serving spoons, circles of different diameter and example food servings. A question regarding time spent in the sunlight was also included to help assess the likely vitamin D status of the participants.

Data were entered into the Foodworks II program (Xyris software, Australia. Pty Ltd, Highgate Hill, Queensland). From the nutrient outputs of this program nutritional inadequacies were ascertained. The percentage of participants with inadequate intakes of calcium, magnesium and zinc were calculated. Nutrient intakes were compared with the Australian RDIs as these are currently used by New Zealand also and the proposed US adequate intake for calcium. The percentage of probable under-reporters (of food consumed within 24 hours) was ascertained using the Goldberg cut-off value which for an n of 20 and using 95% confidence is equal to a ratio of energy intake to estimated basal metabolic rate of <1.37 (Goldberg *et al.* 1991).

2.2.3 Assessment of Physical Activity and Lifestyle

Physical activity was assessed by means of a modified version of the Zutphen Physical Activity Questionnaire [see appendix i] (Casperson *et al.* 1985). Questions concerning odd jobs and sports were defined by giving examples such as knitting, aqua aerobics and tai chi, which the participants may not necessarily consider physical activity. The question referring to bird keeping was changed to general pet-keeping as it was felt that keeping any animal may contribute significantly to one's physical activity level. Physical activity data was assessed in accordance with the method outlined by Casperson *et al.* 1985. The authors suggested the following definitions of exercise intensity: heavy ($16.7 \text{ kJ.kg}^{-1}\text{hr}^{-1}$ or more), moderate (? $8.4 \text{ kJ.kg}^{-1}\text{hr}^{-1}$ but < $16.7 \text{ kJ.kg}^{-1}\text{hr}^{-1}$) and light (< $8.4 \text{ kJ.kg}^{-1}\text{hr}^{-1}$). These definitions were used in the current study. Total times spent doing activity of each intensity was calculated as well as total

hours/day of activity and total kilojoules/kg/day expended. Other questions used to assess lifestyle asked about alcohol consumption and smoking status.

Comparisons were made between men and women and between women living independently and in rest homes. Comparisons between men living independently and in rest homes would not have yielded significant information as only two men from rest homes were recruited. All values are expressed as mean ? standard deviation.

2.3 RESULTS

2.3.1 Recruitment/ Anthropometric Measurements

The number of recruited participants, according to gender and living situation is outlined in table 2.1. A total of fifty-five people, thirty-eight women and seventeen men volunteered to take part in the dietary and lifestyle section of the study. Twenty-seven percent of the volunteers for the dietary and lifestyle section were rest home residents. Anthropometric measurements of the study participants are outlined according to gender in table 2.2. On average men were taller and heavier and had greater maximal handgrips than women ($p<0.001$). There was no significant difference in mean age or BMI between women and men. However the age range of women was greater than that of men.

Table 2.1: Recruited Participants

Participant Description	Men-Independent Living	Men-Rest Home	Men-Total	Women-Independent Living	Women-Rest Home	Women-Total
Number	15	2	17	25	13	38

Table 2.3 explores the measurements of the women participants according to living situation. Mean height, weight and body mass index (BMI) of women living independently were the same as for women living in rest homes. However women living independently were on average ten and half years younger and had greater maximal handgrips than their rest home counterparts ($P<0.001$). There were too few

46

men living in rest homes for a comparison with independently living men to be made. Maximal non-dominant handgrip[2] was negatively correlated with age ($p < 0.01$).

The average BMI of both men and women is above that recommended for the general public (i.e. $>25 \text{kg/m}^2$). In fact 11 out of the 17 men (65%) and 24 out of the 38 women (63%) had BMI values which were over 25 kg/m^2. However the New Zealand Recommendations in the Report of the Nutrition Taskforce (1991) allow for a wider acceptable BMI range ($20-29 \text{kg/m}^2$) for those over 65 years (Ministry of Health, 1996). The average BMI for both men and women participants falls within this wider range. Two men and nine women have BMI values of greater than 29 kg/m^2 and can therefore be described as overweight. Only two women had BMI values below 20 kg/m^2

Table 2.2: Anthropometric Measurements of All Study Participants

Gender	N	Age (years)	Age Range	Height (m)	Weight (kg)	BMI	H/grip N-Dom	Dom
Women	38	79.2 ? 7.3	70-94	1.53 ? 0.05 (n=36)	68 ? 9.8 (n=36)	26 ? 4	16.7 ? 5.2 (n=34)	19 ? 6.0 (n=35)
Men	17	77.7 ?5.7	69-85	1.72 ? 5.74	78 ? 12	27 ? 3	30.1 ? 8..4	31.5 ? 7.2
p-value	-	p=0.42	-	p<0.001	p<0.001	p=0.97	P<0.001	p>0.001

Values are all mean ? standard deviation

Table 2.3 : Anthropometric Measurements of Women Participants According to Living Situation

Living Situation	N	Age (years)	Age Range	Height (m)	Weight (kg)	BMI	H/grip N-Dom	Dom
W-Independent	25	75.6 ? 4.9	70-86	1.57 ? 0.04	66 ? 9.	26 ? 4	18.5 ? 4.1	21.4 ? 4.3
W-Rest Home	13	86.1 ? 1.2	73-94	1.55 ? 0.06	61 ? 11	26 ? 5	10.5 ? 2.7 (n=10)	11.9 ? 4.1 (n=9)
p-value	-	p < 0.001	-	p=0.22	p=0.24	P=0.50	p<0.001	p<0.001

Values are mean ? standard deviation

---

[2] Maximal non-dominant handgrip is used in preference of maximal dominant handgrip. This is because the dominant arm is more likely to be affected by factors such as playing sports such as lawn bowls which can result in one arm/hand being stronger than the other.

2.3.2   Dietary Assessment

2.3.2.1 Energy and Metabolic Measurements

Table 2.4 shows the average metabolic measurements for the group of participants. As would be expected the men had a higher mean predicted basal metabolic rate (BMR_{est}), and energy intake (EI) than the women ($p<0.001$). The average ratio of EI to BMR_{est} for men was not statistically different to the value for women. Figures 2.1 and 2.2 display the EI/BMR_{est} ratios for men and women participants. Values under 1.37, shown as grey bars in the graphs, are ratios indicative of under-reported energy intakes. As can be seen from the two graphs, under-reporting was common amongst both women and men with over half of each, 66% of women and 59% of men under-reporting.

Table 2.4: Energy and Metabolic Measurements for all Participants

Gender	BMR_{est} (MJ/24 hr)	Energy Intake (MJ)	EI/BMR_{est}	EI/BMR_{est} Range	Percentage of Under-reporters
Women	5.2 ? 0.4	6.4 ? 1.3	1.3 ? 0.3	0.8-2.0	66%
Men	6.5 ? 0.7	8.4 ? 1.9	1.3 ? 0.3	0.77-2.0	59%
p-value	P<0.00001	p<0.001	P=0.50		

BMR, energy intakes and EI/MBR values did not differ between women living independently and in rest homes. However there was a greater level of under-reporting amongst rest home women (75%) compared with women living independently (63%).

Figure 2.1 Energy Intake/ BMR for Women Participants
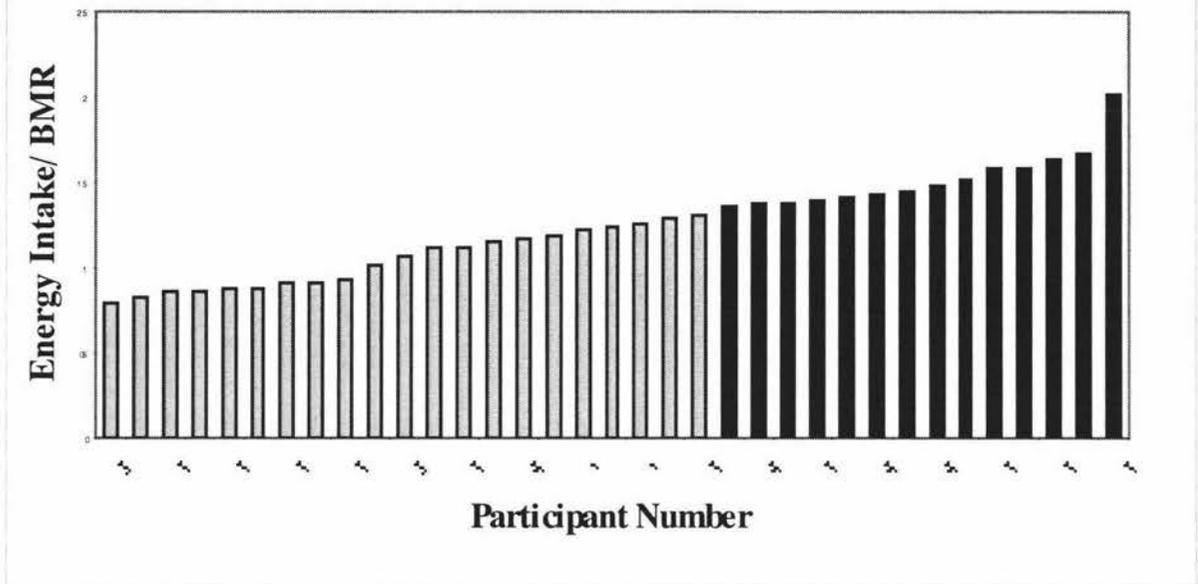
Figrue 2.2 Energy Intake/ BMR for Men Participants
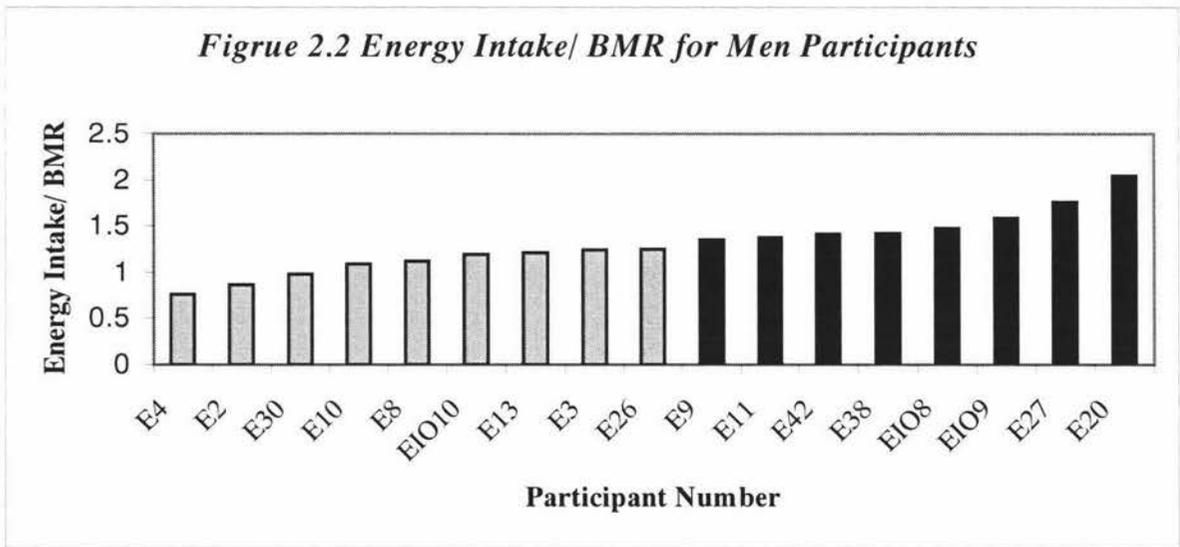
2.3.2.2 Dietary Intake

2.3.2.2.1 Nutrients Related to Bone Health

Average daily intakes for micronutrients particularly relevant to bone health are shown in figure 2.3. Despite men having higher energy intakes than women, intakes of calcium did not differ significantly between the sexes (p=0.79). Men did however have higher magnesium (p=0.0014) and potassium intakes (p=0.003). Phosphorus intakes

appeared to be higher in men although the difference was not quite significant (p=0.11). Zinc and vitamin C intakes were also considered due to their roles in bone health and fracture incidence. Both zinc (p=0.045) and vitamin C (p=0.03) intakes were higher in men than in women.

The current recommended daily intake (RDI), adequate intake (AI) or estimated minimum requirement (EMR) are shown in figure 2.3 as white bars. Intakes of calcium, magnesium and zinc were frequently below these values. Over half of all men and women were below 2/3[3]* of the current USFDA recommended adequate intake for calcium of 1200mg/day and are therefore also under the current Australian RDI for men (800mg/day) and women (1000mg/day). In addition around a third of women and a fifth of men were below 2/3 of the Australian RDIs for zinc (12mg/day) and magnesium (320mg/day for men and 270mg/day for women). However not all zinc RDIs are as high as the Australian recommendation and in a recent report from the Institute of Medicine recommendations for daily zinc intake were lowered to 7.3mg/day for men and 5.5mg/day for women (Institute of Medicine, 2001). Only one woman and two men from our study group were below these lower values. Intakes of phosphorus and potassium were however around twice the RDI for phosphorus and the EMR for potassium. In contrast to the other micronutrients measured no participants were under EMR for potassium and only one woman was below the current RDA for phosphorus.

In general women living independently had higher reported micronutrient intakes than women living in rest homes. Magnesium, potassium and phosphorus intakes were all lower in women from rest homes (p<0.01). Intakes of calcium (p=0.37), zinc (p=0.16), and vitamin C (p= 0.22) were also lower on average although the differences were not statistically significant.

---

[3] The value of 2/3 the RDI/ AI/ EMR was used as a cutoff for defining inadequate intakes as using the actual RDA/AI/EMR value would see those with marginally lower intakes being counted as having inadequate intakes. Therefore a lower cutoff represents a more meaningful estimate of those with low intakes. The actual value of 2/3 was a fairly arbitrary decision, however this value has been used in other studies (Horwath *et al.* 1990).

Figure 2.3 Micronutrient Intakes of Men and Women Participants
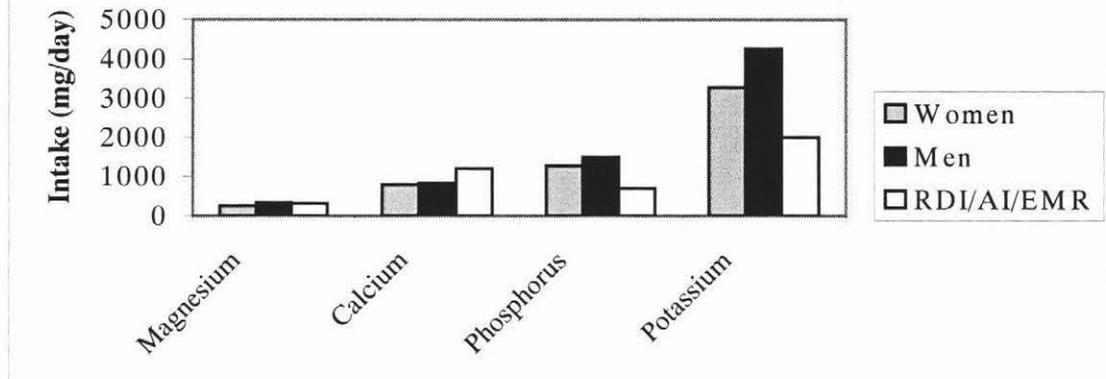
As can be seen in figure 2.4 50% of both men and women were below the 1991 Australian recommended daily intake (RDI) of 800mg/day and 1000mg/d for women. In the United States a new adequate intake (AI) of 1200mg is now being advised for people over the age of 51 years age group (as discussed in section 1.2.2). This AI, based on recent reviews of the evidence, is represented in figure 2.4 as a broken line. The upper quartile of both men and women (representing 75% of men and women) is below this level. 12 out of the17 men (71%) and 23 out of the 38 women (61%) had calcium intakes that were below two thirds of this recommended adequate intake. In addition 15/38 (39%) of women were below two thirds of the Australian RDA for zinc and 16/38 (42%) of women were below two thirds of the US and Australian RDA for magnesium. Close to 100% of participants did not achieve the recommended daily dietary intake of vitamin D for this age group, although this recommendation is more applicable to people who are unlikely to meet their vitamin D requirement through sunlight exposure alone.

Figure 2.4: Calcium Intakes of All Participants
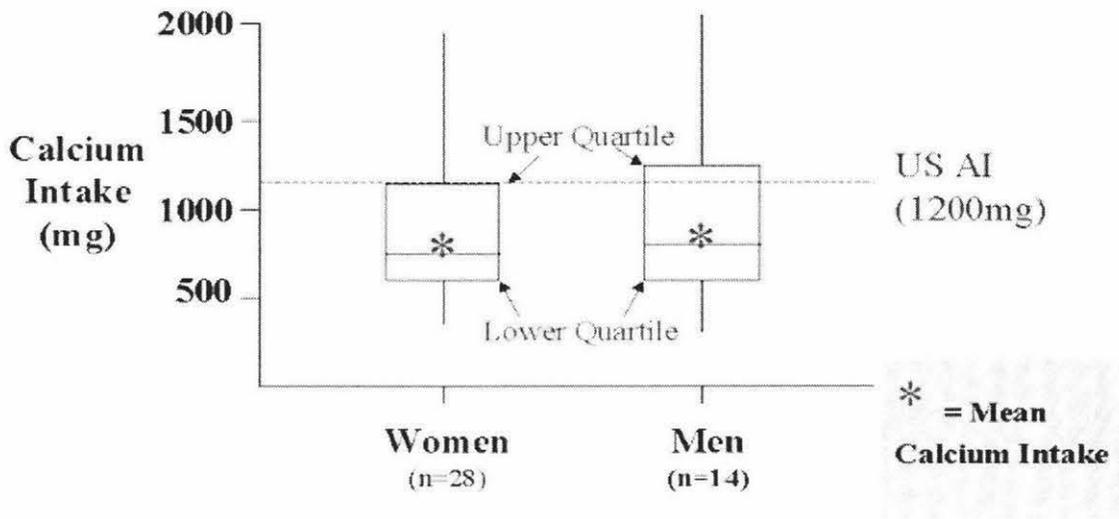
The average protein intakes were 67.9g/day for women and 85.2g/day for men. Although men appeared to have higher intakes the women the difference was not quite significant (p=0.07). Women living independently had significantly higher protein intakes than women living in rest homes, consuming 74.2g/day as compared to 55.8g/day (p=0.01). However there was a negative correlation between the age of women participants and their protein intakes (p<0.05) with older women being more likely to have low protein intakes. A similar relationship was not seen between energy intake and age.

2.3.2.2.2 Solar and Dietary Sources of Vitamin D

Of all the fifty-five participants who completed the survey, fifty-three described their daily exposure to sunlight. Mean (? standard deviation) daily sunlight exposure was 1.5 ? 2.9 hours. Men were exposed to more direct sunlight than women (p<0.05), spending 2.9 ? 2.2 hours in direct sunlight each day and women only 1.4 ? 1.5 hours. Figure 2.5 shows the hours spent exposed to direct sunlight for each of the participants. Twelve, or nearly a quarter, of the participants reported spending less than thirty minutes in sunlight (shown in grey), with 8 of these reporting that they spent very little or no time in direct sunlight. Women living independently had the same average sunlight exposure as those living in rest homes however of the twelve women spending less than thirty minutes in sunlight daily, nine of these, or 75% were rest home residents. This represents nine out of the total of fifteen rest home residents in our

52

sample. Sunlight exposure was related to both total activity ($p<0.05$) and gardening activity ($p<0.05$).

Figure 2.5 Daily Sunlight Exposure for All Participants
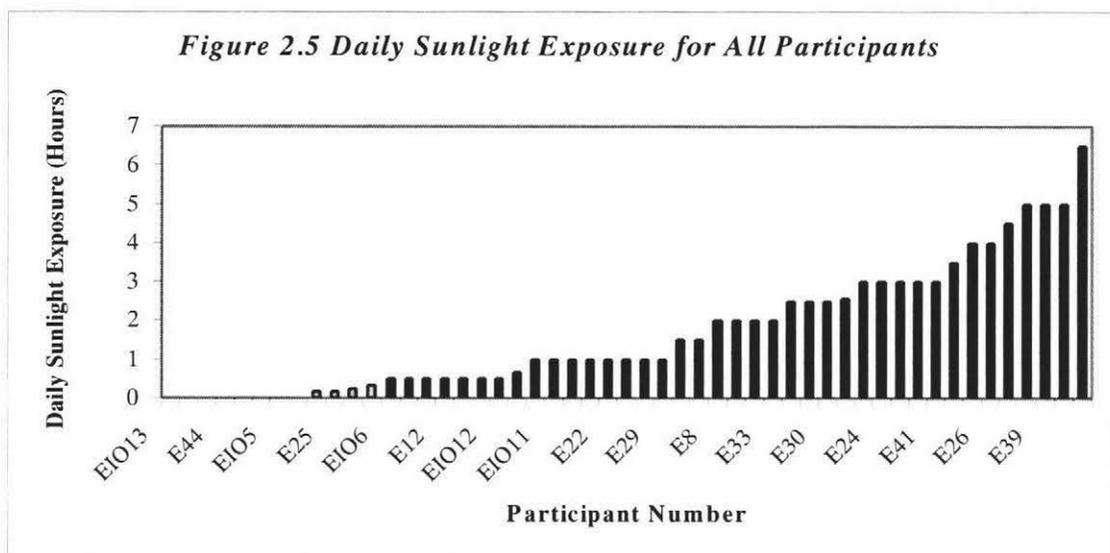
Figure 2.6 displays the dietary intakes of vitamin D for each of the participants.

Figure 2.6 Vitamin D Intake of All Participants
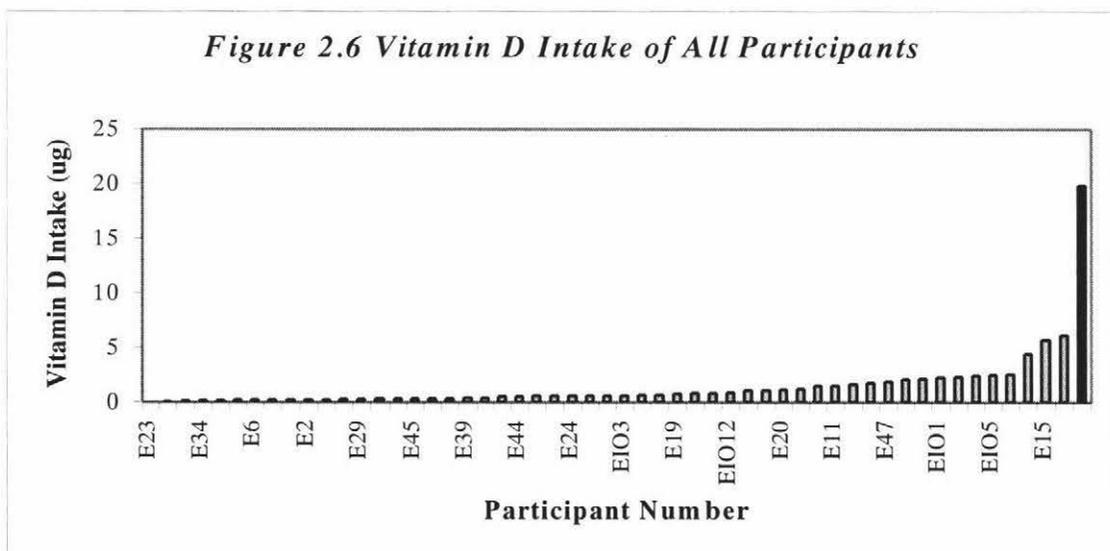
All but one of the participants (shown in black) had vitamin D intakes greater than 50% of the currently recommended 15 ?g for people of this age group who are receiving inadequate sunlight exposure.

2.3.3 Physical Activity Patterns

A total of 37 women and 16 men participated in the physical activity questionnaire. Women spent 3.3 ± 2.2 hours and men 3.4 ± 2.7 hours involved in physical activity. Men appeared to spend more time in physical activities than women but this difference was not significant (p=0.59). Women living independently were more physically active than the women from rest homes (p<0.00001). Independent women spent an average of 4.0 ± 1.9 hours/day and 12.4 ± 7.6 kcal/kg/day in physical activity. In contrast the 13 women from rest homes spent an average of 1.1 ± 1.0 hours/day in physical activity and expended 3.0 ± 3.9 kcal/kg/day.

Figure 2.7 shows time spent in each of three physical activity intensities, defined as follows: light intensity <8.4 kJ/kg/hour; moderate intensity ± 8.4 but <16.7 kJ/kg/hour; heavy intensity ± 16.7 kJ/kg/hour. On average men appeared to engage in a greater proportion of heavy and moderate activities and women a greater proportion in light activities, however these apparent gender differences were not significant (p> 0.2).

Figure 2.7: Time Spent in the Three Intensities of Physical Activity
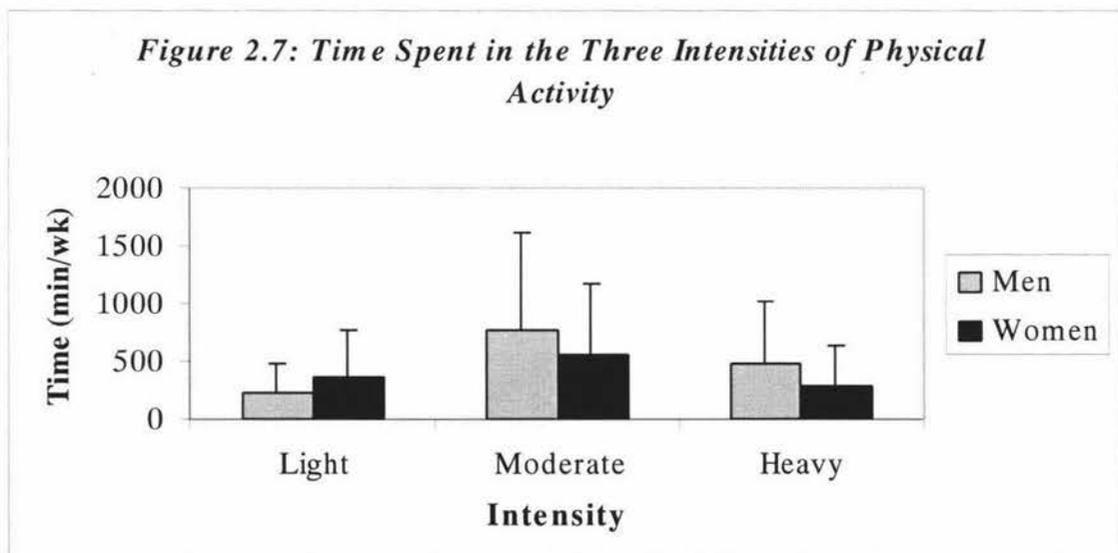
Men also appeared to spend more kJ/kg/hour than women, exerting 46.4 ± 39.7 kJ/kg/hour and women only 36.8 ± 33.4, but this was also not statistically significant (p=0.40).

Figure 2.8 shows the most common physical activities reported and the proportion each represents of total physical activity. Women tended to spend less time

in sports and gardening and more time in household jobs and hobbies than men however all these observations were non-significant. As shown by the large population standard deviations, there were large variations between individuals both across genders and within the same gender.

Figure 2.8: Time Spent in Various Physical Activities
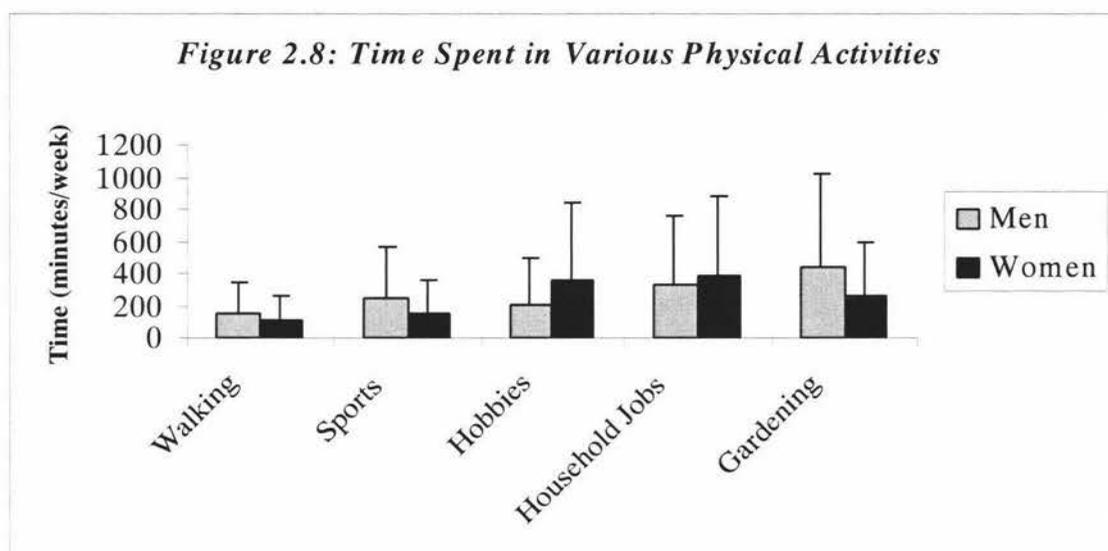
Only two participants reported consuming more than 2 servings of alcohol daily. All but one of the participants were non-smokers.

2.4 DISCUSSION

2.4.1 Recruitment/Anthropometric Measurements

2.4.1.1 Recruitment of Participants

The very elderly are susceptible to nutritional inadequacies and poor bone health (see chapter 1). Therefore they represent a group suitable for milk supplementation. Very few studies looking at bone health in older age have included men in their sample groups. However as elderly men also suffer from bone deterioration and osteoporosis it was decided that men should be included in our study. One study, which included men in the sample group, found that calcium and vitamin D supplements given simultaneously were able to reduce bone loss in both men and women (Dawson-Hughes

et al. 1997) and therefore demonstrates that such supplementation can benefit men as well as women.

Recruitment criteria such as no previous history of bone disease, not taking calcium supplements or receiving hormone replacement therapy were included to rule out obviously confounding factors. Large doses of calcium can interfere with the absorption or action of certain drugs, including some heart medication. Therefore for safety considerations, those who rely on such medicines were excluded from the trial. These recruitment criteria probably ruled out a large portion of the population over seventy. The method of recruitment used also relied on participants volunteering, which required that the participants be interested in the study, motivated enough to contact the research centre and healthy enough to participate. Therefore it is fair to say that the study group was not a group representative of the entire elderly population in the Manawatu. Those who volunteered were probably likely to represent a comparatively healthy, motivated, health conscious and physically active group. Therefore conclusions reached in this study may not be applicable to the wider elderly community in the Manawatu nor in the whole of the country.

2.4.1.2 Anthropometric Measurements

The finding that men were generally larger and stronger than women is to be expected. In terms of bone health heavier body weights are favourable. Therefore the fact that the majority of participants have BMI values on the heavier side of recommendations is positive in terms of their bones. Nine women (24%) had BMI values over the higher upper limit proposed by the Nutrition Taskforce (Ministry of Health, 1996). This may be of some concern for general health as overweight is associated with higher risk of developing conditions such as type II diabetes and cardiovascular disease (Minsitry of Health, 1996).

Women living independently had a stronger mean handgrip than their rest home counterparts. However handgrip strength was negatively correlated with age ($p < 0.01$) and as the women from rest homes were significantly older than those living independently the difference in handgrips is likely to be due in part to their older age. In addition reasons for residing in rest homes probably include lower mobility, frailty or

illness and these too are likely to factor into lower handgrip strength. Although the difference was not significant it appeared that the rest home women were slightly shorter (p=0.22) and lighter (p=0.24). Although it is impossible to see if these women are shorter now than they were when they are younger, it would be interesting, if these women had been measured previously, to note if their apparently shorter stature was related to older age and/or osteoporotic spinal fractures.

2.4.2 Dietary Assessment

2.4.2.1 Energy and Metabolic Measurements

The most striking observation taken from the energy and metabolic measurements is the exceptionally high level of under-reporting amongst both men and women (66% of women and 59% of men). Several factors may help to explain this finding. The success of the 24-hour dietary recall method for assessing relies on the participant having a good memory. As some elderly experience deterioration in memory function it is likely that the participant group would be susceptible to under-reporting due to poor memory. Dietary records, where food is recorded as the time of consumption, may have produced more accurate results however this is a time-consuming and burdensome method, which was beyond the means of our study. It is possible that some of the participants had low energy intakes that were real. Several of the participants especially those women living in rest homes had very low levels of physical activity and may therefore only require a low energy intake.

There is some doubt about the suitability of the Schofield equation for measuring BMR in elderly populations. The population from which the Schofield equations were contrived was "normal" (to a maximum of 84kg) and not obese (Schofield, Schofield and James, 1985 cited in Ministry of Health, 1999). Extrapolating the Schofield equation for those with body weights over 84kg introduces error as lean body mass, which determines BMR, may not increase in proportion to absolute mass. Six out of the seventeen men (35%) in the study sample had body weights that exceeded 84kg and thus require an alternative more suitable means of calculating their BMR before the level of over-reporting among men in the study sample can be confirmed.

Furthermore the Schofield equation is not appropriate for overweight or obese individuals, again because lean body mass may be overestimated by using measures of body weight and height alone. 22% (8/36) of women and 18% (3/17) of men were classed as overweight or obese even under the wider acceptable BMI range attributed to those over 65 years (58% of women and 65% of men were considered overweight or obese under the normal acceptable BMI range). Therefore this poses another source of error when determining BMR from the Schofield equation for this group. In addition, even if overweight and/or obesity were not predominant in this group, it is well known the body composition changes as we age and that the elderly generally have a greater proportion of fat than younger people do. As a result lean body mass and consequently BMR may be overestimated even in those who are not overweight.

Therefore either underestimation of energy intake, overestimation of BMR or more likely a combination of both could partly explain the high level of calculated under-reporting. More experience in dietary recall interviewing may also have benefited the interviewers and enhanced the accuracy of dietary recalls.

As BMR may not be a suitable measurement for validation of dietary intake in this group, it may have been useful to employ another method of validation. Biochemical markers such as 24 hour urinary nitrogen excretion to validate protein intake, or 24 hour excretion of other minerals such as potassium may have been useful to validate the accuracy of the 24 hour dietary recalls.

2.4.2.2 Dietary Intake

2.4.2.2.1 Nutrients Related to Bone Health

The mean calcium intake for men and women in our study were 78mg and 159mg higher respectively than the means found in the most recent National Nutrition Survey for the same age group (MoH, 1999). The mean calcium intakes for both men and women are close to 800mg/day. However for women this is below the New Zealand RDI of 1000mg/day and represents only two thirds of the more recently determined US AI of 1200mg/day. The high levels of calcium intakes below both the current RDI and the recommended AI (see figure 2.4) are of concern and may be jeopardising bone health in this population.

The levels of zinc and magnesium intake below recommended levels are also significant. These micronutrients have commonly been found to be inadequate in the diets of New Zealand elderly (see section1.4.2.2). In all but one case where either zinc or magnesium intakes were low, calcium intakes were below two thirds of the recommended AI. Nearly half of all women and a third of men had either low zinc or magnesium intakes in conjunction with a low calcium intake. In seven of the 38 women (18%) interviewed, intakes of all three micronutrients were below two thirds of the recommended daily intakes (see appendix ii). Where deficiencies of these nutrients occur together, bone health may be significantly compromised. There are some concerns that high calcium intakes may reduce the absorption of zinc (see section 1.1.2.5.3), however low intakes of zinc were only found in conjunction with those that had low calcium intakes this is not likely to be a concern in this group.

Low intakes of calcium, zinc and magnesium occurring together may be indicative of poor diet. However as the level of under-reporting is so high among the study participants, it is possible that many of the participants actually have higher nutrient intakes than they reported (although the inappropriateness of the equation used to determine under-reporting must be considered). The lower reported nutrient intakes of rest home women as compared to women living independently may be due to either the poor diet of rest home residents, the higher level of under-reporting in this group or a combination of the two factors.

Intakes of potassium and phosphorus are more than sufficient in both men and women. Mean intakes are over double the current estimated minimum requirement (EMR) for potassium and the RDA for phosphorous in men and more than one and half times the amounts for women. Therefore inadequacies in these nutrients are not a concern. However the ratio of calcium: phosphorus is also thought to be important for bone health (Calvo, 1994). The ideal ratio of calcium: phosphorous in the diet has been suggested to be 1:1 (Calvo, 1994). The average calcium: phosphorous ratios were 1: 1.16 (0.62) for women and 1:1.18 (0.54) for men. When calcium intakes are low the ratio of calcium: phosphorus becomes more critical, which is concerning as low calcium intakes were common in this group.

Average protein intakes are around 20 g higher than the US RDA. High protein intakes can have a negative impact on bone through increasing calcium excretion. However as an extra 50g of protein only results in a loss of 60mg of calcium, an extra 20g of protein a day is not likely to have a great effect on daily calcium balance except where calcium intakes are very low.

2.4.2.2.2 Solar and Dietary Sources of Vitamin D

All but one participant was below the recommended daily dietary intake of vitamin D for this age group (see figure 2.8). It is well established that the primary source of vitamin D for our bodies is not dietary but from the action of sunlight on skin. The dietary recommendation is therefore only essential for those who do not receive adequate sunlight exposure (at least 15-30 minutes a day[4]) such as those who are bed-ridden or housebound.

Nearly a quarter (12/52) of the participants reported spending less than thirty minutes in sunlight and 8 of these (16%) reported spending very little or no time in direct sunlight. Therefore it seems likely that up to a quarter of the participants are at risk of developing hypovitaminosis D. The high proportion of rest home residents failing to attain adequate sunlight exposure is concerning (60% spent less than 30 minutes in direct sunlight). It seems likely that hypovitaminosis D may be a problem in this particular group especially considering those we recruited were probably among the most physically able and independent of rest home residents. It would have been very interesting to have measured serum vitamin D status in this group to confirm this and to measure the actual prevalence of hypovitaminosis D in this group, however this was beyond the scope of the study.

2.4.3 Physical Activity and Lifestyle Patterns

Although men appear to be more active than women when all subjects are compared, this effect disappears when only those participants living independently are considered. As a larger proportion of women are from rest homes, and women from rest homes had considerably lower levels of energy expenditure and time spent in

---

[4] Public Health Commission recommendations cited in Ley *et al.* 1999)

60

physical activity this effect is hardly surprising. Some of this effect is due to older age (r=0.40) although rest homes may impose factors, other than advancing age, which make certain activities difficult. For example gardening was a common form of physical activity among those living independently but no rest home residents had access to their own garden.

In the original article from which the questionnaire was sourced 863 Dutch men, between 65-85 years old were given the Zutphen Physical Activity Questionnaire (Casperson *et al.* 1985). As modifications made to the Zutphen Physical Activity Questionnaire for our purposes were superficial, a valid comparison between the results of this study and our own may be obtained. The men in Casperson's study had an average reported level of about physical activity of one hour and twenty minutes. This value is much lower than that reported by our participants, which were around three hours and 20 minutes for both men and women. The level of activity reported by the Dutch men was more comparable to the thirteen women from rest homes in our sample (1 hour and 6 minutes reported physical activity/day). However as energy expenditure in kJ/kg/day was not reported in Casperson's study it is hard to say if the time was spent in more vigorous activities.

The proportion of time spent gardening by our study participants was similar to that of the Dutch men. However while cycling was a common activity amongst Dutch men very few of our study participants cycled. This may reflect the popularity of cycling as a means of transport in the Netherlands. Walking was popular among both our study group and the Dutch men, but household jobs and hobbies represented a far smaller proportion of activity in the Dutch men, especially when compared with the women in our study. Although this may reflect a real pattern it may also be partly due to the emphasis placed on these questions in our study. For example in our modified version of the Zutphen Physical Activity Questionnaire a broader range of examples such as knitting and cross-stich were given in the sections referring to household chores and hobbies.

Despite the shorter amount of time spent in physical activity by the Dutch men, they spent a greater proportion of time in heavy, as opposed to moderate and light activities. This greater proportion of heavy exercise may reflect the popularity of

cycling, which is classed as a heavy exercise. The larger proportions of light and moderate activities may be indicative of the greater time spent in sports by the men in our study and in housework and hobbies by the women in our study.

47% of the participants in the New Zealand Health Survey were regarded as being inactive. The definition of inactivity in this study was those who had spent less than 2.5 hours in leisure-time physical activity in the 7 days preceding the interview. Applying this definition to the current study only 3 women (all rest home residents) and two men or about 10% of the whole study population and 23% of all women living in rest homes were considered to be inactive. However the definition of an 'inactive' individual cannot be directly applied to our data as the New Zealand Health Survey used quite a different questionnaire to determine physical activity and did not include physical activity at work or looking after a home. Had housework and other non-leisure physical activity been excluded from our questionnaire the level of 'inactivity' as defined by the New Zealand Health Survey would have been much higher. It is however the opinion of the author that physical activity in the home and in the workplace can make a valid contribution to the physical activity of an individual and as such should be included in physical activity questionnaire.

In a recent Auckland-based study of men and women mean hours on feet/day ranged from 5.82 hrs/day for age-range 70-79 years to 4.55 hrs/day for the age-range 80-89 years to 2.49 hrs/day for those over the age of 90 (Galgali et al. 1998). Except for the above 90 years category of which only 2 of our participants would fall into these values are higher than the times spent in physical activity in our study of 3.3 hours for women and 3.4 hours for men. A finding common to both our study and that of Galgali's group was that those in institutions were less active than those living independently. However their value for institutionalised (2.41 hours/day on feet) was still considerably higher than our value for women from rest homes (1.1 hour/day pent in physical activity). Galgali used time spent 'on feet', rather than time spent in a variety of physical activities as in our study, as a means of defining physical activity. Time spent 'on feet' is likely to include more activities than questions about specific activities and this may account in part for the higher values obtained by Galgali et al.

As only two participants reported consuming more than an average of 2 servings of alcohol daily and only one participant smoked, these lifestyle factors are not likely to have any major influence on the bone health of the study group.

2.5 Conclusions/Summary

In general the group of participants appear to eat fairly well and obtain a good level of physical activity. However the high level of participants with calcium intakes below currently recommended intakes for this age group is concerning. Likewise high numbers of participants with either low magnesium or zinc intakes in combination with low calcium intakes may be compromising bone health in this group. Intakes of vitamin C, potassium, phosphorus and protein appear to be more than sufficient in most, although the negative effects of high intakes of phosphorus and protein may be of some concern.

Women living in rest homes are the most compromised group in terms of bone health. The rest home women are older and tended to be shorter (possibly indicative of an osteoporotic state) and lighter than women living independently. Dietary intake of important nutrients also tended to be lower in this group. Although as under-reporting was also highest in this group it remains to be determined if actual dietary inadequacies really exist. Additionally nine out of the thirteen (69%) rest home women interviewed may be at risk of hypovitaminosis D, which may be impairing their ability to absorb calcium. Furthermore rest home women also reported having the lowest levels of physical activity. This is likely to be due to older age and physical impairment as physical activity was actively encouraged in the rest homes visited.

It must be remembered when deriving conclusions from this work that owing to the means of recruitment and the large number of exclusion criteria, the study group cannot be considered a random or representative sample. However this study points to the susceptibility of institutionalised people to poor bone health and indicates that further research in this area is warranted. Institutionalised people should be encouraged to spend some time outdoors during the day and vitamin D supplementation may be required for those who must remain indoors. For the general group increases in calcium, magnesium and zinc intakes may be warranted for some.

3. THE EFFECT OF MILK ON BONE RESORPTION USING TWO INGESTION STRATEGIES

3.1 INTRODUCTION

Calcium supplementation has been shown to slow the loss of bone mass and reduce fracture risk in older age (see section 1.2.3). Calcium supplementation has also been effective at reducing urinary and serum bone resorption markers, which indicate the rate of bone turnover (see section 1.5.3). Milk is an excellent source of calcium both in terms of quantity and bioavailability. Calcium is absorbed better from supplements when given in divided doses (Heaney *et al*. 1990). Therefore one would expect the calcium in milk to be absorbed better when drunk in smaller portions. However it is not known whether or not the extra calcium absorbed from a serving of milk is enough to have a physiological impact on bone loss. The purpose of this study therefore was to investigate whether the extra calcium absorbed when milk is consumed in divided doses has an additional impact on bone resorption. It was hypothesised that because more calcium should be absorbed when the milk is drunk in smaller portions, dividing the dose of milk would result in a greater reduction in bone resorption than if the milk had been drunk all at once.

3.2 INTERVENTION TRIAL METHODS

3.2.1 Milk composition

Table 3.1 Micronutrient Composition of Milk Supplement (mg/50g of milk powder or 250mls of made up milk)

Micronutrient	Amount (mg)
Calcium	640
Potassium	398
Phosphorous	390
Magnesium	40.8
Aluminium	0.025
Copper	0.003
Iodine	0.278
Iron	0.1
Manganese	0.026
Selenium	0.003
Sodium	110
Sulphur	92.3
Zinc	1.8

3.2.2 Drinking schedule

Participants were alternatively allocated to each of the groups as they presented themselves as volunteers for the trial. Each participant consumed 250mls of the trial milk each evening. The milk-drinking schedule for the two groups is shown in figure 3.1. The single dose group consumed the whole amount of milk within 10 minutes of going to bed, while the divided dose group drank the milk in three portions one every hour before bed. Both groups drank the milk on 14 consecutive nights. On the two evenings before the milk regime began and the last two nights of supplementation, overnight urine collections were taken. Compliance sheets were given at the end of the trial period so that any missed doses of milk could be accounted for.

Figure 3.1. Drinking Schedule for Two Week Intervention

Single Dose Group

Day	0	14
Height, weight and food intake	1 glass milk consumed within 10 minutes just before bed	

Urine

Divided Dose Group

Day	0	14
Height, weight and food intake	1 glass milk consumed in 3 portions, once every hour before bed	
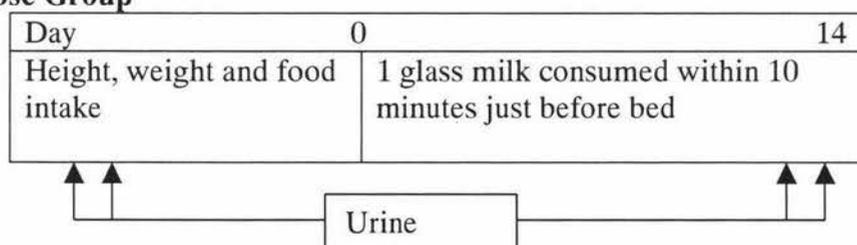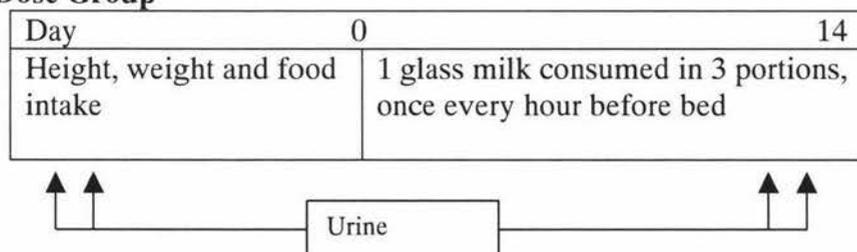
Urine

3.2.3 Collection and Storage

Overnight collections of urine specimens were made by the participants and kept with ice packs in a foil-covered bottle, to protect the samples from light, of which the empty weight was taken. The bottles were kept with ice packs in a small insulated box to protect the samples from heat and light. Specimens were taken in the lab early the next morning where they were prepared for storage. Full weight of the bottles were first taken and then the pH of the specimen. Two fresh aliquots were then taken for storage.

65

Each specimen was then acidified to a pH of between 2.5 and 4.5. Two more aliquots where then taken. All four aliquots were then frozen and kept in a freezer until the time of analysis. The two fresh samples were packed and sent to Canterbury Health Laboratories in Christchurch, New Zealand for creatinine and deoxypyridinoline analysis. Collection and storage of samples was carried out by Caroline Stanley, Jillian Richards and Richard Bunning.

3.2.4 Deoxypyridinoline Analysis

Free deoxypyridinoline (Dpd) was measured by Canterbury Health Laboratories in Christchurch, New Zealand using the Chiron Diagnostics ACS: 180 DPD assay (Chiron Diagnostics, East Walpole, MA 02032). This is a competitive immunoassay using direct chemiluminescent technology that is highly specific for Dpd.

3.2.5 Calcium Analysis

Caroline Stanley measured urine calcium at Massey University with the assistance and supervision of Dr. Phil Pearce. Analysis of urine calcium content was performed using a Cobas Fara discrete centrifugal analyser. The reagent system used was the Roche Reagent for Calcium (Kit # 44033). The reagents used in the assay and their respective concentrations are shown in table 3.2.

Table 3.2 Concentration of Reactants in Reagents

Reagents	Reactants	Concentration
Reagent 1	Arsenazo III	0.33 mmol/L
	2(N-Morpholino) ethanesulfonic acid	60 mmol/L
	Phosphorous acid	15 mmol/L
	pH 5.9	
Reagent 2	Disodium ethylenediamintetraacetic acid	
	pH 5.9	
		50 mmol/L

A rack of diluent (deionised water) was placed in the Cobas Fara to ensure the concentration would read at a suitable level. Controls with known calcium concentrations were also included in the analysis. Samples were pipetted out into small cups in duplicate, placed into the sample rack in recorded positions. Once all reagents,

controls, diluent and samples were correctly placed in the machine the assay was set running. The machine cycle dilutes the samples, adds the reagents and then calculates the calcium content of the samples. Calcium values are then printed out. Duplicate values were checked and any non-matching duplicates were repeated.

3.2.6 Creatinine Analysis

Creatinine analysis was carried out by Canterbury Health Laboratories in Christchurch, New Zealand, who used the Jaffe reaction in an Abbott Aeroset Analyser (Abbott Laboritories, Abbott Park, Illinois, USA).

3.2.7 Data Analysis

Group means for anthropometric measurements, micronutrient and protein intake as well as physical activity levels were compared via 2-sample t-test to determine that there were no differences between the two sample groups.

Both urinary Dpd and calcium were expressed as a ratio to creatinine (Crn) excretion to correct for variation in sample volume. Repeated measures ANOVA, run in the statistical programme SAS, were used to assess Dpd and calcium data. Change in Dpd over time was calculated for each individual and the average change in Dpd for each group ascertained. The presence or absence of relationships between baseline calcium and age with baseline Dpd/Crn were determined by Pearson's correlation in the statistical programme Minitab v. 12. Variability (CV) in overnight creatinine excretion rate was also measured.

All measurements are expressed as mean ? standard deviation (s.d).

3.3   RESULTS OF THE INTERVENTION TRIAL

3.3.1 Anthropometric, Nutritional and Lifestyle Measurements of Intervention Trial Participants

Table 3.3 shows the average anthropometric measurements of the two intervention trial groups. The mean age of the single dose group was not statistically different from the mean age of the divided dose group although the range in ages was slightly larger in the single serve group. Mean height, weight, BMI and handgrip were

67

also the same between groups. The difference in the number of women participants and men participants between groups was not significant as determined by Chi square test.

Table 3.3: Anthropometric Measurements for Single and Divided Dose Groups

Dose	N F M	Age (years)	Age Range	Height (m)	Weight (kg)	BMI	H/grip N-Dom	Dom
Single	13 9	79 ? 6.5	70-94	1.55 ? 0.11	70 ? 16	25 ? 4.3	22.3 ? 8.6 (n=21)	24.7 ? 8.8 (n=21)
Divided	15 5	76 ? 6.4	69-91	1.61 ? 0.08	69 ? 10	26 ? 3.1	21.0 ? 7.7	24.0 ? 7.3

Values are all mean ? s.d

Tables 3.4 displays average metabolic measurements for the trial participants. There were no significant differences in average basal metabolic rate (BMR), energy intake (EI) or EI/BMR between the single dose and the divided dose group.

Table 3.4: Energy and Metabolic Measurements for Single and Divided Dose Groups

Dose	BMR (MJ/24 Hr)	Energy Intake (MJ)	EI/BMR	EI/BMR Range	Number of Under-reporters
Single	5.5 ? 0.95	7.1 ? 1.4	1.2 ? 0.21	0.8-1.52	14/22
Divided	5.6 ? 0.65	6.8 ? 2.2	1.2 ? 0.34	0.77-2.04	10/20

Values are mean ? s.d.

Nutritional and lifestyle measurements of the two groups are outlined in table 3.5. There were no significant differences in any of these measures between the two groups.

68

Table 3.5:Baseline Nutritional and Lifestyle Measurements

Measurement	Single Dose Group	Divided Dose Group
Calcium Intake (mg/day)	843 ? 409	830 ? 425
Zinc Intake (µg/day)	11.0 ? 5.1	9.5 ? 3.9
Magnesium Intake (mg/day)	307 ? 86	299 ? 86
Vitamin C (mg/day)	132 ? 108	113 ? 98
Potassium (mg/day)	3769 ? 1129	3742 ? 1160
Phosphorus (mg/day)	1407 ? 418	1413 ? 607
Protein (g/day)	77.2 ? 26.1	76.7 ? 33.9
Vitamin D (µ/day)	1.0 ? 1.2	2.2 ? 4.5
Sunlight Hours/day	2.5 ? 1.8	1.8 ? 1.8
Physical Activity (hours/day)	2.6 ? 2.1	3.4 ? 2.1

Values are mean ? s.d

3.3.2 Compliance

Participant compliance to the drinking schedules is outlined in table 3.6. Of those that replied 74% of the single serve group and 90% of the divided dose group never missed drinking the full sachet of milk. Three participants (14%) in the single serve group did not complete a compliance form.

Table 3.6: Participant Compliance to Drinking Schedule

Compliance Rating	Single Serve Group	Divided Dose Group
Always Drank Full Sachet	14	18
Always Drank as Instructed (i.e. full amount or divided).	19	15
Missed One Night	2	2
Missed Two Nights	3	0
Missed > Two Nights	0	0
Number of Participants in Group who Replied	19/22	20/20

Of those that did not comply with the drinking schedule four missed drinking the milk on one night only (2 from each group) and three from the single serve group missed taking the milk on two occasions. There were no participants who reported missing the milk on more than two nights. In the divided dose group three participants reported drinking the milk in two portions rather than three. However this never occurred on more than three evenings.

3.3.3 Dpd Analysis

Baseline and 2-week Dpd/Crn are shown in figure 3.2. Values that are statistically the same are denoted by the same letters e.g a, b. Baseline values of Dpd/ Crn did not differ between groups. Analysis of Dpd data by repeated measures anova was unable to show any significant changes over time in either the single serve or the divided dose group, however values in the divided dose group tended to increase (p=0.09). Despite the absence of significant changes over time, there was a significant difference in end (2 week) values of Dpd/Crn (**a** and **b**). At 2 weeks Dpd/ Crn values were higher for the divided dose group than for the single dose group (p<0.002). Baseline calcium intake did not determine baseline Dpd however there was a slightly positive relationship between the age of participants and their baseline Dpd values (p<0.01) (see figure 3.3).

Figure 3.2 Dpd Data for Trial Groups

70
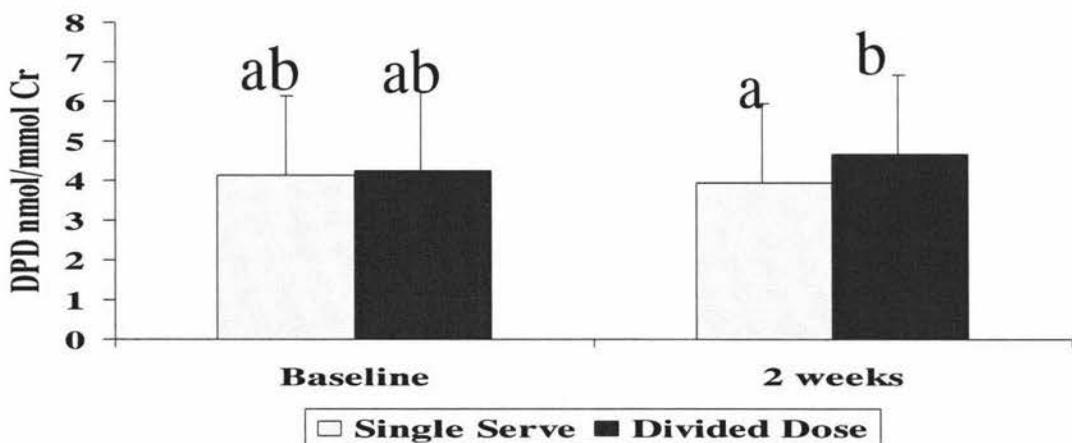
Figure 3.3: Effect of Age on Baseline Dpd/Crn
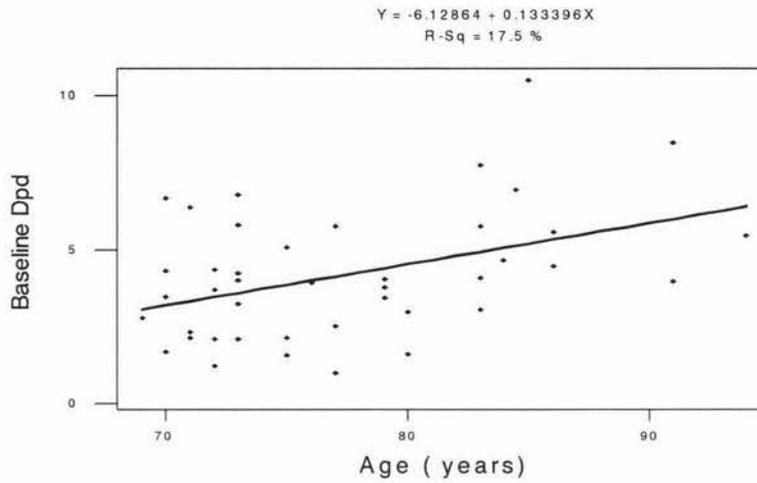
3.3.4 Urinary Calcium Analysis

Both baseline and end values of urinary calcium were statistically the same for members of the single and divided dose group. Figure 3.4 shows calcium data for both trial groups at baseline and at the end of the intervention period. There were no significant changes in urinary calcium with time in either the single or divided dose groups however it tended to increase with milk intervention in the divided dose group (p=0.07). Higher baseline dietary intakes of calcium were associated with elevated urinary calcium excretion (p<0.01). Participant age did not affect the level of urinary calcium excretion (p=0.32).

Figure 3.4 Calcium Data for Trial Groups
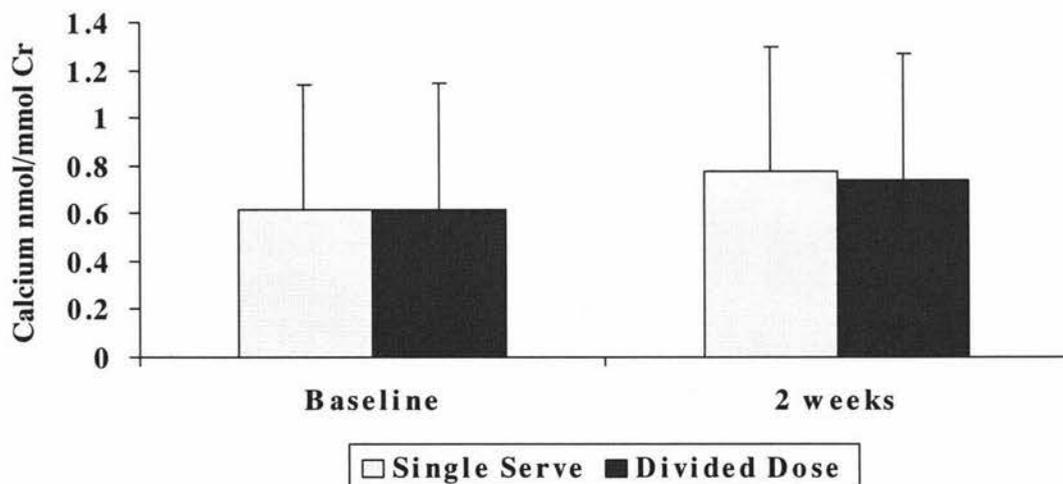
3.3.5 Creatinine Analysis

Figure 3.5 shows box-plots of the rate of creatinine excretion for the group of participants on each of the collection days. Creatinine excretion rates did not differ across collections (p>0.20). Outliers on the graphs are shown as stars and most probably represent inaccurately collected samples.

Figure 3.5 Creatinine Excretion Rate for Each of the Four Collections
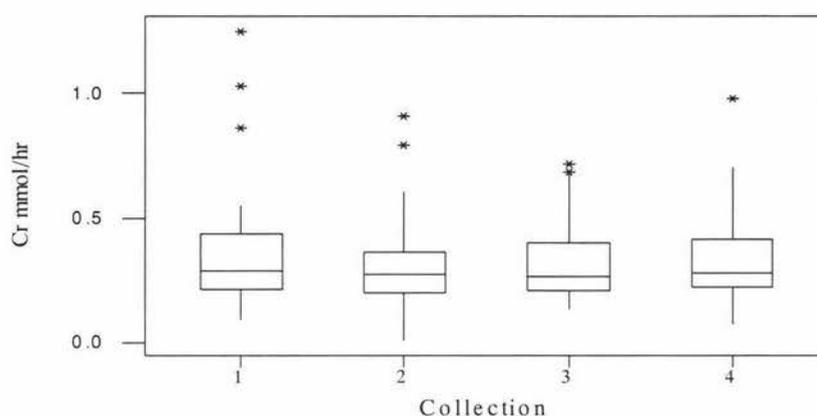
3.4   DISCUSSION

3.4.1 Anthropometric, Nutritional and Physical Activity Data for Trial Groups

The non-random, unblinded manner of allocating participants to each of the groups makes the trial susceptible to bias and error. However the lack of statistically significant differences in anthropometric, nutritional and physical activity data between the single serve and divided dose groups supports a fair comparison.

3.4.2 Compliance

The majority of participants complied to the drinking schedule. Those few that did stray from the drinking schedule did so on no more than two of the fourteen nights.

Therefore the author believes that participant compliance would not have biased the results.

3.4.3 Discussion of DPD Data

Calcium supplementation has successfully reduced urinary Dpd within the same time frame of our study (see section 1.6.3). The average calcium intakes were 843 and 830mg/day for the single serve and divided dose groups respectively. The supplementary dose of 640mg/day therefore brought the average intake up to around 1500mg/day, which is higher than the current US RDA. In theory the supplementary dose should have been enough to cause reductions in urinary Dpd excretion. Because calcium absorption exhibits a threshold at around 1200mg, giving more calcium should not in theory result in more calcium being absorbed or greater physiological changes being seen. However it is interesting to note that studies that show the most convincing evidence of changes in bone resorption markers used very large supplementary doses leading to total calcium intakes much higher than and in some case nearly twice the US RDA (Rubinacci *et al.* 1996; McKane *et al*, 1996; Fardellone *et al.* 1998; Kamel *et al*,. 1998; Prestwood *et al.* 1999). It would be interesting to note whether changes would be produced in these studies had the supplementary dose been smaller.

Some studies have found that the largest changes occur in those participants with low baseline calcium intakes. Fardellone *et al.* found that those participants with intakes below the mean intake of around 600mg/day exhibited the greatest changes in Dpd (Fardellone *et al.* 1998). Woo *et al.* 1991 produced a reduction in hydroxyproline using only 400mg/day for 12 hours (See section 1.6.3). In Woo's study however the elderly women had a mean baseline calcium intake of only 288mg/day. The changes seen in individuals with higher calcium intakes may well be much smaller than in those with very low calcium intakes and would be therefore harder to measure. Although it is important to remember that the difference in race between Woo's participants and the Caucasian population used in the current study as calcium absorption has been shown to be higher in Chinese women than in Caucasian Americans (Kung *et al*, 1998). 62% (26/42) of our participants had values lower than the Australian RDA (800mg/day) and 13 participants (31%) had intakes higher than 1000mg/day. Because of calcium's absorption threshold calcium supplementation would not be expected to benefit those

who already consume sufficient dietary amounts of the mineral. Consequently around a third of our participants would not be expected to benefit from calcium supplementation. However the baseline calcium intakes of our study participants were not related to the change in urinary Dpd/Crn excretion suggesting that another factor may be acting.

It has been suggested that free deoxypyridinoline itself may lack specificity as a marker of bone resorption (see section 1.6.3). Kamel *et al.* 1998 produced changes in DPD in a variety of bone resorption markers but not in free DPD. Therefore it is possible that the free Dpd measured in this study was not sensitive enough to detect the changes in bone resorption that may have resulted. However changes in the secondary resorption marker, urinary calcium produced much the same results as for Dpd (see section 3.3.2). It would however have been useful to have included at least one other marker of bone resorption such as N-telopeptides of type 1 collagen to verify the results of our study. However such an undertaking was beyond the scope of the study.

Another factor that may have prevented a positive outcome from the milk supplementation is the possibility that there was a high level of low vitamin D status. Participants with low vitamin D status would have an impaired ability to absorb calcium and would have therefore responded poorly to treatment. The prevalence of minimal sunlight exposure coupled with low dietary intake of vitamin D was 1 in 22 for the single serve group and 4 in 20 for the divided dose group, however it is possible that the level of hypovitaminosis D is much higher. Assessment of actual vitamin D status in the participants would be necessary to determine the level of hypovitaminosis D in the study population. Reductions in the efficiency of calcium absorption are a common feature of aging (see section 1.3.1.2) and may occur for other reasons than hypovitaminosis D. Therefore it is possible that poor calcium absorption was a factor in the response to calcium supplementation in our study population.

The significant difference that exists between end values is interesting to note. The mean end-value for Dpd/Crn is significantly higher in the divided dose group than in the single dose group even though there were no changes overall within either group. It appears that, if anything, the divided dose group experienced a small increase in Dpd/ Crn. It may be that the one single larger dose of calcium was more effective in altering

bone resorption than each of the smaller divided doses of calcium were. However it is possible that this significant result simply represents the natural variation of Dpd excretion which was slightly down in the single serve group and slightly up in the divided dose group producing a difference between the group values that was large enough to be considered significant. In any case the changes produced were not large enough to be significant enough to demonstrate changes in bone resorption within either the single serve or the divided dose group.

3.4.4 Discussion of Calcium Data

Urinary calcium data follow the trend seen with urinary Dpd in that there were no differences in baseline or end values between the two groups. There were also no significant changes over time in either groups although, in a manner similar to the Dpd data, urinary calcium tended to increase with time in the divided dose group (p=0.07). Therefore the results obtained from this second resorption marker appear to confirm the results seen with urinary Dpd.

3.4.5 Creatinine Data

Because creatinine (and Dpd) are excreted with a circadian pattern (are excreted in more or less volumes depending on the time of day), it is important that samples are taken at the same time every day. Creatinine is used to correct for circadian rhythm, as its excretion is highly consistent. Therefore samples taken at the same time on different days should have very similar rates of creatinine excretion. As creatinine excretion rate did not differ across collections we can assume that any differences in Dpd excretion adjusted against creatinine (for volume) are not due to circadian variation but are caused by some other influence.

3.5   Conclusions/Summary

Milk supplementation did not produce changes in urinary measurements of free Dpd in our study population whether or not the milk was consumed as one amount or in three divided doses. If milk supplementation at the level in this study was able to produce any changes in Dpd, they were not large enough to be measured in either the single or divided dose group. The use of a higher amount of calcium or perhaps a more sensitive bone resorption marker may yield results in further studies.

However it cannot be ruled out that milk supplementation at this level would not produce changes in bone resorption in a different group of participants. Some of the group participants already had sufficient dietary intakes of calcium. Others had low levels of sunlight exposure, indicating that hypervitaminosis D may be present in some.

CHAPTER 4: CONCLUSIONS AND FUTURE WORK

4.1 DIETARY AND LIFESTLYE PATTERNS RELATED TO BONE HEALTH

The high proportion of participants with inadequate calcium intakes in conjunction with moderate to high levels of inadequate magnesium and zinc intakes are of concern and may be compromising bone health in this group of elderly. Further studies are needed to confirm that these nutrients are inadequate in this population. A larger survey of a randomly selected population in which multiple 24 hour recalls or diet records were taken would be necessary to confirm real nutritional inadequacies in this group. Use of another marker of energy intake such as 24 hour urinary nitrogen would be useful to confirm conclusions based on BMR. Should the findings of the current study be confirmed, intervention trials measuring the combined effects of calcium with magnesium, and zinc on bone health would be warranted.

Although most participants appeared to be getting enough sunlight exposure, the low dietary levels of vitamin D meant that any who were not exposed to sufficient sunlight may be at risk of hypovitaminosis D. Especially in rest homes, where sunlight exposure was lowest, measurements of the vitamin D status of the residents would make for a very interesting and pertinent study. Without an adequate vitamin D status even a high calcium intake may not be effective in reducing bone loss. It is therefore crucial that those suffering hypovitaminosis D be diagnosed and treated, in order to maintain their bone health. Most of the participants in our study appeared to have an adequate level of weight-bearing physical activity, although this is not to say that greater amounts would not have had additional benefit.

As rest home residents were the oldest, had the highest level of dietary inadequacy, the lowest level of sunlight exposure, and were the least active among the participants the author suggests that this group may be particularly compromised in terms of bone health and represent an excellent target for intervention.

4.2 EFFECT OF MILK ON BONE RESORPTION USING TWO INGESTION STRATEGIES

The purpose of this study was to see if dividing the dose of a high calcium milk would produce greater changes in bone resorption than consuming the same dose all at once. Supplementation with milk (equivalent of 640mg of calcium) was unable to produce significant changes in bone resorption in our participant group, whether the milk was consumed in one amount or divided into three doses. Therefore dividing the dose did not produce a large enough change in calcium absorption to produce changes in bone resorption in this group of participants. Although this is not a positive outcome, the absence of an effect represents a significant finding and meets the objective of the study. It is possible that a single serve of milk containing 640mg of calcium is not sufficient to elicit physiological changes in bone turnover in the elderly. However a benefit from such a treatment cannot be ruled out as several factors may have impacted on the results that were found.

Vitamin D status was not measured in our study group, but some participants had both low sunlight exposure and dietary intake and may therefore be at risk of vitamin D deficiency. It would be interesting to repeat the trial with a larger number of subjects where half of each group were supplemented with vitamin D to see if this affected the results.

It is possible that the bone resorption marker used may not have been sensitive enough to detect changes in bone resorption that may have occurred in this study. The use of other bone resorption markers alongside the measurement of free Dpd used may have provided a more accurate picture of any physiological occurrences.

Further studies using several bone resorption markers would be required to confirm the conclusions formed in this study.

REFERENCES

1. Aaron, J. E. Gallagher J. C. Anderson J. (1974). Frequency of osteomalacia and osteoporotic fractures of the proximal femur. *Lancet* **1**: 229-33.

2. Abelow, B. J. Holford T. R. Insogna K. L. (1992). Cross-cultural association between dietary protein and hip fracture: a hypothesis. *Calcified Tissue International* **50**: 14-8.

3. Aloia, J. F. (1994). The gain and loss of bone in the human lifecycle. *Advances in Nutritional Research, vol 9.* Editor H. H. Draper, 1-33. Vol. 9. New York: Plenum Press.

4. Bachrach, L. K. Hastie T. Wang M. Narasimhan B. Marcus R. (1999). Bone mineral acquisition in healthy Asian, Hispanic, Black, and Caucasian youth: a longitudinal study. *Journal of Clinical Endocrinology an Metabolism* **84(12)**: 4702-12.

5. Baker, M. R. McDonnell H. Peacock M & Nordin B. E. C. (1979). Plasma 25 hydroxyvitamin D concentration in patients with fractures of the femoral neck. *Lancet* **1**: 589-90.

6. Bauer, D. C. Browner W. S. Cauley J. A. Orwell E. S. Scott J. C. Black D. M. Tao J. L. Cummings S. R. (1993). Factors associated with appendicular bone mass in older women. The Study of Osteoporotic Fractures Research Group. *Annals of Internal Medicine* **118(9)**: 657-51.

7. Bendich, A. Langseth L. (1989). Safety of vitamin A. *American Journal of Clinical Nutrition* **49**: 358-71.

8. Bettica. P., Moro L. Robins S. Taylor A. Talbot J. Singer F. & Baylink D. (1992). Bone-resorption markers galactoyl hydroxylysine, pyridinium cross-links, and hydroxyproline compared. *Clinical Chemistry* **38**: 2313-18.

9. Bettica, P. Taylor A. Moro R. Talamini R. & Baylink D. (1996). Clinical performance of galactosyl hydrosylysine, pyridinoline, and deoxypyridinoline in postmenopausal osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **81:** 542-46.

10. Black, D. Duncan A. & Robins S. (1988). Quantitative analysis of the pyridinium cross-links of collagen in urine using ion-paired reversed-phase high \ performance liquid chromatography. *Analytical Biochemistry* **169:** 197-203.

11. Bleau, G. Roberts K. D. & Chapdelaine A. (1974). The *in vitro* and *in vivo* uptake and metabolism of steroids in human adipose tissue. *Journal of Clinical Endocrinology and Metabolism* **39:** 236.

12. Blumsohn, A. Herrington K. Hannon R. A. Shao P. Eyre D. R. & Eastell R. (1994). The effect of calcium supplementation on the circadian rhythm on bone resorption. *Journal of Clinical Endocrinology and Metabolism.* **79(3):** 730-735.

13. Bouillon, R. Carmeliet G. & Boonen S. (1997). Ageing and calcium metabolism. *Ballière's Clinical Endocrinology and Metabolism* **11(2):** 341-65.

14. Bronner, F. (1997). Calcium absorption-a paradigm for mineral absorption. *Journal of Nutrition* **128:** 917-20.

15. Bronner, F. & Pansu D. (1999). Nutritional aspects of calcium absorption. *Journal of Nutrition* **129:** 9-12.

16. Bronner, F. & Pansu D. & Stein W. D. (1986). An analysis of intestinal calcium transport across the rat intestine. *American Journal of Physiology* **250:** G561-G569.

17. Brot, C. Jørgensen N. R. Sørensen O H. (1999). The influence of smoking on vitamin D status and calcium metabolism. *European Journal of Clinical Nutrition* **53:** 920-926.

18. Buchner, D. M. Beresford S. A. A. Larson E. B., LaCroix, A.Z., Wagner, E.H . (1992). Effects of physical activity on health status in older adults II: Intervention studies. *Annual Review of Public Health* **13:** 469-88.

19. Bullamore, J. R. Gallagher J. C. Wilkonson R. & Nordin B. E. C. (1970). Effect of age on calcium absorption. *The Lancet* **2:** 535-37.

20. Burger, H. de Laet C. E. D. H. van Daele P. L. A. Weel A. E. A. M. Witteman J. C. M. Hofman A. & Pols H. A. P. (1998). Risk factors for increased bone loss in an elderly population. *American Journal of Epidemiology* **147(9):** 871-79.

21. Calvo, M. S. (1994). The effects of high phosphorus intake on calcium homeostasis. In: *Advances in Human Nutrition, Volume 9.* Editor H. H. Draper. New York: Plenum Press.

22. Casperson, C J. Bloemberg B. P. Satis W. H. Merrit R. K. & Kromhout D. (1991). The prevalence of selected activities and their relation with coronary heart disease risk factors in elderly men: The Zutphen Study, 1985. *American Journal of Epidemiology* **133:** 1078-92.

23. Cauley, J. A. Gutal J. P. Sandler R. B. Laporte R. E. Kuller L. H. & Sashin D. (1986). The relationship of endogenous estrogen to bone density and bone area in normal postmenopausal women. *American Journal of Epidemiology`* **124:** 752.

24. Center, J. & Eisman J. (1997). The epidemiology and pathogenesis of osteoporosis. *Ballière's Clinical Endocrinology and Metabolism* **11(1):** 23-62.

25. Channay, A. (1972). Mechanical influences in bone remodelling: experimental research on Wolf's Law. *Journal of Biomechanics* **5:** 173-80.

26. Chapuy, M. C. Arlot M. E. Delmas P. D. Meunier P. J. (1994). Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. *The British Medical Journal* **308(6936):** 1081-82.

27. Chapuy, M. C. Arlot M. E. Duboeuf F. Brun J. Crouzet B. Arnaud S. Delmas P. D. & Meunier P. J. (1992). Vitamin D3 and calcium to prevent hip fractures in elderly women. *New England Journal of Medicine.* **327:** 1637-42.

28. Chiodini, I. Carnevale M. Torlontano M. Fusilli S. Gulglielmi G. Pileri S. Modoni S. Di Giorgio A. Liuzzi A. Minisola S. Cammisa M. Trichitta V. & Scillitani A. (1998). Alterations in bone turnover and bone mass at different skeletal sites due to pure glucocorticoid excess: study in Eumenorrheic patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism.* **83:** 1863-67.

29. Christenson, R. H. (1997). Biochemical markers of bone metabolism: an overview. *Clinical Biochemistry* **30(8):** 573-93.

30. Compston, J. E. (1992). Risk factors for osteoporosis. *Clinical Endocrinology* **36:** 223.

31. Compston, J.E. (1998). Vitamin D deficiency: time for action: Evidence supports routine supplementation for elderly people and others at risk. *British Medical Journal* **317(7171):** 1466-67.

32. Compston, J. Laskey M. Croucher P. Coxon A. Kreitzman. (1992). Effect of diet-induced weight loss on total body bone mass. *Clinical Science* **82:** 429-32.

33. Consensus Development Conference. (1993). Diagnosis, prophylaxis and treatment of osteoporosis. *American Journal of Medicine* **94:** 646-50.

34. Cowell, A. Eastell R. (1996). The renal clearance of free and conjugated pyridinium crosslinks of collagen. *Journal of Bone and Mineral Research* **11:** 1976-80.

35. Cummings, S. R. Nevitt M. C. Browner W. S. Stone K. Fox K. M. Ensrud K. E. Cauley J. Black D. Vogt T. M. (1995). Risk fractures for hip fracture in white women: The Study of Osteoporotic Fractures. *The New England Journal of Medicine* **332(12):** 767-73.

36. Dandona, P. Menon R. K. Shenoy R. Houlder S Thomous M. Malinson W. J. W. (1986). Low 1,25 dihydroxyvitamin D_3, secondary hyperparathyroidism, and normal osteocalcin in elderly subjects. *Journal of Clinical Endocrinology and Metabolism* **63**: 459-62.

37. Dawson-Hughes, B. Harris S. S. Krall E. A. Dallal G. E. (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *The New England Journal of Medicine.* **337(10):** 670-676.

38. Dawson-Hughes, B. Harris S. S. Krall E. A. Dallal G. E. Flaconer G. & Green C. L. (1995). Rates of bone lo in postmenopausal women randomly assigned to one of two dosages of vitamin D. *American Journal of Clinical Nutrition* **61:** 1140-1145.

39. Dawson-Hughes, M. D. Dallal G. E. Krall E. A. Sadowski L. Sahyoun N. & Tannenbaum S. (1990). A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *The New England Journal of Medicine* **323:** 878-73.

40. Delmas, P. (1990). Biochemical markers of bone turnover for the clinical assessment of metabolic bone disease. *Endocrinology and Metabolism Clinics of North America* **19:** 1-18.

41. DeLuca, H. F. (1988). The vitamin D story: a collaborative effort of basic science and clinical medicine. *FASEB Journal* **2:** 224-36.

42. Devine, A. Criddle R. A. Dick I. M. Kerr D. A. & Prince R. L. (1995). A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. *American Journal of Clinical Nutrition* **62:** 740-745.

43. Eastell, R. (1998). Drug therapy: treatment of postmenopausal osteoporosis. *The New England Journal of Medicine* **338(11):** 736-46.

44. Eastell, R. Calvo M. S. Burrit M. F. Offord K. P. Russell G. G. & Riggs B. L. (1992). Abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **74:** 487-94.

45. Eaton S. B., & Nelson D. A. (1991). Calcium in evolutionary perspective. *American Journal of Clinical Nutrition* **54:** 281S-7S.

46. Elmståhl, S. Gullberg B. Janzon L. Johnell O. & Elmståhl B. (1998). Increased incidence of fractures in middle-aged and elderly men with low intakes of phosphorus and zinc. *Osteoporosis International* **8:** 333-40.

47. Erikson, E. Steiniche T. Brixen K. (1996). Biochemistry and histology of osteoporosis. In: *Osteoporosis; Diagnosis and Treatment.* Editor D Satoris. New York: Marcel Dekker.

48. Evans, W. J. & Campbell W. W. (1993). Sarcopenia and age-related changes in body composition and functional capacity. *Journal of Nutrition* **123:** 465-68.

49. Eyre, D. (1992). New biomarkers of bone resorption [editorial]. *Journal of Clinical Endocrinology and Metabolim* **74:** 470A-C.

50. Eyre, D. Dickson I. & Van Ness K. (1988). Collagen cross-linking in human bone and articular cartilage. *Biochemical Journal* **252:** 494-500.

51. Eyre, D. Paz M. & Gallop P. (1984). Cross-linking in collagen and elastin. *Annual Review of Biochemistry* **53:** 717-48.

52. Fardellone, P. Brazier M. Kamel S. Guéris j. Graulet A. Liénard J. & Sebert J. (1998). Biochemical effects of calcium supplementation in postmenopausal women: influence of dietary calcium intake. *American Journal of Clinical Nutrition* **67:** 1273-78.

53. Farmer, M. E. White L. R. & Brady J. A. (1984). Race and sex difference in hip fracture incidence. *American Journal of Public Health* **74:** 1374.

54. Fernyhough, L. K. Horwath C. C. Campbell A. J. Robertson M. C. & Busby W. J. (1999). Changes in dietary intake during a 6-year follow-up of an older population. *European Journal of Clinical Nutrition* **53:** 216-25.

55. Ferrari, S. Rizzoli R. Chevally I. Slosman D. Eisman J. A. Bonjour J. P. (1995). Vitamin D receptor gene polymorphisms and change in lumbar-spine bone mineral density. *The Lancet* **345:** 423-24.

56. Feskanich, D. Willet W. C. Stampfer M. J. & Colditz G. A. (1997). Milk, dietary calcium, and bone fractures in women: a 12-year propective study. *American Journal of Public Health* **87(6):** 992-97.

57. Fiatarone, M. A. O'Neill E. F. Ryan N. D. Clements K. M. Solares G. R. Nelon M. E. Roberts S. B. Kehayias J. J. Lipsitz L. A. Evans W. A. (1995). Exercise training and nutritional supplementation for physical frailty in very elderly people. *New England Journal of Medicine* **330:** 1769-75.

58. Frontera, W. R. Hughes V. A. Evans W. J. (1991). A cross-sectional study of upper and lower extremity muscle strength and mass in 45- to 78-year-old men and women. *Journal of Applied Physiology* **71:** 644-50.

59. Fujita, T. (1994). Osteoporosis in Japan: Factors contributing to the low incidence of hip fracture. In: *Advances in Nutritional Research, Vol. 9.* Editor H. H. Draper. p 89-99. New York: Plenum Press.

60. Fujita, T. & Fukase M. (1992). Comparison of osteoporosis and calcium intake between Japan and the United States. *Proceeding of the Society for Experimental Biology and Medicine* **200:** 149.

61. Galgali, G. Norton R. & Campbell A. J. (1998). Physical inactivity in New Zealanders aged 60 years and older. *New Zealand Medical Journal* **111:** 115-17.

62. Garnero, P. & Delmas P. (1997). Bone markers. *Ballières Clinical Rheumatology* **11:** 517-37.

63. Garnero, P. Weichung J. Gineyts E. Karpf D. Delmas P. (1994). Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *Journal of Clinical Endocrinology and Metabolism* **79:** 1693-700.

64. Gennari, L. Becherini L. Masi L. Mansani R. Gonnelli s. Cepollaro C. Martini S. Montagnani A. Lentini G. Becorpi A. M. & Brandi M. L. (1998). Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. *Journal of Clinical Endocrinology and Metabolism* **83(3):** 939-44.

65. Goldberg, G. R. Black A. E. Jebb S. A., Cole., J.J., Murgatroyd, P.R., Coward, W.A., Prentice, A.M. (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *European Journal of Clinical Nutrition* **45:** 569-81.

66. Goulding, A. (1981). Fasting urinary sodium/creatinine in relation to calcium/creatinine and hydroxyproline/creatinine in a general population of women. *New Zealand Medical Journal.* **93:** 294-97.

67. Goulding, A. Campbell D. R. (1983). Dietary NaCl promotes calciura and bone loss in adult oophorectomised rats consuming a low calcium diet. *Journal of Nutrition* **113:** 1409-14.

68. Green, J. H. (2001). Correlations between biochemical markers of bone resorption and creatinine excretion. *Scandinavian Journal of Clinical and Laboratory Investigation* **60:** 1-8.

69. Greenspan, S. L. Myers E. R. Maitland L. A. Resnich N. M. Hayes W. C. (1994). Fall severity and bone mineral density as risk factors for hip fracture in ambulatory elderly. *Journal of the American Medical Association* **271:** 128-33.

70. Halioua, L. & Anderson J. J. B. (1989). Lifetime calcium intake and physical activity habits: independent and combined effects on the radial bone of healthy premenopausal Caucasian women. *American Journal of Clinical Nutrition* **49:** 534-41.

71. Hathcock, J. N. Hatten D. G. Jenkins M. Y. (1980). Evaluations of vitamin A toxicity. *American Journal of Clinical Nutrition* **52:** 183-202.

72. Heaney, R. P. (2000). There should be a dietary guideline for calcium. *American Journal of Nutrition* **71:** 658-60.

73. Heaney, R. P. McCarron D. A. Dawson-Hughes B. Oparil, S., Berga, S.L., Stern, J.S., Bar., S.I., Rosen, C.J.. (1999). Dietary changes favourably affect bone remodelling in older adults. *Journal of the American Dietetic Association.* **99(10):** 1228-33.

74. Heaney, R. P. Recker R. R. & Saville P. D. (1977). Calcium balance and calcium requirements in middle-aged women. *American Journal of Clinical Nutrition* **30:** 1603-11.

75. Heaney, R. P. Weaver C. M. & Fitzsimmons M. L. (1990). Influence of calcium load on absorption fraction. *Journal of Bone and Mineral Health* **5(11):** 1135-38.

76. Hegsted, D M. (1986). Calcium and osteoporosis. *Journal of Nutrition* **116:** 2316-19.

77. Heikinheimo, R. J. Inkovaara J. A. Harju E. J. Havisto M. V. Kaarela R. H. Kataja J. M. Kokko A. M. L. Kolho L A. & Rajala S. A. (1992). Annual injections of vitamin D and fractures of aged bones. *Calcified Tissue International* **51:** 105-10.

78. Heinonen, A. Kannus P. Sievanen H. Oja P. Pasanen M. Rinne M. Uusi-Rasi K. Vuori I. (1996). Randomised controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. *The Lancet* **348(9038):** 1343-47.

79. Hernandez-Avila, M. Colditz G. A. Stampfer M. J. Rosner B. Peizer F. E. Willet W. C. (1991). Caffeine, moderate alcohol intake and risk of fractures of the hip and forearm in middle-aged women. *American Journal of Clinical Nutrition* **54(1):** 157-63.

80. Herzberg, M. Foldes J. Stenberg R. & Menczel J. (1990). Zinc excretion in osteoporotic women. *Journal of Bone and Mineral Research* **5(3):** 251-57.

81. Hillary Comission for Recreation and Sport. (1991). Volume III Physical Activity. *Life in New Zealand Commission Report.* Wellington: Hillary Comission for Recreation and Sport.

82. Hillary Commission for Recreation and Sport. (1991). *Life in New Zealand Survey.* Wellington: Hillary Commission for Recreation and Sport.

83. Holbrook, T. L. Barrett-Connor E. & Wingard D. L. (1988). Dietary calcium and risk of hip fracture 14-year prospective population study. *The Lancet* **2(8619):** 1046-9.

84. Holick, M. F. Matsuoka L. Y. Wortsman J. (1989). Age, vitamin D, and solar ultraviolet. *The Lancet* **2(8671):** 1104-5.

85. Horwath, C. C. (1990). The nutrition of elderly New Zealanders. *Proceedings of the Nutrition Society of New Zealand* **15:** 83-89.

86. Hu , J. F. Zhao X. H. Jia J. B. Parpia B. Campbell T. C. (1993). Dietary calcium and bone density among middle-aged and elderly women in China. *American Journal of Clinical Nutrition* **58:** 219-27.

87. Inman, C. L. Warren G. L. Hogan H. A. & Bloomfeild S. A. (1999). Mechanical loading attenuates bone loss due to immobilisation and calcium deficiency. *Journal of Applied Physiology* **87(1):** 189-95.

88. Institute of Medicine. (2001). Dietary Reference for Vitamin A, Vitamin K., Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Report of the National Institute of Medicine. January 9, 2001.

89. Jaglal, S. B. Kreiger N. & Darlington G. (1993). Past and recent physical activity and risk of hip fracture. *American Journal of Epidemiology* **138(2):** 107-17.

90. Johnston, C. C. Hui S. L. Witt R. M. Appledorn R. Baker R. & Longcope C. (1985a). Early menopausl changes in bone mass and sex steroids. *Journal of Clinical Endocrinology and Metabolism* **61:** 905.

91. Johnston, C. C. Norton J. Khairi M. R. A. (1985b). Heterogeneity of fracture syndromes in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **61:** 551-56.

92. Jones, G. Wguyen T. Sambrook P. N. (1994). Progressive loss of bone in elderly people: longitudinal findings from the Dubbo osteoporosis epidemiology study. *British Medical Journal* **309:** 691-95.

93. Kamel, S. Fardellone P. Meddah B. Lorget-Gondelmann F. Sebert J. L. & Brazier M. (1998). Response of several markers of bone collagen degradation to calcium supplementation in postmenopausal women with low calcium intake. *Clinical Chemistry* **44(7):** 1437-42.

94. Kanis, J. A. Melton L. J III. Christiansen C. Johnston C. C. Khaltaev N. (1994). The diagnosis of osteoporosis. *Journal of Bone & Mineral Research* **9:** 1137-41.

95. Kelly, P. J. Hopper J. L. Macaskill G. T. Pocock N. A. Sambrook P. N. & Eisman J. A. (1991). Genetic factors in bone turnover. *Journal of Clinical Endocrinology and Metabolism* **72(4):** 808-13.

96. Kerstetter, J. E. O'Brien. K. O. & Insogna K. L. (1998). Dietary protein affects intestinal calcium absorption. *American Journal of Clinical Nutrition* **68:** 859-65.

97. Khaw, K. T. Sneyd M. J. & Compston J. (1992). Bone density, parathyroid hormone and 25-hydroxyvitamin D concentrations in middle aged women. *British Medical Journal* **305:** 273.

98. Khosla, S. Atkinson E. J. Melton L. J III. & Riggs B. L. (1997). Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population study. *Journal of Clinical Endocrinology and Metabolism* **82(5):** 1522-27.

99. Kim, Y. & Linkswiler H. M. (1979). Effect of level of protein intake on calcium metabolism and on parathyroid and renal function in the adult human male. *Journal of Nutrition* **109:** 1399-404.

100. Kriska, A. M. Black Sandler R. Cauley J. A. LaPorte R. E. Hom D. L. & Pambianco G. (1988). The assessment of historical physical activity and its relation to adult bone parameters. *American Journal of Epidemiology* **127(5):** 1053-61.

101. Krølner, B. Toft B. Nielson S. (1983). Physical exercise as prophylaxis against involutional vertebral bone loss: a controlled trial. *Clinical Science* **64:** 541-46.

102. Kung, A.W.C., Luk, K.D.K., Chu, L.W., Chiu, P.K.Y. (1998). Age related osteoporosis in Chinese: an explanation of the response of intestinal calcium absorption and calcitropic hormones to dietary calcium deprivation. *American Journal of Clinical Nutrition.* **68:** 1291-7

103. Kushida, K. Takahashi M. Kawana K. & Inoue T. (1995). Comparison of markers for bone formation and resorption in premenopausal and postmenopausal subjects, and osteoporosis patients. *Journal of Clinical Endocrinology and Metabolim* **80:** 2447-50.

104. Lau, E. M. C. & Woo J. (1994). Osteoporosis in Asia. In: *Advances in Nutritional Research, Vol. 9.* Editor H. H. Draper. p101-18. New York: Plenum Press.

105. Leboff, M. S. Kohlmeier L. Hurwitz S. Franklin J. Wright J. & Glowacki J. (1999). Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *The Journal of the American Medical Association* **281(16):** 1505-11.

106. Ley, S. J. Horwath C. C. & Stewart J. M. (1999). Attention is needed to the high prevalence of vitamin D deficiency in our older population. *New Zealand Medical Journal* **112:** 471-72.

107. Liel, Y. Edwards J. Shary J. Spicer K. M. Gordon L. & Bell N. H. (1988). The effects of race and body habitus on bone mineral density of the radius, hip and spine in premenopausal women. *Journal of Clinical Endocrinology and Metabolism* **66:** 1247-50.

108. Lim, S. K. Park Y S. Park J. M. Song Y. D. Lee E. J. Kim K. R. Lee H. C. & Huh K. B. (1995). Lack of association between vitamin D receptor genotype and osteoporosis in Korean. *Journal of Clinical Endocrinology and Metabolism* **80:** 3677-81.

109. Linder, M. C., Editor. (1991). *Nutritional Biochemistry and Metabolism with Clinical Applications.* 2nd ed. Connecticut: Appleton & Lange.

110. Lipton, A. Demers L. Daniloff Y. Curley E. Hamilton C. Harvey H. Witters L. Seaman J. Van der Giessen R. & Seyedin S. (1993). Increased urinary excretion of pyridinium cross-links in cancer patients. *Clinical Chemistry* **39(4):** 614-18.

111. Looney, J. E. Yoon H. K. Fischer M. Farley S. M. Farley J. R. Wergedal J. E. & Baylink D. J. (1995). Lack of high prevalence of the BB vitamin D receptor genotype in severely osteoporotic women. *Journal of Clinical Endocrinology and Metabolism* **80(7):** 2158-62.

112. Luckey, M. M. Meier D. E. Mandeli T. P. Dalosta M. C. Hubbard M. C. & Goldsmith S. J. (1989). Radial and vertebral bone density in white and black women: evidence for racial differences in premenopausal bone homeostasis. *Journal of Clinical Endocrinology and Metabolism* **69**: 762-70.

113. Lutz, J. (1986). Bone mineral, serum calcium, and dietary intakes of mother/daughter pairs. *American Journal of Clinical Nutrition* **44**: 99-106.

114. Lutz, J. & Tesar R. (1990). Mother-daughter pairs: spinal and femoral bone densities and dietary intakes. *American Journal of Clinical Nutrition* **52**: 872-77.

115. Maggi, S. Kelsey J. L. Litvak J. & Heyse S. P. (1991). Incidence of hip fractures in the elderly: a cross-national analysis. *Osteoporosis International* **1**: 232.

116. Marcus, R. (1996). The nature of osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **81(1)**: 1-5.

117. Matkovic, V. Fontana D. Tominac C. Goel P. & Chesnut C. H. III. (1990). Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *American Journal of Clinical Nutrition* **52**: 878-88.

118. Matkovic, V. & Heaney R. P. (1992). Calcium balance during human growth: evidence for threshold behaviour. *American Journal of Clinical Nutrition* **55**: 992-96.

119. Matkovic, V. Kostial K. Simonavic I. Buzina R. Brodarec A. & Nordin B. E. (1979). Bone status and fracture rates in two regions of Yugoslavia. *American Journal of Clinical Nutrition* **32**: 540-549.

120. Matsuoka, L. Y. Ide L. Wortsman J. MacLaughlin J. A. & Holick M. F. (1987). Sunscreens suppress cutaneous vitamin D_3 synthesis. *Journal of Clinical Endocrinology and Metabolism* **64**: 1165-68.

121. Matsuyama, T. Ishii S. Tokita A. Yabuta K. Yamamori Morrison N. A. Eisman J. A. (1995). Vitamin D receptors and bone mineral density. *The Lancet* **345:** 1239-40.

122. Mazess, R. B. & Mather W. (1974). Bone mineral content of North Alaskan Eskimos. *American Journal of Clinical Nutrition* **27:** 916-25.

123. Mazzeo, R. S. Cavanagh P. Evans W. J. Fiatarone M. Hagberg J. McAuley E. Startzell J. (1998). Exercise and physical activity for older adults. *Medical Science in Sports and Exercise* **30:** 992-1008.

124. Mckane, W. R. Khosla S. Egan K. S. Robins S. P. Burritt M. F. & Riggs B. L. (1996). Role of calcium intake in modulating age-related increases in parathyroid function and bone resorption. *Journal of Clinical Endocrinology and Metabolism* **81:** 1699-703.

125. Melhus, H. Kindmark A. & Ljunghall S. (1994). Author's Reply. *The Lancet* **344(8936):** 1580-1581.

126. Melhus, H. Michaelsson K. Kindmark A. Berstrom R. Holmberg L. Mallmin H. Wolk A. Ljunghall S. (1998). Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Annals of Internal Medicine.* **129(10):** 770-778.

127. Ministry of Health. (1996). *Guidelines for Healthy Older People.* Wellington: Ministry of Health.

128. Ministry of Health. (1999). *NZ Food: NZ People: Key Results of the 1997 National Nutrition Survey.* Wellington: Ministry of Health.

129. Ministry of Health (1999). *Taking the Pulse: the 1996/1997 New Zealand Health Survey.* Wellington: Ministry of Health.

130. Morrison, N. A. Qi J. C. Tokita A. (1994). Prediction of bone density from vitamin D alleles. *Nature* **367:** 284-87.

131. Murphy, S. Khaw K. May H. & Compston J. E. (1994). Milk consumption and bone mineral density in middle aged and elderly women. *British Medical Journal* **308(6934):** 939-41.

132. National Osteoporosis Foundation. (2000). "Osteoporosis." Web page, [accessed June 1929]. Available at http://www.nof.org/osteoporosis/stats.htm.

133. Nelson, M. E. Fiatarone M. A. Morganti C. M. Trice I. Greenberg R. A. Evans W. J. (1994). Effects of high-intensity strength training on multiple risk factors for osteoporotic fractures: a randomised controlled trial. *The Journal of the American Medical Association* **272(24):** 1909-14.

134. New, S. A. Robins S. P. Campbell M. K. Martin J. C. Garton M. J. Bolton-Smith C. Grubb D. A. Lee. S. J. & Reid D. M. (2000). Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health. *American Journal of Clinical Nutrition* **71:** 142-51.

135. NIH Consensus Development Panel on Optimal Calcium Intake (1994). Optimal Calcium Intake. *The Journal of the American Medical Association* **272(24):** 1942-48.

136. Nilas, I. Christiansen C. Rodbro P. (1984). Calcium supplementation and postmenopausal bone loss. *British Medical Journal* **289:** 1103-6.

137. Nordin, B. E. C. (2000). Calcium requirement is a sliding scale. *American Journal of Clinical Nutrition* **71:** 1381-81.

138. Nordin, B. E. C. Need A. G. Bridges A. & Horowitz M. (1992). Relative contributions of years since menopause. age and weight to vertebral density in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **74(1):** 20-23.

139. Nordin, B. E. C. Need A. G. & Chatterton B. E. (1990). The relative contributions of age and years since menopause to postmenopausal bone loss. *Journal of Clinical Endocrinology and Metabolism* **70:** 83.

140. Nordin, B. E. C. Need A. G. Morris H. A. Horowitz M. (1993). The nature and significance of the relationship between urinary sodium and urinary calcium in women. *Journal of Nutrition* **123:** 1615-22.

141. O'Brien, K. O. (1998). Combined calcium and vitamin D supplementation reduces bone loss and fracture incidence in older men and women. *Nutrition Reviews* **56(5):** 148-58.

142. Ohta, A. Ohtsuki M. Baba S Adachi T. Sakata T. Sakaguchi E. (1995). Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. *Journal of Nutrition* **125(9):** 2417-24.

143. Ooms, M. E Roos J. C. Bezemer P. D. van der Vijgh Bouter L. M. Lips P. (1995). Prevention of bone loss by vitamin D supplementation in elderly women: randomised, double-blind trial. *Journal of Clinical Endocrinology and Metabolism* **80:** 1052-58.

144. Ortiz, O. Russell M. Daley T. L. Baumgartner R. N. Waki M. Lichtman S. Wang J. Pierson R. N. Jr. & Heymsfeild S. B. (1992). Difference in skeletal muscle and bone mineral mass between black and white females and their relevance to estimates of body composition. *American Journal of Clinical Nutrition.* **55:** 8.

145. Orwell, E. E. Oviatt S. K. McClung M. R. Deftos L. J. Sexton G. (1990). The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. *Annals of Internal Medicine* **112:** 29-34.

146. Pannemans, D. L. E. Schaafsma G. Westerterp K R. (1997). Calcium excretion, apparent calcium absorption and calcium balance in young and elderly subjects: influence of protein intake. *British Journal of Nutrition* **77:** 721-29.

147. Parfitt, A. M. (1983). Dietary risk factors for age-related bone loss and fractures. *The Lancet* **2:** 1181-85.

148. Parfitt, A. M. Gallagher J. C. Heaney R. P. Johnston C. C. Neer R. & Whedon G. D. (1982). Vitamin D and bone health in the elderly. *American Journal of Clinical Nutrition* **36:** 1014.

149. Polley, K. J. Nordin B. E. C. Baghurst P. A. Walker J & Chatterton B. E. (1987). Effect of calcium supplementation on forearm bone mineral content in paotmenopausal women: A prospective, sequential controlled trial. *Journal of Nutrition* **117:** 1929-35.

150. Pollitzer, W. S. & Anderson J. B. (1989). Ethnic and genetic differences in bone mass: a review with a heredity vs environmental perspective. *American Journal of Clinical Nutrition* **50:** 1244-59.

151. Prestwood, K. M. Thompson D. L. Kenny A. M. Seibel M. J. Pilbeam C. C. & Raisz L. G. (1999). Low dose estrogen and calcium have an additive effect on bone resorption in older women. *Journal of Clinical Endocrinology and Metabolism* **84:** 179-83.

152. Province, M. A. Hadley E. C. Hornbrook M. C. (1995). The effects of exercise on falls in elderly patients. *The Journal of the American Medical Association* **273**: 1341-47.

153. Ramsdale, S. J. Bassey E. J. (1994). Changes in bone mineral density associated with dietary-induced loss of body mass in young women. *Clinical Science* **87:** 843-48.

154. Reid, I. R. (1993). Correction-- Effect of calcium supplementation on bone loss in postmenopausal women. *The New England Journal of Medicine.* **329(17):** 1281.

155. Reid, I. R. Ames R. Evans M. C. Gamble G. D. Sharpe S. J. (1995). Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomised controlled trial. *American Journal of Medicine.* **98(4):** 331-35.

156. Reid, I. R. Ames R. W. Evans M. C. Gamble G. D. Sharpe S. J. (1993). Effect of calcium supplementation on bone loss in postmenopausal women. *The New England Journal of Medicine.* **328(7):** 460-464.

157. Reid, I. R. Mackie M. Ibbertson H. K. (1986). Bone mineral content in Polynesian and white New Zealand women. *British Medical Journal* **292:** 1547-48.

158. Robins, S. Woitge H. Hesley R. Ju J. Seyedin S. Seibel M.(1994). Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone formation. *Journal of Bone and Mineral Research* **9:** 481-86.

159. Rosen, C. J. Chesnut C. H. III. & Mallinak N. J. S. (1997). The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement or calcium supplementation. *Journal of Clinical Endocrinology and Metabolism* **82(6):** 1904-10.

160. Rubinacci, A. Diveti P. Polo R. M. Zampino M. Resmini G. & Tenni R. (1996). Effect of an oral calcium load on urinary markers of collagen breakdown. *Journal of Endocrinology Investigation* **19:** 719-26.

161. Salamone, L. M. Cauley J. A. Black D. M. Simkin-Silverman L. Lang W. Gregg E. Palermo L. Epstein R. S. Kuller L. H. & Wing R. (1999). Effect of a lifestyle intervention on bone mineral density in premenopausal women: a randomised trial. *American Journal of Clinical Nutrition* **70:** 97-103.

162. Schindler, A. E. Ebert A. & Friedrich E. (1972). Conversion of androstenedione to estrone by human fat tissue. *Journal of Clinical Endocrinology and Metabolism* **35:** 627.

163. Schofeild, W N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition: Clinical Nutrition* **39C(suppl 1):** 5-41.

164. Schofield, W N. Schofield C. & James W. P. T. (1985). Basal Metabolic Rate. *Human Nutrition: Clinical Nutrition* **39C(suppl 1):** 1-96.

165. Schutte, S. A. Knowles J. B. Ford H. E. (1989). Effect of lactose or its component sugars on jejunal calcium absorption in adult man. *American Journal of Clinical Nutrition* **50(5):** 1084-87.

166. Schutte, S. A. Zemel M. B. Linkswiler H. M. (1980). Studies on the mechanism of protein-induced hypercalciuria in older men and women. *Journal of Nutrition* **110:** 305-15.

167. Seibel, M. Gartenberg F. Silverberg S. Ratcliffe A. Robins S. & Bilezikian J. (1992). Urinary hydroxypyridinium cross-links of collagen in primary hyperparathyroidism. *Journal of Clinical Endocrinology and Metabolism* **74:** 481-86.

168. Seyedin, S. Kung V. Daniloff Y. Heley R. Gomez B. Nielson L. Rosen H. Zuk R. (1993). Immunoassay for urinary pyridinoline; the new markers of bone resorption. *Journal of Bone and Mineral Research* **8:** 635-41.

169. Sharpe, S. J. Reid I. R. Evans M. C. Ames R. W. (1994). Nutrient intakes of women volunteers for an osteoporosis study. *Proceedings of the Nutrition Society of New Zealand* **19**: 128.

170. Smith, E. L. Gilligan C. Smith P. E. & Sempos C. T. (1989). Calcium supplementation and bone loss in middle-aged women. *American Journal of Clinical Nutrition* **50:** 833-42.

171. Specker, B. L. (2000). Should there be a dietary guideline for calcium intake? No. *American Journal of Clinical Nutrition* **71:** 665-67.

172. Spector, T. D. Keen R. W. Arden N. K. Morrison, N. A. Major P. J. Nguyen T. V. Kelly P. J. Baker J. R. (1995). Influence of vitamin D recepetor genotype on bone mineral density in postmenopausal women: a twin study in Britain. *British Medical Journal* **310:** 1357-60.

173. Suleiman, S. Nelson M. Li F. Buxton-Thomas M. & Moniz C. (1997). Effect of calcium intake and physical activity level on bone mass and turnover in healthy white, postmenopausal women. *American Journal of Clinical Nutrition.* **66:** 937-43.

174. Svensdon, O. Hassanger C. Christiansen C. (1993). Effect of an energy restrictive diet, with or without exercise on lean tissue mass, resting metabolic rate, cardiovascular risk factors and bone in overweight postmenopausal women. *American Journal of Medicine* **95:** 131-40.

175. Takahara, S. Morohashi T. Sano T. Ohta A. Yamada S. Sasa R. (2000). Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. *Journal of Nutrition* **130(7):** 1792-95.

176. Thomas, M. K. Lloyd-Jones D. M. Thadhani R. I , Shaw, A.C., Deraska, D.J., Kitch, B.T., Vamvakas, E.C., Dick, I.M., Prince, R.L., Finkelstein, J.S. (1998). Hypovitaminosis D in medical in-patients. *New England Journal of Medicine* **338:** 777-83.

177. Thompson, F. E. & Byers T. (1994). Dietary assessment resource manual. *Journal of Nutrition* **124:** 2245S-316S.

178. Tokita, A. Kelly P. J. Nguyen T. V. (1994). Genetic influence on type I collagen synthesis and degradation; further evidence for genetic regulation of bone turnover. *Journal of Clinical Endocrinology and Metabolism* **78:** 1461-66.

179. Tortora, G. J. & Grabowski S. R. (1996). *Principles of Anatomy and Physiology.* 8th ed. USA: Harper-Collins.

180. Tucker, K. L. Hannan M. T. Chen H. Cupples L. A. Wilson P. W. F. & Kiel D. P. (1999). Potassium, magnesium, and fruit and vegetable intakes re associated with greater bone mineral density in elderly men and women. *American Journal of Clinical Nutrition* **69:** 727-36.

181. Twomey, L. Taylor J. & Furness B. (1983). Age changes in the bone density and structure of the lumbar vertebral column. *Journal of Anatomy* **136(1):** 15-25.

182. Ubelhart, S. Schlemmer A. Johansen J. Gineyts E. Christiansen C. & Delmas P. (1991). Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium cross-links. *Journal of Clinical Endocrinology and Metabolim* **72:** 367-73.

183. Uprichard, J. E. & Ball M. J. (1996). Fat and calcium intake in women dieters. *American Journal of Clinical Nutrition* **63:** 67-71.

184. Utiger, R. D. (1998). The need for more vitamin D. *The New England Journal of Medicine* **338(12):** 828-29.

185. Välimäki, M. J. Kärkkäinen M. Lamburg-Allardt C. Laitenen K. Alhara E. Heikkinen J. Imipiraara O. Makela P. Palmgren J. Seppanen R. Vuori I. (1994). Exercise, smoking and calcium intake during adolescence and early adulthood as determinants of peak bone mass. *British Medical Journal* **309(6949):** 230-235.

186. van de Heuvel, E. G. Muys T. van Dokkum W. Schaafsma G.(1999). Oligofructose stimulates calcium absorption in adolescents. *American Journal of Clinical Nutrition* **69(3):** 544-48.

187. Villareal, D. T. Cintelli R. Chines A. & Avioli L. V. (1991). Subclinical vitamin D deficiency in potmenopausal women with low vertebral bone mass. *Journal of Clinical Endocrinology and Metabolism* **72(3):** 628.

188. Warden, J. (1998). UK survey shows improved diet in elderly. *British Medical Journal* **317:** 1175.

189. Whiting, S. J. (1999). Excess retinol intake may explain the high incidence of osteoporosis in Northern Europe. *Nutrition Reviews.* **57(6):** 192-98.

190. Woo, J. Swaminathan R. Lau E. MacDonald D. Pang C. P. & Nordin E. C. (1991). Biochemical effects of a single oral dose of calcium on bone metabolism in elderly Chinese women. *Calcified Tissue International.* **48:** 157-60.

191. Wood, R. J. & Zheng J. J. (1997). High dietary calcium intakes reduce zinc absorption and balance in humans. *American Journal of Clinical Nutrition* **65**: 1803-9.

192. Yamagata, Z. Miyamura T. Iijima Asaka A. Sasaki M. Kato J. Koizumi K. (1994). Vitamin D receptor gene polymorphism and bone mineral density in healthy Japanese women. *The Lancet* **344(8928)**: 1027.

193. Yamaguchi, M. Yamaguchi R. (1986). Action of zinc on bone metabolism in rats. Increases in alkaline phosphatase activity and DNA contents. *Biochemical Pharmacology* **35:** 773-77.

194. Yates, A. A. Schlicker S. A. & Suitor C. W. (1998). Dietary reference intakes: The new basis for recommendations for calcium and related nutrients, B vitamins, and choline. *Journal of the American Dietetic Association.* **98:** 699-706.

195. Zhang, J. Feldblum P. J. & Fortney J. A. (1992). Moderate physical activity and bone density among perimenopausal women. *American Journal of Public Health* **82(5):** 736-38.

APPENDIX I: The Zutphen Modified Physical Activity Questionnaire

Modified from the original Zutphen Physical Activity Questionnaire In: Casperson, C. J., Bloemberg, B. P. M., Satis, W. H. M., Merrit, R. K., & Kromhout, D. (1991). The prevalence of selected activities and their relation with coronary heart disease risk factors in elderly men: The Zutphen study, 1985. *American Journal of Epidemiology*, 133: 1078-1092.

Modified Zutphen Physical Activity Questionnaire

1. Can you walk indoors?

   ❑ Yes
   ❑ No, because I use a wheelchair.
   ❑ No, because I am bedridden.
   ❑ No, for a different reason, namely
   If you are **unable** to walk/cycle, you can go on to
   Question number 7.

2. How often did you go for a walk during the last week?

   _____ times

3. How long did such a walk last?
   _____ minutes

4. How would you describe your walking pace?

   ❑ Calm
   ❑ Normal
   ❑ Firm

5. Did you take a walk that lasted longer than 1 hour during the last month?

   ❑ No
   ❑ Yes

5a. How often did you do that?

   _____ times

6. Do you bicycle?

   ❑ No
   ❑ Yes

6a. If yes, how often did you bicycle last week?

   _____ times

6b. How long did you bicycle?
   _____ minutes

6c. How would you describe your bicycling pace?

- ❑ Calm
- ❑ Normal
- ❑ Fast

7. Do you have a garden?

- ❑ No
- ❑ Yes

7a. If yes, how many hours, on average, a week do you spend in your garden?

In summer _____ hours
In winter _____ hours

7b. Do you work in your garden by yourself?

- ❑ No
- ❑ Yes
- ❑ Partly

8. Do you do the odd jobs in and around the house by yourself (e.g., painting and carpentry and including cross-stich, knitting)?

- ❑ No
- ❑ Yes

8a. If yes, for how many hours a month?
_____ hours

9. Did you participate in sports lately? E.g.bowls, golf.

- ❑ No
- ❑ Yes

9a. If yes, what kind of sport?

_____

9b. How many hours, on average, do you spend participating in sports monthly?

- ❑ Less than 1 hour a month
- ❑ _____ hours a month

10. Do you have a hobby (other than gardening or sports)?

- ❑ No
- ❑ Yes

10a. If yes, what kind of hobby?

_____

10b. How many hours a week do you spend on it?

- ❑ Less than 1 hour a week
- ❑ _____ hours a week

11. Did you keep birds as pets for a least 1 year during the past 10 years?

- ❑ No
- ❑ Yes

11a. If yes, what kind of birds?

- ❑ Canaries
- ❑ Parrots
- ❑ Pigeons
- ❑ Chickens
- ❑ Other

12. How often did you perspire during physical exercise in the last week?

- ❑ Never
- ❑ _____ times

13. Do you climb stairs regularly?

   ❑ No
   ❑ Yes

14. Most men and women of your age spend about 1 hour a day doing domestic work, doing odd jobs, gardening, walking, and doing other physical activities. How do you see yourself compared with these men and women?

   ❑ Far more active
   ❑ More active
   ❑ About the same
   ❑ Less active
   ❑ Far less active

14. What do you think of your pace compared with men and women of your age?

   ❑ Much faster
   ❑ Faster
   ❑ About the same
   ❑ Slower
   ❑ A lot slower

16. How many hours, on average, do you sleep at night?

_____ hours

17. How many hours do you sleep during the day (for example, take a nap)?

   ❑ I do not sleep in the daytime.
   ❑ I sleep for _____ hours.

APPENDIX II: Diet and Lifestyle Factors which Contribute to Bone Health

Free-Living Women									
ID	Age	BMI	Ca	Zn	Mg	VitD	PA	Sun	Prot
E5	1	-	-	-	1	-	1	1	1
E6	1		1	1	1	-	1	1	-
E7	1	1	1	1	1	1	1	1	-
E12	1		1	1	1	-	1	1	-
E14	1	-	1	1	-	-	1	-	1
E15	1	-	1	1	1	-	1	1	-
E16	-	1	-	1	1	-	1	1	-
E18	1	-	-	1	1	-	1	1	1
E19	1	1	-	1	1	-	-	1	1
E21	1	1	-	-	1	-	1	1	1
E22	-	-	-	1	-	-	1	1	-
E23	1	-	1	1	1	-	1	1	-
E24	1	-	1	1	1	-	1	1	-
E25	-	1	-	1	1	-	-	-	1
E29	1	1	-	1	1	-	1	1	-
E31	1	1	-	-	-	-	1	1	1
E32	-	N/A	-	-	1	-	-	1	1
E33	1	1	-	-	1	-	1	1	1
E34	1	-	1	1	1	-	1	1	-
E35	1	1	1	1	1	-	1	-	-
E37	-	1	-	1	1	-	1	1	-
E39	1	1	-	1	1	-	1	1	-
E41	-	-	-	-	1	-	1	1	-
EIO2	1	1	1	1	1	-	N/A	1	-
EIO12	1	1	1	1	1	-	1	1	+
Institutionalised Women									
ID	Age	BMI	Ca	Zn	Mg	Vit D	PA	Sun	Prot
EIO11	1	1	1	1	1	-	-	N/A	-
E43	-	-	1	-	-	-	-	-	1
E44	-	1	-	1	-	-	-	-	-
E45	-	1	-	-	-	-	1	-	-
E46	-	1	-	-	-	-	-	-	1
E47	1	-	1	-	-	-	-	-	1
EIO1	-	-	-	-	-	-	-	1	1
EIO3	-	N/A	-	-	-	-	1	-	1
EIO4	-	1	-	-	-	-	-	1	1
EIO5	-	-	-	1	1	-	-	-	1
EIO6	-	-	-	1	1	-	-	1	-
EIO7	-	1	-	-	-	-	-	-	1
EIO13	-	-	-	-	1	-	-	-	1

Men ID	Age	BMI	Ca	Zn	Mg	Vit D	PA	Sun	Prot
E2	-	-	-	-	1	-	-	1	1
E3	1	-	1	1	1	-	-	1	-
E4	-	1	-	1	-	-	1	1	1
E8	-	-	1	1	1	-	1	1	1
E9	1	1	-	-	1	-	1	1	1
E10	-	1	-	-	-	-	1	1	1
E11	1	1	-	1	1	-	1	1	1
E13	1	1	1	1	1	-	1	1	1
E20	1	-	1	1	1	-	1	1	-
E26	1	-	-	1	1	-	-	1	-
E27	1	1	1	1	1	-	1	1	-
E30	-	-	-	1	1	-	-	1	1
E38	-	1	-	1	1	-	1	1	1
E42	1	-	-	1	1	-	1	1	-
EIO10	1	-	-	1	1	-	N/A	1	1
EIO8	-	-	-	1	1	-	-	-	1
EIO9	-	-	-	1	1	-	-	1	1

Criteria For Receiving a point:
Age: Above group mean for age
BMI: Above lower acceptable limit of 20kg/m^2
Nutrients: Above 2/3 of the RDA /EAR / AI assigned to the particular micronutrient/nutrient.
Physical Activity: Greater than the average number of hours spent in physical activity
Sun Hours: More than 30 minutes daily exposure to sun.

N.B: The author acknowledges that not all of these factors that impact on bone health may do so in an equal fashion. E.g. Someone who has a low calcium intake may not automatically be as at risk of osteoporosis as someone with low sun exposure. The points given are not meant to be of equal value but simply to be used as an indication of where deficiencies intersect and are present in combination. Nor are they meant to rate or try to quantify the risk each individual has of developing osteoporosis-this is not possible. The author also acknowledges the reasonably arbitrary assignment of points. This table is not meant as a definitive list of risk factors for osteoporosis as this would have to include many other factors such as genetics and presence of disease states.

APPENDIX III: Consent Form

Massey University
COLLEGE OF SCIENCES

Institute of Food, Nutrition
and Human Health
Private Bag 11 222,
Palmerston North,
New Zealand
Telephone: 64 6 350 4335
Facsimile: 64 6 350 5657

Impact of calcium ingestion strategy on bone resorption

CONSENT FORM

Request for an interpreter			
English	I wish to have an interpreter	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhhakamaori/kaiwhaka pakeha korero	Ae	Kao
Samoan	Oute mana'o ia iai se fa'amatala upu	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea	Io	Ikai
Cook Island	Ka inangaro au I tetai tangata uri reo	Ae	Kare
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	E	Nakai

- I have read the Information Sheet and have had the details of the study explained to me.

- My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

- I understand that I have chosen to take part in this study and that I have the right to withdraw from the study at any time.

- I agree to provide information to the researches on the understanding that my name will not be used without permission.

- I understand that the study will be stopped if it appears harmful to me.

- I understand the compensation provisions for this study.

- I have had time to consider whether to take part.

Te Kunenga ki Pūrehuroa
Inception to infinity: Massey University's commitment to learning as a life-long journey.

- I know I can contact Caroline Stanley or Jill Richards at any time during the study.
- I agree to participate in this study under the conditions set out in the Information Sheet.
- I understand that the Massey University Ethics Committee has approved this project. This means that the committee may check that the study is running smoothly and is following appropriate ethical procedures. Complete confidentiality is assured.
- I consent to the researchers storing specimens for later use on the understanding that I will be contacted for consent if the researchers wish to measure substances other than calcium, deoxypyridinoline, cross-linked N-telopeptides of type I collagen (NTx), C-telopeptides of type I collagen (Crosslaps), hydroxyproline and creatinine.
- I consent to the researchers using data collected in this trial for publication in academic/medical publications.

I would/ would not like one of the researchers to discuss the overall results of the study with me.

I ....(full name)
hereby consent to take part in this study

Signature of Participant ....

Signature of witness ....

Name of witness ....

Principle Researchers:

Caroline Stanley
MSc in Nutritional Science student
Institute of Food, Nutrition and Human Health
Massey University
Palmerston North
Telephone: 06-356-9099 ext.7860
Fax: 06-350-5446
E-mail: C.M.Stanley@massey.ac.nz

Jill Richards
Human Nutrition Studies Laboratory
Institute of Food, Nutrition and Human Health
Massey University
Palmerston North
Telephone: 06-350-6296 (or 06-350-6116)
Fax: 06-350-5446
E-mail: J.K.Richards@massey.ac.nz

Project explained by ....

Role in project ....

Signature ....

Date ....