

School of Public Health

**A Randomised Controlled Trial of Twelve Months Protein
Supplementation on Muscle Mass and Strength in Elderly Women**

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:

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Abstract

Background

Aging is associated with progressive loss of muscle (sarcopenia), which can lead to reduced muscle strength and an increased risk of falls. Sarcopenia exists in otherwise healthy elderly people and its aetiology is not fully understood. Many epidemiological studies have shown that high protein intake is associated with preserving muscle mass and strength in the elderly. To date there have been few randomized trials of sufficient duration and power to examine the effects of dietary protein supplement on muscle mass and strength in the elderly. The objective of this study was to examine the effectiveness of whey protein supplementation on preventing sarcopenia in elderly women.

Methods

A population based, one-year randomized, double blind and placebo controlled trial of protein supplementation was conducted on 219 community-dwelling ambulant women aged 70 to 80 years. Participants in the protein supplement group (n=109) consumed a drink daily which contained 30 g of protein. The control group (n=110) consumed a drink with the same energy (kilojoules) but only contained 2 g of protein. Assessments were taken at baseline and one year. Body composition was assessed by anthropometry and whole body dual-energy x-ray absorptiometry. Peripheral quantitative computer tomography was used to assess calf muscle cross-sectional area. Hand grip, ankle dorsiflexion, knee and hip strengths were assessed using an isokinetic dynamometer. Mobility was assessed by the 'Timed Up and Go' test. Standing balance was assessed by the Romberg test. Dietary intake was assessed by a 3-day weighed food record. Compliance with the dietary intervention was assessed by 24-hour urinary nitrogen and by counting the returned empty supplement containers. Serum insulin-like growth factor one (IGF-1) was also measured.

Results

One-hundred and ninety-five participants aged 74±3 years completed the one year trial. There were no significant differences in baseline characteristics between the

protein supplemented group (n=100) and control group (n=95). Compared to their baseline values, both groups significantly increased whole body lean mass (protein group: +1.6%, $p<0.05$; control group: +2.3%, $p<0.05$), appendicular lean mass (protein group: +1.3%, $p<0.05$; control group: +1.8%, $p<0.05$), body weight (protein group: +0.8%, $p<0.05$; control group: +1.5%, $p<0.05$) and knee strength (protein group: +31%, $p<0.05$; control group: +36%, $p<0.05$) after one year. The total fat mass increased from baseline only in the control group (protein group: +0.7%, $p=0.19$; control group: +1.5%, $p<0.05$). There were however no significant differences between the two drink groups in any of the above mentioned parameters. Over one year serum IGF-1 increased significantly in the protein group but decreased in the control group (protein group: +7.6%, $p = 0.006$; control group: -1.0%, $p = 0.005$), and the changes were significantly different between two drink groups ($p = 0.006$). The protein supplement also showed a protective effect on preserving balance function at one year. The prevalence of 'poor standing balance' and 'fall rates' were significantly increased in the control group at one year.

Conclusion

Muscle mass and strength increased equally in both drink groups. Although fat mass only increased in the control group at one year there was no statistically significant difference in the changes in fat mass between the two groups due to the wide variance in response. Protein supplementation resulted in an increased serum IGF-1 level at one year compared with the control group.

These data are consistent with the concept that in this age group increased energy intake regardless of the macronutrient composition of the supplements improves muscle mass and function. It is possible that achieving this through increased protein rather than carbohydrate may prevent the increase in fat mass noted with the carbohydrate supplement for the control drink perhaps by an effect of the protein to increase serum IGF-1. The metabolic significance of this remains to be explored.

Abbreviations

ADP:	air displacement plethysmography
ALM:	appendicular lean mass
AALM:	adjusted appendicular lean mass
AMA:	arm muscle area
BCM:	body cell mass
BMC:	bone mineral content
BMD:	bone mineral density
BMI:	body mass index
BMR:	basal metabolic rate
CCK:	Gastrointestinal hormones cholecystokinin
CI:	confidence interval
CRP:	C-reaction protein
CT:	computed tomography
CUAMA:	corrected upper arm muscle area
CV:	coefficient of variation
DXA:	dual energy x-ray absorptiometry
DLW:	doubly-labeled water
FAO:	United Nations Food and Agriculture Organization
FFM:	fat-free mass
FM:	fat mass
GH:	growth hormone
HU:	Hounsfield units
ICC:	intraclass correlation coefficient
IGF-1:	insulin-like growth factor 1
IPAQ:	international physical activity questionnaire
IQOLA:	International quality of life assessment
Kcal:	kilocalorie
Mets:	MET-minutes/week
MJ:	megajoule
MMI:	muscle mass index
NHMRC:	National Health and Medical Research Council
PABA:	para-amino benzoic acid
pQCT:	peripheral quantitative computed tomography

rhGH:	recombinant human growth hormone
RDA:	recommended dietary allowance (US/Canada)
RDI:	recommended dietary intake (Australia)
SD:	standard deviation
SE:	standard error
SPSM:	short portable sarcopenia measure
TBK:	total body potassium
TBW:	total body water
TUAG:	Timed Up and Go
UNU:	United Nations University
USDA:	US Department of Agriculture
UWW:	Under water weighing
WHO:	World Health Organization

Definitions

Appendicular lean mass (Baumgartner, Koehler et al. 1998): non-bone lean mass of arms and legs assessed by dual energy x-ray absorptiometry.

Adjusted appendicular lean mass (Baumgartner, Koehler et al. 1998): appendicular lean mass adjusted for height square.

Arm muscle area: is calculated from triceps skinfold thickness and mid-arm circumference by the following equation (Heymsfield, McManus et al. 1982): Arm muscle area (mm^2) = (Mid-arm circumference in millimetre – π (skinfold))² / 4π .

Corrected arm muscle area (Hammond 2004): bone-free for women by the following equation: corrected arm muscle area (cm^2) = arm muscle area (cm^2) – 6.5; bone-free for men: corrected arm muscle area (cm^2) = arm muscle area (cm^2) – 10.

Ceiling effect: In statistics and measurement theory, an artificial upper limit on the value that a variable can attain, causing the distribution of scores to be skewed. For example, the distribution of scores on an ability test will be skewed by a ceiling effect if the test is much too easy for many of the respondents and many of them obtain perfect scores (Colman 2006).

Coefficient of variation: The standard deviation divided by the mean (generally expressed as a percentage). It is used to obtain a measure of relative variation (Dawson and Trapp 2004).

Frankfort plane: a craniometric plane determined by the inferior borders of the bony orbits and the upper margin of the auditory meatus. It passes through the two orbitales and the two tragions (Marfell-Jones, Olds et al. 2006). It is used in positioning the head for the taking of stature.

Intraclass correlation coefficient (ICC): is used to assess consistency or conformity between two or more measurements (Shrout and Fleiss 1979; Muller and Buttner 1994).

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Chapter 1 Introduction

1.1 Background

1.1.1 Public health significance of the problem

Aging is associated with a progressive loss of muscle mass (sarcopenia) (Welle, Thornton et al. 1993; Balagopal, Rooyackers et al. 1997) and bone mineral content (BMC) (Chen, Lin et al. 2008). This can lead to reduced muscle strength and increased risk of physical disability (van den Beld, Blum et al. 2003), falls and fractures (Smith, Khairi et al. 1975; Wasnich, Ross et al. 1989; Wolfson, Judge et al. 1995; Tinetti and Williams 1997; Loeser and Delbono 1999; Fried, Tangen et al. 2001).

Falls are the leading cause of injury-related hospitalisation in elderly people worldwide (Baker and Harvey 1985; Close, Ellis et al. 1999; Cripps and Carman 2001; Scuffham, Chaplin et al. 2003). It is an important public health problem both in Australia and overseas (Gillespie, Gillespie et al. 2003; Osteoporosis Australia 2007). In elderly Australians, 82% of fractures can be attributed to falls (Sambrook, Cameron et al. 2007) and over 90% of hip fractures involve a fall (Gillespie, Gillespie et al. 2003). Hip fracture is the costliest injury (Roudsari, Ebel et al. 2005) and leads to the most devastating consequences including loss of independence and life quality in the elderly (Wallace, Ross et al. 1993; Carey and Laffoy 2005).

Aging populations will raise the burden and costs to public health systems worldwide (Kannus, Sievanen et al. 2005). In Western Australia, fall related hospitalisation among people aged 65 years and over, between 1985 and 1994, increased by 31% (Ashwell, Pinder et al. 1996). In the Eastern Region of Ireland in 2002, there were a total of 2029 hospitalisations due to falls among people aged 65 years and over, 78% were female and the total inpatient costs were 10.6 million Euros (Carey and Laffoy 2005). The mean hospitalisation cost due to falls among people aged 65 or older was 17,483 US dollars in 2004 in the United States of America calculated using the Marked Scan Medicare database. Many developing countries such as China, are also experiencing an increased burden on their public health system due to their aging populations. The direct cost of a hip fracture treated in the United States is estimated to be \$US30,000 (Khan, Liu-Ambrose et al. 2001). The estimated cost to the health system of fall-related injuries in people age 65 or over was \$406.4 million in 1993-

1994 in Australia (Mathers and Penm 1999) and 981 million pounds in the UK in 1999 (Scuffham, Chaplin et al. 2003). The inevitable increasing rate of fall-related injuries in elderly people due to the growing aging population in Australia and in many other countries is imposing a huge burden on health care systems.

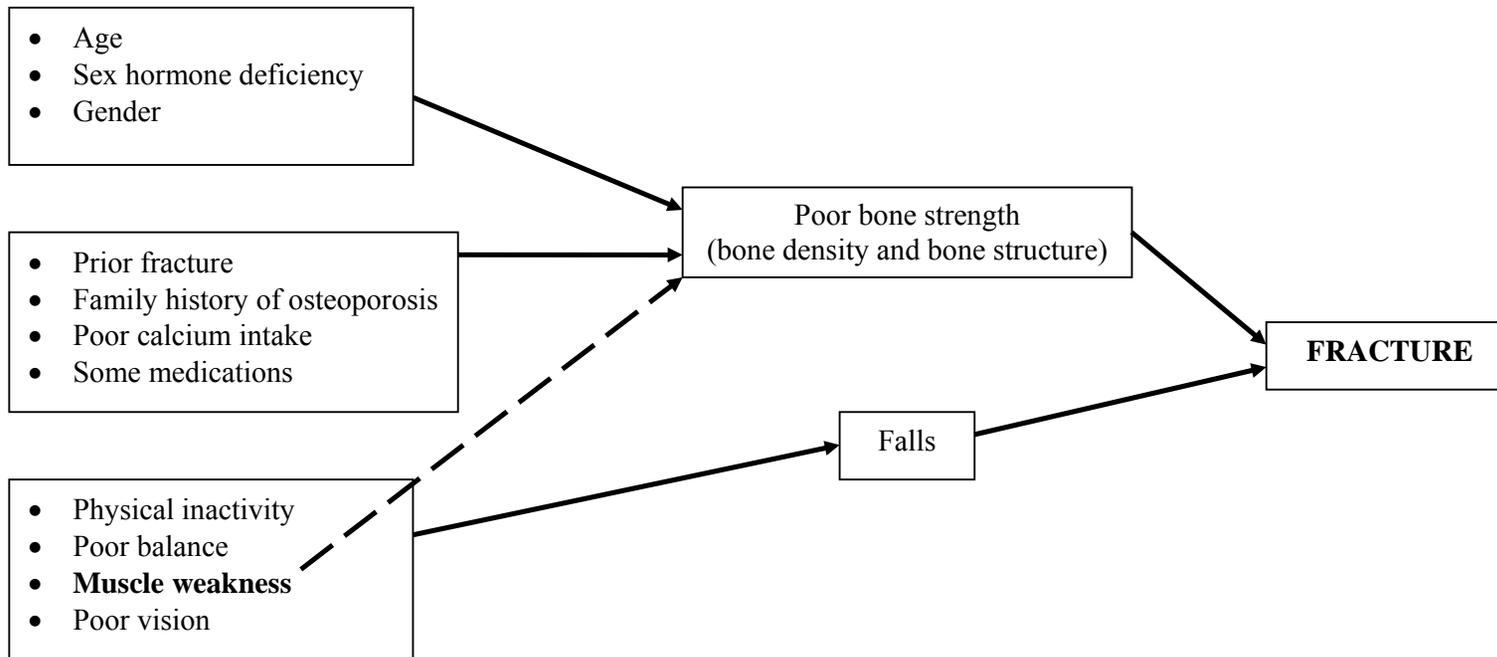
1.1.2 Risk factors for fracture in older people

Fracture risk in the elderly is determined by many risk factors such as osteoporosis related low bone density, impaired balance and loss of muscle mass and strength resulting in a greater risk of falls (Australian National Consensus Conference 1996 1997) as shown in Figure 1.1. These factors also interact with each other. For example, decreased muscle strength, especially lower limb muscle strength, appears to be an important determinant of balance (Lord, Clark et al. 1991) and postural stability in the elderly (Lord, Clark et al. 1991). Balance performance gradually declines with age (Isles, Choy et al. 2004). Lower extremity strength affects balance ability and agility in correcting gait when falling (Woollacott, Shumway-Cook et al. 1986). Studies have shown that ankle dorsiflexion, knee extension and hip abduction strength are inversely correlated with the rate of falls (Wiles, Busse et al. 2006).

In recent years, muscle weakness related falls and poor bone strength in the elderly has become a focus of osteoporosis research. Studies have shown that age related muscle weakness is not only related to the risk of falls but also directly and indirectly affects bone strength and structure (Klein, Allman et al. 2002; Capozza, Cure-Cure et al. 2008). Therefore decreased muscle mass and strength, along with a decline in physical activity in elderly people, may accelerate the progress of osteoporosis.

The aetiology of aging related sarcopenia and its prevention is not well understood. The current study focused on a dietary intervention, a non-pharmaceutical method, to investigate if a protein supplement could improve muscle preservation in elderly women.

Figure 1.1 Fracture risk factors in elderly people.



Adapted from (Australian National Consensus Conference 1996 1997)

1.1.3 The problem of the reduction of muscle mass and strength in older people

1.1.3.1 Aging and decrease of muscle mass and strength

As much as one third of muscle mass can be lost during the three decades after the age of 50 years (Borst 2004). This loss is accelerated after 60 years of age both in men and women (Kyle, Genton et al. 2001). Muscle strength peaks around the third decade of life, remains almost constant to the fifth decade, and then declines with age (Larsson, Grimby et al. 1979; Forrest, Zmuda et al. 2007) at a rate of approximately 8-15% per decade (Young, Stokes et al. 1984; Kallman, Plato et al. 1990; Frontera, Hughes et al. 1991; Lindle, Metter et al. 1997). A recent longitudinal study followed up twelve healthy men (initial mean age 65 years) over a 12-year period (Frontera, Hughes et al. 2000). The computed tomography scans showed that the cross-sectional areas of different sites of muscles all decline, with a reduction rate of 1.4% per year (Frontera, Hughes et al. 2000). The reduction in appendicular or leg fat-free mass was the main predictor of increased disability in older men and women (Fantin, Di Francesco et al. 2007). The decrease in muscle strength in older adults was found to correlate with a decreased proportion of type II muscle fibres (Larsson, Grimby et al. 1979; Lexell and Downham 1992), muscle cross-sectional area (Narici, Maganaris et al. 2003) and other unknown mechanisms including the functional and structural decline in neural regulating system on skeletal muscle (Delbono 2003).

1.1.3.2 Muscle mass, muscle strength and fall risk

Falls are associated with many risk factors as shown in Figure 1.1. Both falls and low body weight have been identified as factors which increase fracture risk in the elderly (Kannus, Sievanen et al. 2005; Sambrook, Cameron et al. 2007; Chen, Simpson et al. 2008). Decreased muscle strength, especially lower limb strength, is one of the most important risk factors associated with increasing risk of falling in the elderly (Owings, Pavol et al. 2001; de Rekeneire, Visser et al. 2003; Sambrook, Cameron et al. 2007). Strong leg strength has been shown to be associated with the ability to prevent a fall after the disruption of gait (Pijnappels, Reeves et al. 2008; Pijnappels, van der Burg et al. 2008). A one year prospective study in 95 older adults living in an aged care hostel in Sydney (mean age 82.7 years) found that

quadriceps strength was poorer in the multiple fallers compared with the non-fallers and once-only fallers (Lord, Clark et al. 1991). Another prospective population-based study followed up 187 community-dwelling women aged 75 years or older for 10 years and found that high muscle strength was associated with a low incidence of fall-related limb fractures (Sipila, Heikkinen et al. 2006). These prospective studies indicate that muscle strength has an important role in falls prevention in the elderly. Muscle power has been reported to be a strong predictor of self-reported functional status in elderly women (Foldvari, Clark et al. 2000). The loss of muscle power, or ‘explosive strength’, with aging has been found to be directly related to an increased number of falls (Lindle, Metter et al. 1997). The Baltimore Longitudinal Study of Aging found that muscle power declined more rapidly than strength after age of 40 years in men (Metter, Conwit et al. 1997).

1.1.3.3 Relationship between body weight, muscle mass and strength with bone health in elderly people

A cross-sectional study investigating 942 community-dwelling (England) men and women aged 65-74 years showed that approximately one third of the decline in bone mineral density with aging could be explained by the associated age-related decline in weight (May, Murphy et al. 1994). Compared to fat mass, lean mass was generally more strongly related to bone density in elderly women (Aloia, McGowan et al. 1991; Gjesdal, Halse et al. 2008). Cross-sectional calf muscle area, leg muscle mass and muscle power were found to be significantly correlated with bone strength in elderly women (Ashe, Liu-Ambrose et al. 2008; Binkley and Specker 2008).

A number of mechanisms have been proposed to explain the close relationship between muscle and bone health in the elderly. Firstly, the weight of muscle mass has a weight-bearing effect on the bone and muscle contraction exerts force on bone (Cifuentes, Johnson et al. 2003; Capozza, Cointy et al. 2004). Muscle contraction by exerting a force on bone may modify its structure (Bemben, Fetters et al. 2000). Muscle also contributes to body mass which requires increased force on the skeleton to counteract the effect of the increased weight (Cifuentes, Johnson et al. 2003). Secondly, muscle and bone also share biological mechanisms and interact with each other (Ferretti, Cointy et al. 2003). For example, insulin-like growth factor 1 (IGF-

1) has been recognized as an important regulator both for muscle (Cappola, Bandeen-Roche et al. 2001; Adamo and Farrar 2006) and bone (Yakar, Rosen et al. 2002; Sacco, Doyonnas et al. 2005). IGF-1 regulates muscle and bone metabolism, including myoblast proliferation, differentiation and protein accretion through multiple mechanisms (Adamo and Farrar 2006) which are not fully understood (detailed in section 2.1.3.2). Studies have also shown that muscle cross-sectional area and structural bone strength share genetic and environmental effects in older women (Mikkola, Sipila et al. 2008; Mikkola, Sipila et al. 2008).

In summary, muscle mass and strength, and bone density decline with age. Body weight, muscle mass and strength are significantly correlated with bone health in the elderly. The decline in muscle mass and strength, especially a decline of lower limb muscle mass and strength, are important risk factors for falls, which are closely related to the incidence of fractures in the elderly.

1.1.4 Possible ways to prevent sarcopenia

Many efforts have been made to try to find effective ways to slow the decrease of muscle mass and strength in the elderly experienced as a part of the aging process. There are three potential approaches to maintaining or increasing muscle mass and function: hormonal therapy, exercise, and nutrition (Wolfe 2006). From the public health perspective for prevention, exercise and dietary interventions are more desirable than using pharmaceutical interventions. Evidence from previous studies suggests that although muscle loss appears inevitable with aging, it could possibly be modified by adequate diet (e.g. adequate protein and energy intake) and exercise (Timmerman and Volpi 2008). To date, these dietary factors have not been adequately researched.

1.1.4.1 Exercise interventions

Most studies have demonstrated that exercise, and especially progressive resistance training, can increase muscle mass and/or strength in the elderly (Slade, Miszko et al. 2002; Figueroa, Going et al. 2003; Miszko, Cress et al. 2003). Progressive resistance training has been thought to be the only effective approach to treat and prevent

sarcopenia to date (Rolland and Vellas 2009). Previous studies have shown that aging muscle can respond to resistance training using evidence from muscle biopsy of the hypertrophy of muscle fibres (Pyka, Lindenberger et al. 1994). However, other studies have shown that sarcopenia also exists in active elderly individuals who are physically active (Westerterp and Meijer 2001; Wiswell, Hawkins et al. 2001; Hughes, Frontera et al. 2002; Marcell 2003). Many other questions remain, such as what kind of exercise is more effective and feasible, and what intensity and duration of exercise is required to overcome the muscle mass decline with aging?

1.1.4.2 Dietary intervention

A decrease in dietary intake is common in the elderly. This may be due to decreased appetite and reduced physical activity which reduces energy requirements. Even when dietary intakes are normal, decreased efficiency of digestion and utilization of nutrients could also play a role in malnutrition in the elderly.

Epidemiological studies have shown that adequate diet or higher protein intake appeared to be able to compensate for the loss of muscle mass with aging (Devine, Dick et al. 2005; Houston, Nicklas et al. 2008). Previous studies have shown energy, protein and other macronutrient intakes decrease in the elderly along with decreasing weight, lean body mass and increasing body fat mass (Nes, Sem et al. 1992). Habitual high protein intake was shown to reduce fracture risk in elderly men and women (Munger, Cerhan et al. 1999; Wengreen, Munger et al. 2004).

Timmerman and Volpi reviewed recent studies and found that age related sarcopenia may be halted or even reversed by changes in daily protein intake and exercise (Timmerman and Volpi 2008). They noted however that further evidence from intervention studies was needed.

There are few long-term protein supplement intervention studies (Lauque, Arnaud-Battandier et al. 2000; Bunout, Barrera et al. 2001; Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003). All of these studies have shown the weight gaining effect of the protein-energy supplements and no significant change in fat-free mass. Only one study found a significant beneficial effect of protein-energy supplements

on increasing muscle strength at three months, but failed to show an effect at nine months (Bonnefoy, Cornu et al. 2003). The study recruited 57 elderly men and women volunteers aged over 72 years in France and grouped participants into four groups: dietary supplemented group, placebo group, physical exercise group and a memory training group (Bonnefoy, Cornu et al. 2003). Although this study had a relatively long study period (9 month), the sample sizes of the subgroups were small. Lanque and colleagues conducted a 60-day randomized controlled nutritional intervention trial in 88 men and women aged 65 and over living in nursing homes in France (Lauque, Arnaud-Battandier et al. 2000). The 88 subjects were divided into four groups: 19 well-nourished subjects received no supplements, 41 who were at risk of malnutrition were randomized into two groups - received no supplements or received supplements, and 28 malnourished subjects received supplements. After 60 days, only those malnourished subjects who had received supplements were found to have a significant increase in weight, but no changes were found in hand grip strength. There were no changes in weight or muscle strength in the remainder of the subjects.

In summary, these studies had small sample sizes (Lauque, Arnaud-Battandier et al. 2000), relatively unwell elderly populations (Lauque, Arnaud-Battandier et al. 2000) and short study duration (Lauque, Arnaud-Battandier et al. 2000; Payette, Boutier et al. 2002). In addition many of the studies combined both dietary and exercise interventions in the one study (Bunout, Barrera et al. 2001; Bonnefoy, Cornu et al. 2003). Most of studies also included both men and women making it difficult to examine gender differences. The proportion of protein to energy varied between studies and most did not mention the protein source used in the supplements (Fiatarone, O'Neill et al. 1994; Gray-Donald, Payette et al. 1995; Wouters-Wesseling, Van Hooijdonk et al. 2003). All of the above factors contribute to a lack of power to draw conclusions and contribute to population based dietary recommendations for the well elderly.

Evidence from amino acid studies on muscle protein metabolism suggested that an effective nutritional intervention aiming to increase muscle mass in the elderly may need to be based on a protein source that has high bioavailability and amino acid utilisation, such as whey protein (Volpi, Ferrando et al. 1998; Walzem, Dillard et al.

2002; Volpi, Kobayashi et al. 2003). This study will investigate if a dietary protein supplement which is based on whey protein can maintain, or improve, muscle mass and strength in healthy elderly women.

1.1.5 Definition of ‘elderly people’

The definition of ‘elderly people’ varied between studies (Al Snih, Markides et al. 2002; Barbosa, Souza et al. 2005; Aagaard, Magnusson et al. 2007). PubMed defines people aged over 65 year as ‘Aged’ (PubMed). Aged over 65 is the widely used criteria to define ‘elderly’, ‘older adult’, or ‘older person’ although there is no physiological basis for this (Binns 1999). During the consultation process of “Dietary Guidelines for older Australians”, the majority also felt that 65 years was the most appropriate age to be considered to be an older Australian (Binns 1999). The recent version of the Nutrient Reference Values of Australia and New Zealand uses age ≥ 70 years to define ‘elderly people’ (National Health and Medical Research Council 2006). The current study recruited elderly women aged between 70 to 80 years.

1.2 Aim of this study

The aim of this study was to undertake a one year randomised, double blind, placebo controlled trial to examine the effectiveness of whey protein supplementation on muscle mass and strength and serum levels of IGF-1 in healthy elderly women.

1.3 Significance of this study

Sarcopenia contributes to loss of independence in the elderly (Doherty 2003). It increases the risk of fractures and falls in the elderly, which are major health problems in the community in terms of disability and cost. The results of this randomised, controlled study will clarify the role of protein on muscle mass and function in the elderly and possible mechanisms of action. By ensuring that the recruitment is population based and by using conservative exclusion criteria it is envisaged that if positive, the results of this research will translate into immediately applicable intervention strategies that are relevant at both a program and an

individual level. Finally the data will directly contribute to the development of a phase three cost effectiveness study of a non-pharmaceutical intervention to prevent sarcopenia in the aging population.

To the best of our knowledge, following a search of the literature, this is the largest randomized controlled trial investigating the effect of whey protein supplementation on the human skeletal muscle system in relatively healthy community-dwelling elderly women.

1.4 Hypotheses

- The muscle mass in the protein supplemented group will increase and there will be significant changes in whole body composition, with a proportional increase in body lean mass and a decrease in body fat mass relative to the control group.
- The mobility, balance and muscle strength in the protein supplemented group will improve relative to the control group.
- The effects of protein supplementation will be associated with increased concentrations of serum IGF-1 (mechanism).

1.5 Objectives

In healthy elderly women, the primary objectives of this study are (assess the effects of a protein intervention):

- 1) To determine the effects of protein supplementation on muscle mass and size and body composition measured using whole body dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) of the mid calf muscle cross-sectional area.
- 2) To examine the effect of protein supplementation on body composition using anthropometry (height, weight, hip and waist circumference, mid upper arm girth, skin fold).
- 3) To examine the effect of protein supplementation on mobility, balance and muscle strength by 'Timed Up and Go', 'Romberg balance' and 'muscle strength' tests.

- 4) To assess the effects of protein supplementation on the risk of falling using self-reported falls in the past three months before assessments.
- 5) To determine if the effects of increased dietary protein on muscle wasting is related to changes in circulating insulin-like growth factor one (IGF-1).
- 6) To assess the effect of the supplements on weight gain.

Secondary objectives (baseline cross-sectional study):

- 7) To assess the dietary intake from 3-day food records, and the relationship between habitual diet with body composition at baseline.
- 8) To validate dietary protein intake assessed by 3-day weighed food record by using 24 hour urine nitrogen as a biomarker.
- 9) To assess the prevalence of sarcopenia, and the relationship of lean body mass with body mobility, balance and upper and lower limb muscle strength at baseline.

Chapter 2 Literature review

2.1 Sarcopenia

2.1.1 Definition (absolute and relative sarcopenia)

In 1988, Rosenberg introduced the term ‘sarcopenia’ for the phenomena of age-related progressive loss of skeletal muscle mass (Rosenberg 1997). Sarcopenia was derived from the Greek terms *sarx* meaning flesh and *penia* meaning loss. The term ‘sarcopenia’ generally refers to a relative status of deficiency of muscle mass with aging.

A number of ways to define sarcopenia in a quantifiable way have been proposed by the research groups as discussed below. Common to these methods was that appendicular lean mass measured by DXA was the key outcome variable for all studies. As appendicular lean mass consists mostly of muscle, this makes a very reasonable index for the quantity of body muscle. Appendicular lean mass has also been reported to be predictor of disabilities in elderly men and women, and its decline accounted for approximately a two-fold increase in disability risk (Fantin, Di Francesco et al. 2007).

There is however, little consistency in the definitions used in different studies. Some studies used the term “skeletal muscle mass (SMS)” instead of “lean body mass”. In most cases, they refer to the same concept, which is the non-bone lean body mass measured by DXA under the assumption that all non-fat and non-bone tissue is skeletal muscle (Houston, Nicklas et al. 2008).

Absolute sarcopenia

In 1998, Rosenberg et al. introduced the term ‘adjusted appendicular lean mass’ (ALM) as an index of relative skeletal muscle mass adjusted for body size which also has been called ‘absolute sarcopenia’ (Baumgartner, Koehler et al. 1998). They took an approach analogous to the use of the body mass index (Weight/height squared) which adjusts mass with height squared to define underweight, overweight, and obesity. That is, ALM is defined as appendicular lean mass in kg divided by height squared in meter, $ALM (kg)/height^2 (m^2)$. Appendicular lean mass is measured by dual-energy x-ray absorptiometry. They defined sarcopenia in women as $ALM/height^2$ less than $5.45 kg/m^2$. $5.45 kg/m^2$ is the two standard deviations below

the mean of ALM/height² of a young reference group. This was later referred to as absolute sarcopenia (Estrada, Kleppinger et al. 2007). The cut-off point used varied slightly between studies. In the Third National Health and Nutrition Examination Survey (USA), using the same definition (ALM/height²), cut-off points of ≤ 5.75 kg/m² and 5.76-6.75 were selected to denote high and moderate physical disability risk in women (Janssen, Baumgartner et al. 2004).

Relative sarcopenia

A number of studies have adjusted skeletal muscle mass for body weight as an index to define sarcopenia, such as using total skeletal muscle mass/body mass (Janssen, Heymsfield et al. 2002) or ALM (kg)/weight (kg) (Estrada, Kleppinger et al. 2007). This was termed relative sarcopenia (Estrada, Kleppinger et al. 2007).

A cross-sectional study of 189 healthy older persons found that the absolute sarcopenia (ALM/height²) was a better indicator of mobility and that the relative sarcopenia (ALM/weight) was a better predictor of isolated muscle group function (Estrada, Kleppinger et al. 2007). The current study used the ALM/height² to define sarcopenia since body weight and BMI are highly collinear with lean body mass (Garn, Leonard et al. 1986).

Residuals method

The residual method uses the residuals obtained from linear regression of appendicular lean mass on height (meter) and body fat mass (kg) to define sarcopenia as the 20th percentile of the residuals. The appendicular lean mass and body fat mass are derived from DXA. The Health ABC study in the US, a longitudinal population based study following up 2976 healthy men and women aged 70-79 for five years, showed that the residuals method was better for predicting disability than the ALM/height² method since it accounts for the effect of body fat mass (Delmonico, Harris et al. 2007). They found that using the original ALM/height² method (absolute sarcopenia index), many obese subjects who were sarcopenic would be misclassified as normal. They defined sarcopenia if the value fell into the gender specific lowest 20% of the distribution of the index which was 5.67 kg/ht² for women. Obese elderly persons with sarcopenia are at high risk of negative health

outcomes including functional limitation and mortality (Stenholm, Harris et al. 2008).

Other methods

Kyle and colleague proposed another sarcopenia index based on whole body counting of potassium (TBK) and body cell mass (BCM) (Kyle, Genton et al. 2001; Kyle, Genton et al. 2001). The reason for using the TBK method is because potassium is the primary intracellular cation and 99.5% of the potassium in the body is rapidly exchangeable with an administered isotope (Moore 1980). Sarcopenia was defined as having values of $BCM/height^2 < -2$ SD (standard deviation) below the sex-specific mean for BCM index ($BCM/height^2$) in healthy control subjects younger than 35 years old ($< 6.96 \text{ kg/m}^2$ in women). TBK was measured by a whole-body scintillation counter (Kyle, Genton et al. 2001). BCM was calculated from TBK by the equation, $BCM \text{ (kg)} = 0.00833 \times TBK \text{ (mmol)}$. A higher proportion of elderly participants were defined as sarcopenic by this method compared to the results obtained using the Baumgartner adjusted appendicular lean mass ($ALM/height^2$) index. The researchers concluded that this was due to the greater decrease in TBK and BCM than the decrease in skeletal muscle mass and fat-free mass. This suggests that there is a change in the composition of fat-free mass with aging. The authors' rationale for using the BCM index was that TBK is the single best predictor of nutritional status because it represents BCM, the 'working' portion of fat-free mass which is mainly muscle and bone (Kehayias, Fiatarone et al. 1997; Kyle, Genton et al. 2001). However, this method includes bone which could be the source of the difference from other methods which exclude this aspect in their definitions. The equipment required for this method, a whole-body scintillation counter, is not widely available compared with DXA which is available in most laboratories.

Miller and colleagues recently developed and validated a sarcopenia measure in the African American health project in which 998 participants aged 49-65 years were involved (Miller, Malmstrom et al. 2009). The method was to combine whole body lean mass measured by DXA (muscle quantity), timed chair rises and hand grip strength/height (muscle function) into a single scale called the 'short portable sarcopenia measure' (SPSM). The SPSM score is based on three weighted component scores: chair stands score weighted 1, lean BMI (derived from

bioelectrical impedance body compositional measurement) weighted 1.5, and grip strength weighted 2. The SPSM scores range from 0 (greatest sarcopenia) to 18 (least sarcopenia) with 0.5 increments. They validated this method by studying the correlation of SPSM scores with knee strength (knee extension and knee flexion), physical performance (4 and 6 meter walk, usual gait speed and standing balance score), and psychological measurements. They found this unidimensional scale was significantly correlated with these measurements, with the strongest correlation with knee strength ($r = 0.61-0.68$, $p < 0.01$). However, this method has not been validated in other populations.

In summary, there is no consensus for a standardized definition of sarcopenia. Appendicular lean mass is the most important component used to define sarcopenia in previous studies. Adjustment for height or height and body fat mass are the major methods used in the previous studies to minimize the influence of body size. The Baumgartner method (which defines sarcopenia as $ALM/height^2 < 2$ standard deviation below a young reference group) has been widely used due to its practical measurement methods and straightforward demonstration. For elderly populations, adjustment for body fat mass should be considered because this method is able to identify obese sarcopenic individuals.

2.1.2 Prevalence of sarcopenia

The prevalence of sarcopenia varies by gender and ethnicity (Baumgartner, Koehler et al. 1998). The prevalence was higher in elderly women than in elderly men, and higher in elderly Hispanic women than in elderly non-Hispanic white women (Baumgartner, Koehler et al. 1998), and is lower in Asians than in white and Hispanic populations (Lau, Lynn et al. 2005). Sarcopenia also varies widely between studies, ranging from 2.8% to 59% (Melton, Khosla et al. 2000; Janssen, Heymsfield et al. 2002; Delmonico, Harris et al. 2007; Tichet, Vol et al. 2008), depending on the age range of the study population and the definition used. The prevalence of sarcopenia was reported to be about 33-36 % in 173 non-Hispanic white women age between 70-80 years (Baumgartner, Koehler et al. 1998). Based on Baumgartner's definition of sarcopenia ($ALM/height^2$ below two standard deviations of the mean of $ALM/height^2$ of a young reference group), the prevalence was 25.9% in 89 healthy

women aged 68 ± 5 in US (Estrada, Kleppinger et al. 2007), 22.6% in 195 women and 26.8% in 142 men aged 64 - 93 years (Iannuzzi-Sucich, Prestwood et al. 2002), and 50.4% in 216 women aged 74 ± 5 years in Italy (Coin, Perissinotto et al. 2008).

In Asia, a study in 527 community-dwelling elderly Chinese men and women showed that the prevalence of sarcopenia was 12.3% in Chinese men and 7.2% in Chinese women aged 70 years and older (Lau, Lynn et al. 2005). This study defined sarcopenia as ALM/height² two standard deviations or more below the normal mean for young Asian men and women which were $7.4 \pm 0.84 \text{ kg/m}^2$ and $6.4 \pm 0.79 \text{ kg/m}^2$ respectively. The mean and SD of ALM/height² were $6.7 \pm 0.8 \text{ kg/m}^2$ for elderly Chinese men and 5.7 ± 0.6 for elderly Chinese women. These values are lower than the New Mexico study where the mean (SD) of ALM/height² was 7.7 (0.7) for men and 5.9 (0.7) for women aged 70 years or over (Baumgartner, Koehler et al. 1998). The lower prevalence of sarcopenia in Asians compared to white or Hispanics may be due to the lower muscle mass in young Asians, which is used as the reference, and then results in a lower threshold for diagnosing sarcopenia (Lau, Lynn et al. 2005). The differences in diet and physical activity may also play a role in the difference in the prevalence of sarcopenia between different ethnic groups (Lau, Lynn et al. 2005).

A population-based epidemiological study of 883 elderly men and women living in New Mexico found that women with sarcopenia had 3.6 times higher rates of disability, and men 4.1 times higher rates, compared with study participants with normal muscle mass (Baumgartner, Koehler et al. 1998). The study also showed that there were significantly greater odds ratios for the use of walking aids and fall history in sarcopenic subjects, which persisted after adjustment for age, race, obesity, income, alcohol intake, physical activity, current smoking and comorbidity. Sarcopenia was independently associated with important health outcomes, including metabolic and functional impairments and disabilities in this relatively healthy ambulatory population.

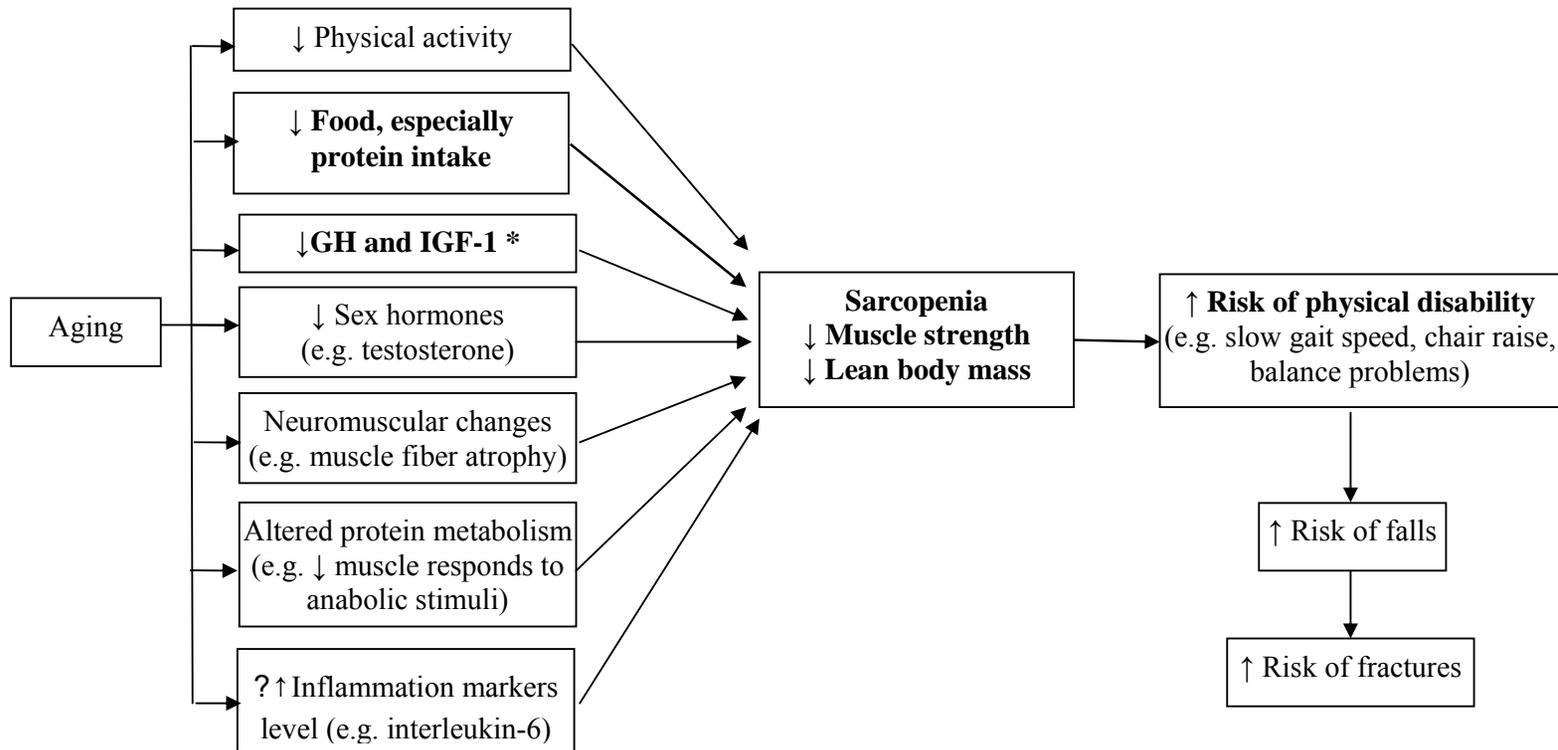
2.1.3 Aetiology of sarcopenia

Sarcopenia is a multifactorial condition (Morley 2003) which is characterised by a progressive loss of muscle mass and strength with aging (Dutta and Hadley 1995;

Dreyer and Volpi 2005). This results in an increased risk of physical disability (van den Beld, Blum et al. 2003), falls and fractures (Loeser and Delbono 1999; Fried, Tangen et al. 2001). All of these factors are incorporated into a model for illustrative purposes in Figure 2.1 and discussed in the following sections.

The aetiology of skeletal muscle loss with aging is not fully understood, but factors have been suggested include decreased energy and protein intake (Chernoff 2004; Walrand and Boirie 2005; Fujita and Volpi 2006), physical inactivity (Baumgartner, Waters et al. 1999), altered protein synthesis and turnover (Carmeli, Coleman et al. 2002), reductions in IGF-1 (Carmeli, Coleman et al. 2002), decreased sex hormones (van den Beld, de Jong et al. 2000; Iannuzzi-Sucich, Prestwood et al. 2002; Roy, Blackman et al. 2002), increased production of inflammatory markers (Visser, Pahor et al. 2002), and neuromuscular changes with aging (Loeser and Delbono 1999; Morley, Baumgartner et al. 2001) (Figure 2.1).

Figure 2.1 Possible etiology of sarcopenia.



* GH: growth hormone; IGF-1: insulin-like growth factor one. This diagram is generated from the following references (Dutta and Hadley 1995; Baumgartner, Waters et al. 1999; Loeser and Delbono 1999; van den Beld, de Jong et al. 2000; Fried, Tangen et al. 2001; Morley, Baumgartner et al. 2001; Carmeli, Coleman et al. 2002; Iannuzzi-Sucich, Prestwood et al. 2002; Roy, Blackman et al. 2002; Visser, Pahor et al. 2002; Morley 2003; van den Beld, Blum et al. 2003; Chernoff 2004; Dreyer and Volpi 2005; Walrand and Boirie 2005; Fujita and Volpi 2006).

2.1.3.1 Decrease in physical activity

In general, physical activity declines with aging. The decrease in muscle mass and strength is not only due to the aging process itself, but also to the complex interaction of aging, disease and muscle disuse (Schwartz and Buchner 1999). Resistance training has been shown to increase muscle mass and/or strength in elderly people (Baumgartner, Waters et al. 1999; Roth, Ivey et al. 2001). However, whether the decline in physical activity is directly associated with a decrease in muscle mass and strength with aging, or whether regular exercise can overcome sarcopenia is still being debated (Marcell 2003).

2.1.3.2 Growth hormone/Insulin like growth factor 1 and skeletal muscle

Physiology of growth hormone/Insulin-like growth factor 1

Insulin-like growth factor 1 (IGF-1) is a polypeptide protein hormone and its chemical structure is similar to insulin. Growth hormone (GH) is the major hormonal determinant of IGF-1 plasma concentrations (Clemmons 2006). The pituitary gland releases GH, and IGF-1 is produced in the liver, endothelial and smooth muscle cells (Sonntag, Lynch et al. 2000; Fanciulli, Delitala et al. 2009). The GH/IGF-1 axis and GH regulation is a complex process in which there are many hormones involved, including GH-releasing hormone, glucocorticoids, gonadal sex and thyroid hormones (Giustina and Veldhuis 1998). The simplified GH/IGF-1 axis and GH regulation is shown in Figure 2.2. All tissues in the body contain IGF-1 receptors (Clemmons 2007). IGF-1 plays a critical role in development, growth, repair, and maintenance of muscle, bone, cartilage and other tissues (Florini, Ewton et al. 1996; Loeser and Delbono 1999; Booth 2006). IGF-1 is also a recognized bone growth promoting factor in elderly persons who have sustained a hip fracture (Schurch, Rizzoli et al. 1998). It also directly regulates bone growth and density in adults (Yakar, Canalis et al. 2009; Yakar, Rosen et al. 2009).

Aging, IGF-1 and muscular skeletal health

Growth hormone and IGF-1 levels decrease with aging (Corpas, Harman et al. 1993). Cross-sectional studies have shown that IGF-1 levels are positively associated with

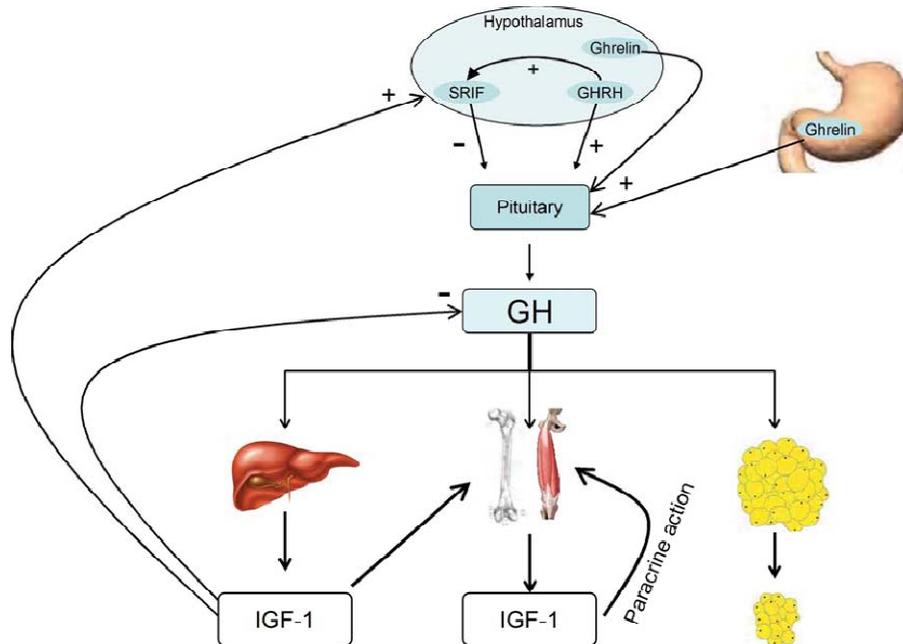
the increased muscle mass and strength in elderly men and women (Baumgartner, Waters et al. 1999; Payette, Boutier et al. 2002; Roubenoff, Parise et al. 2003). Low IGF-1 levels were associated with poorer lower limb strength and functions in a cross sectional study of 619 community-dwelling elderly women aged 70-79 years (Cappola, Bandeen-Roche et al. 2001).

Growth hormone treatment studies in animals and humans

Both animal and human studies have demonstrated the beneficial effect of growth hormone treatment on muscle mass and strength (Barton-Davis, Shoturma et al. 1998; Attanasio, Bates et al. 2002). Research using animal models has found that when IGF-1 was induced to mice muscles, there were significant increases in muscle mass and strength both in young and old mice compared with controls (Barton-Davis, Shoturma et al. 1998). Recombinant human GH (rhGH) has been used in human trials which were based on the rationale of replacing the age-dependent decline in circulating GH (Franco, Andersson et al. 2007; Johannsson 2007). GH dose titration is usually monitored by measuring serum IGF-1 (Fanciulli, Delitala et al. 2009). A 3-year study in 242 adult patients with hypopituitarism showed that lean body mass was significantly increased after 3 years of treatment with human growth hormone (Attanasio, Bates et al. 2002). The rhGH supplementation studies showed an increase in IGF-1, lean body mass, bone density and a decrease in body fat mass both in elderly men (Rudman, Feller et al. 1990; Papadakis, Grady et al. 1996; Hennessey, Chromiak et al. 2001) and in elderly women (Thompson, Butterfield et al. 1995).

The results from previous growth hormone treatment studies on skeletal muscle are inconsistent. A number of studies found that there was no effect of rhGH supplementation on the skeletal muscle system in elderly women (Blackman, Sorkin et al. 2002; Christmas, O'Connor et al. 2002). An animal study in old rats found that the short-term administration of GH late in life did not overcome the functional decline in muscle quality and size and may even accelerate apoptosis in slow-twitch muscles (Marzetti, Groban et al. 2008). To date, there is no definitive evidence to support the hypothesis that GH treatment is beneficial to the elderly to protect against the aging process. Further studies are needed to assess the safety of long-term use of GH (Fanciulli, Delitala et al. 2009).

Figure 2.2 Diagram of growth hormone/insulin-like growth factor 1 axis.



Insulin-like growth factor 1 (IGF-1) axis involves hypophysiotropic hormones controlling pituitary growth hormone (GH) release, IGF-1 production in the liver and elsewhere, and tissue responsiveness to GH and IGF-1. Adapted from (Fanciulli, Delitala et al. 2009).

2.1.3.3 Decrease in food intake

Food intake declines with aging (Clarkston, Pantano et al. 1997; Morley 1997). A 10-year longitudinal study conducted in New Mexico recruited 304 healthy elderly men and women, aged 72 years at the entry to the study in 1980. The participants were seen as outpatients each year and 3-day food records were used to assess their food intake (Vellas, Hunt et al. 1997). For those who dropped out or died, the annually assessed dietary intake data of their last visit were used. The study found that the decrease in energy intake was greater in those who died or dropped out of the study. In 1990, participants who were healthy, frail or sick, or dropped out or died decreased their mean energy intake by 0.13, 0.19 and 0.22 kcal/kg body weight per year, respectively. Therefore those who remained healthy were consuming more energy than all the other participants. The decline in food intake with aging is not fully understood, but several possible peripheral and central mechanisms have been proposed by Morley (2001) are as below:

- Alteration in taste and smell with aging
- Decline in adaptive relaxation of the fundus of the stomach
- Increased cholecystokinin level with aging resulting in increased sensitivity to satiety
- Increased leptin level secondary to the age-related decline in testosterone
- Decrease in physical activity resulting in a decline in the central feeding drive

A study compared eight healthy older and seven healthy young male subjects and found that the effect of a small intestinal lipid infusion on hunger was attenuated and the stimulation of phasic pyloric pressure waves increased in the healthy older participants (Cook, Andrews et al. 1997). The gastrointestinal hormone, cholecystokinin (CCK), is a peptidergic neurotransmitter and is involved in multiple digestive functions including delay of gastric emptying, regulation of insulin secretion, emptying of the gallbladder (Ubilluz 1994). CCK increases with age which may contribute to the decrease in appetite in elderly (MacIntosh, Andrews et al. 1999). A study of 64 healthy postmenopausal women found that serum leptin concentration was negatively correlated with the habitual food intake independent of body fat content (Larsson, Elmstahl et al. 1998). More studies, and especially human trials, are needed to establish and confirm these proposed mechanisms.

However, it is not clear if the decrease in food intake in the elderly is due to aging per se or some other underlying health problem. Lower albumin concentration is associated with decreased muscle mass (Baumgartner, Koehler et al. 1996) and strength (Schalk, Deeg et al. 2005). A recent study of 36 young and older adults who completed three 18-day periods of controlled feeding with three levels of protein intakes (Thalacker-Mercer, Johnson et al. 2007) found that there was no difference in the post-absorptive serum albumin concentration and postprandial albumin synthesis rate between age groups. This suggests that the elderly respond well to protein ingestion and that increasing protein intake could improve nutritional status in the elderly.

2.1.3.4 Protein intake, muscle mass and muscle strength

Animal studies

Animal studies have shown that a high protein diet (50% protein) decreases net muscle catabolism and improves muscle morphology, strength and function in murine (Zdanowicz, Slonim et al. 1995). A high protein diet (23% protein) was sufficient to increase body weight and restore basal values of liver weight and protein content in malnourished adult and old rats (Walrand, Chambon-Savanovitch et al. 2000). This study also found that in old rats, a very high protein diet (27% protein) was more effective than a high protein (23% protein) diet in increasing body weight, albuminemia, muscle weight and protein content, and plasma arginine concentration (Walrand, Chambon-Savanovitch et al. 2000). In another study, whey protein down regulated fatty acid synthesis and increased glycogen content in the liver (Morifuji, Sakai et al. 2005; Morifuji, Sakai et al. 2005), and also increased skeletal muscle in the exercise-trained rats (Morifuji, Sakai et al. 2005). It was proposed that whey protein may reduce accumulation of body fat in rats (Morifuji, Sakai et al. 2005).

Studies in hospitalized patients

Increased protein intake or use of a protein based nutrition supplements have been shown to have a significant benefit in improving muscle mass or strength in very elderly (age \geq 75 years) hospitalized patients (Price, Daly et al. 2005). The hospitalized patients are at greater risk of malnutrition due to pre-existing diseases or reduced physical activity. As malnourished patients are likely to have large

depletion of their body protein, they may respond better to protein and other nutritional supplements than relatively healthy elderly people.

Cross-sectional and longitudinal studies in healthy elderly people

A cross-sectional study in Canada examined the habitual diets and body composition in 38 healthy, normal weight, sedentary women aged 57-75 years (Lord, Chaput et al. 2007). The study found a significant correlation between muscle mass index (MMI = FFM (kg)/height (m)², (FFM is fat-free mass measured by DXA) and muscle protein content, animal and total protein intake. Animal protein intake was the only independent predictor of MMI in the stepwise regression analysis. The authors suggested that protein intake, especially from animal sources, may be associated with a better preservation of MMI. However, a population-based cross-sectional study of 1404 elderly men and women did not find an association between dietary protein intake assessed by food frequency questionnaire and body lean mass assessed by DXA (Mitchell, Haan et al. 2003). Another cross-sectional study of 44 elderly men showed that adequate protein intake did not offset the age-related loss of appendicular lean mass (Starling, Ades et al. 1999).

Two longitudinal studies have shown that a higher protein diet was significantly correlated with reduced loss of lean body mass (Houston, Nicklas et al. 2008) and fewer health problems over the study period in elderly people (Vellas, Hunt et al. 1997). The US study of 2066 community-dwelling men and women aged 70-79 years over three years, found that participants in the highest quintile of protein intake lost approximately 40% less total body lean mass and appendicular lean mass than did those in the lowest quintile of protein intake (Houston, Nicklas et al. 2008). The 10-year longitudinal study of 304 healthy elderly men and women found that women with a protein intake greater than 0.8-1.2 g/kg of body weight had fewer health problems over the next 10 years than those with protein intakes <0.8 g/kg (Vellas, Hunt et al. 1997). Therefore, dietary intake, especially protein intake, may be a modifiable risk factor for sarcopenia in the elderly (Houston, Nicklas et al. 2008).

In summary, previous studies have shown that a diet high in protein content was correlated with higher muscle mass (Meng, Zhu et al. 2009) and strength in the elderly (Price, Daly et al. 2005). However, the results have been inconsistent

between studies. The role of protein in the long-term regulation of body composition in the elderly is not clear and long-term intervention studies are needed.

2.1.3.5 Protein intake, serum IGF-1 and protein metabolism

Protein intake and serum IGF-1

Dietary protein intake has positive effects on the production and action of serum IGF-1 (Bonjour, Schurch et al. 1997). It has been reported that marginal protein intake results in reduced IGF-1 levels and skeletal muscle fibre atrophy in elderly women (Castaneda, Gordon et al. 2000). A dietary protein supplement of 20 g/day has been shown to increase circulating levels of IGF-1 in a study of elderly hip fracture patients (Schurch, Rizzoli et al. 1998). A 9-week study of 32 elderly men and women with usual protein intakes <0.85 g/kg/d showed that the high protein intake group (0.75 g/kg/d protein intake) had significantly higher levels of serum IGF-1 (a bone growth factor) than the low protein intake group (0.04 g/kg/d protein intake) (Dawson-Hughes, Harris et al. 2004). The high protein intake group also had significantly lower levels of urinary N-telopeptide (a marker of bone resorption) than the low protein intake group over the study period. However, the study sample size was small, especially for the subgroups, such as by gender. The study duration was also short. All study participants were on a habitually low protein diet when entered into the study and therefore may not be representative of the general elderly population. Dietary protein intake above habitual intakes does appear to have the ability to raise IGF-1 concentrations. It is not clear however, whether the increase in IGF-1 is of clinical relevance and translates to effects on bone and muscle.

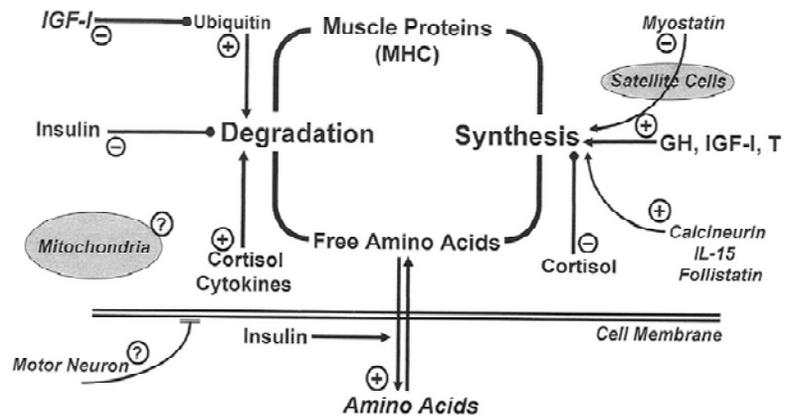
Altered protein metabolism and dietary protein intake

Muscle protein turnover is a complex process of protein degradation and synthesis regulated by several physiological stimuli (Marcell 2003) as shown in Figure 2.3. Altered protein metabolism in the elderly may result from reduced ability of aging skeletal muscle to respond to anabolic stimuli, such as insulin and amino acid (Fujita and Volpi 2006). However, aging does not impair the muscle anabolic response to a protein-rich meal (Symons, Schutzler et al. 2007). This study compared the plasma amino acid concentrations in ten healthy young (41±8 years) and ten elderly (75±5 years) after ingestion of 113g of lean beef. Both groups reached the peak plasma

amino acid concentrations 100 minutes after beef ingestion, but the levels were substantially higher in the elderly (Symons, Schutzler et al. 2007). In a study investigating the response of amino acid transport and protein synthesis in six healthy elderly men age 71 ± 2 years, the free amino acids concentrations and muscle cells (assessed by muscle biopsy) increased significantly during amino acids infusion (Volpi, Ferrando et al. 1998). This study demonstrated that the increased availability of amino acids has positive effects on muscle anabolism in elderly subjects whose muscle mass was reduced (Volpi, Ferrando et al. 1998).

Animal (Pansarasa, Flati et al. 2008) and human studies have showed that aging muscle can respond as well to the excess amino acid as in young adults (Fujita and Volpi 2006). A high protein diet (greater than 0.8-1.2 g/kg of body weight) was associated with a lower loss of lean body mass (Houston, Nicklas et al. 2008) and fewer health problems (Vellas, Hunt et al. 1997) compared with a lower protein diet. However, long-term intervention studies with larger sample sizes are needed to verify the long-term effect of high protein intake on IGF-1 levels in elderly.

Figure 2.3 Skeletal muscle protein turnover.



Adapted from (Marcell 2003)

2.1.3.6 Sex hormones and sarcopenia

Sex hormone status is an important factor for muscle mass and strength level in elderly men but not in women (Baumgartner, Waters et al. 1999). Most of the previous studies have shown that the decline in testosterone with aging in men was associated with a decline in muscle mass and strength (Morley, Kaiser et al. 1997; Baumgartner, Waters et al. 1999) and physical function (Perry, Miller et al. 2000). Testosterone replacement therapy also has been shown to have positive effects on increasing muscle mass and strength in elderly men (Snyder, Peachey et al. 1999). However, a recent 3-year cohort study of 1557 elderly men showed that low levels of testosterone were neither associated with a 3-year decline in physical performance nor with the decline in muscle strength (Schaap, Pluijm et al. 2008). The authors explained that it is possible that their findings are sample-specific as the participants in their study were healthy elderly men, but further studies are needed to confirm these results. Another possibility is the ceiling effect of the physical performance scores used in their study as the physical performance scores may have been set too low to detect changes in the physical activity in these healthy men.

In elderly women, oestrogen was not associated with muscle mass and strength (Baumgartner, Waters et al. 1999). Oestrogen replacement therapy did not show a protective effect against the loss of muscle mass and strength with aging in non-obese, community-dwelling elderly women (Kenny, Dawson et al. 2003). Therefore sex hormone status and sarcopenia appear to be only important in elderly men based on the current evidence.

2.1.3.7 Neuromuscular changes with aging

Loeser and Delbono reviewed the evidence from human and animal studies, and suggested the following pathogenesis of sarcopenia (Loeser and Delbono 1999). They acknowledged that the processes of neuromuscular changes with aging are still poorly understood.

- neuronal alterations (e.g. reduction in number and/or cell size of spinal cord motor neurons)
- neuromuscular transmission alterations (e.g. decrease in nerve terminal numbers)

- primary muscle alterations (e.g. primary muscle fibre atrophy)
- other aging related general mechanisms that involve skeletal muscle (e.g. age-related vasculopathy)

The decrease in protein turnover and repair capacities of aging muscle and impaired mitochondrial functions and energy reserve systems have also been proposed (Carmeli, Coleman et al. 2002). Marzetti and colleague reviewed recent animal and human studies and suggested that the accelerated apoptosis of muscle fibres may be a key mechanism of sarcopenia (Marzetti and Leeuwenburgh 2006). Other factors, such as the lesser stimulation of the mammalian target of the rapamycin (mTOR) signalization pathway and increased proteolysis with aging were also proposed (Schneider, Boirie et al. 2008). mTOR is a serine/threonine protein kinase that regulates cell metabolism (Hay and Sonenberg 2004). However, more studies are needed to establish a clear integrated picture of the interaction of these pathways and their roles in sarcopenia.

2.1.3.8 Inflammatory markers and sarcopenia

An increase in inflammatory markers, such as interleukin-6, C-reactive protein (CRP), and tumour necrosis factor alpha have been found to be associated with aging (Steinle, Sharma et al. 2009) and the risk of cancer incidence and death (Il'yasova, Colbert et al. 2005). High levels of inflammatory markers, such as interleukin-6, CRP, or tumour necrosis factor-alpha were correlated with lower muscle mass and strength in well-functioning older men and women in population based studies (Visser, Pahor et al. 2002; Schaap, Pluijm et al. 2006). CRP is an acute-phase protein found in the blood in response to inflammation, and is produced by liver and fat cells (Pepys and Hirschfield 2003; Lau, Dhillon et al. 2005; Pepys, Hirschfield et al. 2006). CRP is produced by adipocytes (Lau, Yan et al. 2006), and has been shown to be strongly associated with adiposity in both men and women. It is elevated in obese cardiovascular patients and decreases with weight loss (Lau, Yan et al. 2006; Thorand, Baumert et al. 2007). A study in 286 elderly people showed that CRP and interleukin-6 were positively associated with total fat mass and negatively associated with appendicular lean mass (measured by DXA) (Cesari, Kritchevsky et al. 2005). However, a study of 20 young and 26 elderly men showed that

interleukin-6 did not increase with age (Beharka, Meydani et al. 2001). The author suggested that increased interleukin-6 was not a normal consequence of aging but might reflect an underlying, undiagnosed disease state. More studies with larger sample sizes are needed to confirm the relationship between aging and these inflammatory markers. The underlying diseases should be considered when studying the relationship between the inflammatory markers and changes in body composition with aging.

2.1.4 Summary

In summary, sarcopenia is common in aging populations. Sarcopenia increases the risk of physical disability for elderly people. There appear to be multiple factors which contribute to this condition and its aetiology is not fully understood. The interaction of the genetically determined aging process (e.g. decrease in IGF-1 and testosterone), decrease in food intake especially protein intake, and decline in physical inactivity have been suggested. Diet and physical activity are modifiable life style risk factors which appear to play a role in the aetiology of sarcopenia. Based on the above evidence, the current study is based on the hypothesis that long term protein supplementation may have a beneficial effect on preserving skeletal muscle mass and strength in elderly women.

2.2 History of protein

In the middle of the seventeenth century, scientists noticed that there were some gluey substances which existed both in plant and animal foods that have common animal properties (Carpenter 1986). It took scientists another century to find out that these gluey materials were nitrogen based. Swedish chemist Jons Jakob Berzelius and Gerrit Mulder named this material 'protein' in 1838 (Tanford and Reynolds 2001). Justus Liebig, the German organic chemist asserted in 1845, that the various nitrogen compounds such as albumin, fibrin, casein and gluten can convert one to another in the animal body and that protein was the main compound in muscle metabolism (Carpenter 1986). By the early 1900s, scientists understood that protein is a large complex molecule made up of amino acids joined by peptide linkages (Tanford and Reynolds 2001). Max Perutz and Sir John Cowdery Kendrew

discovered the structures of haemoglobin (Perutz, Rossmann et al. 1960) and myoglobin (Kendrew, Bodo et al. 1958) respectively in 1958 (Tanford and Reynolds 2001). They won the Nobel Prize for chemistry in 1962 for their contribution to solving the mystery of the protein structure. By the 1970s, scientists revealed that protein is the protoplasm of all cells and all the process within cells. For instance, enzymes and antibodies are protein and proteins form a substantial part of cell membranes (Tanford and Reynolds 2001). Myosin, a major portion of the muscle protein was isolated for the first time by Willy Kuhne in 1859 but the study of muscle protein was neglected for a long period of time. However in recent decades much evidence has emerged of the critical role of proteins in the structure and function of all living cells.

2.3 Physiology of protein

The main source of body nitrogen is from protein, and about half of this protein is present as skeletal muscle (Lentner 1981; National Health and Medical Research Council 2006). However, protein is not a reserve energy store as fat since only 1% of body protein is available for energy metabolism (Young, Hussein et al. 1968). To avoid depleting the musculoskeletal system to provide energy, sufficient protein and energy intake from the diet is required to maintain human functions (Hansen, Raja et al. 2000).

Proteins are made of twenty-two amino acids and the body can only synthesize fourteen of the twenty-two (Sakami and Harrington 1963). The remaining eight are referred to as essential amino acids which have to be obtained from the diet (Furst and Stehle 2004).

There are three stages for protein metabolism in general (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005; National Health and Medical Research Council 2006). The first stage is the digestion and absorption of protein which ends with the breaking down of protein into amino acids. The second stage is the balance of the 'amino acid pool', and the third stage is the deamination and excretion of amino acids. For the first stage, the quality of dietary protein itself and age related decreased function in digestion and absorption could affect how much

dietary protein would be needed to generate sufficient levels of amino acids. The balance of the amino acid pool is related to the balance of anabolism and catabolism. With aging, both anabolic and catabolic functions are decreasing. The third stage, deamination and excretion of amino acid metabolism mainly through urine, skin (sweat) and faeces. Urea is the principal excretory form of amino acid degradation.

2.4 Clinical effects of inadequate protein intake

Protein as the fundamental component of all body cells, has an essential role in the growth and function of the human body, including as enzymes, hormones and other important molecules (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). Insufficient protein intake and nonprotein energy intake (i.e., carbohydrates, fats) has adverse effects on all organs (Corish and Kennedy 2000). Protein deficiency also has been shown to have adverse effects on the immune system and increased risk of infection (McMahon and Bistrian 1990; Chandra 1997; Chandra 2002). A gene expression study found that short-term inadequate protein intake (0.5 g/kg/d) caused changes in skeletal muscle transcript levels in older people compared with those on an adequate diet (1.2 g/kg/d protein intake) (Thalacker-Mercer, Fleet et al. 2007). Energy metabolism and protein synthesis and proliferation were different between the inadequate protein intake group and the adequate protein intake group. This study also showed the differentially expressed transcripts in functional classes for immune, inflammatory, stress responses, contraction, movement, and development, and extracellular connective tissue between the inadequate protein intake group and the adequate protein intake group.

Protein energy deficiency is common in both children and adults in developing countries (Stephenson, Latham et al. 2000). In industrialized countries, it is more often found in hospitalized patients and elderly people (Bistrian 1990; Allison 1995). The prevalence of protein-energy malnutrition was 5% in a study of 1206 randomly selected free-living elderly Swedish people aged 65 to 80 years (Thorslund, Toss et al. 1990). Their study defined protein-energy malnutrition as having three or more subnormal values (> 2SD below the mean for albumin and prealbumin, or below the tenth percentile for anthropometric measurements, and relative energy) or two or

more very low values ($> 3SD$ below the mean or below the fifth percentile respectively).

Humans consume a wide range of dietary protein and there is limited literature on the adverse effects of having a very high protein diet long term (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). No adverse effects of high protein intake have been found in groups, such as in Eskimos, explorers, trappers and hunters during winters (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). Studies have shown correlations between high protein diets and some chronic diseases, including osteoporosis, renal calculi, renal insufficiency, cancer, coronary artery disease and obesity. However, there is insufficient evidence to permit any recommendation of the upper level of protein intake at the current stage (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). The recent version of Nutrient Reference Values for Australia and New Zealand also agreed that it is impossible to set up the upper limit of intake of protein based on the data available at present (National Health and Medical Research Council 2006).

2.5 Protein requirement assessment methods

There are mainly three methods for studying protein requirements, the factorial method, nitrogen balance method and the amino acid method (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005; National Health and Medical Research Council 2006).

2.5.1 Factorial method (obligate nitrogen loss method)

The factorial method is based on estimating the obligatory nitrogen loss from urine, faeces, sweat, and other miscellaneous losses, such as nasal secretions, menstrual losses or seminal fluid when a person is on an essentially protein free but the energy balanced diet (US panel of macronutrient 2005 P608). This allows the calculation of the amount of dietary protein needed to replace obligatory losses.

The weakness of this method is that the relationship between protein intake and nitrogen retention is curvilinear, and the measurement of miscellaneous losses is inordinately difficult, and these can make significant differences in results between studies (Rand and Young 1999). Moreover, humans can adapt to a large range of protein intakes. For example, a person who had a high protein habitual diet would have higher nitrogen losses in the urine when they switch to a low protein diet, compared to one who was on a habitual low protein diet (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). Therefore, the one who was on a habitual low protein diet would have a lower nitrogen excretion from urine. The estimated obligatory nitrogen loss would be higher for the one who was on a habitual high protein diet, and the estimated protein requirement would be falsely higher than the actual requirements. The differences in the initial nutritional status in individuals and the adaptability of the human body make it difficult to assess protein requirements at the population level.

2.5.2 Nitrogen balance method

The nitrogen balance method is still the most commonly used method to assess protein requirements. Although this method also has serious limitations, there is no validated or acceptable alternative to date (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). This method is based on the assumption that nearly all of total body nitrogen is incorporated into protein (Gibson 2005). The theory is that when the needs are met, the nitrogen balance is zero. When protein intakes are lower than required, nitrogen balance is negative. When the protein intakes are higher than required, nitrogen balance becomes positive. In general, this method involves feeding increasing amounts of protein in a diet that is adequate in total energy to determine the minimal amount required to balance the total nitrogen losses in urine, faeces, skin (sweat), and miscellaneous losses (blood, nails, hair, semen) (Mackenzie, Clark et al. 1985; Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005).

Two different equations have been used to calculate nitrogen balance (Gibson 2005):

1. Nitrogen balance = $I - (U - U_e) + (F - F_e) + S$

I = nitrogen intake (protein intake in g/6.25 as protein contains 16% nitrogen); U = total urinary nitrogen; U_e = endogenous urinary nitrogen; F = faecal nitrogen (as unabsorbed protein); F_e = endogenous faecal nitrogen losses; S = dermal nitrogen losses (nitrogen lost in sweat and sloughed-off skin cells).

2. Nitrogen balance = protein intake (g) / 6.25 – (urinary urea nitrogen (g) + 2 + 2)
The correction of 2g is for the estimation of dermal and faecal losses of nitrogen and another 2 g is for the nonurea nitrogen components of the urine.

The first equation is a theoretical approach because several parameters are not measurable, such as endogenous urinary and faecal nitrogen (Manore and Thompson 2000). Total urinary nitrogen includes urea (86-94% of total urinary nitrogen), creatinine (4.5%), ammonia (2.8%), uric acid (1.7%), and other nitrogen-containing compounds (5%) (Manore and Thompson 2000). Using just urinary urea nitrogen is more common than measuring total urinary nitrogen in routine clinical investigations as it is cheaper and less time consuming for patients and staff (Gibson 2005). However, total urinary nitrogen is more commonly used in research as accuracy is increased by measuring total nitrogen losses from urine instead of using the same fixed estimated losses for all subjects.

Reported nitrogen balance results tend to be biased towards a positive balance (Gibson 2005). This could be due to the underestimation of nitrogen excretion because of unmeasured nitrogen losses, including endogenous urinary and faecal nitrogen losses, losses of faeces and urine on toilet paper and in stool and urine containers, losses from exfoliated cells, sweat, hair and nail growth, menstrual fluid and semen (Gibson 2005).

There are a few limitations of the nitrogen balance method that need to be considered when assessing human protein needs. Firstly, humans can adapt to a wide range of protein intakes to achieve nitrogen equilibrium by increasing or reducing nitrogen excretion and reutilising endogenous amino acids (FAO/WHO/UNU 1985). The concept of a steady state is relative. Each level of protein intake requires several days to reach a new equilibrium. It has been suggested that older people may need more than 10 days to achieve steady state for a given level of protein intake (Morse,

Haub et al. 2001). One objective in determining protein requirements is to define the point at which adaptation is exceeded; beyond this point there will be progressive loss of body protein and deterioration of tissue function (FAO/WHO 1975). A short term nitrogen study usually refers to a study period of 2-3 weeks (Morse, Haub et al. 2001). A long-term study is needed to assess the optimal protein intake level in terms of maintaining muscle mass and strength. In addition, most of the studies either did not measure the miscellaneous losses of nitrogen (e.g. faeces, sweat, and hair etc) or estimated the miscellaneous losses as the same for each individual. Therefore most of the short-term nitrogen balance studies are not convincing.

The 1985 FAO/WHO/UNU Panel suggested adding 5mg of nitrogen per kg body weight to allow for integumental and miscellaneous unmeasured losses (FAO/WHO/UNU 1985). However, some studies have shown that the miscellaneous losses actually could differ significantly between studies and this would affect the estimated protein requirement significantly (Rand and Young 1999). Campbell and colleagues studied 23 younger (aged 21-46 years) and 19 older (aged 63-81 years) men and women for three 18-day trials with protein intakes of 0.50, 0.75, and 1.00 g protein/kg/day (Campbell, Johnson et al. 2008). They concluded that these short-term nitrogen balance results suggested that there is no difference for the dietary protein requirement for healthy older adults and for younger adults. Morse and colleagues conducted a 18-day nitrogen balance study in 11 elderly women aged 70-81 years (Morse, Haub et al. 2001). The study showed that the nitrogen balance was not achieved during week 2 and week 3, and suggested that the total protein needs of elderly women were at or above the current recommended protein intake of 0.8 g/kg/d. They also pointed out that shorter-term nitrogen balance protocols are insufficient to firmly establish the recommendation for protein intake of elderly women.

2.5.3 Amino acid response method

The amino acid method is to measure the balance of selected amino acids at different levels of intake of the amino acids. Techniques include the plasma amino acid response method, the direct amino acid oxidation method, the 24-hour amino acid balance method, and measurement of the indicator amino acid oxidation method

(Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). In general, protein requirements estimated by nitrogen balance derived from amino acid requirements were lower than values derived by the other methods (Young, Bier et al. 1989; Millward 1990). This difference may be due to the excess nonprotein energy used in the nitrogen balance studies and protein requirement is sensitive to the total energy intake (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005).

The leucine oxidation method has been the most common method reported in the literature. The advantage of this method is that adaptations in amino acid metabolism (at least for leucine) can be achieved in 24 hours (Motil, Opekun et al. 1994). This is much quicker than the classical nitrogen balance studies which usually take seven to ten days for urinary nitrogen equilibrium in adults (Rand, Young et al. 1976). It was based on the assumption that values of leucine oxidation and balance reflect the whole body protein oxidation and balance (Kurpad and Vaz 2000). It was thought to be a more sensitive index of the adequacy of protein intake than nitrogen balance (Castaneda, Dolnikowski et al. 1995) though it has limitations (Fuller and Garlick 1994). For example, since it is a measurement only made during a short period where food is given at regular hourly intervals, it cannot represent the whole day status (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005).

2.6 Protein requirements for elderly people

2.6.1 Historical developments of protein requirement

The first known investigation of protein requirements dates back to the 19th century after the discovery of protein. Carpenter (1994) reviewed the historical development of human dietary protein recommendations. In 1853, Lyon Palyfair reported the diets provided to the paupers, prisons and servicemen contained 14 g of nitrogen, which is equivalent to approximately 88 g protein in modern terms (Carpenter 1994). In 1863, Edward Smith, a British physician undertook a dietary survey in prisoners undertaking hard labour in London (Carpenter 1994). He found that the estimated protein intake was 55 g per day with a total energy intake of 2190 kcal per day.

During this historical period there were no published studies targeting protein requirements in the elderly. It was thought that elderly people need less protein than other adult groups because of the decrease in physical activity. However, with increasing knowledge of sarcopenia in the aging process, evidence now suggests that the elderly may need more protein than was previously thought. The following sections summarized the important periods of research in dietary protein requirements in the general population and in elderly people.

2.6.1.1 The Voit standard for protein requirements

The studies in the period of 1890-1920 were mostly on the protein requirements of physical working men. The recommendation at this period was mainly based on observation of how much people ate. German physiologist Dr. Carl Voit (1831-1908) suggested in 1875 that 118 grams of protein intake per day was needed for a physical worker (equivalent to 105 g digestible protein). His theory was based on the observation of eating habits of people with sufficient income to afford almost any choice of foods. The belief that protein was the source of muscle energy and observation of the high protein consumption in the more successful social groups or nations contributed to the preference for high protein intake in this period (Carpenter 1986).

2.6.1.2 Protein recommendations by the United States Department of Agriculture

In the U.S., 1891-1911 is referred to as the “Atwater Period” as Atwater’s work dominated the science of nutrition at this time (Dirks and Duran 1998). Wilbur Atwater was trained in Germany and was greatly influenced by Voit’s techniques. He has been described as the founder of the science of nutrition in the United States. He undertook many studies on the nitrogen and energy intakes in different groups, including mechanics and farmers (Carpenter 1994). Based on those studies, he adjusted Voit’s standard to 100 g protein per day for an elderly man with an energy intake of 2400 kcal, and 118 g protein with 3000 kcal energy intake for a labourer doing moderate work, and 145 g protein with 3300 kcal energy for one doing heavy work.

Hamilton C. Bowie, a visiting worker in Voit's laboratory calculated nitrogen balance in a series of short-term nitrogen balance experiments which were originally designed to evaluate the digestibility of individual foods (e.g. white bread, milk, meat). He claimed that his findings supported Voit's standard. He found that the subject was in negative balance when the protein intake was less than 65 gram digestible protein per day, and was in positive balance when the protein intake was at 120 g or above (Carpenter 1986).

2.6.1.3 Criticism for Voit's standard

The criticism of the Voit's standard is that it was based largely on what men ate rather than what they needed to eat, and did not consider the human body's adaptation to a low protein intake. Initially, adaptability to a low protein intake was found in dogs in Voit's study in 1867 (Carpenter 1986). Then a German physician changed his own diet from high protein to one which contained only 42 to 45 g protein per day. He found his urine nitrogen decreased gradually for the first four days when he changed from a high protein diet to a low protein diet. The results indicated the ability of human body to adapt to low protein diet in the short term (Carpenter 1986).

2.6.1.4 Challenges to high protein intake requirements

Chittenden, R.H. (1856-1943) also raised the question as to whether the previous recommendation (Voit standard) for protein intake was too high (Carpenter 1986). He was concerned that consuming too much protein may impose a greater physiological strain on the kidneys. He experimented on himself, four of his colleagues, eight university students, and thirteen volunteers from the U.S. army. Chittenden found that a 61-64 g/d protein intake could maintain a positive nitrogen balance and remain in good health over five to six months periods. He concluded that 50 g/d was adequate for adults and 'body equilibrium can be maintained on far less than 3000 kcal per day by the brain worker' (Chittenden 1904; Cowgill 1944).

However, Chittenden's conclusion was not fully convincing. The self report about feelings, such as fatigue, was more subjective than based on scientific evidence. Moreover, Atwater reported that almost all subjects reverted to a more traditional diet rich in meat at the end of Chittenden's study. Therefore, Atwater believed that the human body may adapt to a wide range of nitrogen levels but studies were needed to evaluate the optimal level (Carpenter 1994).

2.6.1.5 Recommendations by the World Health Organization (WHO)

The WHO and the Food Agriculture Organization of the United Nations (FAO) have revised the protein intake recommendation several times since 1950s (Table 2.1).

In 1950, protein requirements regained attention after a relatively quiet period from 1920 to 1950 when individual vitamins and amino acids were discovered (Carpenter 1986). Between 1950s and 1970s, the expert committee of FAO and WHO had revised the recommendation for protein requirements several times. Many important points were addressed in these revisions (Carpenter 1994; Weisell 1995). For example, the expert group emphasized the important relationships between total energy intake and protein or other nutrient intakes. They recognized that the quality of nutrients, such as the quality of protein from different resource also needs to be considered while studying protein requirements. The expert committee also clarified that the average requirement suggested by the committee should apply for group of persons rather than individuals. They suggested that other aspects such as differences in age, gender and physical activity level also needed to be taken into account in studies of nutrient requirements. Studies in this period were more focused on protein and energy malnutrition in young children. For adults, most study populations were younger adults.

The report of FAO/WHO in 1973 recommended a safe protein intake of 0.57 g per kg body weight for young men based on an egg protein study (FAO/WHO. 1973). However, later experiments indicated that this level of protein intake was too low for young men (Garza, Scrimshaw et al. 1977). The different results between studies might be because of the following reasons (FAO/WHO/UNU 1985). Firstly, some earlier studies tended to promote weight gain rather than balance. Secondly, some

studies set up the balance point as negative balance. Thirdly, there were differences in the estimation of nitrogen losses other than in feces and urine between studies.

In 1985, FAO/WHO recommended that the safe protein intake for adults age > 19 years (which includes the elderly) should not be lower than 0.75 g/kg/d (FAO/WHO/UNU 1985). This recommendation was made by increasing 25% of 0.605 g/kg/d. 0.605g/kg/d is the average value of protein requirement derived from long-term and short-term nitrogen balance studies, and these studies were mainly conducted in young men. Increasing by 25% is to meet the needs of almost all persons (up to plus 2 standard deviations or 97.5%) of individuals in a population. FAO/WHO also suggested that there was insufficient evidence to support a higher requirement for the elderly (FAO/WHO/UNU 1985).

Table 2.1 Successive recommendations by international groups for protein intake in adult man.

Report	Average for N balance (g/kg/day)	Recommended intake for population (g/kg/day)
FAO 1957	0.35	0.66
FAO/WHO 1965	0.59	0.89
FAO/WHO 1973	0.34	0.75
FAO/WHO/UNU 1985	0.60	0.75

Adapted from (Scrimshaw 1996)

2.6.1.6 WHO recommendation in 1994

In 1994, the Working Group on Protein and Amino Acid Requirements of FAO/WHO/UNU held a meeting in London. The meeting proposed to increase the protein requirement to 0.8 g/kg/d. The participants agreed that the nitrogen balance data for young adults were sufficient but not for older adults (Clugston, Dewey et al. 1996). After reviewing the nitrogen balance data for the elderly, it was realized that 0.75 g/kg/d may not meet the requirements of the elderly, but more data were needed to make a recommendation for a higher protein intake.

The results of nitrogen balance studies in elderly people have been inconsistent. A meta-analysis of data from 19 nitrogen balance studies showed that there was no significant effect of ageing on protein requirements in older adults (Rand, Pellett et al. 2003). Ten healthy men and women aged 55-77 years were able to achieve nitrogen balance after 2 weeks when given 0.8 g/kg/d protein (Campbell, Trappe et al. 2001). They were in positive balance after 8 and 14 weeks, but the thigh muscle area was significantly reduced after 14 weeks. The existence of muscle wasting at nitrogen balance status in the elderly indicated that nitrogen balance itself is not an optimal criterion when assessing the protein requirements in the elderly. Further studies are needed to investigate new criteria for assessing protein requirement in the elderly in addition to the nitrogen balance method.

2.6.1.7 Current recommendations for the elderly

In the United States and Canada, the current Recommended Dietary Allowance (RDA) of protein for all adults groups is 0.80 g/kg/d of good quality protein (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). The RDA is the intake that meets the nutrient needs of almost all (97-98 percent) individuals in a group (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). However, there was evidence that indicates that protein intake greater than the RDA can improve muscle mass, strength and functions in elderly (Wolfe, Miller et al. 2008).

In Australia, the Recommended Dietary Intake (RDI) for protein for the elderly was not specifically addressed until 1999, which was 0.84 g/kg/d for men and 0.75 g/kg/d

for women age over 70 years old. The National Health and Medical Research Council (NHMRC) published the Nutrient Reference Values for Australia and New Zealand in 2006. The RDIs were increased to 1.07 g/kg/d for men and 0.94 g/kg/d for women aged over 70 years old (National Health and Medical Research Council 2006). The NHMRC acknowledged however that the data supporting this increase is limited.

2.6.1.8 Summary of the changing of the protein intake recommendation for the elderly

Dietary protein requirements have been studied for over one hundred years but the protein requirement for the elderly was not specifically addressed until recent decades. The WHO recommended a protein intake of 0.75 g per kg body weight per day for all adults in 1994. Evidence from nitrogen balance studies (Campbell and Evans 1996; Campbell, Trappe et al. 2001) conducted in recent years in the elderly suggested that elderly people may need more protein than younger adults. In 2006 the NHMRC of Australia recommended a protein intake of 1.07 g/kg/d for men and 0.94 g/kg/d for women aged over 70 years but acknowledged that more studies are needed in supporting this recommendation. Therefore there is a need for further research on protein requirements in the elderly.

2.6.2 Studies of protein requirement in elderly people

2.6.2.1 Short term and long term nitrogen balance studies

Few nitrogen balance studies have been undertaken in the elderly as shown in Table 2.2 (Cheng, Gomez et al. 1978; Uauy, Scrimshaw et al. 1978; Zanni, Calloway et al. 1979; Gersovitz, Motil et al. 1982; Campbell, Crim et al. 1994; Castaneda, Charnley et al. 1995; Campbell, Trappe et al. 2001; Morse, Haub et al. 2001; Kortebein, Ferrando et al. 2007). These nitrogen balance studies generally have small sample sizes and are of relatively short duration which may explain the lack of consistent findings. Most of the recent studies have shown that a protein intake of 0.8 g/kg body weight per day was not adequate for elderly adults to maintain muscle mass (Campbell, Trappe et al. 2001; Kortebein, Ferrando et al. 2007). A study conducted in 15 young and older men showed that there were no significant differences between

younger and older men in protein requirements (Cheng, Gomez et al. 1978). This may be due to the efficiency of protein use or the ability to adapt to changes in protein intake levels (Cheng, Gomez et al. 1978).

Two short-term studies were carried out in resistance training participants (Campbell, Crim et al. 1994; Welle and Thornton 1998). The first study with twelve participants aged 56-80 years showed that a high protein intake (1.6 g/kg vs. 0.8 g/kg) did not influence changes in body composition in a 10 day period (Campbell, Crim et al. 1994). For studying changes in body composition, longer study durations are needed. In another study of nine men and nine women aged over 60 years, the acute consumption of a high protein content meal (28% of energy intake) did not enhance myofibrillar synthesis after resistance exercise, compared to those had a low protein meal (7% of energy) (Welle and Thornton 1998).

Table 2.2 Nitrogen balance studies of protein requirement in the elderly.

Studies	Subjects number, sex	Age (year)	Study period	Protein intake (g/kg/d)	Results	*Adequacy of protein intake of 0.8 g/kg/d
Kortebein 2007	6 M 6 F	67±5	10 days bed rest	0.8	<ul style="list-style-type: none"> • Nitrogen balance - negative throughout the study • Protein synthesis ↓ • Whole body lean mass ↓ • Lower extremity lean mass ↓ • Muscle strength ↓ 	Not adequate
Morse 2001	11 F	75±4	3x18 days	0.50, 0.75, 1.00	<ul style="list-style-type: none"> • From week 2 to week 3, more positive nitrogen balances occurred at all three protein intake levels • shorter-term nitrogen balance protocols are insufficient to firmly establish the recommendation 	At or above 0.76 g/kg/d

Studies	Subjects number, sex	Age (year)	Study period	Protein intake (g/kg/d)	Results	*Adequacy of protein intake of 0.8 g/kg/d	
Campbell 2001	10 M&F	66±3	14 weeks	0.8	<ul style="list-style-type: none"> • Urinary nitrogen - decreased • Whole body protein metabolism – no change • Whole body composition – no change • Mid-thigh muscle area – decreased 	Not adequate	
Castaneda 1995a	12 F	66-79	9 weeks	0.45 vs. 0.92	Protein intake <ul style="list-style-type: none"> • Nitrogen balance • Lean tissue • immune response • muscle function 	0.45g/kg/d negative ↓ ↓ ↓ 0.92g/kg/d balance no change improved improved	Not adequate
Campbell 1994	12 M&F	56-80	10 days	0.8 vs. 1.6	Protein intake vs. nitrogen balance <ul style="list-style-type: none"> • 1.0 g/kg/d is required to achieve nitrogen equilibrium 	0.8 g/k/g/d negative 1.6 g/kg/d positive	Not adequate

Studies	Subjects number, sex	Age (year)	Study period	Protein intake (g/kg/d)	Results	*Adequacy of protein intake of 0.8 g/kg/d
Gersovitz 1982	7 M 8 F	75±4 78±9	30 days	0.8	<ul style="list-style-type: none"> • Men: 4 had distinctly negative nitrogen balance in the 2nd period and 3 men were negative in the final period • Women: 4 were negative in the 1st and final periods and 7 were negative during the 2nd period. 	Not adequate
Zanni 1979	6 M	63-77	17 days	Protein-free diet with adequate energy intake	<ul style="list-style-type: none"> • Nitrogen losses from fecal and urinary were 0.79±0.18 and 2.27±0.22 g/d respectively. • Nitrogen requirement for balance: 95 mg nitrogen or 0.59 g protein/kg ideal body weight 	Adequate

Studies	Subjects number, sex	Age (year)	Study period	Protein intake (g/kg/d)	Results	*Adequacy of protein intake of 0.8 g/kg/d
Uauy 1978	7 M 7 F	68-74 70-84	10 days	M: 0.57, 0.70, 0.85 F: 0.52, 0.70, 0.80	<ul style="list-style-type: none"> • 0.52 g/kg/d: all women were negative balance • 0.83 g/kg/d was required for elderly women 	Not adequate
Cheng 1978	7 M 8 M	61-73 23-29	3x11 days	0.4, 0.8, 1.6	<ul style="list-style-type: none"> • 0.4 g/kg: all subjects had negative nitrogen balance. • 1.6 g/kg: all subjects had positive nitrogen balance. • 0.8 g/kg: 3 young men and 4 aged men had positive balance • No significant differences in protein requirement between young and aged men 	Adequate

M: male, F: female. * Original conclusion of the authors

2.6.2.2 Reviews and meta-analysis studies of nitrogen balance studies

Many research groups have reviewed recent nitrogen balance studies as shown in Table 2.3 (FAO/WHO/UNU 1985; Prothro 1989; Garlick, McNurlan et al. 1999; Rand, Pellett et al. 2003; Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005; Morais, Chevalier et al. 2006; National Health and Medical Research Council 2006). Generally, the findings are consistent and indicate that protein requirements in the elderly are higher than the previously suggested recommendation of 0.75 g per kg body weight per day. Most reviews support the recommendation for a protein intake of 0.8 g/kg/d or above for the elderly (Prothro 1989; Rand, Pellett et al. 2003; Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). The most recent review suggested that a protein intake of 1.0 – 1.3 g/kg/d is required to maintain nitrogen balance in the elderly (Morais, Chevalier et al. 2006).

Reviews and meta-analyses of available studies show that previous nitrogen balance studies have not provided sufficient information to estimate protein requirements for the elderly. This is due to the limited number of available nitrogen balance studies in the elderly and the limitations of the nitrogen balance method itself as discussed in the previous section (2.1.5.2).

Table 2.3 Review studies of protein intake and nitrogen balance studies.

	Recommended protein intake for elderly people by reviewers	Other results	Study types
Morais 2006	1.0-1.3 g/kg/d	Elderly may need more protein intake due to: <ul style="list-style-type: none">• Lower energy intake• Impaired insulin action• Reduced muscle mass• Lower rates of myofibrillar protein turnover	Review
National Health and Medical Research Council 2006	M>70y: 81g/d (1.07g/kg) F>70y: 57g/d (0.84g/kg)		Review and recommendation
Panel on Macronutrients 2005	0.8 g/kg	<ul style="list-style-type: none">• No sufficient data to suggest the upper limit of protein intake of total energy intake• Usually is about 15% of total energy intake	Review and recommendation
Rand 2003	132 mg nitrogen/kg/d (0.83 protein g/kg/d)		Meta-analysis

	Recommended protein intake for elderly people by reviewers	Other results	Study types
Garlick 1999		No strong evidence supported that high protein diets confer any advantage for muscle strength or health	Review
Prothro 1989	1g/kg/d (12-14% of total calories should be provided by protein)	<ul style="list-style-type: none"> • Factorial method underestimated the protein requirement • Results were inconsistent for recommended 0.8g/kg/d 	Review
FAO/WHO/UNU 1985	0.75 g/kg/d	The recommended value could be too low as the value was based on nitrogen balance studies conduct at high energy intakes.	Review and recommendation

M: male, F: female

2.6.2.3 Cross-sectional and longitudinal studies of protein intake and body composition in relative healthy elderly population

Results from protein intake and body composition cross-sectional studies in the elderly are inconsistent as shown in Table 2.4 (Vellas, Hunt et al. 1997; Starling, Ades et al. 1999; Mitchell, Haan et al. 2003; Devine, Dick et al. 2005; Houston, Nicklas et al. 2008), which could be due to the differences in the study population, assessment methods, and study duration. For example, in some studies subjects were a population based sample and have large sample sizes (Mitchell, Haan et al. 2003; Devine, Dick et al. 2005; Houston, Nicklas et al. 2008). Others studies however, had relatively small sample sizes and were recruited through advertisements, which introduces a new set of biases (Vellas, Hunt et al. 1997; Starling, Ades et al. 1999). Some studies included both men and women (Mitchell, Haan et al. 2003; Houston, Nicklas et al. 2008) and other studies only investigated men (Starling, Ades et al. 1999) or women (Devine, Dick et al. 2005). As can be seen from Table 2.4, the age ranges of study participants also varied between studies. Although DXA was used by most of the studies, dietary intakes were assessed by different methods. Some studies used food-frequency questionnaire, and other studies used 3-day food records (Vellas, Hunt et al. 1997; Starling, Ades et al. 1999). Study designs ranged from cross-sectional surveys to a 10-year longitudinal study.

Two earlier cross-sectional studies found that protein intake and physical activity were not correlated with age-related muscle loss in elderly people (Starling, Ades et al. 1999; Mitchell, Haan et al. 2003). However, a population based cross-sectional study (Devine, Dick et al. 2005) and two longitudinal studies found that high protein intake was correlated with higher lean body mass and higher bone mineral density (Houston, Nicklas et al. 2008) and fewer health problems over a 10-year period (Vellas, Hunt et al. 1997) in elderly people. A study of 1077 elderly Australian women aged 75 ± 3 years showed that women in the lowest protein intake tertile had significantly lower hip bone mineral density compared to those in the highest protein intake tertile (Devine, Dick et al. 2005). The population based Health ABC longitudinal study in the US found that the community-dwelling older people in the highest quintile of protein intake lost about 40% less lean mass and appendicular lean

mass than did those in the lowest quintile of protein intake over a 3-year period (Houston, Nicklas et al. 2008). Vellas and colleagues followed up 304 healthy elderly men and women for 10 years and found that women with protein intake greater than 0.8 – 1.2 g/kg/d tended to have fewer health problems over the study period than those with protein intake < 0.8 g/kg/d (Vellas, Hunt et al. 1997). For assessing the correlation between diets and changes in body composition, longitudinal studies are preferable. The evidence from these longitudinal studies suggests that protein intake may be a modifiable risk factor for sarcopenia.

Table 2.4 Cross-sectional and longitudinal studies in protein intake and body composition in elderly people.

Studies	Subjects number, sex	Age (year)	Study population	Study type	Method	Results
Houston 2008	2066 M & F	75±3	Population-based random sample	3-year longitudinal	FFQ DXA	Participants in the highest quintile of protein intake lost about 40% less lean mass and appendicular lean mass than did those in the lowest quintile of protein intake. The associations were attenuated slightly after adjustment for change in fat mass, but the results remained significant.
Devine 2005	1077 F	75±3	Population-based random sample	Cross- sectional and longitudinal	FFQ QUS	The mean protein intake 80.5±27.8 (1.19±0.44 g/kg/d). Subjects in the lowest tertile (<66g/d) had significantly lower hip BMD (2.6%) than did the subjects in the higher tertile (>87g/d).

Studies	Subjects number, sex	Age (year)	Study population	Study type	Method	Results
Mitchell 2003	1404 M & F	elderly	Population-based sub-sample	Cross- sectional	FFQ DXA PAQ	Lean muscle mass was not associated with dietary intake and physical activity.
Starling 1999	44 M	67±11	Healthy volunteers	Cross- sectional	3-day food record, DXA, accelerometer	Maintaining regular physical activity and adequate protein intake did not offset the age-related loss of appendicular skeletal muscle mass.
Vellas 1997	304 M & F	72 (median) at entry	Healthy volunteers	10-year longitudinal	3-day food record	Women with protein intake>0.8-1.2 g/kg/d tended to have fewer health problems over the next 10 years than those with protein intake<0.8 g/kg/d.

M: male, F: female; FFQ: food frequency questionnaire; DXA: dual energy x-ray absorptiometry; PAQ: physical activity questionnaire; QUS: quantitative ultrasound.

2.6.2.4 Randomized controlled trials of protein and energy supplementation

There are a few studies on the effects of dietary protein supplementation on body composition and muscle mass and function in elderly participants as shown in Table 2.5 (Fiatarone, O'Neill et al. 1994; Gray-Donald, Payette et al. 1995; Bunout, Barrera et al. 2001; Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003; Wouters-Wesseling, Van Hooijdonk et al. 2003; Bunout, Barrera et al. 2004; Maesta, Nahas et al. 2007; Aquilani, Opasich et al. 2008). Most of the studies combined dietary supplements with resistance training in the study protocol and assessed the combined effect of supplements and exercise. The sample size of these studies was generally small (around 40 to 80), with only two studies having more than 100 study participants (Bunout, Barrera et al. 2001; Bunout, Barrera et al. 2004). Protocols also varied between studies, such as differences in the total energy or protein content of the supplements and study duration. For example, the supplement sources varied from essential amino acids (Aquilani, Opasich et al. 2008) to soy protein (Maesta, Nahas et al. 2007), but most of studies did not detail the type of protein source. The amount of protein supplements varied from around 15 g (Bunout, Barrera et al. 2004) to 30 g (Bonnefoy, Cornu et al. 2003) per day, and the energy intake from protein supplements varied from 14% (Wouters-Wesseling, Van Hooijdonk et al. 2003) to 90% (Payette, Boutier et al. 2002). The study population varied largely between studies, including different age ranges of the participants, ranging from 45-70 (Maesta, Nahas et al. 2007) years to 72-98 years (Fiatarone, O'Neill et al. 1994). The differences in the study populations and study protocols make analysis of pooled results difficult and contributes to the inconsistent results between the studies.

Most protein supplement studies were conducted in unwell older people, such as hip fracture patients (Avenell and Handoll 2006) or hospitalized patients with other illnesses, such as congestive heart failure (Milne, Potter et al. 2005). There were very few long term protein supplement studies undertaken in relatively healthy community free-living older populations. Frequently the study subjects have been malnourished or frail (Lauque, Arnaud-Battandier et al. 2000; Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003).

The impact of an 18-month nutritional supplementation and a resistance training program was studied in 149 healthy free-living Chilean elderly age ≥ 70 years (Bunout, Barrera et al. 2001). Body weight and fat-free mass did not change in either group, but fat mass increased in all groups. The rate of bone loss was less in the supplemented group compared to the control group. Muscle strength and physical function improved in the trained subjects. A total of 98 participants completed the follow-up at 18 months (31 supplemented and trained, 26 supplemented, 16 trained and 25 with no intervention). The supplement was of soup or porridge consistency and given twice a day as a snack. The total energy from the supplement per day was 873kJ with 13% energy (13g) from protein. Compliance with taking the supplement was 48%. The daily activities remained constant in the supplemented subjects and decreased in the other groups over the period of the study. Body weight did not change in any of the groups. Although the study duration was long, the low protein content in the supplement in their study may explain the lack of an effect on body weight observed in this study.

Fiatarone et al. conducted a 10-week randomized, placebo-controlled trial in 100 frail elderly men and women (63) aged 87.1 ± 0.6 years living in nursing homes (Fiatarone, O'Neill et al. 1994). The study compared four groups: progressive resistance training group, multi-nutrient supplementation group, a group with both interventions, and a control group with neither intervention. The 250ml nutritional supplement was given once daily in the evening. It contained 1512 kJ (360 kcal) in the form of 17% of soy-based protein (15 g), 60% of carbohydrate and 23% of fat. They found that the nutritional supplement had no effect on muscle strength or muscle size during the 10 weeks study period in any of the groups. However, the study duration of 10 weeks may not be long enough to detect the changes in muscle strength. The significant reduction in the voluntary food intake after the trial in the supplemented only group may also explain the lack of effect of the supplement on muscle strength and size as the total energy intake did not change from baseline.

A 9-month randomized controlled trial conducted in France investigated whether a progressive exercise program and nutritional supplements would increase muscle mass and strength in 57 elderly men and women aged over 72 years from six retirement homes (Bonneyfoy, Cornu et al. 2003). There were four groups in the

study. The dietary supplements group was compared with a placebo group, and the physical exercise group was compared to the non-exercise group. Muscle strength was assessed by a leg-extensor machine and was significantly increased by 57% at 3 months in the supplement group, and it wasn't significant at 9 months. Whole body fat-free mass did not increase significantly (2.7%). They found exercise did not improve muscle power but improved functional tests (the 'five-time-chair rise').

In summary, few studies have shown any benefit on improvement in muscle strength and physical function by taking protein supplements alone. One possibility for the lack of effect of the protein intervention observed in most studies is that an insufficient dose and/or the inefficient source of protein supplement may have been used. As discussed in section 2.1.3.3, elderly people tend to eat less. As the digestibility of protein is a critical determinant of protein utilisation (addressed in detail in section 2.8), the protein source and dose of protein supplement are important in nutritional intervention trials. There has been no consensus on the effective dose of protein supplement or the protein source for dietary intervention studies. Exercise training has been shown to improve some physical function with or without nutritional supplements in some studies. The nutritional status of the subjects, known to influence protein requirements may also influence the response. The study populations in most of these studies were relatively unwell or very frail or undernourished elderly people. Because of these limitation it is difficult to extrapolate these results to the well elderly.

Table 2.5 Randomized controlled trials of protein-energy supplementation with or without resistance training.

Studies	Subjects number & sex	Age (years)	Study period	Study group	Study population	Supplement	Fat mass or anthropometric measurements	Fat-free mass	Muscle strength	Physical functional tests
Aquilani 2008	27M 11F	74±4	8 weeks	2 groups: Supp Non-supp	Chronic heart failure with severe depletion of muscle mass	Essential amino acids 8g/day (however, all patients had adequate energy- protein intake)	N/A	N/A	N/A	↑ in supp group
Maesta 2007	46F	61±5	16 weeks	4 groups: Supp Training Both Placebo	Post- menopausal Brazilian women	25 g soy protein or 25 g maltodextrine- (placebo)	↓ in training group	↑ in training group	NA	NA

Studies	Subjects number & sex	Age (years)	Study period	Study group	Study population	Supplement	Fat mass or anthropometric measurements	Fat-free mass	Muscle strength	Physical functional tests
B. Bunout 2004a	101 M&F	≥70	1 year	4 groups: Supp Training Both Neither	2 outpatient clinics	Total energy: 400 kcal Protein: 15 g	Fat mass ↑ in all groups	No change in either group	↑ in training group with or without supplemented	Improved in training group with or without supplement
Bonnefoy 2003	57 M&F	>72	9 months	4 groups: Supp Placebo Exercise Non-exercise	16 retirement home, frailty	Twice daily of 200ml drink: 1686 kJ(total) 30% protein (30g) 50% carbohydrate (50g) 20% lipids (8.8g)	BMI ↑ in supp group, decreased in placebo group	↑ in supp group, but not significant	↑ in supp groups at 3 months but only a tendency at 9 months. No change in exercise group.	Improved in exercise group

Studies	Subjects number & sex	Age (years)	Study period	Study group	Study population	Supplement	Fat mass or anthropometric measurements	Fat-free mass	Muscle strength	Physical functional tests
Wouters- Wesseling 2003	68 M&F	82±7	6 months	2 groups: Supp Placebo	Nursing home, BMI≤25kg/m ²	Twice daily of 125ml drink: 1050 kJ (total) 14% protein (17.5g) 46% carbohydrate (57g) 40% fat (22.5g)	Anthropometric measurements– no change		No difference between groups	No difference between groups
Payette 2002	83 M&F	80±7	16 weeks	2 groups: Supp Control	Community- living, under- nourished	2x235 ml commercial drink (Ensure or Ensure Plus) Total 710 kcal 90% protein	Weight↑ in supp group; Other Anthropometric indexes–no differences between groups		No difference between groups	No difference between groups.

Studies	Subjects number & sex	Age (years)	Study period	Study group	Study population	Supplement	Fat mass or anthropometric measurements	Fat-free mass	Muscle strength	Physical functional tests
D. Bunout 2001	149 M&F	≥70	18 months	4 groups: Supp Training Both Neither	3 public outpatient clinics	Twice daily of soup: Total energy: 873kJ Protein: 13g Carbohydrate: 62.4g Fat: 11g	Fat mass↑ in all groups; Weight–no change in either group.	No change in either group	Improved in trained groups	Improved in trained groups.
Fiatarone 1994	63F 37M	87 (72- 98)	10 weeks	4 groups: Training Supp Both Neither	725 nursing homes	240ml liquid daily: 360kcal (total), 60% carbohydrate 23% fat 17% soy-based protein		Cross- sectional muscle area –no differences between groups	Improved in exercise group	Improved in exercise group.

Studies	Subjects number & sex	Age (years)	Study period	Study group	Study population	Supplement	Fat mass or anthropometric measurements	Fat-free mass	Muscle strength	Physical functional tests
Gray-Donald 1995	50 M&F	78±7	12 weeks	2 groups: Supp Control	Community- living, under- nourished	Twice daily of 235ml liquid:1045-1480 kJ (total), 14-14.7% protein 31.5-32% fat 54.5-53.3% carbohydrate	Weight ↑ in supp group			No difference between groups. Significantly fewer falls in supp group.

M: male, F: female; Supp: supplemented group.

2.6.2.5 Amino acid supplementation and muscle protein metabolism

Supplementation with infusions of pure amino acids has shown positive effects on muscle protein synthesis both in young and elderly individuals (Volpi, Ferrando et al. 1998; Paddon-Jones, Sheffield-Moore et al. 2004). A study conducted in six healthy males aged 71 ± 2 yr found that protein breakdown did not change during an amino acid infusion (Volpi, Ferrando et al. 1998). Thus a positive net balance of amino acids across the muscle was achieved (Volpi, Ferrando et al. 1998). This indicated that adequate protein intake may be protective against muscle wasting in the elderly. Volpi and colleagues further compared the muscle metabolism after supplementing with 18 g of essential amino acids and 40 g balanced amino acids (18 g essential amino acids + 22 g nonessential amino acids) (Volpi, Kobayashi et al. 2003). They found no difference in muscle protein metabolism between the two groups. Therefore, they concluded that essential amino acids are primarily responsible for muscle metabolism. Supplementation of the diet with essential amino acid plus arginine for 16 weeks has been shown to improve lean body mass, muscle strength and physical function in 12 glucose intolerant elderly individuals (Borsheim, Bui et al. 2008). This implies that the protein source is important and that protein rich in essential amino acids is more efficient for muscle metabolism. Increased protein intake especially high quality protein intake may slow the onset of sarcopenia in the elderly.

Recently, a one year randomized controlled trial compared the effect of essential amino acids supplements (β -hydroxy- β -methylbutyrate, L-arginine, L-lysine) to an isonitrogenous control-supplement (mixture of nonessential amino acids: alanine, glutamic acid, glycine, serine and ascorbic acid) on protein metabolism in elderly people (Baier, Johannsen et al. 2009). A total of 38 men and 39 women aged 76 ± 1.6 years living in senior citizen centres and adult assisted-living and care facilities participated. Subjects were included in the study if their 'Get-up-and-go' test scores were greater than 10 seconds but less than 20 seconds. They found that subjects supplemented with essential amino acids had increased their protein turnover and body lean tissue significantly compared to subjects in the control group at one year, but there were no significant differences in changes in fat mass or

percentage body fat. In their study, the amount of supplements was not fixed but varied by participants' baseline weight and no information was provided about the total energy content of the supplements. In contrast, a 3-month randomized placebo controlled trial conducted on 30 healthy elderly women aged 71 ± 4 years found that long-term leucine supplementation (7.5g/d) did not increase skeletal muscle mass and strength (Verhoeven, Vanschoonbeek et al. 2009). The inconsistent results between these two randomised controlled studies (Baier, Johannsen et al. 2009; Verhoeven, Vanschoonbeek et al. 2009) may be due to the differences in their study population and study duration. The study subjects in Baier's study were living in nursing homes and were older (76 ± 1.6 years) than the study subjects in Verhoeven's study (71 ± 4 years). Baier's study had a one year duration, which was longer than Verhoeven's study of three months duration. Therefore, the results are not directly comparable.

In summary, it is the essential amino acids that are primarily involved in muscle metabolism. In the studies reported above essential amino acid supplementation has a positive effect on protein turnover and lean body mass gain in elderly people and can improve muscle strength and physical function in glucose intolerant elderly individuals. More long-term studies are needed into the effect of amino acid supplements on the musculoskeletal system in relatively healthy elderly.

2.6.2.6 Cochrane systematic review of protein supplement studies in hip fracture elderly patients

Avenell and Handoll undertook a comprehensive review for the comparison of nutritional supplements versus control studies in hip fracture elderly patients aged over 65 years (Avenell and Handoll 2006). Twenty-one randomised trials involving 1727 participants were included in their meta-analysis. Of these twenty-one trials only 4 trials (Tkatch, Rapin et al. 1992; Schurch, Rizzoli et al. 1998; Espauella, Guyer et al. 2000; Neumann, Friedmann et al. 2004) compared high protein supplements with low protein or non-protein containing supplements. Most of the studies involved nutritional supplements, which provided non-protein energy, some vitamins and minerals orally, or feeding nasogastrically. They concluded that the overall trial quality was poor. The meta-analysis showed that there was no

statistically significant effect of protein supplementation on mortality, but protein supplements may reduce ‘unfavourable outcomes’, such as death or complications compared to not using supplements in hip fracture patients (risk ratio 0.78, 95% CI 0.65 to 0.95) as shown in Table 2.6.

Table 2.6 Comparison of mortality and unfavourable outcomes of studies with high protein containing supplements versus low protein or non-protein supplements in hip fracture patients.

Outcome	No.of studies	No.of participants	Effect size*
Mortality by end of study	4	361	1.42 [0.85, 2.37]
protein v non-protein supplement	3	315	1.38 [0.82, 2.34]
High protein v low protein supplement	1	46	2.18 [0.21, 22.42]
Unfavourable outcome (deaths or complications) at end of study	2	223	0.78 [0.65, 0.95]
Protein v non-protein supplement	2	223	0.78 [0.65, 0.95]
High protein v low protein supplement	0	0	Not estimable

Adapted from: (Avenell and Handoll 2006). * Effect size: risk ratio [99% confident interval]

2.6.2.7 Cochrane system review of randomized and quasi-randomized trials of oral protein and energy supplementation studies

Milne and colleagues reviewed 55 randomized and quasi-randomized controlled trials of oral protein and energy supplementation compared with placebo or control groups in older people (aged over 65 years) (Milne, Avenell et al. 2006). However, most of the study subjects were unwell and had been hospitalized due to various illnesses including fracture, heart failure or stroke, or had just been discharged from hospitals. For dichotomous variables, such as mortality or number of patients with complications, the effect size was calculated as the risk ratio with 95% confidence intervals. For continuous variables, such as percentage of weight change and hand grip strength, the effect size was calculated as weighted mean differences and 95% confidence intervals using a fixed effects model which assumes the same underlying effect in all studies and considers any heterogeneity between trials to be due to random errors.

Their meta-analysis as shown in Table 2.7 showed that weight gain was common in supplemented subjects, and there was a reduced mortality in the supplemented compared with control groups (relative risk 0.74, 95% CI 0.59 to 0.92). There was no significant functional benefit, such as hand grip strength and no difference in the risk of complication between supplemented and control groups (relative risk 0.95, 95% CI 0.81 to 1.11).

Table 2.7 Comparison of oral protein-energy supplements versus routine care.

Outcome or subgroup title	No. of studies	No. of participants	Effect size
Mortality	34	3021	0.74 [0.59, 0.92]
Mortality by nutritional status	34	3021	0.74 [0.59, 0.92]
Undernourished	21	1825	0.72 [0.55, 0.64]
Nourished	13	1196	0.78 [0.53, 1.15]
Mortality by kcal offered per day	31	2905	0.74 [0.60, 0.93]
≥400 kcal	19	2177	0.71 [0.56, 0.90]
<400 kcal/day	12	728	0.98 [0.52, 1.82]
Mortality by age group	32	2940	0.72 [0.58, 0.90]
Mean age ≥75 years	24	2033	0.69 [0.54, 0.87]
4.2 Mean age <75 years	8	907	0.99 [0.51, 1.82]
Mortality by period of supplementation	30	2600	0.73 [0.57, 0.94]
<35 days supplementation	10	1063	0.70 [0.44, 1.11]
≥35 days supplementation	20	1537	0.75 [0.56, 1.00]
Mortality by wellness	34	3021	0.74 [0.59, 0.92]
Well	6	393	0.98 [0.25, 3.78]
Unwell	28	2628	0.73 [0.59, 0.92]
Mortality by hospital or community	31	2799	0.73 [0.58, 0.92]
In-patients	19	2022	0.67 [0.52, 0.86]
Community	12	777	1.07 [0.64, 1.78]
Mortality by diagnostic group	32	2933	0.74 [0.59, 0.92]
Geriatric conditions	20	2314	0.72 [0.57, 0.92]
Hip fracture	7	337	0.89 [0.47, 1.68]
Congestive heart failure	1	22	1.44 [0.10, 20.21]
Chest conditions	2	120	3.07 [0.13, 73.30]
Perioperative	1	100	1.33 [0.16, 11.38]
Stroke	1	40	0.29 [0.07, 1.21]
Complications	14	1169	0.95 [0.81, 1.11]
% weight change	36	2474	2.32 [1.93, 2.0]
% arm muscle circumference change	15	1246	1.22 [0.44, 1.99]
Handgrip	4	246	-0.27 [-1.10, 0.56]

(Adapted from (Milne, Avenell et al. 2006))

2.6.2.8 Summary of protein requirement studies

Evidence from short and long term nitrogen balance studies suggests that elderly people may need more protein than the current US/Canadian recommended 0.8 g/kg/d (Panel on Macronutrients, Panel on the Definition of Dietary Fiber et al. 2005). Most of researchers recommend a protein intake of 1.0-1.3 g/kg for elderly people. This is the position taken by the Australian NHMRC when making its Nutrient Reference Value Recommendations. However, there has been no universal consensus due to lack of sufficient evidence from long term studies.

Evidence from cross-sectional studies and longitudinal studies are not consistent in the beneficial effect of high dietary protein intake on preventing lean body mass loss with aging in elderly people.

There have been a limited number of randomized controlled trials of protein supplementation in non-hospitalized elderly people. Most of them have shown a weight gain benefit from protein supplements but few have shown that protein supplements improve muscle mass, strength and physical functions in the elderly. Moreover, the study population, source and amount of protein supplement and study duration varied considerably between studies.

2.6.3 Factors affecting the estimation of protein requirement in the elderly

Total energy intake needs should be considered when evaluating protein requirements because protein metabolism and the nitrogen balance method are sensitive to total energy intake and balance (Bier, Motil et al. 1981; Blackburn 1984; Fukagawa and Young 1987; Young, Yu et al. 1991). If a nitrogen balance study conducted with a high energy intake, the body still can achieve nitrogen balance or even positive balance with a relatively low protein intake. Elderly people tend to eat less food than their younger counterparts, and it is likely that there is a decrease in total energy intake in elderly people (Evans 2004). Therefore, the provision of adequate energy intake is important in nitrogen balance studies in the elderly. In order to minimize the differences in protein metabolism induced by the differences in total energy intake, for cross-sectional studies energy intake should be adjusted in the analysis.

Body size is the major determinant of the absolute requirements for energy and protein (FAO/WHO 1975). Provided that energy intake is adequate, nitrogen losses are closely related to body weight and basal metabolic rate (Scrimshaw, Hussein et al. 1972; Castaneda, Charnley et al. 1995; Castaneda, Dolnikowski et al. 1995). Thus, body size needs to be adjusted when evaluating protein requirements in long term studies. BMI gives a measure of weight for height that is largely independent of height (FAO/WHO/UNU 1985).

2.7 Protein and energy intake in relatively healthy elderly people

In general both American and Australians consume diets with a moderate to high protein intake, but some sub groups of the population, including the elderly, consume diets with less protein (Morley 1997; Australian Bureau of Statistics 1998; Morley, Baumgartner et al. 2001). There are few available data on dietary protein intake in elderly populations in developing countries. Details of the studies found are shown in Table 2.8 (Munro, McGandy et al. 1987; Woo, Cheung et al. 1988; Deschamps, Astier et al. 2002; Correa Leite, Nicolosi et al. 2003; Watanabe, Hanamori et al. 2004; Aghdassi, McArthur et al. 2007; Feart, Jutand et al. 2007; Fulgoni 2008; Meng, Zhu et al. 2009). The recommended dietary intakes for protein differ between countries.

In a US survey using 3-day food diaries in 691 American men and women aged 60-98 years, the daily protein intake was 1.02-1.05 g/kg body weight. This is much higher than the current US/Canada RDA (Recommended Dietary Allowance), which is 0.8 g/kg good quality protein (Munro, McGandy et al. 1987). However, in the 2003-2004 National Health and Nutrition Examination Survey, protein intake (as assessed by 24-hour recall) was 67 ± 19 g/d amongst women aged 51-70 years and 59 ± 15 g/d amongst women aged 71 years and older. Approximately 8% of older adult women reported consuming protein levels below their estimated average requirement (Fulgoni 2008).

In Canada, 3-day food records were obtained from 407 elderly men and women with an average age of 85 ± 8 years. Their average BMIs were 24 ± 6 kg/m² and they were

living in 11 long-term care facilities. The average daily energy intake was 1513 ± 363 kcal and protein provided 15% of energy. A total of 29.5% of the respondents had protein intakes below the US/Canada RDA which is 0.8 g/kg (Aghdassi, McArthur et al. 2007).

In France, 1786 subjects aged between 68-95 years completed a 24 hour dietary recall. Protein provided 18% of energy intake. The total energy intake was lower in women and in older participants (≥ 85 years). High energy intake was associated with higher income, but not with education level. The study suggests that being female, older, being widowed and low income level were the risk factors for inadequate dietary intake (Feart, Jutand et al. 2007). Another study conducted in France investigated 169 elderly people free-living in the community and found that the median protein intake was 1.33 g, but in this study no correlation was found between poor nutrition and mortality (Deschamps, Astier et al. 2002).

In Italy, a study by 24-hour dietary recall in a random sample of 257 independent-living elderly aged above 75 years found that the average daily energy intake was 6.3 ± 2.1 MJ (1508 ± 502 kcal) in women (Nes, Sem et al. 1992). The energy from fat was 41.7% in women which is higher than the usually recommended level. In this study 14% of the women had a BMI below 18.5 kg/m^2 , and 2% were below 16.

In Japan, a survey was conducted in 2001 in 57 (women 26) healthy free-living people aged over 70 years (Watanabe, Hanamori et al. 2004). The energy and protein intake for women were 38.1 ± 7.6 kcal/kg/d and 1.51 ± 0.26 g/kg/d respectively. In this study protein provided 16% of energy.

In a population based study in Australia, dietary intake was assessed using a food frequency questionnaire in 1077 women aged 75 ± 3 years who were randomly selected from the community (Devine, Dick et al. 2005). The protein intake was 81 ± 28 g/d (or 1.2 ± 0.4 g/kg/d), which was higher than the current Australian/New Zealand RDI (Recommended Dietary Intake) of 0.94 g/kg/d protein for women aged over 70 years. The energy intake was 7155 ± 2192 kJ/d, slightly higher than the Australia National Nutrition Survey of 1995-1996 for the age group of 70-79, which

was 6200 kJ/d (1482 kcal) (Australian Bureau of Statistics 1998). Protein provided 19±3% of energy intake.

In summary, few studies have examined protein intakes in elderly living in developing countries. Protein intake in the elderly varied between countries which ranged from 59 to 81 g/d in elderly females (Munro, McGandy et al. 1987; Woo, Cheung et al. 1988; Deschamps, Astier et al. 2002; Correa Leite, Nicolosi et al. 2003; Watanabe, Hanamori et al. 2004; Aghdassi, McArthur et al. 2007; Feart, Jutand et al. 2007; Fulgoni 2008; Meng, Zhu et al. 2009). In general, elderly men consume more protein than elderly women but the percentage energy intake from protein intake was similar in both genders and across countries and ranged from 15-19% of total energy.

Table 2.8 Dietary protein intakes in relatively healthy elderly population.

	Country	Dietary assessment method	Subjects number, sex	Age of subjects (years)	Protein intake mean or mean \pm SD	% Protein intake (%) mean or \pm SD
Fulgoni 2008	America	24-hour recalls	618 F	51-70	67 \pm 19 g/d	16 \pm 3
			419 F	71+	59 \pm 15 g/d	16 \pm 2
Munro 1987	America	3-day food diary	619 M&F	60-98	1.02-1.05 g/kg/d	NA
			289 F	60-75	1.02 \pm 0.02 g/kg/d†	18 \pm 0.2 †
			158 F	\geq 76	1.06 \pm 0.03 g/kg/d†	18 \pm 0.4 †
Meng 2009	Australia	FFQ	862 F	75 \pm 3	81 \pm 28 g/d 1.2 \pm 0.4 g/kg/d	19 \pm 3
Aghdassi 2007	Canada	3-day food record	108 M	84 \pm 8	0.9 \pm 0.3 g/kg/d	16 \pm 2
			299 F	86 \pm 8	1.0 \pm 0.4 g/kg/d	15 \pm 2
Feart 2007	France	24-hour recall	1786 M&F	68-95	75 \pm 27 g/d	18 \pm 5
			666 M		85 \pm 27 g/d	17 \pm 4
			1120 F		69 \pm 25 g/d	19 \pm 5
Deschamps 2002	France	3-day food record	169 M&F	75.4 *	1.33 g *	NA
Correa 2003	Italy	FFQ	847 M	\geq 65	106 g/d	17.4
			1465 F		89 g/d	17.2
Watanabe 2004	Japan	3-day	57 M&F	74		
		weighed food	31 M		1.80 \pm 0.35 g/kg/d	16
			26 F		1.51 \pm 0.26 g/kg/d	16
Woo 1998	China (Hong Kong)	3-day food recall	417 M&F	\geq 60	1.2 g/kg/d	NA

M: male, F: female; FFQ: food frequency questionnaire; * median.

† mean \pm standard error.

2.8 Protein source for dietary intervention

2.8.1 Digestibility of protein

Digestibility determines the utilisation of protein by the body. Ageing does not impair the anabolic response to a high-quality protein meal and therefore Paddon-Jones proposed including 25-30 g of high quality protein per meal for the elderly to maximize muscle protein synthesis (Paddon-Jones and Rasmussen 2009).

In general, animal based protein has higher digestibility (FAO/WHO/UNU 1985). However, the results are inconsistent. A study in 38 normal weight, sedentary women aged between 57-75 years showed that protein intake particularly from animal sources was associated with a better preservation of muscle mass index ($MMI = \text{Fat-free mass (kg)}/\text{height(m)}^2$) (Lord, Chaput et al. 2007). Fat-free mass was measured by DXA, and muscle protein content was measured by the use of creatinine excretion. In contrast, a prospective study with a mean duration of 7.0 ± 1.5 years on 1000 community-dwelling elderly women aged greater than 65 years suggested that a high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women (Sellmeyer, Stone et al. 2001). However, Heaney suggested that there was inconsistency in Sellmeyer's study, that is the subjects with the highest ratios of animal to vegetable protein intake had marginally higher bone mineral densities (BMDs) on entry into their study (Heaney 2001). The positive correlation between protein intake and BMD at baseline and negative correlations between protein intake and BMD at follow-up need to be further investigated.

It has been suggested that rapidly digested protein, such as whey protein, may be more efficient for maintaining muscle mass in the elderly than slowly digested protein (Dangin, Guillet et al. 2003). This may be due to the fact that whey protein is rich in essential branched-chain amino acids and thus it has different digestion and absorption properties than other proteins (Dangin, Boirie et al. 2002; Hall, Millward et al. 2003; Layman 2004; Bowen, Noakes et al. 2006). The speed of protein digestion and amino acid absorption from the gut has a major effect on whole body protein anabolism after one single meal (Boirie, Dangin et al. 1997). The stimulation of protein synthesis depends on the sensing of the concentration of extracellular

amino acid availability (Bohe, Low et al. 2003). To date, there is no consensus on optimal protein sources for elderly in term of skeletal muscle metabolism, but high quality protein rich in essential branched-chain amino acids are suggested by the majority of studies.

2.8.2 Whey protein

Whey protein is a by-product of cheese making. The major components of dairy whey are several proteins (α -lactalbumin, β -lactoglobulin, immunoglobulins, bovine serum albumin, glycomacropptides and lactoperoxidase), peptides, and branched-chain amino acids (Walzem, Dillard et al. 2002). It is particularly rich in essential branched-chain amino acids (BCAA) (26% in whey protein compared to soy protein isolate 18% and wheat protein 15%). Whey protein appears to play a significant role in preserving lean body mass (Walzem, Dillard et al. 2002). The whey protein products also contain some non-protein components, such as lactose, minerals, and traces of milk fat.

There are three main types of whey protein supplements: hydrolysed whey protein, whey protein concentrate, and whey protein isolate. The physical composition of whey protein depends on the manufacturing procedure. With the hydrolysed whey protein, the protein is broken down into peptides, which gives it a bitter taste and makes it costly to manufacture. Whey protein concentrate products have between 25%-89% of protein, but the 80% protein content form is the most commonly used. Whey protein isolate (WPI) is the purest form which contains 90% to 95% protein and has very little lactose (less than 1%) (Toba, Takada et al. 2001). On the above basis, whey protein isolate was selected as the test product in the current study.

2.9 Ageing and body composition

2.9.1 Body composition in the general population

Body composition varies by age, gender, racial/ethnic groups. In general, men have a higher total body mass and higher percentage of fat-free mass, muscle mass and bone mass and lower percentage of body fat mass than women (McArdle 2006).

White females have lower fat-free body density than other ethnic groups, including native American, African American and Hispanic (McArdle 2006).

2.9.2 Changes in body soft tissue composition with aging

With aging, there is a loss of lean body mass, and increased body fat mass and abdominal fat mass (Bunout, de la Maza et al. 2007; Fantin, Di Francesco et al. 2007; Chen, Lin et al. 2008). Studies in different populations have shown similar trends (Guo, Zeller et al. 1999; Chen, Lin et al. 2008). A study of 301 men and women aged 65-97 years in the US found that age related loss of muscle mass could occur in relatively healthy well-nourished elderly men and women and had a multifactorial basis (Baumgartner, Waters et al. 1999). Physical activity, underlying disease, IGF-1 level and body fat mass were found to be significantly associated with muscle mass (Baumgartner, Waters et al. 1999).

Lean body mass peaks during the 5th decade (Chen, Lin et al. 2008) and the decline is accelerated after 60 years of age (Kyle, Genton et al. 2001; Chen, Lin et al. 2008). A cross-sectional study of 433 healthy ambulatory Caucasian men and women from 18 to 94 years of age showed that whole body lean mass measured by DXA decreased by 11.8% and 9.7% in men and women aged over 75 year compared to the 18 to 34 years old subjects, respectively (Kyle, Genton et al. 2001). The appendicular skeletal muscle mass decreased 16.4% and 12.3% in men and women respectively. This indicates that the loss of skeletal muscle mass was greater than the loss of non-skeletal muscle mass. Another cross-sectional study of 316 healthy Chinese women aged 20 to 74 years found that bone mineral content (BMC) and whole body lean mass measured by DXA declined 18.1% and 5.2% respectively at age 74 years (Chen, Lin et al. 2008). The differences in the definition of body lean mass between these two studies may explain the differences in the rate of loss of body lean mass. In Kyle's study, whole body lean mass includes bone mineral content, but Chen's study whole body lean mass referred to bone-free lean mass. A cross-sectional study in the US used the whole body magnetic resonance imaging to measure skeletal muscle mass in 468 men and women (Janssen, Heymsfield et al. 2000). The majority of participants were Caucasian (67%), 17% were African-American, 8% were Asian and 7% were Hispanic. The study observed a small reduction of skeletal muscle

mass in the third decade and an increased rate of loss that started at the end of the fifth decade. They also found a curvilinear correlation between skeletal muscle mass and body weight. Total skeletal muscle mass decreased about 17% and 18% in women and men respectively after age of 70 years compared to those of age of 39 years or younger.

Several longitudinal studies have also shown that muscle mass decreases by approximately 1% per year after age 65 (Frontera, Hughes et al. 2000; Visser, Pahor et al. 2003). A study examined the changes in skeletal muscle size and functions in twelve sedentary men (initial mean age 65 years) over a period of 12 years (Frontera, Hughes et al. 2000). The cross-sectional area of the thigh, all thigh muscles, quadriceps femoris muscle and flexor muscles assessed by the computed tomography showed reductions ranged from 12.5% to 16.1%. A population based study assessed the body composition in 2040 well-functioning black and white American men and women aged 70-79 years at baseline and after 1 year and 2-year follow-up (Visser, Pahor et al. 2003). In women, total body mass decreased by 0.4% at 2 years, and fat-free mass decreased 0.6% after 2 years with no change in body fat mass. Among men, total body mass decreased by 0.3%, fat-free mass decreased by 1.1% and there was an increase in total fat mass of 2%.

Mazariegos investigated the body composition in 19 weight- and height- matched pairs of young (age 19-35 years) and older (age>65 years) healthy white females using isotope dilution, dual photon, whole-body counting, hydrodensitometry and anthropometric method (Mazariegos, Wang et al. 1994). They also found that the older females had significantly high total body fat, significantly less fat-free body mass and total body water when compared with the younger females (Mazariegos, Wang et al. 1994). Age-related decline in fat-free mass reached significance in white women at age 75-79 years (Obisesan, Aliyu et al. 2005). Skeletal muscle mass decreased with aging in both men and women (Gallagher, Visser et al. 1997; Fantin, Di Francesco et al. 2007). The decreases in fat-free mass with aging were smaller in women than in men, and had wide inter-individual variation depending on the degree of weight change (Gallagher, Visser et al. 1997; Hughes, Frontera et al. 2002). In the elderly, with weight loss, there was significantly more lean mass loss than fat mass loss (Newman, Lee et al. 2005).

Both weight gain and weight loss are common in the elderly. The factors involved in the changes of body composition with age are a decline in physical activity, a decline in basal metabolic rate, a decrease in the synthesis rates of muscle protein (Nair 2005; Short, Bigelow et al. 2005), a decrease in IGF-1 levels and inadequate nutritional intake.

2.10 Body composition assessment

2.10.1 History and overall of assessing human body composition

Human body composition can be assessed directly or indirectly. There are two methods used to assess body composition directly: chemical analysis and cadaver dissection (McArdle 2006). Both are costly, time-consuming, and also involve ethical issues (Driskell 2000). The most complete cadaver dissection study was conducted by Clarys and colleagues in 1984 (Clarys, Martin et al. 1984). A total of 25 cadavers sampled from an elderly Belgian population were dissected into gross tissue masses of skin, adipose tissue, muscle tissue, bone, and residuals. The results showed that females had higher percentage of adipose tissue and less muscle tissue than males. There were 40.5% and 28.1% of adipose tissue in females and males respectively, and 28.6% and 37.4% of muscle tissue in females and males respectively.

All other body composition methodologies provide indirect assessment of body composition. Body composition can be assessed at five levels: atomic level, molecular level, cellular level, tissue-organ level and whole-body level (Wang, Pierson et al. 1992). Beside anthropometry measurements, currently available methods for research include underwater weighing (UWW), hydrometry (total body water), whole-body counting (total body potassium) and neutron activation analysis, dual-energy x-ray absorptiometry (DXA), bioelectrical impedance analysis, and computed tomography and magnetic resonance imaging (Shen, St-Onge et al. 2005). These methods are mainly based on the most common approach, which is to consider body mass as two compartments: fat and fat-free mass. It is based on the knowledge

that lipids are hydrophobic and thus free of water. Therefore, fat mass, percentage body fat and fat-free mass can be calculated from total body water (Schoeller 2005).

Underwater weighing was long considered as the ‘gold standard’ for assessing body composition and used as the reference criterion to validate other body composition assessment methods. Recently, other techniques have been shown to be as reliable and precise as UWW. These include total body water (TBW); total body potassium (TBK); and dual energy x-ray absorptiometry (DXA). DXA is now the most commonly used method to assess body composition. This is due to its relatively simple operating procedure, low cost, and wide availability in many institutions. In the current study, DXA was used to assess the body composition.

2.10.2 Dual-energy x-ray absorptiometry

2.10.2.1 DXA history and types

Dual-energy x-ray absorptiometry (DXA) was developed from dual-photon absorptiometry. It was originally designed to estimate bone mineral content (BMC) and bone mineral density (BMD). A dual-energy x-ray absorptiometry has an x-ray tube with low- and high-energy peaks which allow it to measure fat-free mass (FFM) and fat mass (FM). FFM is also called lean soft tissue mass.

There are two types of DXA: pencil-beam DXA and fan-beam DXA. The current study used the Hologic 4500A, an advanced version of the fan-beam DXA. The analyses are reported as fat mass, bone-free lean mass, lean mass combined with bone mineral content (BMC), BMC and BMD. The analysis of fat mass and lean mass is reported for whole body and for regional (head, arms, trunk and legs).

There are two assumptions used in the DXA which are related to the assessment of whole body soft tissue (Lohman and Chen 2005). Firstly, DXA assumes the constant attenuation of pure fat and of bone mineral-free lean tissue. Secondly, it is assumed that DXA measurements are not affected by body thickness. However, DXA is unable to distinguish the thickness of soft tissue but uses a Step Phantom to adjust for tissue thickness.

The advantages of using DXA to assess body composition are the very low radiation exposure, wide availability and low cost (Wang, Wang et al. 1999). The radiation exposure ranges from 0.02 to 1.5 mrem depending on the instrument and the scan speed and can be used for subject of all ages (Lohman and Chen 2005). This dosage is very low compared with yearly background radiation (e.g. radiation from gas from ground and cosmic rays), which is about 1.9 mSv (equals to 190 mrem) in Perth, Australia. 1 mrem equals to 0.01 mSv (Australia and New Zealand Bone and Mineral Society 2005).

2.10.2.2 Validation of DXA measured fat mass and fat-free mass

There is a general agreement that DXA is a precise method to estimate whole body and regional body composition including lean soft tissue and appendicular muscle mass, fat mass and bone mineral density. Although the precision for estimation of regional composition is less than for total body composition (Lohman and Chen 2005).

The accuracy of DXA depends on the subjects' body thickness and size, calibration procedures, instrument and software version, company and model. The coefficients of variation (CV) for DXA measured fat mass, lean mass and fat mass vary between labs depending on the DXA machine type and brand. The CVs of DXA (Hologic 4500A) for total lean mass and total fat mass of our lab were 1.1% and 1.6% respectively.

Salamone and colleagues evaluated the accuracy of total body fat mass (FM) and leg fat-free mass (FFM) measured by Hologic 4500A in 60 healthy elderly (aged 70-79 years) compared with computed tomography (CT) (Visser, Fuerst et al. 1999; Salamone, Fuerst et al. 2000). They suggested DXA is an accurate method for the measurement of fat mass and fat-free mass for the elderly though there is a systematic overestimation of fat-free mass and underestimated of fat mass. Schoeller, Tylavsky and colleagues had the similar results using the Hologic QDR-4500A DXA scanner (Tylavsky, Lohman et al. 2003; Schoeller, Tylavsky et al. 2005). Schoeller and colleagues compared DXA measured body composition (fat

mass and fat-free mass) from seven studies, with total body water from four centres, densitometry from one centre, and four-compartment analysis at two centres (Schoeller, Tylavsky et al. 2005). The study cohort included 1195 subjects, 602 men and 593 women aged 18-82 years with a body mass index of 16-44 kg/m². They found that the fan-beam DXA significantly overestimated fat-free mass ($p < 0.05$), and there was a significant difference in all seven data sets for the fat-free measurements (mean \pm SE was 5 \pm 1%). They recommended that the FFM estimate with the Hologic QDR 4500A be reduced by 5% and for FM be increased by 5%.

2.10.2.3 Validation of DXA measured regional body composition

DXA measured regional body composition such as abdominal fat and limb muscle mass have also been validated. Svendsen and colleagues found that DXA measures of abdominal fat accounted for 80% of the variance in the intra-abdominal fat assessed by computed tomography (CT) (Svendsen, Hassager et al. 1993). Snijder and colleagues studied 3075 black and white men and women age 70-79 years (Snijder, Visser et al. 2002). They found the trunk fat measured by Hologic QCR 1500 DXA was strongly correlated with total abdominal fat derived from CT scan at the L4-L5 level ($r = 0.84$ in white women).

2.10.2.4 Validation of DXA measured changes in body composition

The ability of DXA to detect the changes in body composition has been validated by comparing the DXA measurements to other criterion body composition measurements, such as densitometry and UWW. DXA estimated percentage change of body fat mass was found to have comparable accuracy to estimates obtained using other methods (Evans, Saunders et al. 1999). A study in 17 subjects during a dehydration-rehydration protocol found that DXA is able to detect small individual changes in the total body mass and soft-tissue mass (Going, Massett et al. 1993). A 16-week intervention study of combined diet and exercise found that the Coefficients' of Variation of changes in percentage of body fat measured by DXA, bioelectrical impedance analysis and body mass index were similar, ranging from ± 2.0 -2.4% (Evans, Saunders et al. 1999).

DXA was more accurate in assessing body fat mass (FM) in elderly men and women when compared to underwater weighing (UWW) and the total body water method (TBW) (Goran, Toth et al. 1998). When using UWW, there was significant overestimate of fat mass among leaner subjects and an underestimate in fatter subjects. For TBW, there was a significant underestimation of fat mass among leaner subjects and overestimation in fatter subjects. In Goran's study (1998), a four compartment model was used as a criterion method. The four compartment model is based on the chemical model of body composition, that is, water, mineral, protein and fat, presented by Heymsfield et al (Heymsfield, Lichtman et al. 1990). As all indirect methods are based on assumptions (detailed in the following sections), the four compartment model combines the components which can be measured directly without assumptions in different methods and then combined using a complex model. This model assumes densities of 0.9 g/cc for fat, 0.99g/cc for water, 3.042 g/cc for bone mineral and 1.34 g/cc for the unmeasured fraction of the body composed of protein and glycogen (Baumgartner, Heymsfield et al. 1991). Body water can directly be measured by the isotope dilution method without any assumptions and bone mineral is measured by DXA. The four compartment model combines these direct measures with a direct measure of total body density by hydrodensitometry in a model to estimate body fat mass with minimal assumption.

Although several studies have shown a good agreement between DXA and other criterion methods, since all methods are indirect, it is difficult to determine which method gives the best agreement with the true tissue masses.

2.10.2.5 Validation of DXA measured muscle cross-sectional area

Computed tomography (CT) and magnetic resonance imaging are the current criterion methods for measuring muscle cross-sectional area. DXA is not a validated method for measuring muscle cross-sectional area, and especially for evaluating any changes (Hansen, Williamson et al. 2007). A study compared mid-thigh muscle mass estimated by DXA, a relatively cheaper and more accessible method, with the mid thigh muscle cross-sectional area measured by CT in 30 elderly hospitalized patients (aged 81 ± 8 years) recovering from hip fracture (Hansen, Williamson et al. 2007). Fat-to-lean soft tissue ratios were calculated in both methods. They found

that the baseline ratios of mid-thigh fat-to-lean ratios were similar in the two methods, but the DXA measured follow-up changes of fat-to-lean ratio was only weakly correlated with CT measurements. The authors suggested that DXA measured muscle cross-sectional area should not apply to individuals or to evaluate the response to interventions (Hansen, Williamson et al. 2007).

2.10.2.6 Animal validation studies of DXA

Several animal studies evaluated the accuracy of body composition measurement by DXA (Hologic and Lunar instruments) compared with the direct method of chemical analysis. Often these studies are difficult to compare as different DXA machines and different versions of software have been used. A study assessed the accuracy of body-composition measured by Lunar DPX (3.2) in seven pig carcasses compared to the chemical analysis after post-mortem homogenization (Svendsen, Haarbo et al. 1993). The study showed that the correlation r values between DXA measurements and chemical analysis for fat mass, lean body mass, and percent fat were all >0.97 . The mean differences in body composition measurements between the DXA measurements and those by chemical analysis were all insignificant (all $p>0.05$: $-2.2\pm 1.0\%$, $-1.7\pm 0.8\text{kg}$, and $0.4\pm 1.2\text{kg}$ for percentage of fat mass, fat mass and lean body mass, respectively).

Another study assessed the accuracy and reproducibility of the Hologic QDR 2000 in 13 piglets (Picaud, Rigo et al. 1996). The authors found that the DXA measured body weight, bone mineral content, and fat mass were significantly correlated with body weight, ash weight, chemical calcium, and chemically measured fat. Body weight was accurately measured by DXA, but fat content was overestimated by DXA. The precision of DXA was 0.23% for body weight and 4.44% for calcium content. The precision of DXA measured fat content was 8.85% compared to the chemical fat analysis.

2.10.3 Underwater Weighing

The Underwater weighing (UWW) method is based on Archimedes's principle of buoyancy. It measures body density and calculates fat mass and fat-free mass using

equations. When a subject is submerged in water; body volume is equal to the loss of water corrected for the density of water corresponding to the temperature of the water at the time of the submersion (Going 2005). The equation is shown as below:

Body volume = (weight in air – weight in water) / water density

Water density depends on water temperature. It is 0.9937 when the water temperature is at 36°C (Brozek, Grande et al. 1963).

Once volume is known, body density can be calculated as following equation (Going 2005):

$$\text{Body density} = \text{body mass} / \text{body volume}$$

Air in the lungs and flatus in the gastrointestinal tract need to be corrected in the final calculation. The residual volume is the volume of air remaining in the lungs after maximal expiration (Rhoades 2008). It is commonly measured by the closed circuit nitrogen/oxygen dilution method of Wilmore (Wilmore 1969).

When the constant densities of fat (0.09 g/mL) and non-fat (1.10 g/mL) at 36°C are assumed (Brozek, Grande et al. 1963), percentage of body fat can be calculated as following commonly used equations proposed by Siri (Siri 1993) or Brozek et al (Brozek, Grande et al. 1963):

Siri: $\% \text{ Fat} = (4.95/D - 4.50) \times 100$

Brozek et al: $\% \text{ Fat} = (4.570/D - 4.142) \times 100$

Where D = body density

Going reviewed previous studies and found that in adults, within-subject coefficients of variation for percent body fat have ranged from 1.7-4.5% within a day, and from 2.0-2.3% between days (Going 2005).

The limitations of UWW include biological error and technical error. The major source of biological error is likely to be due to the assumption of constant density of the fat-free mass (Adams, Mottola et al. 1982). In a study in 29 Canadian professional football players, negative values for percent body fat were obtained by

using the Siri's formula (Adams, Mottola et al. 1982). This was because of the very high density of fat-free mass of these athletes. The major source of technical error is due to the variation of the entrapped air in the lungs (measured as residual volume) and gastrointestinal tract (Goldberg, Black et al. 1991).

2.10.4 Air displacement plethysmography

More recently, whole body air-displacement plethysmography body composition system, known commercially as BODPOD has been developed and commercially available since 1994 (Dempster and Aitkens 1995). The BOD POD is based on the air displacement plethysmography (ADP) measurement of body volume (Dempster and Aitkens 1995). It is based on similar principles to hydrostatic weighing or underwater weighing (McCrory, Mole et al. 1998; Fields, Higgins et al. 2005). The difference is that the method uses air displacement which is more convenient and comfortable than water displacement (Dempster and Aitkens 1995), and therefore, this method can be used to measure infant body composition (Urlando, Dempster et al. 2003). This method has been validated in overweight and obese middle age populations (Ginde, Geliebter et al. 2005; Minderico, Silva et al. 2006), healthy middle age adults (Iwaoka, Yokoyama et al. 1998), and in children (Sainz and Urlando 2003). A study of a 16-month weight loss intervention in 93 healthy female aged 39 ± 6 years found that the change in total body fat mass measured by DXA and ADP were similar (Minderico, Silva et al. 2006). A validation study compared BOD POD and under-water weighing in assessing total body density, and compared the BOD POD with the four-compartment model in assessing percentage body fat (Fields, Wilson et al. 2001). In their study, total body water was determined by isotope dilution and bone mineral was determined by dual-energy x-ray absorptiometry, body density and percentage of body fat were determined by BOD POD and under-water weighing. The four-compartment model was used as the criterion measure of body fat. The study showed there was no significant difference in total body density assessed by BOD POD compared with under-water weighing. However, the percentage of body fat measured by BOD POD was significantly lower than was assessed by the four-compartment model (2.2% lower, $p < 0.01$), which indicates that BOD POD under predicted body fat when compared with the four-compartment model. A review of validation studies of the ADP method suggested

that this method appeared to be a suitable and reliable instrument for the assessment of body composition in a wide range of populations and more studies are needed to understand sources of measurement error (Fields, Higgins et al. 2005).

2.10.5 Total body water

Total body water (TBW) measures total body water by measuring the dilution of isotopic tracers. It can estimate body composition at three levels: molecular, cellular and tissue. There are four assumptions, in the measurement of total body water by dilution (Schoeller 2005):

1. The tracer is distributed only in body water
2. the tracer is equally distributed in all anatomical water compartments
3. The rate of equilibration of the tracer is rapid
4. Neither the tracer nor body water is metabolized during the time of tracer equilibration.

The widely used two-compartment TBW and other hydrodensitometry methods to predict fat-free body mass (FFM) are based on the following equation which assumes that everybody has a constant hydration level which is 0.73 of body fat-free mass (Mazariegos, Wang et al. 1994):

$$\text{TBW/FFM} = 0.73 \text{ kg/kg}$$

$$\text{The density of FFM} = 1.100 \text{ g/cubic centimetre}$$

This assumption is not applicable for people who are dehydrated or who have oedema (Schoeller 2005). Since there is a general loss of total body water from tissues with aging (Schoeller 1989; Aloia, Vaswani et al. 1998), the error would occur due to using the same defined parameter to predict body composition for elderly people. The precision of the total body water method is between 1% and 2% (Schoeller 2005). It can be achieved by paying careful attention to details of each aspect of the measurement procedures, such as subject preparation, dosing, sample collection, and isotope analysis (Schoeller 2005).

2.10.6 Whole body counting

Whole body counting uses nuclear based techniques to measure whole body content of chemical components, such as potassium calcium, phosphorus, sodium, chlorine, nitrogen, hydrogen and carbon (Ellis 2005).

Total body potassium (TBK) is the commonest used whole body counting method for assessing body composition, because more than 90% of the body's potassium is found in fat-free tissues (Hammond 2004). Natural potassium is distributed in three isotopic states: 93.1% ^{39}K , 6.9% ^{40}K and 0.0118% ^{40}K . Only ^{40}K is radioactive. Whole-body counter detects ^{40}K signal from the body and then the signal converts to a quantitative assay for the total body potassium. To convert TBK to the body's fat-free mass (FFM), the TBK-FFM ratio of 68.1 mmol/Kg FFM (Forbes and Lewis 1956) is commonly used. Recent studies showed that the ratio could be lower and differ by gender and age (Wang, Pi-Sunyer et al. 2001; He, Heo et al. 2003). Thus, caution is needed when TBK is used solely to assess FFM. The TBK precision range is between 2% and 5% (Cohn and Parr 1985). However, the accuracy of the TBK estimate for each whole-body counter is less firmly established (Ellis 2005).

Recently, the whole body potassium method also has been used to assess skeletal muscle mass. Wang and colleagues proposed ratios of total body potassium to whole body skeletal muscle (SM) for men and women, which are 119.9 ± 6.7 mmol/kg and 118.7 ± 8.4 mmol/kg respectively (Wang, Zhu et al. 2003). The ratio tended to be lower in subjects aged 70 years or over. They studied 300 men and women of various ethnicities and found a strong correlation between TBK and skeletal muscle mass ($r = 0.97$, $P < 0.001$).

2.10.7 Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) assesses the relationship of body composition to the water content of the body (Chumlea and Sun 2005). It requires a regression equation validated against a criterion body composition method, such as TBW or DXA. This is the only way to interpret the BIA value. However, cross-validation studies assessing BIA and other body composition measurement methods are few. BIA was found to have systematically higher value for fat-free mass

(Boneva-Asiova and Boyanov 2008), and lower values for fat mass and percentage of fat mass than DXA. Variation of the difference also depends on gender and body weight status (Boneva-Asiova and Boyanov 2008; Volgyi, Tylavsky et al. 2008).

The ability of BIA to estimate changes in body composition over time is debatable. Phillips and colleagues found BIA provided accurate estimates of the change in both fat-free mass and percentage of body fat over time in 196 non-obese girls (Phillips, Bandini et al. 2003). Lohman and colleagues found BIA is less reliable than skinfolds in assessing body composition changes over 3 years (Lohman, Thompson et al. 2003).

2.10.8 Computed tomography and magnetic resonance imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are considered the most accurate methods for assessing body composition at the tissue-organ level (Ross and Janssen 2005). They are used as criterion methods to calibrate other methods of assessing adipose tissue and skeletal muscle in vivo (Ross and Janssen 2005).

The principle of CT is to measure x-ray attenuation relative to air and water. They are defined as -1000 to 0 Hounsfield units (HU) for air and water respectively. The density of adipose tissue is lower than water, therefore adipose tissue pixels normally range from -190 to -30 HU (Kvist, Chowdhury et al. 1988; Chowdhury, Sjostrom et al. 1994; Yoshizumi, Nakamura et al. 1999). The density of skeletal muscle is greater than water, thus its pixels ranges are from 30 to 100 HU (Chowdhury, Sjostrom et al. 1994). MRI uses a magnetic field to align the nuclear magnetization of hydrogen atoms in water in the body (Ross and Janssen 2005). Radio frequency fields are used to systematically alter the alignment of this magnetization and produce a rotating magnetic field detectable by the scanner to construct an image of the body (Tidwell and Jones 1999).

Both CT and MRI can measure the quality of skeletal muscle. The average Hounsfield units of skeletal muscle from CT can be used as an index of skeletal muscle lipid content and it was reported to be well correlated with skeletal muscle

lipid level determined by muscle biopsy samples (Goodpaster, Kelley et al. 2000). The total muscle fat assessed by imaging was strongly correlated with total muscle fat determined by magnetic resonance spectroscopy (Schick, Machann et al. 2002). Despite the high accuracy of CT and MRI in body composition assessment, the high cost and technical skill required in both methods limit their use in large population studies.

2.10.9 Peripheral quantitative computed tomography (pQCT)

Peripheral quantitative computed tomography (pQCT) can be used to assess cross sectional muscle structure although it was originally designed to assess cross-sectional bone structures. The advantage of using pQCT is the low dose of radiation exposure.

The conventional site for studying leg cross-sectional muscle area by using pQCT is at 66% of the tibia length, proximal to the terminal end of the tibia (Miljkovic-Gacic, Gordon et al. 2008). The reason to use this site is because it is the region of the largest circumference of the calf with very little variability across individuals (Simonsick, Maffeo et al. 1997).

There are different models of scanner that can be used depending on the research purpose, such as the radius scan model, the tibia scan model or the muscle scan model. The scan image can be analysed by using the software the pQCT producer provided to generate data of bone area and volumetric bone density. If the muscle scan model is chosen at the scan stage, the scan image can be analysed using the software to generate data of muscle area and intermuscular fat content. However, when the bone scan model is used at the scan stage, although the scan image contains muscle image, muscle area is not able to be generated automatically by using the analysis software. For the current study, since the primary interest of the intervention study was bone structure, the bone scan model was used, therefore, the muscle area was analysed by tracing muscle edge manually, and this method is not able to analyse the intermuscular fat content.

2.10.10 Anthropometry

Anthropometry can assess body size and composition. The anthropometry instruments are relatively inexpensive, the measurements non-invasive and suitable for the field. Anthropometry can provide valuable information when done by a trained or experienced anthropometrist. However, the accuracy and sensitivity of using anthropometry to monitor small changes in muscle mass associated with weight loss or gain in an individual are not clear (Lukaski 2005).

The commonly used anthropometric measurements related to body composition assessment include height, weight, skinfolds and, girth measurements. In general, anthropometry is less accurate and sensitive in predicting body composition. Anthropometry also has many limitations in measuring and predicting regional body composition when used alone (Bellisari and Roche 2005).

2.10.10.1 Height, weight and BMI

Anthropometric variables of height and body weight are most commonly reported as various indices with the most common being the Body Mass Index (BMI). Height is positively correlated with fat-free mass (Kerr, Papalia et al. 2007), but has low correlation with percentage body fat (Bellisari and Roche 2005). Neither height nor weight is an effective predictor of fat mass and lean body mass when used alone but best reported as an indice.

The BMI can be calculated from height and weight as the following equation: $BMI = \text{weight (kg)} / \text{height}^2 (\text{m}^2)$. BMI has been widely used as an indicator of relative fatness and body frame size in many epidemiological studies. According to World Health Organization, the classification of BMI is as shown below regardless of sex (2000):

Underweight:	<18.5
Normal:	18.5 – 24.9
Overweight (or pre-obese):	25 – 29.9
Obese level 1:	30 – 34.9
Obese level 2:	35 – 39.9
Obese level 3:	≥ 40

Although BMI is an acceptable approximation of total body fat at the population level, caution is needed when interpreting the correlations between BMI and chronic disease. This is because some subjects can be misclassified by this method, such as obese individuals (Rothman 2008), muscular individuals (Witt and Bush 2005), the elderly (Baumgartner, Heymsfield et al. 1995; Miller and Wolfe 2008) and Asian populations (Dudeja, Misra et al. 2001). Older adults have more fat than younger adults at any BMI due to the loss of muscle mass with age (Baumgartner, Heymsfield et al. 1995).

2.10.10.2 Skinfold thickness for assessing body fatness

Skinfold thickness measures subcutaneous adipose tissue (Khatoon, Tapadar et al. 2008). A skinfold caliper reading is the measurement of the thickness of a double fold of skin and compressed subcutaneous adipose tissue. Skinfold readings can be measured at different sites of the body. The triceps and biceps skinfolds are commonly used in clinical research for predicting total body fatness. Skinfold thicknesses have high correlations with body fat mass and percent of body fat mass (Khatoon, Tapadar et al. 2008). There are more than 100 equations to predict percentage body fat from skinfolds, but these equations are age, sex, population and site specific (Lohman 1981; Johnston 1982). As well as the error inherent in using prediction equations, error also occurs in measuring skinfold itself.

The precision of the skinfold measurement is highly dependent on technician skill, type of skinfold caliper used and subject factors (e.g. a person's unique body fat distribution) (Heyward, Cook et al. 1992). By far the most variable factor appears to be skinfold compressibility. Significant inter-subject and inter-site differences in skinfold compressibility were observed in cadavers (Martin, Ross et al. 1985). Skinfold measurements may also be less reliable in older subjects with less connective tissue or in obese individual with large folds (Irwin 2006). Minimising measurement error is therefore an important issue in skinfold assessment. Training of the anthropometrist, use of a standard protocol and calibrated skinfold callipers are all important ways to minimise measurement error (Marfell-Jones, Olds et al. 2006).

2.10.10.3 Waist circumferences and waist-to-hip ratio for assessing body fatness

Age and population specific waist circumference and waist-to-hip ratio (WHR) have been used to assess the distribution of body fat in overweight and obesity (Molarius, Seidell et al. 1999). They have also been used as predictors of cardiovascular risk factor in different populations as these measures have been found to provide an indirect measure of internal visceral body fat (Huang, Lin et al. 2002; Shahraki, Shahraki et al. 2008). The cut-off points related to health are related to different gender and ethnic groups (Mohan, Deepa et al. 2007).

For women, a waist circumference greater than 80 cm indicates excess internal fat deposition and an increasing risk of non-communicable diseases (Han, van Leer et al. 1995; Expert Panel on the Identification Evaluation and Treatment of Overweight in Adults 1998). Waist circumference had higher sensitivity and specificity for identifying cardiovascular risk factors when compared to WHR or with other anthropometry measurements (Esmailzadeh, Mirmiran et al. 2006). Studies showed that waist circumference was highly correlated with intra-abdominal fat volume (Han, McNeill et al. 1997), and its reduction in obese subjects was significantly correlated with the fall of total cholesterol, low-density lipoprotein, and diastolic blood pressure (Han, Richmond et al. 1997). Height has little influence on using waist circumference to predict intra-abdominal fat volume in women (Han, McNeill et al. 1997).

WHR was reported to be a poor indicator of abdominal visceral fat in women (Rankinen, Kim et al. 1999). A randomized controlled trial showed that the changes in WHR were not associated with changes in any cardiovascular risk factors in 110 overweight or obese women (Han, Richmond et al. 1997). A cross-sectional study of a random sample from 18 populations in UK aged 25-64 years found that the age standardized mean waist and hip circumferences and WHR ranged 78-91 cm, 97-108 cm, and 0.76-0.84 in women (Molarius, Seidell et al. 1999). It is still being debated as to whether waist circumference or WHR is the better predictor of cardiovascular risk.

2.10.10.4 Arm, thigh and calf circumferences

Arm muscle area or corrected arm muscle area have been used to predict whole body lean mass, and has been used as indicator of malnutrition in the elderly (Miller, Crotty et al. 2002).

Arm muscle area (AMA) is calculated from triceps skinfold thickness and mid upper arm circumference as in the following equations (Heymsfield, McManus et al. 1982):

$$\text{Arm muscle area (mm}^2\text{)} = (\text{Mid upper arm circumference} - \pi (\text{skinfold}))^2 / 4\pi$$

Mid upper arm circumference and skinfold are in millimetre (mm)

Corrected arm muscle area (CAMA) which corrects for bone is:

$$\text{For women: Corrected arm muscle area (mm}^2\text{)} = \text{arm muscle area (mm}^2\text{)} - 6.5$$

$$\text{For men: Corrected arm muscle area (mm}^2\text{)} = \text{arm muscle area (mm}^2\text{)} - 10$$

The within-subject errors of corrected arm muscle area were 7-8% compared to computed tomography values (Heymsfield, Olafson et al. 1979).

Total body muscle mass can be predicted by the following equation:

$$\text{Total body muscle mass (kg)} = (\text{height cm}^2) (0.0264 + 0.0029 \times \text{corrected AMA})$$

Limb composition, especially leg muscle mass, is important in the elderly, because it has been found to be related to the prevalence of falls and fractures (Bellisari and Roche 2005). The US Health ABC study in 3075 well-functioning black and white men and women aged 70-79 found that lower cross-sectional thigh area measured by computed tomography was associated with increased risks of self-reported mobility loss (Visser, Goodpaster et al. 2005).

However, thigh or calf girth measurements provide limited information about the leg muscle composition, such as cross-sectional muscle size. It is difficult to predict subcutaneous fat or fat infiltration in the muscle based on limb girth measurements alone.

Mid-arm circumference was not reliable in predicting lean tissue mass in 354 elderly Chinese females aged 69-82 years when compared to DXA measurements (Kwok, Woo et al. 1997). A 3-year longitudinal study of 2032 Hong Kong Chinese aged 70 and older failed to find any significant change in corrected upper arm muscle area over the study period (Woo, Ho et al. 2001). This may be due to the short study duration. Calf circumference was found to be positively correlated to plantar flexor maximum voluntary contraction strength in 39 premenopausal women (Bamman, Newcomer et al. 2000). Their study also found that the cross-sectional muscle size determined by magnetic resonance imaging (MRI) correlated to strength better than the limb anthropometric measurement (leg circumference).

A study used computed tomography to assess the composition of muscle cross-sectional area of 13 young and 11 elderly men (Overend, Cunningham et al. 1992). The results showed that there was no difference in the total thigh cross-sectional area between the young and elderly men. Compared with the young men, the elderly men had significantly smaller muscle areas. The elderly men also had significantly greater cross-sectional thickness of skin plus subcutaneous fat, and non-muscle tissue within muscles.

Hilton and colleagues compared the leg muscle composition of 6 obese, diabetic and peripheral neuropathy patients to 6 healthy obese subjects by magnetic resonance imaging (Hilton, Tuttle et al. 2008). They found that although the leg muscle and fat volumes were similar in the two groups, the calf intermuscular adipose tissue volumes were significantly higher in the subjects with obesity, diabetes and peripheral neuropathy. The excess fat infiltration in leg skeletal muscle was associated with low calf muscle strength, low calf muscle power, and impaired physical function.

When using circumference measurements to assess the change of muscle mass, the proportionality between size and composition is not always constant (Heymsfield, Stevens et al. 1982). This means that the changes in girth do not always represent the changes in muscle size, because the girth measurement cannot distinguish the changes that are due to the changes in subcutaneous fat or muscle. To assess sarcopenia in elderly, therefore, girth measurements should be combined with other

measurements such as DXA or computed tomography to assess the changes in muscle size.

2.11 Muscle strength in the elderly

2.11.1 Aging and muscle strength

In general, muscle strength progressively declines from 30 years (Vianna, Oliveira et al. 2007) and with greater losses in the elderly (Mateo Lazaro, Penacho Lazaro et al. 2008). A cross-sectional and longitudinal study in 847 men and women aged 20-100 years showed that hand grip strength increased into the thirties and then declined at an accelerated rate after 40 years (Kallman, Plato et al. 1990). Knee extension strength declined 14% per decade and knee flexion strength declined 16% per decade (Hughes, Frontera et al. 2001). The age-associated differences in muscle strength are highly correlated with the age-related differences in muscle mass (Kallman, Plato et al. 1990).

Previous studies showed that there is a gender difference in the rate of loss in muscle strength between the upper and lower extremities. A study reviewed medical records collected from 1994 to 2005 in 3648 men and women aged between 18 and 90 years (Vianna, Oliveira et al. 2007). The study found that handgrip strength declined with age and differed between genders. A faster decline in handgrip strength occurred at age 30 years for men and 50 years for women.

A longitudinal study examined the changes in elbow and knee strength in 120 community-dwelling men and women initially aged 46 to 78 years (Hughes, Frontera et al. 2001). Men had greater strength than women in both upper and lower extremities at baseline and at follow-up. After 9.7 ± 1.1 years, the losses in lower limb strength were similar in men and women. However, the percent losses for upper limb strength were greater in men than in women. Men had similar rates of decline in upper and lower limb strength at follow-up. Women had a greater loss in knee extensor strength ($-11.8 \pm 15.5\%$ per decade) than in elbow flexor strength ($2 \pm 33.8\%$ per decade). The difference in muscle distribution in men and women such that women have a smaller percentage of muscle in their arm (Janssen,

Heymsfield et al. 2000) may partially explain the gender differences in the rate of loss in muscle strength between the upper and lower extremities.

2.11.2 Muscle strength determinants

Muscle size and volume are the major determinants of muscle strength among healthy young adults (Trappe, Trappe et al. 2001; Holzbaur, Delp et al. 2007) and elderly people (Frontera, Hughes et al. 1991; Frontera, Hughes et al. 2000). Muscle cross-sectional area was found to have significant positive correlation with muscle strength both in males and females (Maughan, Watson et al. 1983; Bamman, Newcomer et al. 2000). In a cross-sectional study of 3075 well-functioning men and women aged 70-79 years, mid-thigh muscle cross-sectional area obtained from computed tomography was strongly positively correlated with leg performance assessed by 6-meter walk and repeated chair stands (Visser, Kritchevsky et al. 2002).

Lower cross-sectional thigh muscle area and greater fat infiltration into the muscle were associated with increased risk of mobility loss in older men and women (Visser, Goodpaster et al. 2005). The decrease in muscle strength was significantly related to the reduction in muscle cross-sectional area in older men and women (Frontera, Reid et al. 2008).

Aging related decreases in muscle mass (strength) are associated with a reduction in both the number and size of muscle fibres, mainly a reduced size of type II fibres (Lexell 1995; Porter, Vandervoort et al. 1995; Deschenes 2004). Type II fibres have fast-switch nature and are efficient for short bursts of speed and power and Type I fibre are slow to fatigue and suited for endurance. power (The Muscle Physiology Laboratory 2009). The results from many studies on muscle fibre type composition using muscle biopsies are rather consistent, that is type II fibre size is reduced with aging while type I fibre is reduced much less or no change (Aniansson, Hedberg et al. 1986; Poggi, Marchetti et al. 1987; Lexell, Taylor et al. 1988; Lexell and Taylor 1991). The loss of lean tissue, in addition to other factors in the elderly contributes to the decrease of muscle strength and function (Landers, Hunter et al. 2001).

Muscle fibre composition studied by muscle biopsy indicated a poor correlation between fibre type and maximal isometric force production (Trappe, Trappe et al.

2001). A 10-year longitudinal study of 120 men and women aged 46-78 showed that although age-related changes in muscle mass influenced the magnitude of the strength changes over time, muscle strength declines in spite of muscle mass maintenance or even gain in some subjects (Hughes, Frontera et al. 2001). This means other factors such as cellular, neural or metabolic mediators are also involved in changes in muscle strength with aging (Hughes, Frontera et al. 2001).

2.11.3 Muscle strength assessment methods

There are several ways to assess muscle strength, including manual muscle testing or using an isokinetic dynamometer. However the most common method is the one-repetition maximum (1RM) test with an isotonic contraction using free or machine weights. Previous studies showed that the 1RM test is reliable for testing muscle strength in elderly men, and the coefficients of variation were 4.2-9.0% (Schroeder, Wang et al. 2007). However, another study showed that elderly women required significantly more testing sessions (8-9 sessions) to achieve the same consistency of measurement compared with young women (3-4 sessions) (Ploutz-Snyder and Giamis 2001). In their study, both young and elderly women had a similar absolute increase in strength between the first and final testing sessions, which was 11-13 kg. The older women had a significantly greater relative increase ($22\pm 4\%$) in strength than the younger women ($12\pm 5\%$). Therefore, it is important to consider the learning effect when using 1RM to assess the magnitude of strength increases in elderly women.

In general, it is hard to compare measurements between studies due to the variation of test conditions and test subjects (Keating and Matyas 1996). A review which included more than 200 articles suggested that subject factors which lead to measurement variation, include age, gender, weight, athletic background, disability and limb dominance (Keating and Matyas 1996). Test conditions that led to measurement variation include the range of movement, and the type of contraction or movement, such as concentric, axes alignment and preload. Other factors include pretest procedures such as starting position, stabilization and preload, test conditions such as speed, test sequence and rest intervals; and type of data analysis (the data selected and how they are manipulated). The authors concluded that most of the

publications failed to give these details and therefore lacked replicability (Keating and Matyas 1996).

2.11.3.1 Manual muscle test

The manual muscle test has been widely used clinically to identify abnormal muscle strength, such as muscle weakness or asymmetry of the muscle groups. In a traditional manual muscle testing, the examiner assesses how much resistance a subject can tolerate and then grades it. The commonly used scoring system has six category levels scores from grade 0 (not contractile activity can be felt in the gravity eliminated position) to grade 5 (patient can hold position against maximum resistance and through complete range of motion) (Hislop and Montgomery 2007). In the new manual muscle testing, the examiner uses a hand-held dynamometer to test a patient's muscle strength. The new method has reasonable agreement with the traditional method (Bohannon 1986; Mulroy, Lassen et al. 1997). For the traditional muscle test which uses word scores, the grading system combines subjective and objective factors. For the hand-held dynamometer method, the examiner's strength or push force would affect the results (Mulroy, Lassen et al. 1997). A recent review of studies showed that the correlation coefficient of manual muscle testing ranged from 0.63 to 0.98 for individual muscle groups, and from 0.57 to 1.0 for a total manual muscle testing score (Cuthbert and Goodheart 2007).

2.11.3.2 Isokinetic and other muscle test methods

The isokinetic dynamometer and isometric torque methods are commonly used methods in research. Hand grip dynamometry is an easy, cheap and efficient method to assess the functional limitations (Arroyo, Lera et al. 2007). A cross-sectional survey of 377 men and women aged 65 years or over found that the self-reported functional limitation, such as in the basic activities of daily living, mobility or advanced activities were associated with lower handgrip strength in both sexes (Arroyo, Lera et al. 2007). The coefficient of variation of handgrip strength assessed by Jamar handgrip dynamometer was 6.3% (Trutschnigg, Kilgour et al. 2008). A reliability study in 31 men and women aged 76 to 87 years showed that the ankle

dorsiflexion strength measured by isometric spring gauge had acceptable reliability which was 9.7% (Menz, Tiedemann et al. 2003).

Montgomery and colleagues assessed the reliability of an isokinetic test of knee extension and flexion muscle strength in 20 adults (11 males) on three separate occasions and 2-4 days apart (Montgomery, Douglass et al. 1989). The subjects were assessed by the velocity spectrum test (5 repetitions at velocities ranging from 90-330 degrees/sec). They found the reliability was generally higher at lower velocities and higher for knee extension than flexion. The mean intraclass correlation coefficients for peak torque were 0.88 for knee extension and 0.79 for knee flexion.

A study assessed the test-retest reliability of using an isokinetic dynamometer to test hip adductor and flexor strength (Emery, Maitland et al. 1999). In this study it was found that eccentric peak hip adductor torque can be reliably measured at a speed of 60 degrees/second, but hip flexor torque measurement was less reliable. Hip extensor was shown to have better test-retest reliability than hip abductors; having higher interclass correlation coefficient (ICC) and lower coefficient of variation (CV) (Pua, Wrigley et al. 2008).

Wyse et al. also showed that there was a significant variation of muscle strength due to time-of-day effects (Wyse, Mercer et al. 1994). That is, leg muscle strength had significantly higher mean scores which were achieved during 18.00-19.30 pm compared to the mean scores achieved during morning and noon time. Many factors contribute to the large variation of muscle strength measurements, including the differences in study subjects, test conditions, test muscle group, data analysis methods, and time-of-day effects. A validation study is essential for any proposed muscle strength test method.

2.12 Assessment of mobility and balance function in elderly people

Studies have shown that muscle strength is positively correlated with body functional performance in elderly women and men (LeBrasseur, Sayers et al. 2006; Misic, Rosengren et al. 2007). Lower extremity performance, such as walking speed and

time to complete five chair-stands decreased dramatically with aging in older women (Forrest, Zmuda et al. 2006). The study subjects aged 65-69 years decreased 11% in walking speed and increased 38% in chair-stand time ten years after entering the study. Women aged 80 years or older decreased 37% in walking speed and increased 38% in chair-stand time 10 years later. A one year randomized controlled clinical trial included 80 elderly women aged 75 ± 5 years and showed that leg power was a strong predictor of self-reported functional status in elderly women (Foldvari, Clark et al. 2000). The tests used to assess the physical functions varied between previous studies, and the tests chosen depend on the study population and purpose of the study. The “Timed Up and Go” test and the Romberg test are widely used in testing mobility and standing balance in the elderly.

2.12.1 Timed Up and Go Test

The ‘Timed Up and Go’ test (TUAG) is the simplest performance-based measurement of mobility and balance (Zimmer, Rothenberg et al. 1997) and was developed by Mathias and colleagues in 1986 (Mathias, Nayak et al. 1986). It is also called the ‘Get Up and Go’ Test. The original scoring system was a 5-point categorical scale (1=normal, 2=very slightly abnormal, 3=mildly abnormal, 4=moderately abnormal, and 5=severely abnormal) (Mathias, Nayak et al. 1986). Normal healthy elderly usually complete the task in ten seconds or less and a score more than or equal to fourteen seconds has been shown to indicate high risk of falls (Jankovic and Tolosa 2007). Podsiadlo and Richardson further developed and tested the reliability of a modified scoring system which was a continuous time scale (Podsiadlo and Richardson 1991). The test times a subjects from getting up from an armrest chair, walking 3 meters, turning around and walking back and sitting down. The test has been shown to be highly correlated with gait speed, balance, functional level, and the ability to go outside alone safely (Podsiadlo and Richardson 1991). It is a sensitive measure for identifying community-dwelling adults who are at risk for falls (Shumway-Cook, Brauer et al. 2000).

2.12.2 Romberg Test

Age-related decline in balance also contributes to the increased risk of falls and fractures in the elderly along with other risk factors, such as gait and lower-extremity muscle strength (El Haber, Erbas et al. 2008).

The original Romberg test requires the patient to stand with their feet together and close their eyes. The test is recorded as positive if the patient is stable when their eyes are open but falls when they shut their eyes, and it indicates a dorsal column lesion (Rogers 1980). Many versions have been developed from the original Romberg test, such as semi-tandem eye-open, tandem eye-open, semi-tandem eye-close, tandem eye-close (also called Sharpen Romberg test). In these tests, the subject is asked to maintain a given position for a specific time. The researcher's judgement will influence the scoring, such as the variation in judgment of swaying. A study showed that it had a larger intra-individual variation in both young and old adults (Black, Wall et al. 1982). Although the Sharpen Romberg test has been reported to have reasonable inter-rater reliability and test-retest reliability (Franchignoni, Tesio et al. 1998), a study of 132 healthy adults concluded that the Romberg performance was too variable, especially for subjects younger than 20 or older than 50 years. Therefore, caution is needed when applying the Romberg test to older populations (Yim-Chiplis and Talbot 2000).

2.13 Physical activity

2.13.1 Aging and physical inactivity

The prevalence of physical inactivity increases with age and many older Australian are predominantly sedentary (Binns 1999). Physical inactivity increases the risk of coronary heart disease, non-insulin dependent diabetes mellitus, and also play an important role in the health of muscles, bones and joints (Binns 1999; Department of Health and Aging 1999). American national data indicates that the prevalence of no leisure-time physical activity was 18.9% for persons aged 18-24 years and increased to 39.1% for those aged ≥ 75 years, and inactivity was more common in women than in men (Ahluwalia, Mack et al. 2003). The leisure-time physical activity includes golf, gardening or walking. A study in 1993 showed that 14% of all deaths in the

United States were attributed to insufficient activity and inadequate nutrition (McGinnis and Foege 1993).

2.13.2 Physical activity guidelines for Australian

The National Physical Activity Guidelines for Australians recommends that all Australian adults engage in at least 30 minutes a day of moderate-intensity exercise on most days, or a total of at least 150 minutes of moderate activity per week (Department of Health and Aging 1999). The 30 minutes can be continuous or a combination of several short sessions of different activities (at least 10 minutes) together. Moderate-intensity activity includes things such as a brisk walking or cycling. The guidelines emphasise that the recommendation refers to the minimum level of physical activity required for the attainment of good health and a healthy body weight. A healthy eating pattern should be combined with physical activity in order to achieve the best result. The American College of Sports Medicine and the American Heart Association updated the physical activity guidelines for American in 2007 (Nelson, Rejeski et al. 2007). The recommendations for the elderly are to do moderately intensive aerobic exercise 30 minutes a day, five days a week, or do vigorously intense aerobic exercise 20 minutes a day, 3 days a week, and do eight to ten strength-training exercise, 10-15 repetitions of each exercise twice to three times per week, and to do balance exercises if you are at risks of falling and have a physical activity plan (Nelson, Rejeski et al. 2007).

2.13.3 The role of physical activity in maintenance of muscle mass, strength and physical function

Studies have shown that the physical activity level was significantly positively associated with muscle strength (Rolland, Lauwers-Cances et al. 2004) and lean body mass (Foo, Zhang et al. 2007). Physical activity is an important predictor of muscle mass both in elderly men and women (Baumgartner, Waters et al. 1999). However, whether the decline in physical activity is directly associated with a decline in muscle mass and strength with aging or whether regular exercise can prevent or minimise the effect of sarcopenia is still debatable (Marcell 2003).

Many studies have shown a positive correlation between physical activity and muscle strength and mass (Guo, Zeller et al. 1999; Daly, Ahlborg et al. 2008; Goodpaster, Chomentowski et al. 2008; Peterson, Giuliani et al. 2009). A one year randomized controlled trial found that regular physical activity prevented both the age-associated loss of muscle strength and the increase in muscle fat infiltration in 42 elderly men and women with moderate functional limitations (Goodpaster, Chomentowski et al. 2008). A 10-year prospective population-based study of 152 men and 206 women aged 50-80 found that those who maintained a habitually active lifestyle had lower bone loss and retained better balance than those who were inactive (Daly, Ahlborg et al. 2008). A longitudinal study (average follow up 8.95 years) in 108 women found that physical activity was positively associated with increases in fat-free mass and decrease in total body fat and percentage body fat in elderly women (Guo, Zeller et al. 1999). A 5-year follow-up population-based study of 2964 older people showed that sedentary individuals had a significantly increased risk in developing frailty compared with the physically active group (Peterson, Giuliani et al. 2009). The study assessed frailty by a gait speed of less than 0.60 m/s and or inability to rise from a chair without using one's arms.

Long term disuse of muscle groups can lead to a marked loss of muscle strength and muscle mass in the elderly (Suetta, Aagaard et al. 2007). However, not all studies have found this relationship. A study in 120 men and women aged 46-78 years followed up for 10 years found that although the change in leg strength was directly related to the change in muscle mass with aging, the decline in physical activity was not directly associated with strength changes (Hughes, Frontera et al. 2001). A study in 136 women and 180 men over 20 years showed that increasing age was associated with decreasing activity and lower fat-free mass, but physical activity levels were not related to fat-free mass (Westerterp and Meijer 2001). Starling's study in 44 healthy white men age 49-85 years suggested that maintaining regular physical activity and adequate protein intake may not offset the age-related loss of appendicular skeletal muscle mass (Starling, Ades et al. 1999).

2.13.4 Physical activity assessment methods

To assess the habitual physical activity level is important in research because it is a potential confounder for many lifestyle-health related issues. There are many self-report methods, such as physical activity records, physical activity logs, recalls and questionnaires (Denkinger, Coll-Planas et al. 2007). A physical activity record is a diary which records the different types of activities and the time spent on each activities during a given time period (Levin, Jacobs et al. 1999). Physical activity logs report the time spent doing different types of activity during a given time period (Lamonte and Ainsworth 2001). Both the self-report record and log methods may influence subjects' behavior due to the act of recording (Lagerros and Lagiou 2007). Questionnaires are the most widely used method in research especially when large numbers of study subjects are involved. In addition, they are easy to administer, take less time, and do not influence participants' physical activity behavior as subjects are required to recall their activity.

The International Physical Activity Questionnaire (IPAQ) was developed by a multinational working group and tested for reliability and validity in many countries (Craig, Marshall et al. 2003). There are two versions, a short form and a long form. The short form provides information of time spent on vigorous activity, moderate activity, walking and sedentary. The long form covers time spent in leisure, work, household, yard, and sedentary activity and self-powered transport. The reliability has been shown to be high. However, over-reporting has been found to be related to lower education level (Fogelholm, Malmberg et al. 2006). The current study used the IPAQ short form to assess the habitual physical activity level of the participants.

2.13.5 Effect of resistance training on muscle mass, strength and physical functions in the elderly

Previous studies have shown that progressive resistance training appears to be an effective and feasible way for preventing muscle wasting in elderly people (Fiatarone, O'Neill et al. 1994; Bonnefoy, Cornu et al. 2003; Bunout, Barrera et al. 2004; Daly, Dunstan et al. 2005). When lifting the weight, the muscle is shortened (concentric contraction) in order to produce force, and when lowering the weight, the muscle is forced to lengthen (eccentric contraction) to produce force (Evans 2002).

Muscle damage (microscopic tears in contractile proteins muscle cells) has been shown to occur during these lengthening muscle contractions (Evans 2002). This muscle damage may cause an acute phase response including increased circulation of skeletal muscle interleukin-1, mobilization of neutrophils and an increase in muscle protein synthesis and degradation (Evans 2002). These repeated muscle contractions stimulate muscle protein turnover and muscle hypertrophy (Evans 2002). Muscle quality and quantity are not the only determinants for the muscle strength but the nervous system is also involved in activating the muscle (Sale 1988). Some individuals experience little or no change in muscle cross-sectional area but have large gains in muscle strength following a period of resistance training (Evans 2002). The proposed explanation is that the neural adaptation occurs after a period of resistance training (Bandy, Lovelace-Chandler et al. 1990) and the electromyographic (EMG) studies have provided the most direct evidence that the increases in muscle strength are associated with the increased muscle activation (motor unit activation) (Sale 1988). Many intervention trials have demonstrated that 2-3 sets of 8-15 repetitions of moderate to high intensity resistance training can enhance muscle strength and mass in the elderly (Kongsgaard, Backer et al. 2004; Reeves, Narici et al. 2004; Levinger, Goodman et al. 2007; Henwood, Riek et al. 2008; Kukuljan, Nowson et al. 2009; Kukuljan, Nowson et al. 2009). Few studies have shown the beneficial effects on improving body functions, such as stair climbing time, the number of chair stand in 30 seconds, lung function (Kongsgaard, Backer et al. 2004), and balance by resistance training solely in the elderly (Orr, Raymond et al. 2008).

Epidemiological studies

Epidemiological studies have shown that resistance training can effectively increase muscle strength, muscle mass and muscle function in postoperative patients (Suetta, Magnusson et al. 2004), in elderly men and women after long-term unilateral disuse (Suetta, Aagaard et al. 2004) and in healthy elderly men and women (McCartney, Hicks et al. 1995; Kongsgaard, Backer et al. 2004). A study in older adults age 68-78 years found that muscle strength was significantly greater in those who had undertaken endurance or strength training for more than 50 years than those untrained elderly subjects (Aagaard, Magnusson et al. 2007). The muscle fibre size

and mechanical muscle performance, particularly the rate of force development were consistently elevated in those lifelong trained elderly.

Clinical trials

Clinical trials have shown that strength training can increase muscle strength or muscle size or both, but the effect of traditional resistance training on physical function is limited in the elderly (Skelton, Young et al. 1995; Harridge, Kryger et al. 1999; Campbell, Joseph et al. 2002). A 12-week strength training study in eleven very elderly men and women aged 85-97 years showed that the cross-sectional muscle area significantly increased after training and the cross-sectional muscle area was positively correlated with muscle strength (Skelton, Young et al. 1995).

Progressive resistance exercise can produce significant increases in muscle strength after adjusted for body weight in healthy, independent and very elderly women (age ≥ 75 years). However, the increase in strength may have limited benefit on functional abilities in this group of women.

Clinical studies have not reached a consensus on the mechanism of exercise in preserving age related decline in muscle mass and strength in the elderly. A study in people aged 68-78 years found muscle fibre size and mechanical muscle performance were consistently elevated by strength training (Reeves, Narici et al. 2004; Aagaard, Magnusson et al. 2007). A gene expression study in healthy older (62-75 years old) and younger (20-34 years old) men demonstrated an impaired response to exercise (resistance training) in the older subjects (Jozsi, Dupont-Versteegden et al. 2000). A study showed that 6-months endurance training did not alter serum IGF-1 in healthy older man and women (Vitiello, Wilkinson et al. 1997). A three month resistance training study showed that although the muscle strength improved by training both in young and old subjects, there was no significant change in myofibrillar synthesis rate in either the young or elderly subjects (Welle, Thornton et al. 1995). The researchers concluded that the disuse cannot explain the slower myofibrillar synthesis rate in the elderly subjects.

Randomized controlled trials

Randomized controlled trials have shown that resistance training significantly increased muscle mass and strength (Pyka, Lindenberger et al. 1994; Binder,

Yarasheski et al. 2005), increased muscle cross-sectional area (Sipila and Suominen 1995; McCartney, Hicks et al. 1996), improved physical function (Sipila and Suominen 1995) and daily living capacity (Levinger, Goodman et al. 2007) in elderly women. The increased muscle strength obtained by resistance training was correlated with significantly increased body lean mass (Binder, Yarasheski et al. 2005). Muscle biopsies in 25 elderly men and women found that the increased muscle strength by resistance training were accompanied by hypertrophy of both type one and type two muscle fibers (Pyka, Lindenberger et al. 1994).

In summary, studies have shown that resistance training is an effective way to prevent aging related muscle wasting through increasing muscle mass and strength, however, the mechanism is not fully understood. The results were inconsistent for the effect of resistance training on improving physical functions, such as balance.

2.14 Dietary assessment

2.14.1 Dietary assessment methods

Assessing dietary intake is important but problematic (Black and Cole 2001) as there is no one method that can record the 'true intake'. The most important direction of bias is the underestimation of dietary intake which occurs with all methods of dietary assessment (Black 2000).

The most commonly used methods to assess the food intake in individuals are food records, 24-hour recall, food frequency questionnaires, and diet histories (Thompson and Subar 2001; Bingham 2007). The food record is a prospective method, where a person records everything that they eat and drink at the time of consuming by either weighed or household measures, such as cup or teaspoon, for 3 to 14 days. The other methods, 24-hour recall, food frequency, and diet history, are retrospective methods and therefore rely on memory, which can be an issue in the elderly. Each method has its strengths and weaknesses, but all the methods depend on the ability of the subject to provide accurate information (Nelson and Bingham 1997). The features of all these dietary assessment methods have been well summarized (Black, Bingham et al. 1997; Nelson and Bingham 1997; Willett 1998; Thompson and Subar 2001).

2.14.1.1 Food records (food diary)

Food records require the subject to record all food and drinks consumed for a specified number of days. The subject weighs all the food and drinks or estimates the amount eaten using household measures, such as cups and spoons. The weighed food record has been regarded as the 'gold standard' (Livingstone and Black 2003) and are used to calibrate other dietary assessment methods. The advantage of food records are that they provide accurate quantitative information; have lower degree of error of portion size (especially if a weighed food record); and do not rely on memory (Bingham, Cassidy et al. 1995; Willett 1998; Trabulsi, Troiano et al. 2003). The main limitation of food records is the underreporting or misreporting of energy intake and representativeness of the overall diet (Livingstone, Prentice et al. 1990).

Validation of food records is undertaken by comparison to external biomarkers, such as doubly-labeled water (Schoeller and van Santen 1982) or urine nitrogen (Isaksson 1980; Bingham, Williams et al. 1988). A study validated dietary assessment methods in 156 free-living women over one year at 3-monthly intervals using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers (Bingham, Gill et al. 1997). The study showed a poor correlation between urine nitrogen and the food frequency questionnaire (FFQ) and 24-hour recall ($r = 0.10-0.27$), but high correlation between 24-hour urine excretion and dietary nitrogen intake from the weighed food record ($r = 0.78-0.87$) and 7-day food diary ($r = 0.60-0.70$) (Bingham, Gill et al. 1997).

To keep an accurate food record, the respondent needs to be trained and the record needs to be reviewed by a trained interviewer to clarify what's recorded and to probe for forgotten foods and drinks. There are a few weaknesses of this method. First is the bias due to sample selection since the participants need to be motivated and literate (Willett 1998; Gibson 2005). It also increases the burden on the participants especially if the numbers of days are greater than three to four and if the subject is required to weigh all food and drink consumed. Research has shown that the incomplete record increases with the number of recording days, especially for recording more than 7 days which could generate respondent fatigue (Gibson 2005).

Moreover, the act of recording can alter participant's dietary behaviours (Trabulsi and Schoeller 2001). The high data processing burden and cost make it not suitable for studies with more than 500 people (Thompson and Subar 2001). In the current study, study subjects were provided with electronic food scales and were asked to record their intake for three days only to minimise subject burden.

2.14.1.2 24-hour recall

A 24-hour recall requires subjects to recall all food and beverage consumed in the previous 24 hours (Gibson 2005). The recall is usually conducted as an interview by trained interviewers. It is often used in large cross-sectional or longitudinal studies. Compared to the food record method, a 24-hour recall doesn't require literacy. It takes between 30 minutes to 60 minutes to complete. It has a lower burden both on subjects and researchers as the form has pre-coded foods lists which aid the speed of recording and processing. Moreover, participants are less likely to change their dietary behaviour while being monitored. More recently, the US Department of Agriculture (USDA) has developed computer systems, including the Automated Multiple Pass Method (AMPM) for collecting food intakes, the Post-Interview Processing System (PIPS) for reformatting data and assigning food codes, and Survey Net for final coding, quality review, and nutrient analysis (Raper, Perloff et al. 2004).

The main limitation of the 24-recall is it requires trained interviewers, preferably dietitians. People may not report food accurately, such as type and amounts of foods eaten. However, the main limitation of this method is a single 24-hour recall cannot account for day to day variation in food intake and the previous day may be an atypical day. Therefore, caution is needed when using the data from 24-hour recall to represent the habitual intake. Conducting several 24-hour recalls on non-consecutive day for the same individuals can improve the reproducibility of the usual intake of an individual (Gibson 2005).

2.14.1.3 Food frequency questionnaires (FFQ)

A Food Frequency Questionnaire obtains information on the frequency of consumption and serving size of each specified food and drink for a specific period, such as one week or three months (Horwath and Worsley 1990). They were developed for use in large epidemiological studies linking diet and disease (Nelson and Bingham 1997). It can be specific to nutrients of interest, such as calcium or protein intake. There are three formats: qualitative, semi-quantitative and quantitative. The qualitative method only requires information on the frequency of specified foods. The semi-quantitative method requests serving size information for some foods but not all foods. When the quantitative method is used information on the amounts of food eaten for all foods is included in the questionnaire.

The strengths of the FFQ are the lower burden on the subjects and researchers. They are easier to code and enter data since most are computer-scannable questionnaires that can be completed in 20-30 minutes. The FFQ can be self-administered or by a non-nutritionist and is therefore cheaper to use in large epidemiological studies (Zulkifli and Yu 1992; Thompson, Moler et al. 1997). The semi-quantitative questionnaire is especially useful to examine changes in diet due to disease (Willett 1998), and also provides good estimation of energy intake in free-living and nonobese adults (Koebnick, Wagner et al. 2005). An advantage of the FFQ is that it does not influence food intake during the study period.

The weaknesses of the FFQ are the inaccuracies in the quantification of intake. It is especially unreliable for energy intake. It was reported that there was significant underreporting of energy intake from FFQ in higher BMI women (Olafsdottir, Thorsdottir et al. 2006). It was also reported that FFQ lacked reliability for estimating the absolute amounts of dietary fats and cholesterol intakes (Schaefer, Augustin et al. 2000). The inaccuracies are due to the incomplete listing of all possible foods in the questionnaire, the error in frequency estimation and the errors in estimation of usual serving size. Some researchers think that FFQ is more like a measure of attitude than a measure of real consumption (Drewnowski 2001). Nevertheless the advantages of the FFQ have led to its widespread use in epidemiological studies.

2.14.1.4 Diet history

The diet history method is usually conducted by trained interviewers. It records detailed information of an individual's usual dietary intake over an extended period of time from several weeks or months to one year (Nelson and Bingham 1997). The major strength of this method is that it can better estimate the habitual diet pattern and details of food intake than other methods (Nelson and Bingham 1997). The major disadvantage of this method is the necessity for a lengthy face-to-face interview and the consequent costs including coding the data (Nelson and Bingham 1997). This limits its use in epidemiological studies, and it is more suited to clinical situations used by dietitians (Nelson and Bingham 1997).

2.14.2 Dietary assessment in the elderly

The advantage of dietary assessment in elderly populations is that their diets are likely to be more structured when compared to young adults. Previous studies suggest that the validity of dietary information collected from the elderly is comparable to that collected from young adults (Nes, Frost Andersen et al. 1992; Goldbohm, van den Brandt et al. 1994). The dietary-history method also showed good agreement with a weighed record in the elderly (van Staveren, de Groot et al. 1994). Diet Recall and FFQ may be inappropriate if memory is impaired (Thompson and Subar 2001; Pope, Kritchevsky et al. 2007). Cognitive ability can be assessed prior to the dietary assessment if necessary.

2.14.3 Why validate dietary assessment methods?

There is no one dietary assessment method that can provide 'true' information of dietary intake. Underestimation of food intake occurs in most of the current dietary assessment methods. Previous studies reported that 16-81% of women underreported their energy intake when their reported energy intake was compared energy requirements assessed by basal metabolic rate or by the doubly-labeled water method (Briefel, Sempos et al. 1997; Lafay, Basdevant et al. 1997; Pryer, Vrijheid et al. 1997; Johansson, Solvoll et al. 1998; Zhang, Temme et al. 2000). Fewer than 9% of

subjects over-reported their energy intake compared to their actual energy expenditure as measured by the doubly-labeled water method, and the over-reporters were characterised with having a lower BMI (Mertz, Tsui et al. 1991; Johansson, Solvoll et al. 1998; Zhang, Temme et al. 2000). The reason for assessing underreporting is to evaluate the validity of reported total energy intake which provides a check of the quality of the dietary data. The validation of dietary assessment can help to exclude subjects whose measures are believed to be flawed.

2.14.4 Underreporting in dietary assessment

Underreporting refers to a failure in reporting all food eaten or misreport food portions or incorrectly describe the food (Livingstone and Black 2003). Long term metabolic studies have identified underreporting between self-reported energy intake and the energy intake required to maintain body weight (Mertz, Tsui et al. 1991; Lichtman, Pisarska et al. 1992).

The extent of underreporting can be assessed by comparison with external biomarkers. 24-hour urinary nitrogen excretion is used to validate protein intake (Isaksson 1980; Bingham and Cummings 1985; Bingham, Cassidy et al. 1995), and doubly-labelled water (DLW) validate free-living energy expenditure (Schoeller and van Santen 1982; Schoeller, Ravussin et al. 1986; Schoeller 1988; Livingstone, Prentice et al. 1992). A study validated protein intake assessed by 18-day dietary records by eight 24-hour urine nitrogen in eight health subjects (Bingham and Cummings 1985). The study found that the within person variation in dietary intake ranged from 14 to 26% (CV), and within individual CV of urine nitrogen varied from 11 to 18%.

Another approach made by Goldberg, Black and his colleagues can be used when biomarkers have not been collected. They developed Goldberg 'cut-off' technique (Goldberg, Black et al. 1991). By using this method they found strong bias of underreporting habitual energy intake by the dietary assessments (Black, Goldberg et al. 1991).

2.14.5 Factors associated with underreporting

Underreporting may be characteristic of some subjects regardless of what dietary assessment method is used (Black and Cole 2001). Females are more likely to underreport their food intake compared to males (Livingstone, Prentice et al. 1990; de Vries, Zock et al. 1994; Johnson, Goran et al. 1994). Subjects may record a socially desirable dietary intake rather than real intake (Westerterp and Goris 2002).

Weight status is the most significant variable associated with underreporting (Livingstone and Black 2003). Obese subjects are more likely to underreport their protein intake (from diet history) (Steen, Isaksson et al. 1977; Prentice, Black et al. 1996). Body weight, percentage of body fat and BMI were positively associated with underreporting (Johnson, Goran et al. 1994; Johansson, Solvoll et al. 1998; Johnson, Soultanakis et al. 1998; Zhang, Temme et al. 2000). Underreporting may also occur due to under eating or a change in food intake during the food recording period (Livingstone and Black 2003). Study participants may simplify their food choices to ease the reporting burden when keeping a food record (Livingstone and Black 2003). Recording dietary intake itself could alter eating behaviour even in lean women due to the burden of recording (Goris and Westerterp 1999).

2.14.6 Validation of dietary assessments methods

2.14.6.1 Doubly-Labeled Water method for assessing energy intake

There is no biomarker available for energy intake (Livingstone and Black 2003). The method of using doubly-labeled water to validate reported energy intake was developed by Schoeller in 1982 (Schoeller and van Santen 1982). The assumption of this method is that the habitual energy intake is equal to the habitual energy expenditure when weight is stable. The principle of the method is that carbon dioxide production can be estimated from the difference in the elimination rates of body hydrogen and oxygen (Frary and Johnson 2004). The subject is given a dose of isotopes deuterium and oxygen-18. Deuterium is lost in water only, and oxygen-18 is lost in both water and CO₂. The elimination rates of the two isotopes are measured and the difference between the two elimination rates is the carbon dioxide production. The total energy expenditure is calculated from carbon dioxide production by using

the classical indirect calorimetric equations (Schoeller, Ravussin et al. 1986). It is the current gold standard for measuring energy expenditure and has a precision of 2% to 8% (Schoeller 1988). However, the high cost of laboratory equipment and the expertise required to operate a spectrometer and analyse the isotopes make it is impractical and expensive for routine use (Frary and Johnson 2004).

2.14.6.2 24-hour urine nitrogen for assessing reported protein intake

24 hour urinary nitrogen excretion is the most commonly used biomarker to validate protein intake. It also provides a more economical alternative to assess the underreporting of dietary assessment methods (Nelson and Bingham 1997; Bingham 2003). The assumptions of this method are that subjects are in nitrogen balance and the 24-hour urine sample are completely collected (Bates, Thurnham et al. 1997). If the above assumptions are met, the following relationship is applied (Gibson 2005):

$$\text{24-hour protein intake (g)} = \text{24-hour nitrogen (g)} + 2 \text{ (g)}$$

The used of 2 g is to account for extra-renal nitrogen losses, such as from faeces and skin. This simplified fixed correction may not be appropriate because of the large variation both in faecal and dermal losses between individuals (Gibson 2005). The alternative approach is to account for the extra-renal losses as a fixed proportion (e.g. ~80-81%) of total urinary nitrogen excretion (Bingham 2003).

Early studies showed a good correlation between 24 hour urine nitrogen excretion and protein intake estimated from the dietary history method (van Staveren, de Boer et al. 1985) and the 24-hour recall (Isaksson 1980; Slimani, Bingham et al. 2003). In 1985, Shirley Bingham's group studied four women and four men in a metabolic suite over a 28-day period. The study subjects were provided duplicate diets each day which matched their usual varied diets (Bingham and Cummings 1985). The study found that despite the daily variation in dietary intake, the average 28-day urine nitrogen was highly correlated with dietary intake ($r = 1.00$). The study also found that urine nitrogen underestimated dietary intake in all subjects and was progressively greater at higher intakes. The average 24-hour urine nitrogen constantly accounted for $81 \pm 2\%$ of nitrogen intake (Bingham 2003).

The within-subject variation of 24-hour urinary nitrogen is related to the number of the samples collected. The correlation of nitrogen intake and 24-hour urinary nitrogen based on a single day collection is about 0.5 with a CV of 24%. The correlation was increased to 0.95 with a decreased CV of 5% when 18 days 24-hour urine samples were collected (Bingham 2003), however, this greatly increases the subject burden.

Para-amino benzoic acid (PABA) can be used to verify the completeness of the 24-hour urine collection (Bingham and Cummings 1983). Three tablets of 80 mg PABA are taken with meals and its excretion within 24 hours measured. Those that contain less than 85% of the PABA are classified as unsatisfactory and a cause of underestimation of 24-hour urine nitrogen (Bingham, Cassidy et al. 1995). In the current study, 24-hour urine nitrogen and 3-day food records were collected.

2.14.6.3 Goldberg EI:BMR ‘cut-off’ method

When biomarkers are not measured, the Goldberg method allows a checking of the dietary intake data quality. When comparing reported energy intake (EI) to energy expenditure (EE) measured by doubly-labelled water, Black and colleagues used the ratio of EI:EE to evaluate the validity of dietary assessment. They defined underreporting, acceptable-reporting and over-reporting as EI:EE ratio of < 0.76 , $0.76-1.24$ and >1.24 respectively (Black 2000). They considered the Goldberg cut-off point as useful to identify the underreporting at a group level. This method also can provide valuable information on the characteristics of under reporters (Macdiarmid and Blundell 1997).

The principle of the Goldberg EI:BMR ‘cut-off’ method is to define the minimum cut-off limits for energy intake below which a person of a given sex, age, and body weight could not live a normal life-style (Goldberg, Black et al. 1991). From the mean of all of the very inactive calorimeter protocols, they defined that $1.27 * \text{BMR}$ (basal metabolic rate) as the ‘survival’ limit. They adapted the value of 1.35 at CUT-OFF 1 as the lowest value for habitual energy intake of an individual that is compatible with a normal (not bedbound) life style. They suggested that reported energy intakes below $1.35 * \text{BMR}$ either in individuals or populations was most unlikely to represent the habitual physical activity level. However, BMR has been

measured (by DLW) rather than predicted. By using predicted BMR by weight or height, they tabulated the CUT-OFF 2 which is the lowest value for the ratio of reported energy intake to the estimated BMR ($EI:BMR_{est}$) according to the sample size and study period. For calculating CUT-OFF 2, $1.55 * BMR$ was used as energy requirement for a sedentary life style.

The following equations summarized the Goldberg EI:EMR 'cut-off' method:

- Assumption: $EI = EE$
- $EE = BMR * PAL$ (PAL is the physical activity level)
- Energy balance equation: $EI = BMR * PAL$
- For a person who has a sedentary life style, the equation is: $EI = BMR * 1.55$
- The reported EI is not plausible when $EI < BMR * 1.55$

Basal metabolic rate (BMR) can be estimated by the Schofield Equation (Schofield 1985) which was included in the 1985 FAO/WHO/UNU report. BMR is predicted from weight with age and gender is taken into account. For woman aged over 60 years the equation is:

$$BMR = 0.038 \text{ weight} + 2.755$$

BMR is in MJ/day (megajoule/day) and weight is in kg.

A similar approach to the Goldberg cut-off method was investigated by comparing reported dietary energy intake with energy requirements estimated by using total energy expenditure predicted from age, weight, height and sex using a previously published equation (McCrory, McCrory et al. 2002). Two 24-hour recalls, height and weight were assessed in 3755 men and women aged 21-45 years. The results showed that the reported energy intake was 77% and 64% of predicted total energy expenditure in men and women respectively. The authors suggested excluding subjects whose reported energy intakes fell outside the ± 1 SD of predicted energy expenditures (<70% or >130%) when studying the relationships between diet and health.

Chapter 3 Methods

3.1 Study design

This study was a double blind, placebo controlled, one year protein supplementation intervention study using a whey protein drink. Major assessments were made of the participants at baseline and one year, with continuous delivery of the intervention and control products and monitoring of consumption.

3.2 Subjects

3.2.1 Recruitment

Two hundred and nineteen women were recruited during April and September 2007. Letters were sent to 2356 individuals aged between 70 and 80 years selected randomly from the Western Australian Electoral Roll, which has the names and addresses of all women of this age range who have registered to vote. Since voting is compulsory in Australia this is the most complete population information available which ensured a population based study. Telephone screening ensured the inclusion criteria for each subject was met. Eligible volunteers were sent a study outline and informed consent document and booked for a baseline clinic visit. The study was approved by the Human Ethics Committee of Sir Charles Gardiner Hospital and Curtin University (Appendix 1).

3.2.2 Randomisation

Two hundred and nineteen eligible study participants were assigned to either the study or control groups using a computer-generated randomisation program with a block size of four to assign participants to protein drink or control drink in a ratio of 1:1. They were allocated a number and assigned to one of the treatment groups. One hundred and nine women were randomised to the protein supplement group, and 110 to the control supplement group (Figure 4.1). The randomisation code were generated by an independent research fellow and placed in sealed envelopes. The study participants and the study staff remained blinded to the treatment code until all the data had been entered and evaluated for accuracy and the a priori hypothesis reviewed.

3.2.3 Interventions

Participants in the protein supplement group were asked to consume the test drink daily for the duration of the study. Every day the trial group drank 250 mls of the whey protein based high protein supplement product reconstituted with cold water. The reconstituted protein drink provided 30 g of protein, 600 mg of calcium (given as calcium lactate) and contained 810 KJ. The protein source was whey protein isolate (Alacen 894). Participants in the control group received an isocaloric placebo drink similarly reconstituted with cold water from the placebo drink powder which has the same energy (KJ) and calcium content but only contains 2 g protein per 250 ml. The high protein drink and the placebo drink were similar in taste, consistency and ‘mouth feel’.

Participants were instructed to have the assigned drink in the morning before breakfast. The reason for advising participant to have the drink first in the morning is that it was expected that the participants would be more compliant with this routine and less likely to forget to take the drink. The participants were provided supplements with batches on a regular basis. Each batch contained three month supplies (90 small individual containers). Each small container contains one day’s dose of supplement. They were advised to place all the powder from the container provided into the provided milkshake maker (Figure 3.1), and add cold water (from the refrigerator) to the 1 cup level (250ml) to the level marked on the container, then immediately shake the container with two hands until the powder was well mixed.

Figure 3.1 The shaker maker.



3.2.4 Inclusion criteria

Ambulant women aged between 70 and 80 years who were able to comply with the requirements of the protocol were included in the study. This age range was chosen because women in this range are declining in muscle mass but are still capable of fulfilling the study requirements. A signed and dated written informed consent was obtained prior to participation.

3.2.5 Exclusion criteria

Subjects were excluded if they had the following conditions:

- Participation in another clinical trial in the 12 weeks prior
- Previous osteoporotic fracture
- Currently or within last year taking medication for osteoporosis (Bisphosphonates, such as Fosamax, Actonel, and Zoledronate, Evista, Teriparatide, Protos, HRT (hormone replacement treatment)) apart from calcium or vitamin D
- Taking steroid tablets (e.g. Cortisone) in the past 3 months or have taken more than 7 g in total in their lifetime
- Metabolic bone disease (e.g. Paget's disease) apart from osteoporosis
- Total hip bone density more than 2SD below the mean for their age.
- Lactose intolerance or do not like milk products
- High protein intake as assessed by food frequency questionnaire (equivalent to protein intake more than 1.5 g/kg body weight per day)
- Cognitive impairment (Mini mental state exam < 24) (Appendix 10)
- BMI > 35 kg/m²
- Bowel surgery resulting in difficulty absorbing food or other reasons caused difficulty absorbing food
- Coeliac disease
- Clinical hepatic insufficiency
- Clinical diagnosis of diabetes
- Renal insufficiency – creatinine more than twofold the upper limit of normal

3.3 Study assessments

All the assessments listed below were made at baseline and repeated at 12 months.

3.3.1 Body composition and muscle mass by dual energy X-ray absorptiometry (DXA)

3.3.1.1 Instrument and quality control

Lean body mass (total and regional) were derived from the whole body dual energy x-ray absorptiometry (DXA) scan on a Hologic Discovery 4500A Bone mineral densitometer (Hologic Corp., Boston, MA, USA). The spine phantom was scanned daily. The whole body step phantom was scanned weekly. Follow-up scans were done on the same machine as used for the baseline scans. The CVs for total lean mass and total fat mass were 1.1% and 1.6% respectively.

3.3.1.2 Whole Body DXA Scanning

Participants removed their shoes, socks and bra, and changed into a light hospital gown. They were asked to take off all metal objects including jewellery, glasses, wrist watches, and rings. If a ring could not be taken off, a note was made in the checklist, and consistency was kept at the one year visit.

Participants were asked to lie down on the scanning table with their body centered on the table. The feet were placed together but without the ankles and knees touching using an elastic band to keep the two big toes together. The subject's head was 3cm below from the horizontal line marked at the top of the mattress so that the first few scan lines above the patient's head are entirely in air.

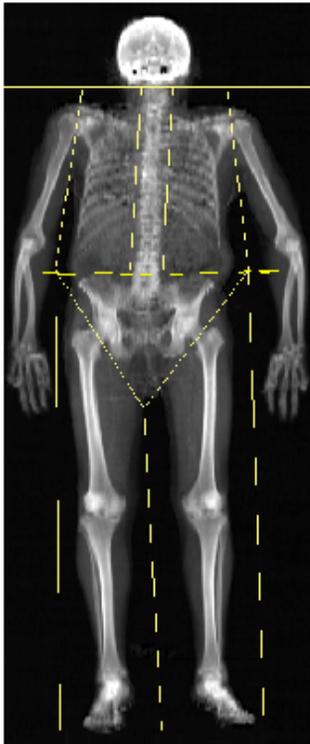
Participants were scanned without a pillow, as the pillow may affect the body composition results. The subject's entire body was within the edges of the scanning field marked on the mattress. The hands were placed palms down with the fingers extended and together alongside the body. There should be a gap between the hands and the hips. If the subject was too large the hands were positioned vertically with the thumbs in line. The participants was instructed to keep very still until the scan was finished.

3.3.1.3 Scan analysis

The scan image analysis was done by trained researchers. The scans were analyzed using Hologic Discovery version 12.6.1. Ten lines were used to separate the body into six parts: head, left and right arms, trunk (spine is separated from trunk and divided into thoracic and lumbar spine) and left and right legs (Figure 3.2). Firstly, a line is positioned just below the chin to separate the head from the rest of the body. Secondly, two lines are drawn to separate the left and right arms from the trunk. The cuts are passed through the centre of the left and right humeral socket. Another two parallel lines are placed close to the spine to separate the spine from the trunk. Thirdly, a line is placed above the tip of the pelvis to separate the trunk from the lower limbs. Fourthly, two angled lines are positioned to pass through the femoral necks to separate the left and right legs from the trunk. Finally, one line is placed in the middle of the two legs. The analysis technique followed the instructions in the “Clinical bone densitometry training course” provided by the Australia and New Zealand Bone and Mineral Society (ANZMBS) (Australia and New Zealand Bone and Mineral Society 2005).

The head was excluded from the scanned area and the following data analysis as recommended by the manufacturer as bone density of skull and brain fat content are not associated with the body composition analysis. The soft tissue was partitioned into fat and lean by the machine derived algorithms. The lean mass of arms and legs was then summed to provide the appendicular lean mass.

Figure 3.2 Sample of DXA scan image showing the placement of lines for the image analysis.



3.3.1.4 Data analysis for DXA

In the SPSS database, data for the head was excluded from all the analysis in this study. Lean mass refers to bone-free lean mass. Whole body lean mass refers to whole body head-free bone-free lean mass. Whole body fat mass refers to head-free whole body fat mass.

Percentage of whole body fat mass = Whole body fat mass / Total body mass

Percentage of whole body lean mass = Whole body lean mass / Total body mass

Total body mass refers to head-free body mass.

The lean mass of arms and legs were summed as appendicular lean mass (ALM).

The Baumgartner's adjusted appendicular lean mass was calculated as following equation (Baumgartner, Koehler et al. 1998):

Adjusted appendicular lean mass = appendicular lean mass (kg) / height² (m²)

3.3.2 Calf muscle cross-sectional area measured by Peripheral Quantitative Computed Tomography (pQCT)

3.3.2.1 Instrument quality control

The calf muscle cross-sectional areas was measured by the peripheral quantitative computed tomography (pQCT) using a Stratec XCT 2000 (Stratec Medizintechnik GmbH, Pforzheim Germany). The position was chosen at 38% of the tibia length proximal to the ankle joint. A standard phantom scan was scanned every week and a core phantom scan was scanned every two weeks.

3.3.2.2 Patient positioning

The scans were taken on the participant's left leg. If the participant had a fracture on her left ankle, the scans were taken on the right leg. The participant's leg was measured from inside ankle (medial malleolus) to the anterior tibial tuberosity of the knee using a steel segmometer (Rosscraft, Canada) and the data were entered in the computer connected with the pQCT scanner.

The participants sat in a wheelchair and were instructed to sit back in the chair comfortably and lift their leg and point their toe. The foot was placed in a support and a Velcro strap used to keep the foot in position. A cushion was placed under their knee and the leg clamp was tightened (Figure 3.4). The permission for displaying this picture in this thesis has been obtained from this participant.

Figure 3.3 Segmometer (Rosscraft, Canada).



Figure 3.4 Peripheral quantitative computed tomography scan at 38% length of tibia.



3.3.2.3 Scan sites

The scan site was at 38% of tibia length. The reference line was placed at the top of the distal of the tibia. The reference line was placed at the same site for baseline and one year by performing the pre CV scan which shows the ankle joint.

The primary outcomes of this study were bone and muscle. For studying bone structure, the 4% and 15% of tibia length are the conventional sites measured, and 66% is often used to study muscle structure. For XCT2000 machine, the 66% site scan only can be performed under a setting called “Muscle Mask” which is not suitable for measuring bone structure. The scans were taken at 38% of tibia length in this study instead of scanning at the conventional 66% site to study both bone and muscle estimates at the same time, which minimized the radiation exposure to the patient. The disadvantage of this method is that because of not using the Muscle Mask and the scans were taken by using bone parameters, only the muscle area can be obtained from the scan. It is unable to generate the muscle density from the scans using the company provided analysis software. As the automatic muscle analysis setting is not applicable for these scans, we used the manual tracing method to obtain the area of the cross-sectional muscle. Fat infiltration is therefore not available.

3.3.2.4 The analysis of pQCT scan of muscle area at 38% site of tibia

The pQCT scans were analyzed using the Stratec 2000 software version 6.0. The first step was to manually trace the 38% site of tibia muscle total area which included calf muscle, tibia bone and fibula bone (Figure 3.5A). The second step was to manually trace the area of tibia bone and fibula bone (Figure 3.5B&C). Then the cross-sectional muscle area of 38% site of tibia was calculated by the following equation:

Muscle area (cm²) = Total muscle cross-sectional area of the leg (cm²) – area of tibia bone (cm²) – area of fibula bone (cm²).

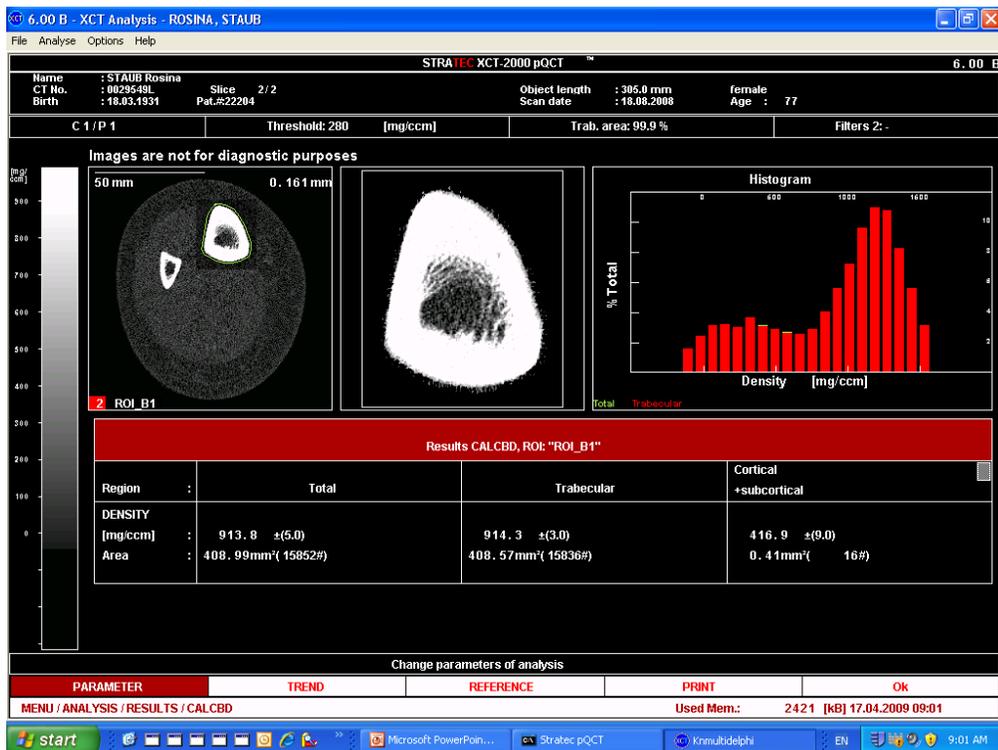
All scans were done by trained operators. All analysis was done by one researcher (the candidate). The inter- and intra- operator and subject variation were assessed by repositioning and reanalysis of the scans on thirty participants. The CV of muscle area at 38% site of tibia length was 1.51%.

Figure 3.5 (A-C) Sample of pQCT scan imagine analysis

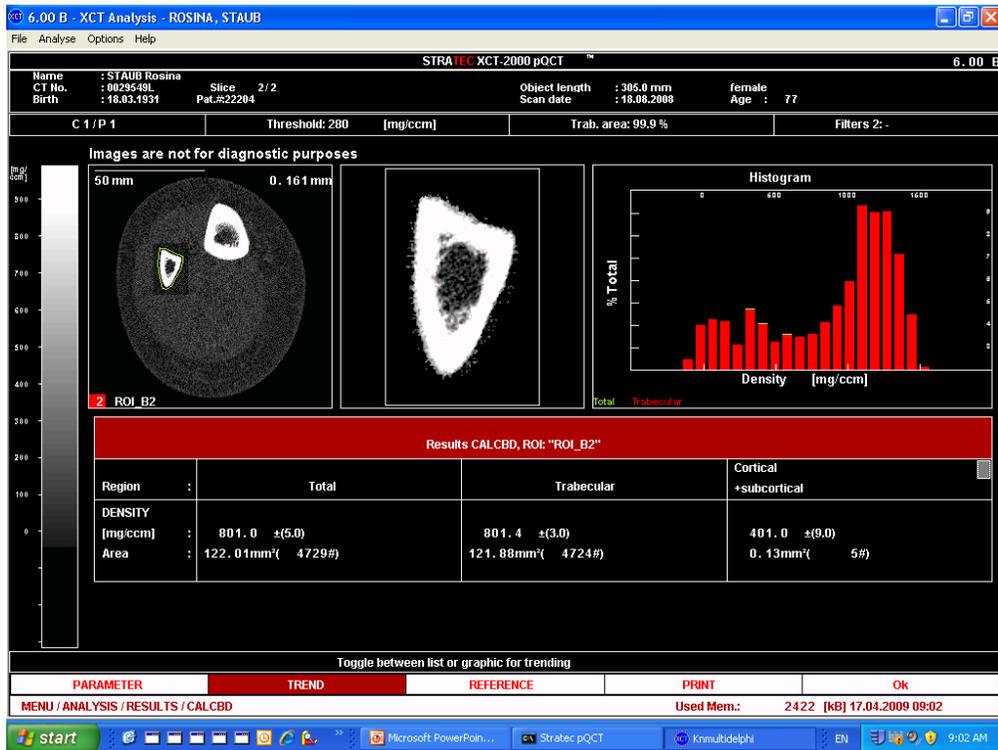
A. Cross-sectional area of calf muscle plus bones as traced by the green line



B. Cross-sectional area of tibia bone as traced by the green line



C. Cross-sectional area of fibula bone as traced by the green line



3.3.3 Muscle strength

3.3.3.1 Hand grip strength

Hand grip strength was assessed by a hand dynamometer (TTM, Original, Tokyo) on the dominant hand (Figure 3.6A). The dominant hand was found by asking the participants the question of ‘Are you right-handed or left-handed?’. The dynamometer was calibrated at baseline and at one year. The trigger of the dynamometer was adjusted to fit each participant’s hand size before the measurement. The participants were in a standing position, held the dynamometer with their dominant arm extended, as straight as possible above the head. They then squeezed the trigger as hard as possible while dropping their arm to the front (Figure 3.6B). The arm was always kept straight. The research assistant demonstrated the test to the participants first. The participant repeated the test three times (including the first time as a practice) and the maximum reading in kg was recorded.

The coefficient of variation (CV) of hand grip strength was 6.7%. It was assessed by repeating the measurement on 30 participants by an interval of 30 minutes between two tests for each person.

Figure 3.6 Hand dynamometer and hand grip strength test.

A. Hand dynamometer (TTM, Original, Tokyo)



B. Procedure of hand grip strength test.

1. Start position: dominant hand held the dynamometer upright above the head.



2. Arm dropped from the start position.



3. Finish position: arm was placed aside to the body.



3.3.3.2 Ankle dorsiflexion strength

Ankle dorsiflexion strength was measured on the dominant leg. The participants were asked to remove their right shoe and sit on the chair with their foot positioned on the footrest device (Figure 3.7A). The foot was strapped to a plate with a spring gauge attached. The ankle was positioned directly over the hinge (i.e. the back of the heel was positioned on the line below the hinge). The knee joint angle was angled at 120-degrees determined using the 120-degree metal angle (Figure 3.7B). This ensured the ankle position was at the same level for every participant because a previous study showed that the ankle position could significantly influence the ankle dorsiflexion torque (Geboers, van Tuijl et al. 2000). The strap was then tightly applied to the foot where the foot can exert the greatest force just proximal to the base of the fifth toe. When the patient's foot was in place they were asked to cross their arms in front of their chest. The participants kept their heel on the footrest and raised the front of their foot as forcefully as they could (Figure 3.7C). The research assistant held the device in position by placing their foot on the front of the base plate. The participants repeated the task three times (including the first time as a practice) and the highest results in kg were recorded.

Figure 3.7 (A-C) Footrest device, metal angle and ankle dorsiflexion strength test.

A. Footrest device used for testing ankle dorsiflexion strength. A Velcro strap was attached to the foot plate, and the spring gauge was attached to the foot plate.



B. Metal angle. The 90-degree metal angle was used to ensure the knee angle was 90-degree before testing knee strength. The 120-degree metal angle was used to ensure the knee angle was 120-degree before testing ankle dorsiflexion strength (see picture C3)



C. Procedure of ankle dorsiflexion test

1. Participants' dominant foot were strapped on the foot plate by the Velcro strap.



2. Participants raised the front of their foot from the footrest.



3. The 120 degrees metal angle was used to ensure the knee angle was at 120-degrees before starting the test.



4. Participants were asked to cross their arms in front of their chest while testing ankle dorsiflexion strength



3.3.3.3 Knee extension and flexion strength tests

The knee extension and flexion strength test measured the strength of the muscles that flex and extend the knee on the dominant leg. The measurements were taken using a dynamometer (TTM, Original, Tokyo) attached to a metal bar on the wall (Figure 3.8A-C).

To measure the knee flexion, the participants were asked to stand facing the wall. An adjustable strap was attached on the participant's calf (at the end of the calf muscle) and the dynamometer. The dynamometer was moved up or down to ensure that the long strap was horizontal. Keeping the horizontal strap tight, the participant was asked to sit down on a chair facing towards the wall on which the dynamometer is attached. The participants sat upright with their hip and knee at 90-degree measured using the metal 90-degree angle. The participants were instructed to hold on to the chair for support and slowly and forcefully pull the lower leg toward the back of the thigh as strongly as possible sliding the ball of the foot along the floor holding it for as long as possible. The participants were given two attempts (including the first time as a practice) with an interval of 30 seconds and the maximum result in kg was recorded.

To measure the knee extension, the participants were asked to stand up and turn to face away from the wall while keeping the horizontal strap tight. The chair was moved so it was behind the participants, facing away from the wall with the back against the metal bar. The participants were instructed to hold on to the chair for support, and slowly and forcefully push against the strap as strongly as they could. The tested foot was kept on the floor. The participants were given two attempts (including the first time as a practice) with an interval of 30 seconds and the maximum results in kg were recorded.

Knee flexion strength and knee extension strength were summed and reported as the total knee strength.

Figure 3.8 (A-C) Wall-attached dynamometer and the procedure of knee strength tests.

A. Wall-attached dynamometer which can move up and down along the metal bar.



B. Procedure of knee flexion strength test

1. Participants' dominant leg was attached with the wall-attached dynamometer by the Velcro strap. Knee was at 90-degrees before starting the test.



2. Participants held hands on the chair and pull the lower leg toward the back of the thigh.



C. Knee extension strength test: participants pushed against the strap attached to the wall-attached dynamometer.



3.3.3.4 Hip strength (extension, abduction, flexion and adduction)

The hip strength was tested on the participant's dominant leg. The participants were given two attempts (including the first time as a practice) with an interval of 30 seconds. The maximum results in kg were recorded. The following procedures described the measurements taken on the right leg for example (Figure 3.9A-D).

Hip extension strength

To measure the strength of hip extensors, participants were asked to stand straight, facing the wall. The strap was attached to the lower edge of the right thigh which was just above the participant's right patella. The strap also was attached to the dynamometer and was adjusted to the height of the dynamometer to a position that is horizontal to the strap attached to the participant. The participants stood so that the strap was taut but not causing a reading on the dynamometer. The participant had their back and left leg vertical and they steadied themselves by holding their right hand on the chair (the chair was stabilised by the research assistant). The participants were instructed to forcefully pull their leg back from the wall at a moderate pace but as strongly as they could.

Hip abduction strength

To measure the strength of hip abductors, the participants were asked to turn 90-degree to their right with the strap still in place on their thigh so that the strap was running across their left leg to the dynamometer. They were asked to stand upright holding their hands on the chair (the chair was stabilised by the research assistant). When ready, the participants were asked to slowly and forcefully pull their right leg away from their left leg as strongly as they could.

Hip adduction strength

To measure the strength of hip adductors, the participants were asked to turn 180 degree to their left with the strap still in place on their thigh. The participants were asked to stand with their feet together then abduct the left leg to shoulder width to allow their right leg room to adduct. They were asked to stabilise themselves by holding their left hand on the chair (the chair was stabilised by the research

assistant). They were instructed to slowly and forcefully pull their right leg away from the wall towards their left leg as strongly as possible.

Hip flexion strength

To measure the strength of hip flexors, the participants were asked to turn 90 degree to their left again facing away from the wall. A chair was placed in front of them to hold on to while doing the test. The chair was stabilised by the researcher during the test. Participants were asked to stand with their left and right legs and body upright holding onto the chair with their hands. The participants were asked to transfer their weight to their left leg and then to slowly and forcefully push their right leg against the strap (moving foot away from the wall) as strongly as they can.

Hip strength for extension, abduction, flexion and adduction were summed and reported as the total hip strengths.

Figure 3.9 (A-D) Procedure for the hip strength tests.

A. Hip extension strength: participants' leg was attached with the wall-attached dynamometer by the strap, and they pulled their leg back from the wall.



B. Hip abduction strength: participants pulled their leg away from their left leg.



C. Hip flexion strength: participants were facing away from the wall and pushed their right leg against the strap.



D. Hip adduction strength: participants pulled their right leg towards their left leg.



3.3.3.5 The test-retest reliability of leg strength tests

As pointed out by Keating and Matyas, there is considerable variation between studies using dynamometry to test muscle strength (Keating and Matyas 1996). The potential factors causing variations are the subjects factors and test procedures. The subject factors include age, gender, weight, athletic background, disability and limb dominance. The test conditions includes the range of movement; the type of contraction or movement, such as concentric, eccentric, isokinetic or isometric etc, pre-test procedures such as warm-up, starting position and stabilization etc; the test speed, sequence and test intervals.

The coefficients of variation (CV) for the lower limb strength tests were assessed by repeating the measurements on 30 participants with a 30 minutes interval between two tests for each person. The test-retest was assessed twice. First test-retest was taken at the beginning of the study and the results were shown in Table 3.1. Since the CVs were too large, the methodology of the tests were reviewed promptly and finalized as described above. The method was improved to reduce the compensation of other muscle groups and the consistency of the methods. The test-retest reliability was reassessed and the CVs were improved as shown in Table 3.1.

Previous studies have found that the reliability of knee extension indices generally exceeds that of flexion indices (Montgomery, Douglass et al. 1989; Gleeson and Mercer 1992). The current study also found the larger CV for knee flexion strength (13.0%) than for knee extension strength (9.5%).

In the data analysis, to reduce the variation of single group of muscle strength, the knee flexion and extension were summed up as the total knee strength; and the hip extension, abduction, flexion and adduction were summed up as the total hip strength. The CV for total knee strength and total hip strength were 9.0% and 6.9% respectively, which improved compared to the single muscle group strength.

Table 3.1 CVs of the test-retest of lower limb muscle strength on 30 patients at the beginning of the study and the improved tests method.

Muscle strength (kg)	CV of first time test-retest on the original test method	CV of second time test-retest on the improved test method
Ankle dorsiflexion	15.0%	7.0%
Knee flexion	21.8%	13.0%
Knee extension	13.7%	9.5%
Hip extension	14.0%	8.6%
Hip abduction	20.5%	9.3%
Hip flexion	15.2%	9.8%
Hip adduction	15.6%	12.1%
Total knee strength	-	9.0%
Total hip strength	-	6.9%

3.3.4 Mobility and balance

3.3.4.1 Timed Up and Go Test (TUAG)

Mobility function was measured by the Timed Up and Go Test. It is a test of basic functional mobility for elderly persons (Podsiadlo and Richardson 1991). The TUAG test requires the participant to be timed while getting up from a chair, walking 3m, turning, returning to chair and sitting down again.

The test score was recorded in seconds to the nearest 0.1 second using a sport stop watch (Sportline digital watch as shown in Figure 3.10 A). The chair with arms of approximately 46 cm in height was placed 3 metres from a marked green line (Figure 3.10 B). The participants wore their regular footwear and used their usual walking aids if required. The participants started seated with their back against the back of the chair, arms on the chair arms, walking aids were within ready if needed. The participants were instructed on the word “GO” to get up, walk at a comfortable safe pace to the 3 metre mark, turn, return to the chair and sit down again. The participants walked through the test once as practice then being timed.

The coefficient of variation (CV) of TUAG is 5.7%. It was assessed by repeating the measurement on 30 participants with a 30 minutes interval between two tests for each person.

Figure 3.10 Stop watch and TUAG test.

A. Stop watch for testing TUAG (Sportline sport digital watch)



B. Timed Up and Go test settings: a chair was placed three meters away from the green line marked on the ground.



3.3.4.2 Romberg test

Standing balance was assessed by a modified test (Guralnik, Ferrucci et al. 1995) based on the Romberg test. The participants were asked to attempt to maintain their feet in the side-by-side, semi-tandem (heel of one foot beside the big toe of the other foot), and tandem (heel of one foot directly in front of the other foot) positions for 10 seconds each. If the participants were not able to hold side-by-side for 10 seconds, they were given a score of zero. They were given a score of 1 if they could hold this position for 10 second, a score of 2 if they could hold semi-tandem position for 10 seconds, a score of 3 if they could perform tandem position but only hold the position for less than 10 seconds, and a score of 4 if they could hold tandem position for 10 seconds. The participants were asked to attempt these tests first with their eyes open, then with their eyes closed. The final scores of tests with the eyes-open and the eyes-closed were recorded on the checklist sheet (Appendix 12). The participants completed the tests with their shoes on. For each stand, the demonstration of the task was given first. The participants were allowed to hold on to the support (the metal bars beside them) whilst they got into position. When the participants were ready, they released the support and the researcher started timing. The research assistant stopped timing when the participant moved their feet or grabbed for support.

3.3.5 Anthropometry

The anthropometry measurements were height, weight, mid upper arm girth, triceps skin-fold, calf girth, hip girth and waist girth. The measurements were taken prior to the DXA scan on the morning clinic visit in a hospital gown. All the measurements followed the standard procedures as detailed in the International Standards for Anthropometric Assessment (Marfell-Jones, Olds et al. 2006) and described below. All anthropometrists underwent training conducted by a level four accredited anthropometrist. A flexible steel tape (Lufkin, Executive Thinline, W606PM) was used to measure all girths. The Technical Error of Measurement was <7.5% for skinfolds and <1.5% for girths; as required for Level 1 anthropometry accreditation for the International Society for the Advancement of Kinanthropometry (Marfell-Jones, Olds et al. 2006).

Figure 3.11 (A-C) Anthropometry measurement devices.

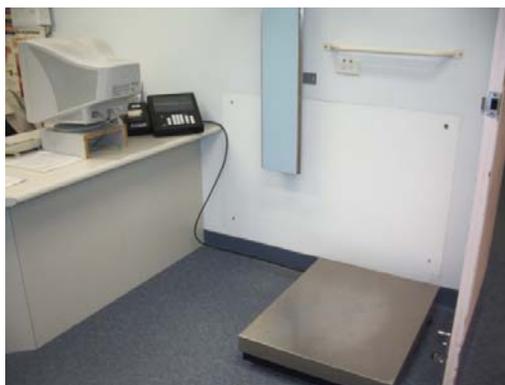
A. Lufkin steel tape (Lufkin, Executive Thinline, W606PM) and Harpenden skin fold caliper (John Bull British Indicators LTD, England): used to measure skin-fold and girth.



B. Stadiometer (Veeder-Root, Elizabethown, N.C., USA): used to measure height



C. Electronic scale (August Sauter GmbH D-7470 Albstadt 1 Ebingen, West Germany): used to measure weight.



3.3.5.1 Height

Standing height was measured using a wall-mounted stadiometer (Veeder-Root, Elizabethown, N.C., USA) (Figure 3.11B) to the nearest 0.1 cm. The participant removed their shoes and socks and stood against the wall with the heels together and the heels, buttocks and upper part of back touching the scale. The head was positioned in the Frankfort plane (orbitale is in the same horizontal plane as the tragion), and the participant was instructed to take a deep breath and hold. The head board was placed firmly down on the vertex, compressing the hair as much as possible, and the reading was recorded to the nearest 0.1 cm.

3.3.5.2 Weight

Body weight was measured in a light hospital gown by an electronic scale (August Sauter GmbH D-7470 Albstadt 1 Ebingen, West Germany) (Figure 3.11C) to the nearest 0.1 kg. The electronic scale was calibrated yearly. Body mass index was calculated as body weight kg/height m².

3.3.5.3 Triceps skinfold

Triceps skinfold was measured by a Harpenden skinfold caliper (John Bull British Indicators LTD, England) to the nearest 0.1 mm (Figure 3.11A). The measurement was taken parallel to the long axis of the right arm at the triceps skinfold site, which is the point on the posterior surface of the arm, in the mid-line, at the level of the marked mid-acromiale-radiale landmark. The participants assumed a relaxed standing position with the arm hanging by the side in the mid-prone position.

3.3.5.4 Mid upper arm girth

Mid upper arm girth was measured at the level of the mid-acromiale-radiale site on the right arm by a tape to the nearest 0.1 cm. The participants assumed a relaxed standing position with the arms hanging by the sides.

3.3.5.5 Upper arm muscle area and corrected upper arm muscle area

Upper arm muscle area was derived from the measurements of mid upper arm girth and triceps skin fold using the following formula (Hammond 2004):

$$\text{Upper arm muscle area (cm}^2\text{)} = (\text{mid upper arm girth in cm} - \pi (\text{triceps skinfold in cm}))^2 / 4\pi$$

$$\text{Corrected upper arm muscle area (cm}^2\text{)} = \text{arm muscle area} - 6.5$$

3.3.5.6 Waist girth

Waist circumference was the narrowest point of the abdomen girth. The participants assumed a relaxed standing position with the arms folded across the thorax. The participants were instructed to breathe normally and the measurement was taken at the end of a normal expiration by a tape to the nearest 0.1 cm.

3.3.5.7 Hip girth

Hip girth was measured at the level of the greatest posterior protuberance, perpendicular to the long axis of the trunk by a tape to the nearest 0.1 cm. The participants assumed a relaxed standing position with the arms folded across the thorax and feet together.

3.3.5.8 Waist-to-hip ratio

Waist-to-hip ratio (WHR) was calculated by dividing the waist girth by hip girth.

3.3.5.9 Calf girth

Calf girth was measured on the right leg at the level of the medial calf skinfold site which is the calf at the level of the maximal calf girth. The participants assumed a relaxed standing position with the arms hanging by the sides and feet separated.

3.3.6 Biochemical markers

Fasting blood and urine samples and a 24-hour urine collection were taken at baseline and 12 months. The venous blood sample was collected in the morning after an overnight fast from 10pm. The participants were instructed to empty the bladder and drink at least two glasses of water before they came to the clinic. The serum was separated from the blood within 30 minutes after collection and stored in a -79 Celsius freezer until analyzed.

3.3.6.1 Serum insulin-like growth factor one (IGF-1)

Serum IGF-1 was measured by IMMULITE 2000 IGF-1 Analyzer (Catalog Number: L2KGF2) in Royal Perth Hospital. IMMULITE 2000 IGF-1 is a solid-phase, enzyme-labeled chemiluminescent immunometric assay. It determined if the effects of increased dietary protein on muscle mass and strength was related to changes in IGF-1.

3.3.6.2 24-hour urine nitrogen

24-hour urine nitrogen was used as a biomarker of the validation of dietary protein intake. The participants were given written (Appendix 14) and verbal instructions on the collection of the 24-hour urine specimens. The participants were asked to collect a 24 hour urine sample on days two and three of the food recording period. They were asked to discard the first urine specimen at the first morning of collection and collect all specimens for the following 24 hours including the first urine specimen of the next day. They were provided with a urinary hat and a 5 L plastic collection bottle which contained 20 ml of 1M HCL as a preservative. They were asked to record the day and time of the start and end on a provided checklist (Appendix 14) and to keep the bottle in a cool place. The returned urine samples were weighed (minus the bottle weight) and recorded on the checklist (Appendix 12). Five ml sample was dispensed into a 5 ml screw cap tubes and stored in -20 Celsius freezer until analyzed.

The urine nitrogen concentration was measured by the Kjeldahl method which involves three steps: digestion, neutralization and titration (McClements 2003). The

analysis was completed by the laboratory staff at Curtin University. In the Kjeldahl method, protein is digested (oxidized) and the nitrogen is converted to $(\text{NH}_4)_2\text{SO}_4$. The alkaline was added to the mixture to convert the NH_4 to free NH_3 . Then the solution is titrated to determine the amount of NH_3 captured. The 24 hour total urinary nitrogen in grams was calculated as multiplying the weight of 24 hour urine with the urinary nitrogen concentration. The urinary nitrogen refers to all sources of urinary nitrogen, which included urea urinary nitrogen and nonurea nitrogen, such as ammonia and creatinine.

The estimated dietary protein intake in gram equals $6.25 \times (\text{N} + 2)$, where N is the number of grams of total urinary nitrogen in the 24 hour urine, and 2 (g) is the estimated nitrogen excretion by routes other than urine (i.e. faeces and sweat) (Carlson 2004; Gibson 2005).

3.3.7 Dietary assessment

At the baseline screening visit, participants completed a simplified, self-administered, quantitative food frequency questionnaire (FFQ) requesting information on the normal eating pattern over the last year (Appendix 13). The daily dietary intake of protein was calculated. Participants with protein intakes greater than 1.5 g/kg/d were considered as high protein intake, and were excluded from the study.

The participants' diets were also assessed by 3-day food record (Appendix 13) at baseline and 12 months. The participants were asked to record everything they ate and drank for three consecutive days which included two week days and one weekend day (Thursday, Friday and Saturday, or Sunday, Monday and Tuesday). They watched a training video on how to complete their food record, and they were also provided with electronic food scales (Philips, HR 2385/A, Hungary). They were instructed not to alter what they ate or drank during this time and record as accurately as they could using either the food scales provided or household measures (for example cups and spoon measures). On the day they returned the food record, the participants were interviewed by a trained research assistant to clarify types and amount of food recorded. A dietitian and university students majoring in nutrition,

who had undergone advanced competency training in dietary assessment completed all analysis of the food and drink records. The food record was analyzed for protein, energy and other nutrients intakes using the AusNut database (Foodworks Professional edition version 3.02). Vitamin and mineral supplements were included in the analysis.

3.3.8 Physical activity assessment

Physical activity level was assessed by the International Physical Activity Questionnaire (IPAQ) (www.ipaq.ki.se) short form (Appendix 7). The participants completed the questionnaire during the morning clinic.

One metabolic energy turnover (MET) equals to one kcal per kg body mass per hour (Denkinger, Coll-Planas et al. 2007). The MET-minutes/week (Mets) was calculated as below and used as the continuous scores of total physical activity level (<http://www.ipaq.ki.se/scoring.pdf>). The calculation method is described as below:

Walking Mets/week = 3.3 * walking minutes * walking days

Moderate Mets/week = 4.0 * moderate-intensity activity minutes * moderate days

Vigorous Mets/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days

Total physical activity Mets/week = sum of Walking + Moderate + Vigorous MET-minutes/week scores.

Physical activity was also evaluated as category variables as low, moderate, and high following the IPQA user instructions (<http://www.ipaq.ki.se/scoring.pdf>). The low was defined as the total physical activity less than 600 Mets/week, moderate was defined as the physical activity between 600-3000 Mets/week, and high was defined as at least 3000 Mets/week.

3.3.9 General health status assessment

General health status was assessed by the SF-36 questionnaire, IQOLA (International Quality of Life Assessment), English (Australia), version 1.0 (Appendix 8) designed

by Medical Outcomes Trust (<http://www.outcomes-trust.org/instruments.htm>). This is self-administered or by trained interviewers either in person or by telephone. The questionnaire contains 36 questions in 8 domains. The eight health concepts are physical functioning, role limitation due to physical health problems; bodily pain, general health, vitality (energy/fatigue), social functioning, role limitations due to emotional problems and mental health (psychological distress and psychological well being) (<http://www.outcomes-trust.org/instruments.htm#SF-36>). There are two versions: standard (4-weeks recall) and acute (1-weeks recall).

The current study used Standard Australian version 1.0 which is a 4-week recall version. Study participants completed the questionnaire at the clinic visit and were assisted by research staff where needed. The completion was checked by research staff at interview section.

The data was analyzed by Stata software (Intercooled 9.0) using a designed program for calculation of summary statistics for SF-36 (Ryan 1999). The results gave the raw scores and transformed scores for each of the eight domains and country-weighted (such as Australia, UK or USA) two summary scores (physical health scores and mental health scores). Since the raw data could be negative and eight domains also have different scales, the transformed scores are preferable by researchers. The transformed scores for each of eight domains and two summary scores are from 0-100 (Ware). The two summary scores are national specific. For Australia, the Stata program uses zero to represent the worst health status and 100 to represent the best health status. The standard population normal health score is mean of 50 and standard deviation of 10. The current study used transformed scores to describe the participants' general health status.

3.3.10 Compliance with test drink consumption

To assess compliance with the test drink consumption, study participants were asked to return their emptied containers at the one year visit. They were also provided with a drink record calendar to cross the date they consumed the drink.

3.4 Sample size calculation

Sample size was calculated by 'Power and Sample Size Calculation' Version 2.1.31 (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>). A sample size of 85 in each group would be sufficient to detect a difference of 3% on change in total body lean mass, the primary outcome variable, assuming a standard deviation of 6% based on the previous study (Bonney, Cornu et al. 2003), at 90% power and 5% level of significance. This sample size would also allow the detection of a 16.5 µg/L (16.5 ng/ml) difference in serum IGF-I concentration (SD 33 µg/L) (Schurch, Rizzoli et al. 1998), a main biochemistry outcome, at 90% power and 5% level of significance. This number has been increased to 110 per group (total of 220) to allow for a predicted drop-out from treatment of around 30% which we have reported in previous studies of a similar age group (Prince, Devine et al. 2006).

3.5 Data management and Statistical analysis

3.5.1 Data management

Data was entered from the hard copy source data into a Microsoft Access database designed for the study and then into SPSS (version 15.0). Stata Intercooled 9.0 software (StataCorp, College Station, Texas) was used to analyze SF-36 data by a designed program for calculation of summary statistics for SF-36 (Ryan 1999).

The initial screening of data revealed the extreme and missing values. These values were checked with the original hard records and the Access database. The entry errors were corrected and data re-exported to SPSS. The doctoral candidate was responsible for this process.

The normality of all the numerical variables have been examined by normality test, histograms and normal q-q plots, showing no severe skewness of these variables except physical activities. Physical activity (Mets/week) was transformed to $\log(\text{Mets/week})$ due to the severe right skewness.

3.5.2 Data analysis

All data were analyzed by SPSS (version 15.0; SPSS Inc, Chicago, IL) and graphs were made using Graph Pad PRISM (Version 4, Graph pad software, San Diego, CA) and Microsoft Office Excel 2003. Primary outcome measures in this study were DXA measured lean body mass, pQCT measured cross-sectional muscle mass, muscle strength, mobility and biochemistry variables. Explanatory covariates were the drink test consumption and 24-hour urine nitrogen. The homogeneity of variance and normality assumptions were checked by Levene and Kolmogorov-Smirnov test. Spearman's rank correlation test was used to examine the correlation between baseline participants' characteristics and muscle mass and size measurements (whole body lean mass, appendicular lean mass, calf muscle cross-sectional area and adjusted upper arm muscle area).

The 'intention to-treat' principle (U.S. Food and Drug Administration 1998) was used in analysis to measure the effectiveness of the protein supplementation while ignoring the lack of full compliance.

Intention-to-treat analysis (ITT) analysis is to

“Includes all randomized patients in the groups to which they were randomly assigned, regardless of their adherence with the entry criteria, regardless of the treatment they actually received, and regardless of subsequent withdrawal from treatment or deviation from the protocol”(Fisher, Dixon et al. 1990).

It has been widely used in analysis of randomized clinical trial data. Because, theoretically, in randomized clinical trials, patients randomly assigned in different treatment groups should have equal chance to be non-compliant. Therefore, ITT is the first analysis expected to be performed in a randomized clinical trial (Fisher 1999).

The primary outcome variables was analyzed using general linear model (GLM) repeated measures analysis consisting of time effect (within-subjects variables of baseline and one year) and treatment effect (between-subjects factors: drink group)

and the interactions of treatment and visits time. The advantage of GLM is that it allows the missing values and the variable intervals between visits. If the GLM analysis indicated a significant treatment effect or a significant effect of treatment and visits interaction, the treatment effects (changes since baseline) at year one was analyzed by univariate analysis of covariance (ANCOVA), with drink groups as the fixed factor and baseline values as covariates. The time effect was then evaluated by ANOVA paired-sample t-test in the treatment and control groups separately. Differences in categorical variables between groups were analyzed by chi-square test or logistic regression where appropriate. The significance level for test statistics was set at $P < 0.05$.

3.6 Ethic issues

The Medical and Allied Health and the Human Research Ethics Committee have approved this study. The major ethical issues of this study related to consent, confidentiality and the potential risks and discomforts to the participants.

Consent: All participants have received an explanation sheet to inform them in lay terms the purpose, demands, risks, methods, inconveniences and possible outcomes of the study by mail before they come to the screening visit. At the screening visit, an investigator explained the benefits and risks of participation in the study to each participant. Participants who agreed to participate signed the consent form with investigator's witness before undertaking any study related procedures. The consent form also states that they may withdraw from the study at any time without reason or justification.

Confidentiality: All information has been stored in a locked filing cabinet and all the blood and urine samples have been stored in a locked freezer. All identifiable information has been coded. All personal information such as name, address and phone number have been used for contact purpose only. The proposed publication will have no means of being able to identify.

Potential risk and discomfort: There are no substantial risks involved in this study. The radiation dose involved in this study is about 5.7 mSv. This is below the

NHMRC established guidelines for individuals participating in a medical research programme which states that the annual effective dose received by volunteers should be limited less than 5 mSv per year. The intervention in this study is the natural food supplement (whey protein based drink). Previous studies have showed that it is benefit to bone and muscle health in the elderly. The control group may also benefit from the study since the placebo drink contains the same amount of calcium as in the test drink but without additional protein. Blood sampling via venipuncture may involve mild physical and psychological discomfort to the participants. The physical discomfort is comparable to a pinprick.

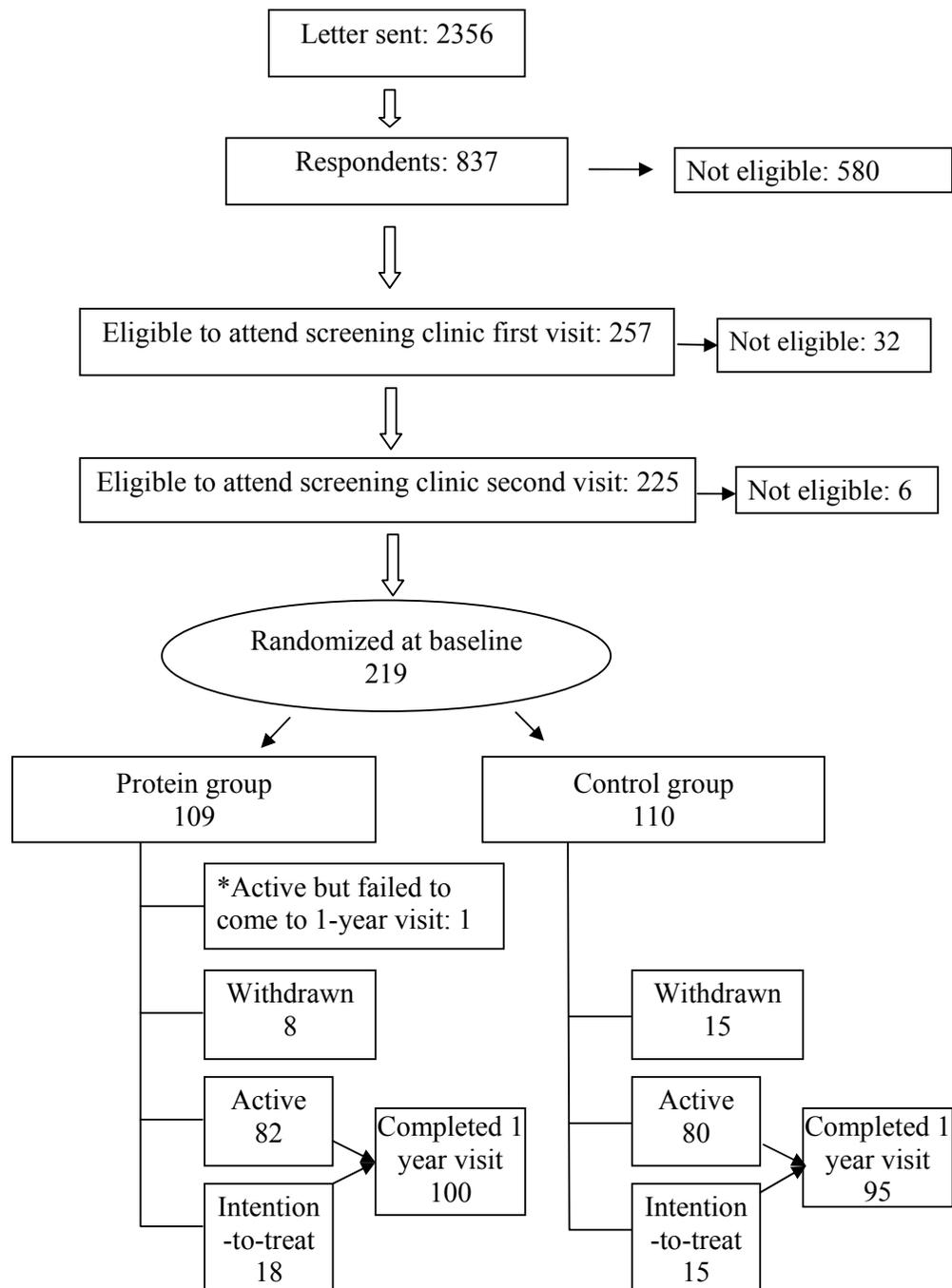
Chapter 4 Results

4.1 Baseline data analysis

4.1.1 Baseline data overview

A total of 219 elderly women were recruited from the Western Australian general population of women aged between 70 and 80 years. Letters were sent to 2356 individuals selected randomly from the electoral roll. A total of 837 (36% of letters sent) women responded, and 254 (30% of the respondents) women were eligible to attend the clinic screening. After the screening visits one and visit two, 35 subjects were found to be ineligible for the study and were excluded. Initially, as planned there were a total 220 women enrolled in the study, but one woman was excluded from the study after she had been randomized because she was found to be taking hormone replacement treatment. The final sample size was 219 women, who commenced the study, and 109 were in the protein supplement group and 110 allocated to the control group. These two groups are referred to as the protein group and the control group. The detail is shown in Figure 4.1.

Figure 4.1 Flowchart of the study population.



* The primary study is a 2-year trial. This subject stayed in the study and will attend the 2-year study, but was excluded from one year data analysis.

4.1.2 Management of baseline data analysis

Baseline data were examined the following way:

- Descriptive statistics of baseline characteristics, including demographic, general health status, anthropometry, body composition, physical activity, muscle strength, mobility, balance, and dietary intake.
- Correlations between baseline characteristics, including correlation between body composition, muscle strength, mobility and balance.
- Assessment of baseline sarcopenia by three defined methods.
- Relationship between baseline protein intake and baseline characteristics.
- Underreporting of baseline dietary intake and comparison to urinary nitrogen excretion.

The data was first explored to check the outliers and extreme values. All the outliers and extreme values were then checked. The original records and the entered errors were corrected. The distribution of each variable has been examined. Except for the physical activity results that are highly skewed, all other variables were normally distributed. Physical activity (Mets/week) was transformed into $\log(\text{Mets/week})$ to achieve a normal distribution. There were six participants who had Mets/week equal to zero. These participants were treated as missing value when the results were transformed to $\log(\text{Mets/week})$.

4.1.3 Baseline characteristics

4.1.3.1 Demographic characteristics at baseline

At baseline, the average age of the participants was 74.2 ± 2.7 years (mean \pm SD). There were no significant differences in age between the two groups ($p = 0.96$ in t-test). Among the 219 participants, 67% were born in Australia or New Zealand, 16% originally came from United Kingdom of Great Britain and Northern Ireland, and the remainder were from twenty different countries. Almost one half were married and one third were widowed. For the majority (73%) the highest education level attained was high school or equivalent. Thirty-five percent of them were smokers, but only

three of them (1% in the total number of participants) were current smokers. Most of them (93%) stated that they went outside on most days. Only four participants (2%) were using a walking aid. The mean age of the last menstrual period was 48 ± 7 (SD) years. The average number of children were 3 ± 2 (SD), ranging from zero to nine. Details are shown in Table 4.1. There were no significant differences between the two drink groups in any of their demographic variables (all p values >0.05 in chi-square test).

The majority (90%) of the participants had not experienced a fall in the past three months and only four (2%) had had multiple falls in the last three months. However, there was a significant difference in the fall rate between the two groups ($\chi^2 = 10.4$, $p = 0.02$ in Pearson chi-square test). It can be seen from Table 2.1, the protein group had greater frequencies of falls in the categories of fallen once (10 cases versus 2 cases) and multiple falls (4 cases versus 0) than the control group at baseline.

4.1.3.2 General health status at baseline

The self-reported general health status was assessed using the SF-36 questionnaire. The participants' mean score for each health domain was above or similar to the normal health level (Ware, Kosinski et al. 1994) as shown in Table 4.2. The participants had a high level of social functioning with a mean score of 90 out of 100. After adjustment for Australian population data, the summary standardised physical health score was 46 ± 10 , slightly lower than the normal Australia national level for adults which is 50 ± 10 (mean \pm SD) (Ware, Kosinski et al. 1994; Ryan 1999). Mental health status was 54 ± 8 , just above the normal Australia national mental health level which is 50 ± 10 (mean \pm SD) (Ware, Kosinski et al. 1994; Ryan 1999). There were no significant differences in these scores between the two drink groups (all p values >0.05 in the t-test).

Table 4.1 Baseline demographic data.

	Number (% of total)		
	Protein group	Control group	Total
Country of birth (n=219)			
Australia	72 (33%)	74 (34%)	146 (67%)
United Kingdom	17 (8%)	17 (8%)	34 (16%)
Others	20 (9%)	19 (8%)	39 (17%)
Marital status (n=219)			
Married	54 (25%)	50 (23%)	104 (48%)
Divorced	16 (7%)	13 (6%)	29 (13%)
Never married	1 (1%)	7 (3%)	8 (4%)
Separated	3 (1%)	2 (1%)	5 (2%)
Widowed	35 (16%)	38 (17%)	73 (33%)
Education level (n=219)			
Primary school	9 (4%)	8 (4%)	17 (8%)
High school or equal	75 (34%)	84 (39%)	159 (73%)
≥ Tertiary degree	25 (11%)	18 (8%)	43 (19%)
Frequency of going out (n=219)			
2-3 days	2 (1%)	7 (3%)	9 (4%)
4-5 days	2 (1%)	4 (2%)	6 (3%)
Most days	105 (48%)	99 (45%)	204 (93%)
Use a walking aid (n=219)			
No	107 (49%)	108 (49%)	142 (98%)
Yes	2 (1%)	2 (1%)	77 (2%)
Frequency of fall in the past 3 month (n=216)			
0	91 (42%)	104 (48%)	195 (90%)
1	10 (5%)	2 (1%)	12 (6%)
2	2 (1%)	3 (1%)	5 (2%)
≥3	4 (2%)	0 (%)	4 (2%)

Table 4.2 Baseline general health status assessed by SF-36 questionnaire.

	Mean \pm SD		
	Protein group	Control group	Total
Physical Function (n=219)	76 \pm 19	73 \pm 21	74 \pm 20
General Health (n=217)	76 \pm 17	76 \pm 14	76 \pm 15
Vitality (energy/fatigue) (n=219)	67 \pm 19	65 \pm 19	66 \pm 19
Mental Health (n=219)	82 \pm 14	82 \pm 15	82 \pm 14
Role limitation due to physical health problems (n=219)	75 \pm 36	73 \pm 38	74 \pm 37
Role limitation due to emotional problem (n=218)	86 \pm 26	84 \pm 30	85 \pm 28
Social Function (n=219)	90 \pm 19	90 \pm 17	90 \pm 18
Bodily Pain (n=219)	69 \pm 23	70 \pm 21	70 \pm 22
Standardised Physical Health Summary (n=216)	46 \pm 9	46 \pm 10	46 \pm 10
Standardised Mental Health Summary (n=216)	54 \pm 8	54 \pm 9	54 \pm 8

* The scores were adjusted for the Australia population data.

4.1.3.3 Anthropometry, body composition and physical activity level at baseline

Among the 219 study participants, 32% were in the normal range for the BMI (18.5 – 24.9 kg/m²), 44% of participants were classified as overweight (25 – 29.9 kg/m²), and 23% moderately obese (30 – 34.9 kg/m²). Only 1% were underweight (<18.5 kg/m²). As shown in Table 4.3, there were no significant differences in body composition measured by DXA and pQCT or any anthropometry measurements between the two drink groups, except that the control group had a slightly larger (0.8 cm) calf girth than the protein group at baseline (p = 0.049).

The physical activity level of participants was calculated as Mets (Met-minutes) per week. A few of the participants were extremely active, but the majority had Mets/week ranges from 855 to 4095 (interquartile range). There were 6 participants who had Mets/week equal to zero. Since the physical activity data was significantly positively skewed (skewness = 3.116), log transformation was used. The transformed data then had a normal distribution (skewness = -0.129). When the participants were grouped into three IPAQ physical activity levels, 15% of them had low levels of physical activity (<600 Mets/week), 48% of them had moderate levels of physical activity (600 – 3000 Mets/week) and 37% of them had high levels of physical activity (>3000 Mets/week).

Table 4.3 Baseline anthropometry, body composition and physical activity comparing the protein group with the control group.

	Mean \pm SD		
	Overall n=219	Protein group n=109	Control group n=110
Anthropometry			
Height (cm)	159.9 \pm 6.0	160.0 \pm 6.3	159.8 \pm 5.8
Weight (kg)	68.5 \pm 11.3	67.4 \pm 11.1	69.7 \pm 11.5
BMI (kg/m ²)	26.8 \pm 3.9	26.3 \pm 3.8	27.3 \pm 3.9
Waist circumference (cm)	88.7 \pm 9.6	87.5 \pm 9.5	90.0 \pm 9.6
Hip circumference (cm)	104.6 \pm 9.0	103.8 \pm 8.5	105.5 \pm 9.5
Waist-to-hip ratio	0.85 \pm 0.06	0.84 \pm 0.06	0.85 \pm 0.06
Arm girth (cm)	31.5 \pm 3.4	31.2 \pm 3.3	31.8 \pm 3.5
Triceps skinfold (mm)	28.7 \pm 8.3	28.2 \pm 8.1	29.1 \pm 8.5
CUAMA (cm ²) ^a	34.3 \pm 10.3	33.9 \pm 10.2	34.6 \pm 10.4
Calf girth (cm)	35.7 \pm 2.9	35.3 \pm 2.8	36.1 \pm 3.1*

Body composition measured by DXA and pQCT

Whole body lean mass (kg)	37.5 \pm 4.7	37.2 \pm 4.7	37.8 \pm 4.7
Appendicular lean mass (kg)	16.5 \pm 2.4	16.3 \pm 2.4	16.6 \pm 2.4
Whole body fat mass (kg)	26.1 \pm 7.5	25.2 \pm 7.2	27.1 \pm 7.7
% of whole body fat mass (%)	39.4 \pm 5.7	38.8 \pm 5.4	40.1 \pm 6.0
Calf muscle cross-sectional area measure by pQCT (cm ²)	31.2 \pm 5.6	30.7 \pm 5.1	31.7 \pm 5.9

Physical activity assessed by International Physical Activity Questionnaire

Mets/week ^b	2101 (855-4095)	2160 (1120-5201)	1826 (740-3779)
log(Mets/week)	3.31 \pm 0.46	3.38 \pm 0.44	3.26 \pm 3.3

^a CUAMA: corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π (triceps skinfold in cm))²/4 π – 6.5; ^b Values are median (interquartile range); * p < 0.05 in the independent sample t-test of the differences between two drink groups.

4.1.3.4 Muscle strength, mobility, balance and self-reported falls at baseline

There were no significant differences in hand grip strength or in any of the lower limb muscle strength measures between the two drink groups at baseline (see Table 4.4). Overall, the total mean knee and total hip muscle strength were 24.9 ± 8.4 and 51.2 ± 17.9 kg respectively. In the following analysis, the summed lower limb muscle strength (total knee strength and total hip strength) were also examined.

As shown in Figure 4.2, the majority of the participants (87%) were able to hold the tandem position (heel of one foot directly in front of the other foot) more than 9 seconds with their eyes open (scored 4). With their eyes closed, only about 28% could hold the tandem position for more than 9 seconds. There were no differences in the distributions of Romberg scores with the eyes-open test between the two drink groups ($p = 0.61$ in chi-square test). There was a significant difference in the distributions of Romberg scores with the eye-closed test between the two drink groups ($p = 0.03$ in chi-square test). The details of these distributions are shown in Appendix 20.

The overall prevalence of self-reported falling during the preceding three months was 10% (detail data is shown in Appendix 20). The prevalence of falls during the preceding three months was 15% in the protein group and 5% in the control group, and the difference was significant between the two groups ($\chi^2 = 5.0$, $p = 0.02$).

Table 4.4 Baseline muscle strength, mobility and balance tests comparing the protein group with the control group.

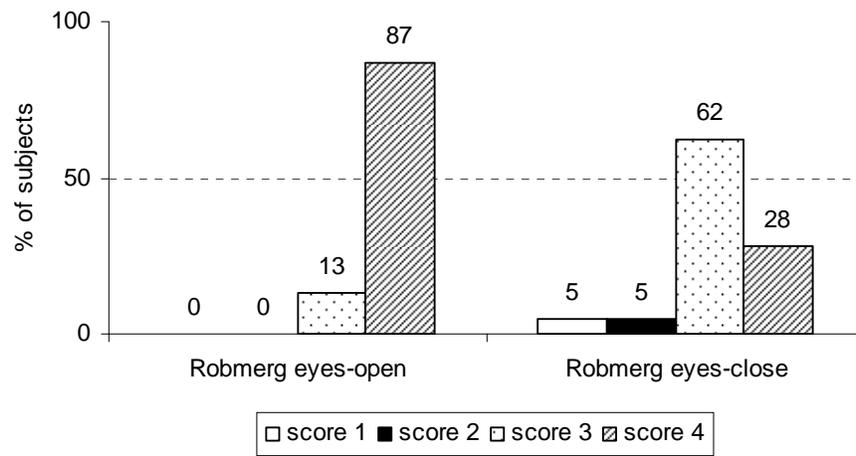
	Mean \pm SD		
	Overall n=219	Protein group n=109	Control group n=110
Muscle strength (kg)			
Hand grip strength	21.6 \pm 5.4	21.9 \pm 5.1	21.4 \pm 5.7
Ankle dorsiflexion	10.5 \pm 4.6	10.9 \pm 5.1	10.1 \pm 4.1
Knee flexion	9.3 \pm 3.8	9.2 \pm 4.0	9.4 \pm 3.7
Knee extension	15.6 \pm 6.2	15.4 \pm 5.4	15.8 \pm 6.9
Hip extension	16.5 \pm 6.5	17.0 \pm 6.6	16.0 \pm 6.3
Hip abduction	11.0 \pm 4.6	11.2 \pm 4.2	10.9 \pm 4.9
Hip flexion	11.9 \pm 4.5	12.1 \pm 4.5	11.6 \pm 4.4
Hip adduction	11.8 \pm 5.6	11.9 \pm 5.5	11.8 \pm 5.6
Total knee strength ^a	24.9 \pm 8.4	24.6 \pm 7.9	25.2 \pm 8.9
Total hip strength ^b	51.2 \pm 17.9	52.2 \pm 17.9	50.2 \pm 18.0
Mobility and balance tests			
Timed Up and Go (second)	8.0 \pm 1.4	7.9 \pm 1.3	8.0 \pm 1.5
Romberg (eyes-open) (0-4) ^c	4 (4 – 4)	4 (4 – 4)	4 (4 – 4)
Romberg (eyes-close) (0-4) ^c	3 (3 – 4)	3 (3 – 4)	3 (3 – 3)

^a Total knee strength = knee flexion + knee extension

^b Total hip strength = hip extension + hip abduction + hip flexion + hip adduction

^c Values are median (interquartile range)

Figure 4.2 The distribution of subjects of Romberg tests at baseline (n = 219).



4.1.3.5 Dietary intake at baseline

Baseline dietary protein intake was assessed by a 3-day food record. A 24-hour urinary nitrogen was assessed as an external biomarker of the protein intake. One of the participants in the protein group did not complete the 24-hour urine and the 3-day food diary.

Protein intake assessed by the 3-day food record was higher (76 ± 19 g/day or 1.14 ± 0.33 g/kg/day) than the estimation from the 24-hour urine nitrogen (66 ± 19 g/day or 0.98 ± 0.30 g/kg/day). Total energy intake was 7140 ± 1518 KJ/day and protein provided 19 ± 4 % of energy intake. Carbohydrate and fat intake provided 46 ± 7 % and 33 ± 6 % energy intake respectively. The details of other nutrient intakes are shown in Table 4.5. There were no significant differences in any of the nutrient intakes or 24-hour urinary nitrogen between the two drink groups at baseline (all p value > 0.05 in the independent sample t-test).

Table 4.5 Baseline dietary intake comparing the protein group with the control group.

	Overall n=218	Protein group n=108	Control group n=110
24-hour urine nitrogen (n = 218)			
24 hour urine nitrogen (g)	8.6 ± 3.0	8.8 ± 3.0	8.4 ± 3.0
Protein intake assessed by urine nitrogen (g/day) †	66 ± 19	67 ± 19	65 ± 19
Protein intake assessed by urine nitrogen (g/kg/day)*	0.98 ± 0.30	1.02 ± 0.32	0.95 ± 0.28
Dietary intake assessed by 3-day food record (n = 218)			
Energy (KJ/day)	7140 ± 1518	7166 ± 1590	7114 ± 1451
Protein (g/day)	76 ± 19	77 ± 22	76 ± 17
Protein (g/kg/day)	1.14 ± 0.33	1.17 ± 0.34	1.12 ± 0.31
Carbohydrate (g/day)	189 ± 47	187 ± 49	191 ± 45
Fat (g/day)	62 ± 19	63 ± 18	61 ± 20
Saturated-fat (g/day) (% of fat intake)	24 ± 9 (43%)	25 ± 9	24 ± 8
Polyunsaturated-fat (g/day) (% of fat intake)	9 ± 4 (17%)	9 ± 4	9 ± 4
Monounsaturated-fat (g/day) (% of fat intake)	22 ± 8 (40%)	23 ± 7	22 ± 9
Cholesterol (mg/day)	244 ± 103	245 ± 103	243 ± 103
Calcium (mg/day)	904 ± 382	927 ± 420	881 ± 342

	Overall n=218	Protein group n=108	Control group n=110
% of energy intake from protein (%)	19 ± 4	19 ± 4	19 ± 3
% of energy intake from fat (%)	33 ± 6	33 ± 5	32 ± 6
% of energy intake from saturated fat (%)	13 ± 3	13 ± 4	13 ± 3
% of energy intake from carbohydrate (%)	46 ± 7	45 ± 6	47 ± 7
% of energy intake from alcohol (%)	3 ± 4	3 ± 4	3 ± 4

Values are mean ± SD. † Protein intake (g) = 6.24 x (urinary nitrogen (g) + 2), * g/kg/day refers to gram per kilogram body weight per day.

4.1.3.6 Prevalence of sarcopenia at baseline

The prevalence of sarcopenia was calculated using the widely used cut-off point of appendicular lean mass (ALM)/height² < 5.454 kg/m² to define sarcopenia. The average of ALM/height² was 6.4 ± 0.8 (kg/m²) in the current study. Only 20 participants (9% of total study sample) were categorized as having sarcopenia according to this criterion. As can be seen in Table 4.6, both groups had a similar number of subjects who were classified as sarcopenic (Fisher's Exact test, F=0.36, p = 0.24), which means there was no significant difference in the prevalence of sarcopenia between two groups at baseline.

Table 4.6 Baseline prevalence of sarcopenia in two drink groups.

	Number of cases (%)		
	Protein group	Control group	Total
Sarcopenic	12 (11%)	8 (7%)	20 (9%)
Non-sarcopenic	97 (89%)	102 (93%)	199 (81%)
Total	109 (50%)	110 (50%)	219 (100%)

4.1.4 Baseline cross-sectional study one - the relationship of body composition with mobility, balance and upper and lower limb muscle strength

4.1.4.1 Correlation of body lean mass and muscle strength

Age, weight, BMI, physical activity, and protein and energy intake

Age was only negatively correlated with knee extension strength ($r = -0.21$, $p < 0.01$). Weight and BMI were positively correlated with hand grip strength and all lower limb strength measurements except hip abduction and adduction strengths (Table 4.7). No correlation was found between measures of muscle strength and physical activity, or protein or energy intake.

Body composition

Hand grip strength was positively correlated with all of the muscle mass and size measurements, including whole body lean mass, appendicular lean mass, adjusted appendicular lean mass, calf muscle cross-sectional area and corrected upper arm

muscle area, as shown in Table 4.7. Hand grip strength had the strongest correlation with appendicular lean mass ($r = 0.38$, $p < 0.01$) and the weakest correlation with whole body fat mass ($r = 0.14$, $p < 0.05$). After adjustment for age and BMI in linear regression, hand grip strength remained significantly associated with whole body lean mass (Beta = 0.44, $p < 0.001$) and appendicular lean mass (Beta = 0.44, $p < 0.001$), but its correlation with calf muscle cross-sectional area and upper arm muscle area disappeared.

Similar patterns were found in the relationships between lower extremity muscle strength values (ankle dorsiflexion, knee and hip strengths) and body composition as shown in Table 4.7. The lower limb muscle strength values had higher correlations with lean mass measurements (ranged from $r=0.16$ to 0.38) than with fat mass measurements (ranged from $r=0.07$ to 0.21) as expected. After adjusted age and BMI in the linear regression model, total knee strength remained significantly correlated with whole body lean mass (Beta = 0.23, $p = 0.007$), appendicular lean mass (Beta = 0.20, $p = 0.02$) and calf muscle cross-sectional area (Beta = 0.21, $p = 0.002$). The total hip strength was also significantly associated with whole body lean mass (Beta = 0.22, $p = 0.12$), appendicular lean mass (Beta = 0.22, $p = 0.01$), and calf muscle cross-sectional area (Beta = 0.15, $p = 0.04$) after adjustment for age and BMI. After the adjustment, ankle dorsiflexion was not associated with calf muscle cross-sectional area, but remained significantly associated with whole body lean mass (Beta = 0.24, $p = 0.005$) and appendicular lean mass (Beta = 0.20, $p = 0.03$).

In summary, hand grip strength was significantly correlated with whole body lean mass, appendicular lean mass and the association remained significant after adjustment for age and BMI. Lower limb strength including ankle dorsiflexion, total knee strength and total hip strength were significantly associated with whole body lean mass, appendicular lean mass and calf muscle cross-sectional area and the association remained significant after adjustment for age and BMI. The one exception was that no association was found between ankle dorsiflexion and calf muscle cross-sectional area.

Table 4.7 Correlation of muscle strength with baseline characteristics and body composition (n=219).

	Hand grip	Lower limb muscle strength (kg)								
	strength (kg)	Ankle dorsiflexion	Knee flexion	Knee extension	Hip extension	Hip abduction	Hip flexion	Hip adduction	Total knee	Total hip
Age (years)	-0.10	-0.11	-0.01	-0.21**	-0.07	0.01	-0.11	-0.06	-0.15*	-0.06
Height (cm)	0.26**	0.15*	0.18*	0.05	0.14*	0.10	0.11	0.11	0.11	0.13
Weight (kg)	0.25**	0.20**	0.24**	0.15*	0.19*	0.13	0.24**	0.11	0.22**	0.20**
BMI (kg/m ²)	0.14*	0.14*	0.20**	0.17*	0.17*	0.10	0.22**	0.09	0.22**	0.17*
Physical activity (log(Mets/week))	-0.01	-0.04	-0.07	0.03	-0.02	0.03	0.05	-0.03	0.00	0.02
Protein intake (g/d) †	0.07	-0.01	0.06	-0.01	-0.04	-0.05	-0.07	-0.02	0.04	-0.04
Energy intake (kcal/d) †	0.06	-0.02	0.01	0.08	0.01	-0.01	-0.05	-0.07	0.08	-0.03
Body composition ^a										
Whole body lean mass (kg)	0.36**	0.24**	0.32**	0.19**	0.25**	0.19**	0.24**	0.16*	0.28**	0.25**
ALM (kg)	0.38**	0.20**	0.33**	0.19**	0.26**	0.18**	0.24**	0.18**	0.28**	0.26**
Whole body fat mass (kg)	0.14*	0.15*	0.16*	0.10	0.13	0.07	0.21**	0.06	0.14*	0.14*
% of fat mass (%)	-0.03	0.07	0.05	0.03	0.03	-0.02	0.13	-0.03	0.04	0.04

	Hand grip	Lower limb muscle strength (kg)								
	strength (kg)	Ankle dorsiflexion	Knee flexion	Knee extension	Hip extension	Hip abduction	Hip flexion	Hip adduction	Total knee	Total hip
Calf muscle cross-sectional area (cm ²)	0.17*	0.14*	0.23**	0.22**	0.20**	0.15*	0.12	0.20**	0.26**	0.20**
CUAMA (cm ²)	0.18**	0.06	0.17*	0.05	0.07	0.09	0.12	0.08	0.12	0.11

Values are Spearman rank correlation coefficients. * p < 0.05; ** p < 0.01. † assessed by 3-day food record.

^a Whole body lean mass and fat mass, appendicular lean mass (ALM) and percentage of fat mass were derived from DXA; Calf muscle cross-sectional area was derived from pQCT measurements at 38% length of tibia; CUAMA: Corrected upper arm muscle area (cm²) = (Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.

4.1.4.2 Correlation of ‘Timed Up and Go’ (TUAG) and Romberg tests with body lean mass and muscle strength

Age, weight, BMI, physical activity, dietary protein and energy intakes

As shown in Table 4.8, age was significantly positively correlated with TUAG ($r=0.24$, $p < 0.01$) and negatively correlated with the Romberg eyes-open and the eyes-closed tests ($r = -0.15$, $p < 0.05$ and $r = -0.18$, $p < 0.01$ respectively).

Participants who were older were slower and took longer to complete the TUAG task, and had worse balance. The TUAG and Romberg tests were not correlated with height, weight, BMI, physical activity level, or dietary protein or energy intakes.

Body composition

The TUAG and Romberg tests were not correlated with any of the absolute and adjusted muscle mass derived from DXA measurements, or muscle size measured by calf girth and pQCT (calf cross-sectional muscle area). However, percentage body fat was significantly positively correlated with the TUAG test ($r = 0.14$, $p < 0.05$).

This means that participants with a higher percentage of body fat took a longer time to accomplish the TUAG task. After adjustment for age and height in the linear regression model, the percentage body fat remained significant. Romberg balance performance was not related to percentage body fat.

Muscle strength

The TUAG was inversely correlated with almost all lower extremity muscle strength tests which included ankle dorsiflexion ($r = -0.16$, $p < 0.05$), total knee strength ($r = -0.32$, $p < 0.001$) and total hip strength ($r = -0.20$, $p < 0.01$). The participants with stronger lower extremity strength were faster in performing the TUAG test. Knee extension strength had the highest correlation with TUAG ($r=0.34$, $p < 0.001$). No correlation was found between hip adduction and TUAG. Compared to hip strength, knee strength had a stronger correlation with TUAG performance (Table 4.8).

After adjustment for age in the linear regression model, the significant correlation between TUAG with ankle dorsiflexion strength (Coefficients Beta = -0.14 , $p = 0.03$), total knee (Coefficients Beta = -0.32 , $p < 0.001$) and total hip strength (Coefficients Beta = -0.20 , $p = 0.002$) remained.

The 'Romberg eyes-open' test was positively correlated with ankle dorsiflexion and hip extension strength ($r = 0.22$, $p < 0.001$ and $r = 0.14$, $p < 0.05$ respectively). After adjusting for age in the linear regression model, the significant correlation between 'Romberg eyes-open' and ankle dorsiflexion remained (Coefficients Beta = 0.20, $p = 0.002$) but the correlation with hip extension strength disappeared. The Romberg eyes-closed scores were not correlated with any of the muscle strength measurements. Hand grip strength was not correlated with mobility or balance performance in this study population.

In summary, both mobility (TUAG) and balance (Romberg) were inversely correlated with age, but not correlated with any lean body mass measurement including whole body lean mass, appendicular lean mass or calf muscle cross-sectional area. Mobility was significantly inversely correlated with percentage body fat, but significantly positively correlated with almost all lower extremity strength measurements after adjustment for age. The Romberg eyes-open test was only significantly positively correlated with ankle dorsiflexion after adjusted for age, but not with other lower extremity strengths.

Table 4.8 Correlation of Timed Up and Go (TUAG) and Romberg tests with baseline characteristics (n=219).

	TUAG (second)	Romberg (eyes open)	Romberg (eyes close)
Age (years)	0.24**	-0.15*	-0.18**
Height (cm)	-0.10	0.07	0.03
Weight (kg)	0.01	0.07	-0.03
BMI (kg/m ²)	0.06	0.06	-0.03
Physical activity (log(Mets/week))	-0.09	0.11	0.08
Protein intake (g/d) †	-0.03	0.04	-0.02
Energy intake (kcal/d) †	-0.09	-0.00	-0.02
Body composition ^a			
Whole body lean mass (kg)	-0.08	0.03	-0.01
% of whole body lean mass	-0.13	-0.07	0.07
Appendicular lean mass (kg)	-0.05	0.06	-0.02
Whole body fat mass (kg)	0.07	0.09	-0.05
% of whole body fat mass (%)	0.14*	0.06	-0.08
Calf cross-sectional muscle area (cm ²)	0.02	0.10	0.13
Muscle strength			
Hand grip strength (kg)	-0.11	0.11	-0.04
Ankle dorsiflexion (kg)	-0.16*	0.22**	0.10
Knee flexion (kg)	-0.21**	0.02	0.03
Knee extension (kg)	-0.34**	0.10	0.10
Hip extension (kg)	-0.24**	0.14*	0.11
Hip abduction (kg)	-0.14*	0.07	0.12
Hip flexion (kg)	-0.21**	0.08	0.06
Hip adduction (kg)	-0.07	0.07	0.13
Total knee strength (kg)	-0.32**	0.07	0.08
Total hip strength (kg)	-0.20**	0.11	0.11

Values are Spearman rank correlation coefficients. * p<0.05; ** p<0.01. † assessed by 3-day food record. ^a Whole body lean mass and fat mass, appendicular lean mass (ALM) and percentage of fat mass were derived from DXA; calf muscle cross-sectional area was derived from pQCT measurements at 38% length of tibia.

4.1.5 Baseline cross-sectional study two: assessing underreporting of dietary intake at baseline

Assessing dietary intake is always a difficult task that requires a systematic approach to minimizing measurement error. The most common error in dietary assessment is the underreporting or misreporting of food intake. Doubly-labeled water (DLW) which measures energy expenditure, is an excellent biomarker of energy intake. In this study, DLW was not measured. The EI:EE (ratio of energy intake and energy expenditure) provides an estimation of the degree of underreporting of energy both at the group and individual level (Black, Bingham et al. 1997). The ratio of urinary nitrogen and dietary nitrogen intake provides an estimation of underreporting of protein intake and identifies the most obvious under-reporters of energy (Black, Bingham et al. 1997).

Underreporting assessment method

For the analysis of underreporting, the energy intake and protein intakes were assessed by a 3-day food record. These results were examined using the Goldberg EI:BMR 'cut-off' method (Goldberg, Black et al. 1991) and 24-hour urinary nitrogen.

The reported energy intake (EI) by the 3-day food record was compared to the energy expenditure (EE) which was calculated using the adjusted basal metabolic rate (BMR). Firstly, the BMR for women aged over 60 years was calculated using the Schofield Equation (Schofield 1985) which was used in the 1985

FAO/WHO/UNU report:

$$\text{BMR} = 0.038 \times \text{weight} + 2.755$$

BMR is in MJ/day (megajoule/day) and weight is in kg.

In order to calculate the minimal energy intake for survival, BMR was adjusted by 1.27 (Goldberg, Black et al. 1991). The current study used 1.27 x BMR as the cut-off point for energy expenditure (EE). Although Goldberg and colleagues suggested that reported energy intakes of below 1.35 x BMR (known as CUT-OFF point 1 in their study) either in individuals or populations was most unlikely to represent

habitual intake, they used $1.55 \times \text{BMR}$ (known as CUT-OFF point 2 in their study) as the energy requirement for a sedentary life style (Goldberg, Black et al. 1991).

The ratio of EI:EE in this study can be calculated from the following equation (BMR $\times 1000$ was used in order to convert BMR to kJ from MJ from):

The ratio of EI:EE = Energy intake (kJ) / (BMR $\times 1000 \times 1.27$) (kJ)

The final equation for the ratio of EI:EE is as below:

The ratio of EI:EE = Energy intake (kJ) / ((0.038 \times weight + 2.755) $\times 1000 \times 1.27$) (kJ)

Participants were then grouped into three groups using the Black method, which is to defines underreporting, acceptable-reporting and over-reporting as EI:EE <0.76 , $0.76-1.24$ and >1.24 respectively (Black 2000) (EE in their study was measured by doubly-labelled water).

Characteristics of under-reporters

Using the method described above, 24 participants (11%) were categorized as under-reporters in the current study (Table 4.9). Compared to the over-reporters, the under-reporters had significantly higher body weight, BMI, waist and hip girth, triceps skinfold, arm girth, and calf girth. The under-reporters also had a significantly higher total body fat mass than acceptable-reporters and over-reporters. However, there were no significant differences in weight, BMI, waist and hip girths or percentage of whole body fat mass between under-reporters and acceptable-reporters. There were no differences in age and height between the groups. Compared to the acceptable-reporters and over-reporters, the under-reporters had significantly lower total energy intake, and protein, fat and carbohydrate intakes. However, the 24-hour urinary nitrogen showed no statistical differences across the three groups.

In summary, the under-reporters had larger body size, higher proportion of whole body fat mass compared to the over-reporters. They reported significantly lower total energy, protein, fat and carbohydrate intakes than both acceptable-reporters and over-reporters. However, 24-hour urinary nitrogen showed that protein intakes were

not significantly different across the groups, indicating that underreporting of protein intake was likely to have occurred in this sample.

The agreement between protein intake assessed by 3-day food record and 24-hour urinary nitrogen by BMI groups

There was no difference in the 24-hour urine nitrogen between the three groups Table 4.10. As this method is used as external validation of dietary protein intake, the assumption is it more accurately reflects dietary protein intake and will show the degree of underreporting or misreporting. Comparing the protein intake assessed by the 3-day food record with the protein intake assessed by 24-hour urinary nitrogen by BMI groups, no significant differences were found between groups (Table 4.10). There were no significant differences in the ratio of protein intake assessed by the two assessment methods across the BMI groups either ($p = 0.45$). Therefore, by using 24-hour urinary nitrogen as external recovery biomarker representing a more accurate assessment of protein intake, participants with a larger body size should have been consuming more protein than they reported eating. As shown in Table 4.9, the reported protein intake (57.0 ± 12.6 g) was lower than protein intake assessed by 24-hour urinary nitrogen (61.1 ± 19.1) in the under-reporters. In contrast, the reported protein intake was higher than protein intake assessed by 24-hour urinary nitrogen in those acceptable-reporters (74.4 ± 15.9 g vs. 65.7 ± 16.4 g) and over-reporters (92.2 ± 20.2 g vs. 70.0 ± 24.6). The reason for underreporting may be due to the recording process which could affect eating habits during the recording period, known as the 'Hawthorne' effect. Subjects improve an aspect of their behaviour simply in response to the fact that they are being studied (McCarney, Warner et al. 2007).

Table 4.9 Baseline characteristics of under-reporters, acceptable reporters, and over-reporters defined by the EI:EE ratios (n=218) *.

	Under- reporters EI:EE <0.76 n = 24	Acceptable- reporters EI:EE 0.76–1.24 n = 145	Over-reporters EI:EE >1.24 n = 49	P value of ANOVA test
Age (year)	73.3 ± 2.3	74.5 ± 2.7	74.0 ± 2.8	0.10
Height (cm)	160.3 ± 8.1	159.9 ± 5.8	159.4 ± 5.2	0.83
Weight (kg)	74.1 ± 13.4 ^c	69.5 ± 10.6 ^c	62.8 ± 10.2 ^{ab}	<0.001
BMI (kg/m ²)	28.7 ± 3.6 ^c	27.2 ± 3.6 ^c	24.8 ± 4.1 ^{ab}	<0.001
Waist girth (cm)	93.1 ± 10.1 ^c	89.5 ± 8.9 ^c	84.4 ± 10.1 ^{ab}	<0.001
Hip girth (cm)	109.9 ± 10.3 ^c	105.5 ± 8.5 ^c	99.6 ± 7.8 ^{ab}	<0.001
Waist-to-hip ratio	0.85 ± 0.06	0.85 ± 0.06	0.85 ± 0.06	0.96
Triceps skinfold (mm)	29.3 ± 8.0	29.7 ± 8.2 ^c	25.4 ± 8.1 ^b	0.006
Arm girth (cm)	32.8 ± 3.4 ^c	31.9 ± 3.2 ^c	29.7 ± 3.4 ^{ab}	<0.001
Calf girth (cm)	36.5 ± 3.6	35.8 ± 2.8	34.8 ± 2.7	0.04
Physical activity (log(Mets/week))	3.31 ± 0.45	3.31 ± 0.48	3.34 ± 0.41	0.95

Dietary intake assessed by 3-day food record

Energy intake (kJ/day)	4696 ± 607 ^{bc}	6929 ± 986 ^{ac}	8959 ± 908 ^{ab}	<0.001
Protein intake (g/day)	57.0 ± 12.6 ^{bc}	74.4 ± 15.9 ^{ac}	92.2 ± 20.2 ^{ab}	<0.001
Protein intake (g/kg/day)	0.78 ± 0.17 ^{bc}	1.08 ± 0.24 ^{ac}	1.49 ± 0.30 ^{ab}	<0.001
Fat intake (g/day)	36.5 ± 7.8 ^{ab}	60.0 ± 14.6 ^{ac}	80.9 ± 16.0 ^{ab}	<0.001
Carbohydrate intake (g/day)	130.7 ± 26.5 ^{ab}	183.4 ± 33.3 ^{ac}	234.6 ± 49.9 ^{ab}	<0.001
% energy from protein	21.3 ± 4.7 ^{bc}	18.7 ± 3.2 ^{ac}	17.8 ± 3.2 ^{ab}	<0.001
% energy from fat	29.5 ± 4.9 ^{bc}	32.5 ± 5.6 ^a	34.1 ± 5.9 ^a	0.005
% energy from carbohydrate	48.2 ± 6.6	45.8 ± 6.4	45.0 ± 7.3	0.16

24-hour urinary nitrogen

24-hour urinary nitrogen (g/day)	7.77 ± 3.06	8.51 ± 2.63	9.20 ± 3.94	0.15
Protein intake assessed by	61.1 ± 19.1	65.7 ± 16.4	70.0 ± 24.6	0.15

	Under-reporters EI:EE <0.76 n = 24	Acceptable-reporters EI:EE 0.76–1.24 n = 145	Over-reporters EI:EE >1.24 n = 49	P value of ANOVA test
urinary nitrogen (g/day)				

Body composition †

Whole body lean mass (kg)	38.4 ± 5.5	37.6 ± 4.7	36.5 ± 4.1	0.21
Appendicular lean mass (kg)	16.8 ± 3.1	16.5 ± 2.3	16.0 ± 2.2	0.35
Whole body fat mass (kg)	30.7 ± 8.2 ^{bc}	26.9 ± 6.8 ^{ac}	21.4 ± 7.0 ^{ab}	<0.001
% of whole body fat mass	42.9 ± 4.5 ^c	40.3 ± 5.0 ^c	35.2 ± 6.3 ^{ab}	<0.001
Calf muscle cross-sectional area (cm ²)	30.1 ± 5.6	31.1 ± 5.0	32.1 ± 7.0	0.35
Corrected upper arm muscle area (cm ²)	38.4 ± 11.0 ^c	34.4 ± 10.3	31.6 ± 9.5 ^a	0.03

Results are mean ± SD. * EI (energy intake in kJ) was assessed by 3-day food record. EE (energy expenditure in kJ) = ((0.038 x weight + 2.755) x 1000 x 1.27) (kJ).

† Whole body lean and fat mass, appendicular lean mass and percentage of whole body fat mass were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.

^a significantly different from the 1st tertile, ^b significantly different from the 2nd tertile,

^c significantly different from 3rd tertile (ANOVA with Tukey's test, p<0.05).

Table 4.10 Baseline protein intake assessed by 24-hour urinary nitrogen and by 3-day food record by BMI groups.

	Protein intake measured by 24-hour urinary nitrogen (g)	Protein intake assessed by 3-day food record (g)	Ratio of protein intake assessed by two methods
BMI <25 (n=73)	64.7 ± 20.1	77.4 ± 18.9	0.86 ± 0.27
BMI 25-29.9 (n=95)	65.4 ± 15.5	74.9 ± 20.5	0.92 ± 0.32
BMI ≥30 (n=50)	69.7 ± 22.4	78.1 ± 17.9	0.91 ± 0.30
P value of ANOVA test	0.58	0.30	0.45

Values are in mean ± SD.

The distribution of the under-reporters at baseline

As underreporting may affect the results relating to the dietary intake, the distribution of the under-reporters at baseline was assessed by chi-square test. As can be seen in Table 4.11, the proportion of under-reporters, acceptable reporters and over-reporters were equally distributed in the two drink groups ($\chi^2=1.217$, $p = 0.544$).

Table 4.11 The distribution of the under-reporters in the protein supplement group and the control group at baseline.

	Number (%)		
	Protein group	Control group	Total
Under-reporters	13 (12%)	11 (10%)	24 (11%)
Acceptable reporters	68 (63%)	77 (70%)	145 (66.5%)
Over-reporters	27 (25%)	22 (20%)	49 (22.5%)
Total	108	110	218

4.1.6 Baseline cross-sectional study three – the relationship between the habitual dietary protein intake and body composition at baseline

Participants were grouped into three groups according to their protein intake as assessed by the 3-day food record. Protein intakes were <69 g/day, 69-83 g/day, and >83 g/day in the 1st, 2nd, and 3rd tertiles respectively. Total energy intake, other macronutrient intakes and calcium intake had similar patterns to the protein intake. That is, the nutrient intakes were significantly different across the protein groups with the lowest energy and nutrient intakes in the 1st tertile and highest energy and nutrient intakes in the 3rd tertile as shown in Appendix 17.

Whole body lean mass and appendicular lean mass were significantly lower in the 1st tertile compared to the 2nd and 3rd tertiles of baseline protein intake (Figure 4.3 A&B). There were no differences in whole body fat mass or percentage whole body fat across the tertile of protein intake groups. The adjusted appendicular lean mass (ALM/height²) and calf muscle cross-sectional area showed similar patterns as the whole body lean mass. That is, the participants in the 1st tertile of protein intake had a significantly lower adjusted appendicular lean mass and calf muscle cross-sectional area than those in the 1st tertile than in the 3rd tertile (Figure 4.3 C&D). There was no difference in the corrected upper arm muscle area across the tertile of protein intake groups.

There were no significant differences in age, weight, BMI, self-reported health status, physical activity level, mobility or balance functions, or any of the muscle strength measurements across the tertile of protein intake groups (details are shown in Appendix 17). However, participants in the 2nd tertile were significantly taller than those in 1st tertile (161.2 ± 5.3 cm and 158.3 ± 6.4 cm respectively, $p < 0.05$).

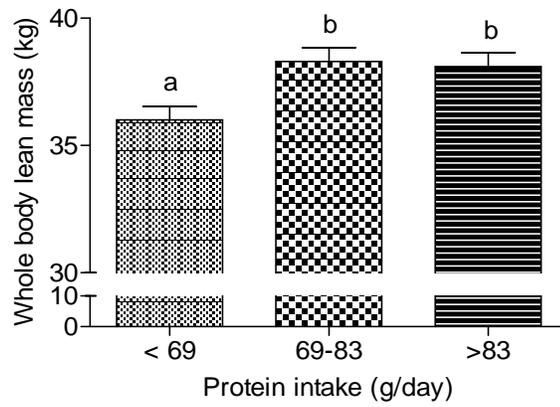
ANCOVA analysis with Bonferroni test was therefore used to further examine muscle mass and size by tertile of protein intake adjusted for height. The differences in these muscle mass and size measurements between the tertile of protein intake groups disappeared (Appendix 18).

As underreporting dietary intake may affect the analysis results associated with the dietary intake, all the analyses in this section have been repeated after the exclusion of the under-reporters. There was no difference in the results when the underreporters were included or excluded. The exception is that, in the ANCOVA analysis with Bonferroni test, after adjusted for height, the lowest protein intake group remained significantly lower in whole body lean mass than the highest protein intake group (Figure 4.4 A), and also remained significantly lower in appendicular lean mass than the acceptable protein group (Figure 4.4 B). However, when total energy intake was entered into the ANCOVA model as a covariate, the positive correlation between protein intake and whole body and appendicular lean mass disappeared.

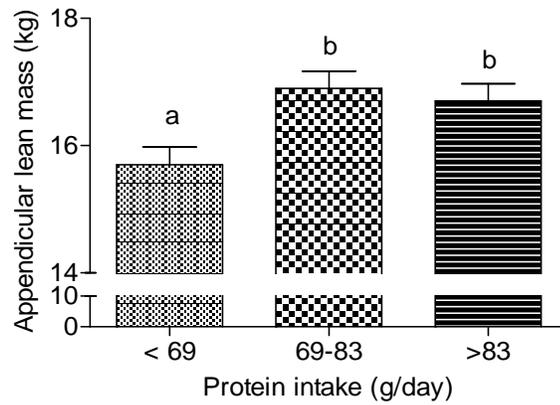
In summary, participants in the lowest protein intake group (1st tertile) had significantly lower whole body lean mass and appendicular lean mass than those in the acceptable protein intake group (2nd tertile) or highest protein intake groups (3rd tertile). This relationship was independent of total body fat mass. These differences disappeared after adjustment for height and total energy intake. The results were similar in these analyses after excluding the under-reporters. There were no significant differences in body functional tests or muscle strength measurements between participants who were having a lower or higher protein intake in their habitual diet at baseline.

Figure 4.3 (A-D) Baseline body composition by tertiles of protein intake.

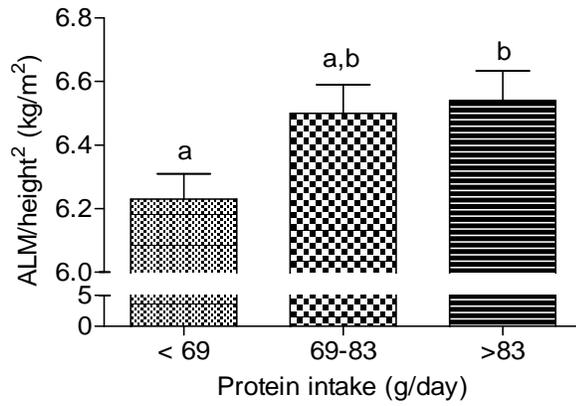
A. Whole body lean mass by tertiles of protein intake



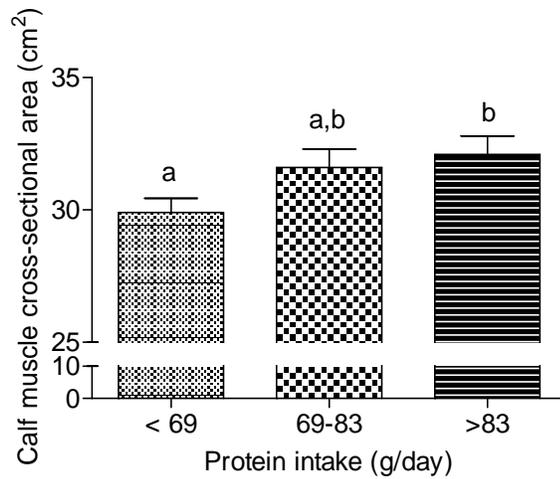
B. Appendicular lean mass by tertiles of protein intake



C. ALM/height² by tertiles of protein intake



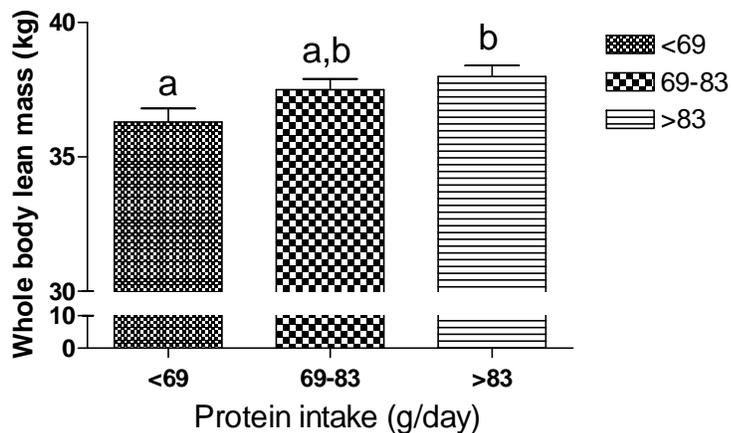
D. Calf muscle cross-sectional area by tertiles of protein intake



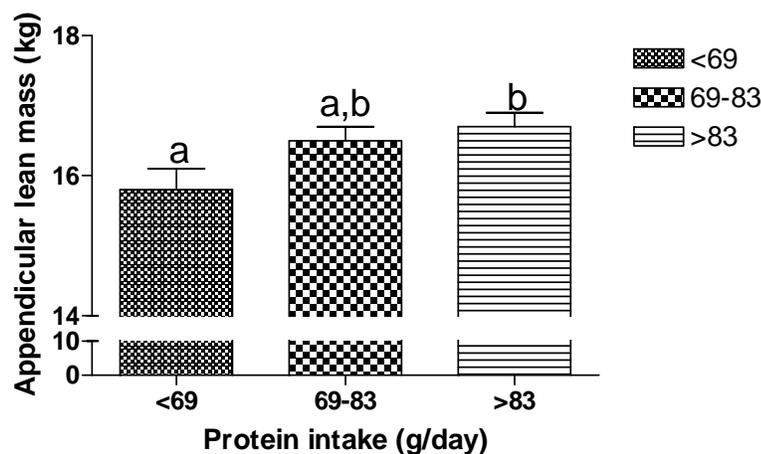
Body composition (whole body lean mass, appendicular lean mass, ALM/height²) was assessed by DXA, calf muscle cross-sectional area was assessed by pQCT, and protein intake was assessed by 3-day food record. Values are reported as mean \pm SD. Groups with different lower case letters are significantly different, $p < 0.05$ (ANOVA with Tukey test)

Figure 4.4 (A-B) Baseline body composition by tertile of protein intake after excluded the under-reporters in the analysis and adjusted for height.

A. Whole body lean mass by tertiles of protein intake



B. Appendicular lean mass by tertiles of protein intake



Estimated marginal mean (SE) of whole body lean mass and appendicular lean mass by baseline protein intake in tertiles (after excluding the under-reporters in the analysis). The values were adjusted for baseline height. Groups with different lower case letters are significantly different, $p < 0.05$ (ANCOVA with Bonferroni test).

4.1.7 Differences in baseline characteristics and compliance between the two drink groups and between those who withdrew and those who completed the one year intervention

4.1.7.1 Differences between the two study groups in baseline characteristics of subjects who completed the one year study

There were no significant differences in the baseline characteristics (e.g. age, physical activity, anthropometry measurements, muscle mass and size, muscle strength and body functional tests) between the two drink groups (details are shown in Appendix 21). The exception was when participants in the protein supplementation group were compared to the control group at baseline, they had significantly lower waist girths (protein group: 87.1 ± 9.4 ; control group: 90.3 ± 9.6 ; $p=0.02$) and whole body fat mass (protein group: 24.8 ± 7.2 ; control group: 27.0 ± 7.6 ; $p=0.04$). These differences would not affect the following comparison analysis of drink effects because for the primary outcomes, body lean mass and muscle strength, baseline values are adjusted for in the analysis models as covariates.

4.1.7.2 Differences in compliance at one year between two drink groups

Assessed by returned drink bottles

Compliance was calculated based on the number of the drink bottles that participants returned at one year. There were a total of 179 participants (91 in the protein group and 88 in the control group) who returned the consumed drink bottles including those who reported consuming zero bottles of drink. There were 16 participants who failed to return any drink bottles and they were treated as missing values in the analysis.

The mean consumption of drinks at one year in the protein group and the control group were 190 ± 94 and 152 ± 92 bottles respectively. The protein group consumed 38 (11, 66) (mean and 95% CI) more bottles than the control group. To be considered 100% compliant, participants were required to consume a total of 365 bottles over the period of a year. The overall compliance was 47% and the distribution was shown in Figure 4.5. The compliance of the protein group and the control group were 52% and 42% respectively. The compliance was significantly

different between the two groups ($p=0.007$ in two-sample t-test). Among 179 participants who were in the compliance analysis, 141 (79%) participants' recorded a compliance of less than 70%, and 38 (21%) participants' had a compliance greater than 70% or higher. Among these 38 high compliance participants, only eight (4% out of 179 participants) consumed more than 90% of their allocated drinks. Of these participants, 25 were from the protein drink group, and 13 were from the control drink group.

Further ANCOVA analysis, which included adding the BMI as a covariate, showed that there was a similar decline in the trend of drink consumption with increasing baseline BMI in both groups (Figure 4.6). However there was no significant differences in compliance by BMI groups within the same drink group.

Compliance to the Test Drink as Assessed by 24-hour urinary nitrogen

Compliance was also tested by 24-hour urinary nitrogen measurements at one year. The 24-hour urinary nitrogen of the protein group had increased significantly over the placebo group (protein group: +1.3 g/day, placebo group: -0.5 g/day, $p<0.05$) at one year as anticipated (detail is shown in Table 4.12). There was no difference in baseline urinary nitrogen between the two drink groups (protein group: 8.8 ± 3.1 g/day, $n=100$; placebo group: 8.7 ± 3.0 g/day, $n=93$; $p=0.96$). As there was no difference at baseline between the two drink groups, the significant difference in 24-hour urinary nitrogen at one year between the two groups indicates a moderate compliance of the participants to the protein drink.

Figure 4.5 The distribution of compliance with drink consumption (n=179).

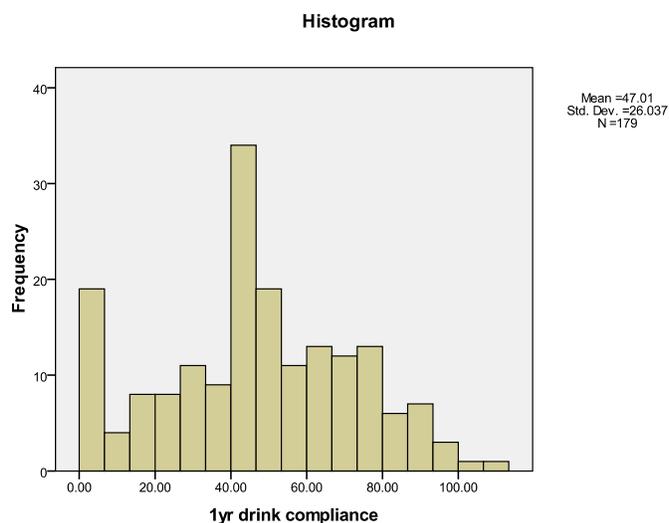
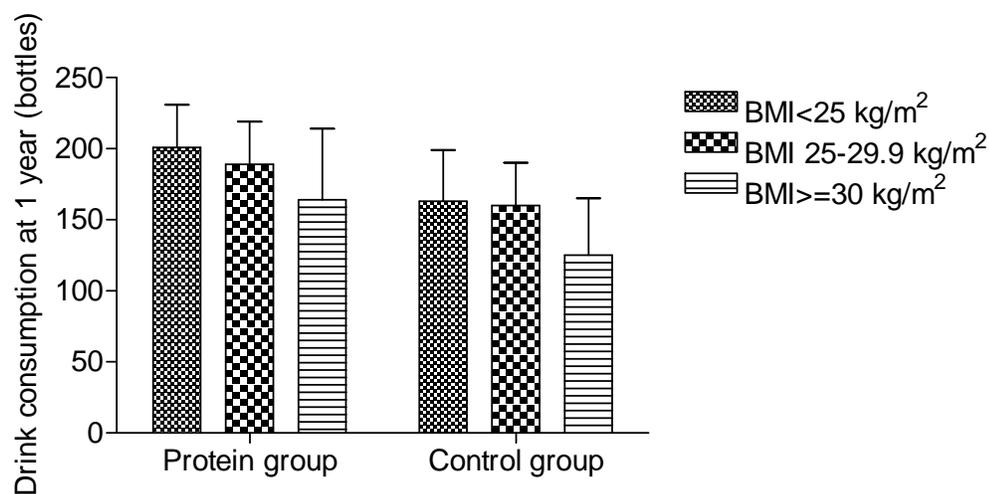


Figure 4.6 Drink consumption at one year by drink groups and baseline BMI.



Drink consumption was estimated by the number of empty drink bottles that participants returned after one year (protein group n = 91, placebo group n = 88). Values are estimated marginal mean and 95% confidence interval (ANCOVA with Bonferroni test) after adjusted BMI as covariate.

Table 4.12 Changes in 24-hour urinary nitrogen at one year in two drink groups.

	Protein group (n = 99)			Placebo group (n = 94)		
	Value at 1 year	Absolute change from baseline	% change from baseline (%)	Value at 1 year	Absolute change from baseline	% change from baseline (%)
24-hour urinary nitrogen (g)	10.1 ± 3.0 ¹ *	1.3 (0.5, 2.0) ² *	26 (15, 38)*	8.2 ± 3.1	-0.5 (-1.2, 0.2)	0.1 (-8.2, 8.0)
Protein intake assessed by 24-hour urinary nitrogen (g/day) †	75.8 ± 18.5*	8.0 (0.5, 12.7)*	19 (11, 27)*	63.9 ± 19.6	-3.1 (-7.3, 1.1)	-1 (-7, 5)

¹ Mean ± SD (all such values). ² Mean (95% confidence interval). Absolute change = value at 1 year – value at baseline. Percentage change = (value at 1 year – value at baseline)/value at baseline x 100. † Protein intake (g) = 6.24 x (urinary nitrogen (g) + 2). * p < 0.05 compared to the control group tested by the independent-sample t-test.

4.1.7.3 Differences in baseline characteristics between those who withdrew and those who completed the one year intervention

A total of 23 participants (protein group n=8, control group n=15) withdrew and 195 completed the study at one year. Twenty-one percent of those who withdrew (a total of 5 participants: 3 in the protein group and 2 in the control group) stated they did so because they felt ill after taking the drink, (this included nausea, constipation and gastro-intestinal discomfort). Thirteen percent withdrew due to not liking the drink (a total of 3 participants: 2 in the protein group and 1 in the control group). Eight percent (one participant in each of the drink groups) withdrew due to medical reasons. The majority (54%: 3 in the protein group and 10 in the control group) withdrew without giving any specific reasons. Compared to those who completed the one year study, the withdrawals had significantly lower scores in self-reported physical health status (withdrawals: 40.9 ± 11.0 , completed: 46.4 ± 9.2 , $p=0.007$ in t-test), and significantly lower 24-hour urinary nitrogen (withdrawals: 7.07 ± 2.09 , completed: 8.77 ± 3.06 , $p=0.009$ in t-test). The withdrawals also had lower physical activity level and lower ankle dorsiflexion strength than those who completed the study but the differences were not statistically significant (both p values =0.08). There were no significant differences in other baseline characteristics between the participants who withdrew and those who completed the one year intervention (details are shown in Appendix 22).

4.2 Data analysis after one year of intervention

At one year, of the 219 participants randomized at baseline, 23 women (11%) had withdrawn from the study (8 from the protein group and 15 from the control group). One participant could not participate in the one year examination because of a hip replacement operation and was excluded from the one year data analysis. The final number of participants who completed the one year study was 195. Amongst the 195 participants, 100 were in the protein supplement group and 95 were in the control group. The details are shown in Figure 4.1, the flow chart of the study participants.

4.2.1 The effect of one year protein supplement intervention on anthropometry, physical activity level, and self-reported health status

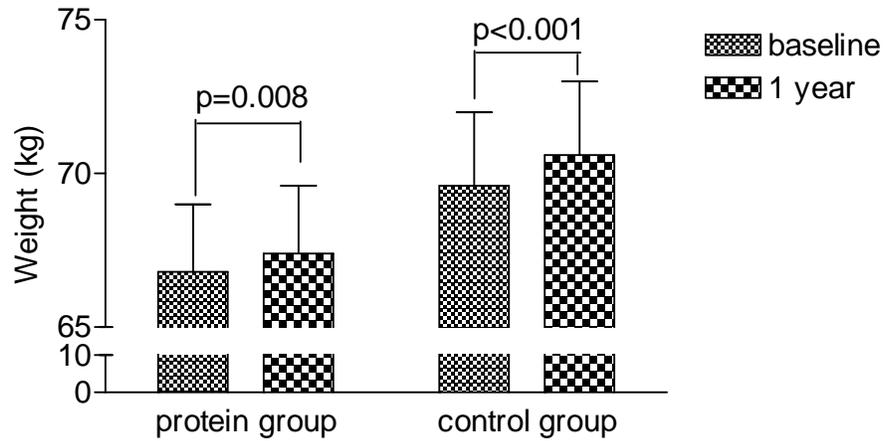
Weight ($p < 0.001$), BMI ($p < 0.001$), triceps skinfold ($p=0.007$) and calf girth ($p<0.001$) showed a significant time effect in the GLM (general linear model) repeated measures tests. Therefore the study participants overall had increased significantly in these variables at one year. When the drink groups were analysed separately using a paired sample t-test, both groups showed a significant increase in weight (Figure 4.7), BMI and calf girth at one year (all p values <0.05).

There were a significant drink treatment effect for BMI ($p = 0.04$), waist ($p = 0.02$) and waist-to-hip ratio ($p = 0.04$) at one year as shown in Table 4.13. ANCOVA analysis was performed for these three variables. The drink treatment effect on BMI disappeared ($p = 0.36$) after adjustment for age and baseline BMI. After adjustment for baseline waist, age and height, the treatment effect on waist ($p = 0.83$) and waist-to-hip ratio also disappeared ($p = 0.52$). There was no difference for any other anthropometry measurements (including weight), physical activity, or self-reported health status at one year between the two drink groups using the GLM (general linear model) repeated measures test (all p values >0.05 in the models).

These results indicate that there was no protein treatment effect for the following: weight, height, BMI, waist or hip girth, waist-to-hip ratio, physical activity and self-reported health status. Furthermore there were no significant interactions of the drink treatment and the time effect for anthropometry, physical activity and self-reported health status.

The details of the absolute and percentage changes of these variables at one year are shown in Table 4.13. A subgroup (participants whose compliance $>70\%$) analysis was performed using GLM repeated measures test. There was a significant time effect on weight gain but there was no protein treatment effect ($F = 1.411$, $p = 0.237$). Other results were similar to the results as using the whole study population data in the analysis.

Figure 4.7 Weight at baseline and one year in participants who completed one year study (comparing the protein group with the control group).



(The protein group $n = 100$, and the placebo group $n = 95$. Values are mean and 95% confidence interval. The within drink group changes between baseline and one year were tested by the paired sample t-test.)

Table 4.13 The absolute and percentage changes in anthropometry, physical activity and health status after one year intervention for the protein group and the control group.

	Absolute change		Percentage change (%)		P values	P values	P values
	Protein group	Control group	Protein group	Control group	(1)	(2)	(3)
Height (cm)	-0.2 (-0.2, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	-0.1 (-0.1, 0.0)	0.99	0.22	0.03
Weight (kg)	0.6 (0.2, 1.0)	1.0 (0.6, 1.5)	0.8 (0.2, 1.5)	1.5 (0.9, 2.2)	0.06	0.15	0.13
BMI (kg/m ²)	0.3 (0.1, 0.5)	0.4 (0.3, 0.6)	1.0 (0.4, 1.7)	1.6 (1.0, 2.3)	0.04	0.23	0.55
Waist girth (cm)	0.3 (-0.6, 1.2)	0.3 (-0.5, 1.3)	0.4 (-0.6, 1.5)	0.4 (-0.6, 1.5)	0.02	0.91	0.80
Hip girth (cm)	-0.1 (-0.7, 0.5)	-0.2 (-0.8, 0.4)	-0.1 (-0.7, 0.5)	-0.2 (-0.7, 0.3)	0.16	0.81	0.32
Waist-to-hip ratio	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.6 (-0.4, 1.6)	0.7 (-0.3, 1.7)	0.04	0.79	0.51
Physical activity (log(Mets/week))	0.0 (-0.1, 0.1)	-0.0 (-0.1, 0.1)	-0.3 (-3.5, 2.8)	2.1 (-1.1, 5.3)	0.26	0.30	0.62
SF-36 standard physical health (0-100)	0.5 (-1.0, 2.0)	-0.2 (-1.6, 1.2)	2.2 (-1.5, 5.8)	1.3 (-2.3, 4.8)	0.54	0.50	0.19
SF-36 standard mental health (0-100)	-1.4 (-3.2, 0.3)	-0.5 (-2.4, 1.3)	-1.4 (-5.3, 2.4)	0.9 (-3.2, 5.0)	0.69	0.49	0.58

Values are mean and 95% confidence interval. The protein group n =100, and the control group n =95. Absolute change = value at 1year – value at baseline. Percentage change = (value at 1 year – value at baseline)/value at baseline x 100. P values (1) were for the treatment effect derived from General Linear Model (GLM) repeated measures tests using the whole study population data (n = 195), p values (2) were for the interaction of treatment and visit time in the model. P values (3) were for the treatment effect derived from GLM model using data of participants (n = 38, 25 in the protein group and 13 in the control group) whose compliance > 70%.

4.2.2 The effect of one year protein supplement intervention on muscle mass and size and body composition

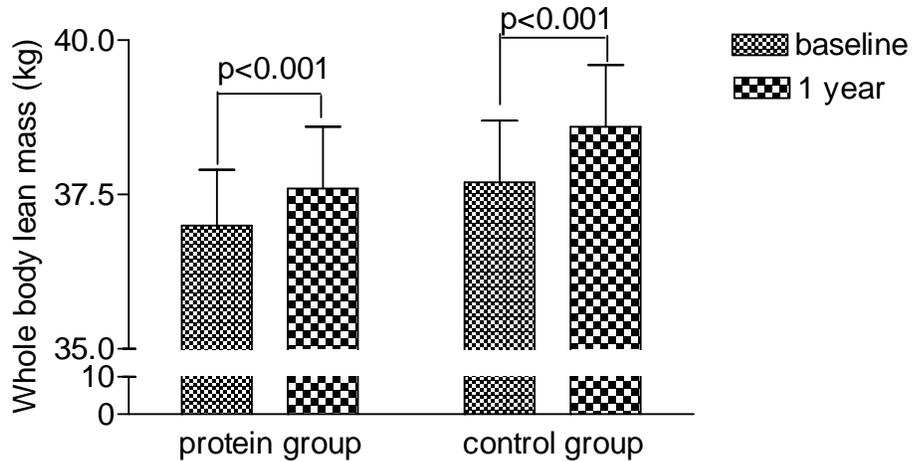
Whole body lean mass ($p < 0.001$), appendicular lean mass ($p < 0.001$), and total fat mass ($p < 0.001$) showed a significant time effect in the GLM (general linear model) repeated measures tests. This means that the participants overall (both in the protein supplemented group and the control group) increased in these measurements as a result of the one year intervention. The percentage of whole body lean mass and fat mass measured by DXA or calf muscle cross-sectional area did not change at one year.

There was no difference for whole body lean mass, appendicular lean mass, or calf muscle cross-sectional area at one year tested between two drink groups using the GLM (general linear model) repeated measures test (all p values > 0.05 in the models) as shown in Table 4.14, which means there was no protein treatment effect for any of these variables.

There were no significant interactions of treatment and time for whole body lean mass, appendicular lean mass and calf muscle cross-sectional area. When the trial and control drink groups were analysed separately using a paired sample t-test, both groups showed a significant increase in whole body lean mass (protein group: +1.6%, $p < 0.05$; control group: +2.3%, $p < 0.05$) (Figure 4.8) and appendicular lean mass (protein group: +1.3%, $p < 0.05$; control group: +1.8%, $p < 0.05$) (Figure 4.9). However, only the control group had a significant increase in total fat mass, (+1.5%, $p = 0.04$) as shown in Figure 4.10, and the changes between two drink groups were not significant. ANCOVA analysis was performed by adjusting for baseline age and height and controlled for baseline total body fat mass, and this showed there was no difference in changes between the two drink groups at one year (drink effect: $F = 1.23$, $p = 0.27$). The percentage of body fat mass slightly decreased in both group by 0.5% at one year (Table 4.14), but the changes were not statistically significant for either drink group.

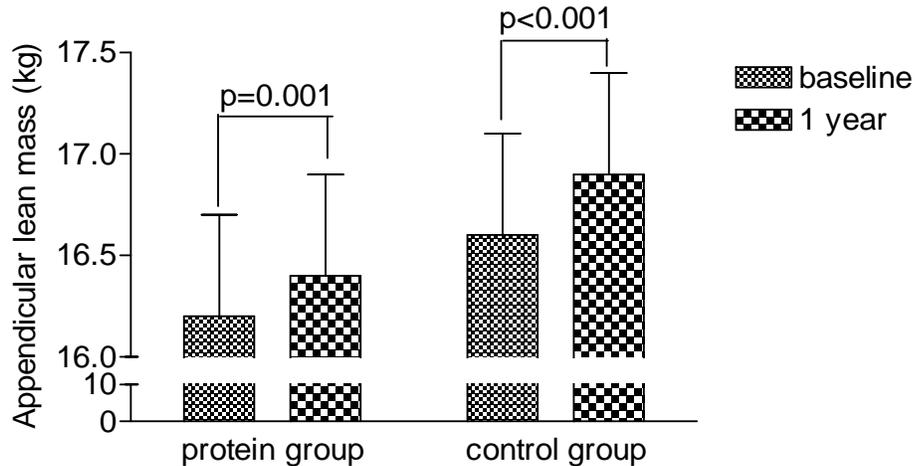
The details of the absolute and percentage changes at one year are shown in Table 4.14. The subgroup analysis which only included the high compliers (compliance >70%) was performed to assess the effect on these outcomes using the GLM repeated measures test, but there were no significant findings (p values of protein treatment effect were reported in Table 4.14).

Figure 4.8 The whole body lean mass at baseline and one year in participants who completed the one year intervention.



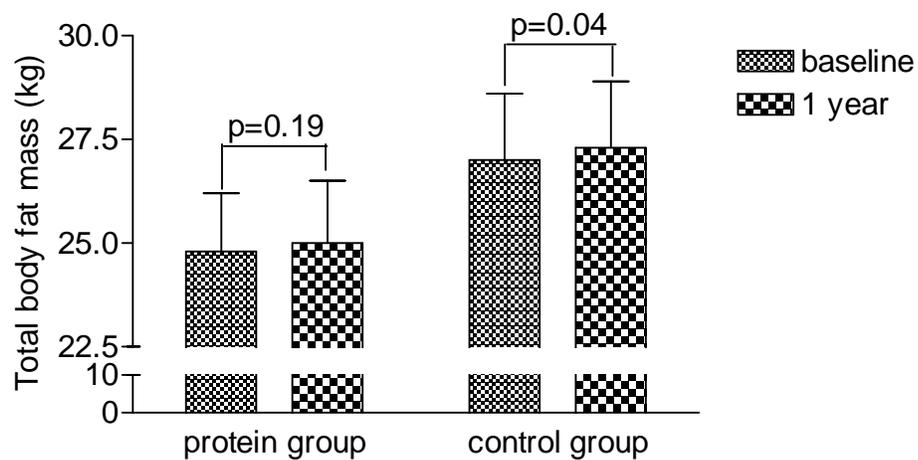
(The protein group n = 100, and the placebo group n = 95. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Figure 4.9 The appendicular lean mass at baseline and one year in participants who completed the one year intervention.



(The protein group n = 100, and the placebo group n = 95. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Figure 4.10 The total body fat mass at baseline and one year in participants who completed the one year intervention.



(The protein group n = 100, and the placebo group n = 95. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Table 4.14 The absolute and percentage change in body composition and muscle size after one year intervention comparing the protein group with the control group.

	Absolute change		Percentage change (%)		P values (1)	P values (2)	P values (3)
	Protein group	Control group	Protein group	Control group			
Whole body lean mass (kg)	0.6 (0.4, 0.8)	0.9 (0.6, 1.1)	1.6 (1.0, 2.1)	2.3 (1.7, 2.9)	0.22	0.08	0.14
% of whole body lean mass	0.2 (-0.1, 0.6)	0.2 (-0.1, 0.5)	0.4 (-0.1, 1.0)	0.4 (-0.1, 1.0)	0.08	0.93	0.45
Appendicular lean mass (kg)	0.2 (0.1, 0.3)	0.3 (0.1, 0.4)	1.3 (0.5, 2.0)	1.8 (1.0, 2.6)	0.23	0.42	0.48
Whole body fat mass (kg)	0.2 (-0.1, 0.6)	0.4 (0.0, 0.8)	0.7 (-0.7, 2.1)	1.5 (0.1, 2.9)	0.054	0.16	0.11
% of whole body fat mass	-0.2 (-0.5, 0.1)	-0.2 (-0.5, 0.1)	-0.5 (-1.4, 0.3)	-0.5 (-1.3, 0.4)	0.08	0.96	0.53
Calf muscle cross-sectional area (cm ²)	0.0 (-0.3, 0.2)	0.0 (-0.2, 0.4)	0.0 (-0.9, 0.9)	0.4 (-0.5, 1.4)	0.13	0.45	0.52

Values are mean and 95% confidence interval. The protein group n=100, and the control group n=95.

Absolute change = value at 1year – value at baseline. Percentage change = (value at 1 year – value at baseline)/value at baseline x 100. Whole body and percentage of whole body lean and fat mass and appendicular lean mass were derived from DXA. Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia.

P values (1) were for the treatment effect derived from General Linear Model (GLM) repeated measures tests using the whole study population data (n = 195), and p values (2) were for the interaction of treatment and visit time (DO YOU MEAN LENGTH OF INTERVENTION?) in the model. P values (3) were for the treatment effect derived from GLM model using data of participants (n = 38, 25 in the protein group and 13 in the control group) whose compliance > 70%.

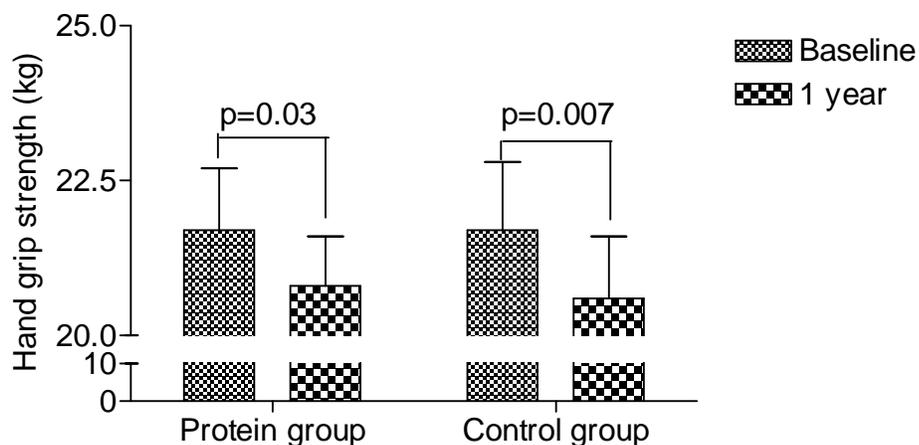
4.2.3 The effect of one year protein supplement on muscle strength

Participants overall (both in the protein and the control group) had a significant decrease in hand grip strength ($p=0.001$), and a significant increase in ankle dorsiflexion ($p=0.005$) and total knee strength ($p<0.001$) at one year using the GLM (general linear model). When the two drink groups were analysed separately using a paired sample t-test, both drink groups showed a significant decline in hand grip strength (Figure 4.11), but a significant increase in ankle dorsiflexion strength (Figure 4.12) and total knee strength (Figure 4.13). There was no difference for total hip strength at baseline and one year between the two drink groups ($p=0.29$ for time effect in the model). There was no significant interaction between drink treatment and time for any of these variables except for the hip extension strength ($p = 0.02$ in GLM model for interaction of treatment and visit time). When the two drink groups were analysed separately using a paired sample t-test, only the control group showed a significant increase ($p = 0.04$), and there was no significant difference between group differences for changes in hip extension strength (treatment effect $p = 0.76$ in GLM model).

The changes in hand grip, ankle dorsiflexion, knee and hip strength at one year were not significantly different between the two drink groups, using the GLM (general linear model) repeated measures test (all p values for drink treatment were >0.05 in the models as shown in Table 4.15).

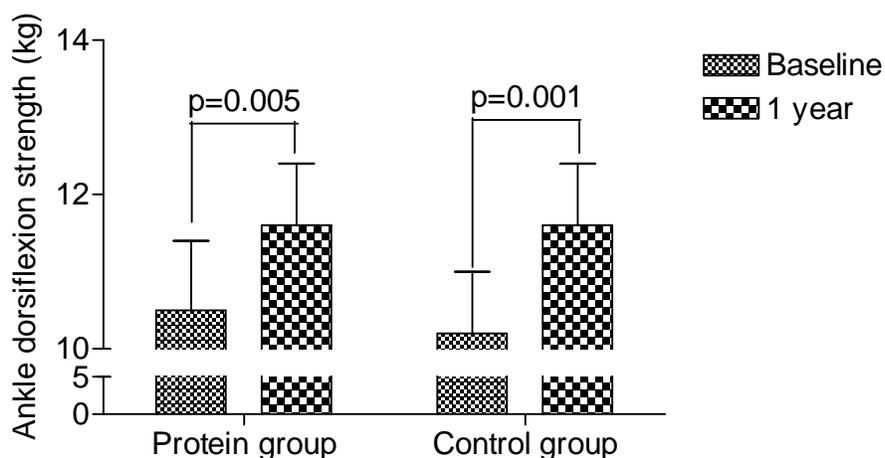
The absolute and percentage changes at one year for the two drink groups are shown in Table 4.15. The subgroup analysis including only the high compliers ($>70\%$ drink compliance) was performed, but there were no significant differences found between the control and trial drink groups in any of these muscle strength tests at one year (Table 4.15).

Figure 4.11 The hand grip strength at baseline and one year in participants who completed the one year study.



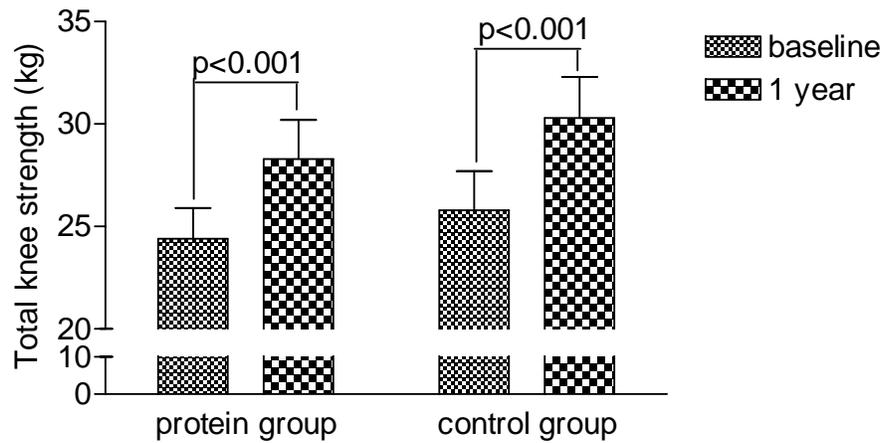
(The protein group n = 98, and the placebo group n = 93. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Figure 4.12 The ankle dorsiflexion strength at baseline and one year in participants who completed the one year study.



(The protein group n=98, and the placebo group n=94. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Figure 4.13 The total knee strength at baseline and one year in participants who completed the one year study.



(The protein group n = 98, and the placebo group n = 94. Values are mean and 95% confidence interval. The within drink group change between baseline and one year were tested by the paired sample t-test.)

Table 4.15 The changes in muscle strength and TUAG after one year intervention comparing the protein and the control group.

	Absolute change		Percentage change (%)		P values	P values	P values
	Protein group	Control group	Protein group	Control group	(2)	(2)	(3)
Hand grip strength (kg)	-0.8 (-1.7,-0.1)	-1.1 (-1.9,-0.3)	-1 (-6, 4)	-3 (-7, 2)	0.83	0.67	0.85
Ankle dorsiflexion (kg)	1.1 (0.3, 1.9)	1.4 (0.6, 2.2)	27 (14, 40)	32 (17, 46)	0.80	0.55	0.54
Knee flexion (kg)	1.8 (1.1, 2.6)	1.6 (0.8, 2.5)	37 (23, 52)	28 (18, 38)	0.18	0.78	0.58
Knee extension (kg)	2.1 (1.0, 3.2)	2.8 (1.5, 4.2)	17 (9, 25)	30 (18, 42)	0.21	0.39	0.35
Hip extension (kg)	-0.6 (-1.7, 0.5)	1.4 (0.1, 2.7)	4 (-4, 11)	21 (9, 34)	0.76	0.02	0.29
Hip abduction (kg)	0.7 (-0.1, 1.5)	0.9 (-0.1, 1.8)	15 (6, 24)	23 (11, 35)	0.91	0.77	0.42
Hip flexion (kg)	1.5 (0.6, 2.4)	2.2 (1.2, 3.2)	21 (11, 31)	30 (19, 41)	0.89	0.29	0.28
Hip adduction (kg)	-2.1 (-3.1, -1.0)	-1.6 (-2.8, -0.4)	-15 (-25, -5)	-11 (-24, 2)	0.50	0.56	0.73
Total knee strength (kg)	3.9 (2.4, 5.3)	4.5 (2.8, 6.2)	20 (12, 27)	23 (15, 32)	0.15	0.60	0.37
Total hip strength (kg)	-0.5 (-3.2, 2.2)	2.8 (-0.6, 6.3)	4 (-2, 9)	14 (5, 23)	0.77	0.13	0.34
TUAG (second)	-0.1 (-0.4, 0.1)	-0.2 (-0.5, 0.1)	-1 (-4, 2)	-2 (-5, 2)	0.64	0.91	0,34

Values are mean and 95% confidence interval. The protein group n=99, and the control group n=94.

Absolute change = value at 1year – value at baseline. Percentage change = (value at 1 year – value at baseline)/value at baseline x 100.

P values (1) were for the treatment effect derived from General Linear Model (GLM) repeated measures tests using the whole study population data (n = 193), and p values (2) were for the interaction of treatment and visit time in the model. P values (3) were for the treatment effect derived from GLM model using data of participants (n = 37, 25 in the protein group and 12 in the control group) whose compliance > 70%.

4.2.4 The effect of one year protein supplement on mobility and balance

Mobility (Timed Up and Go)

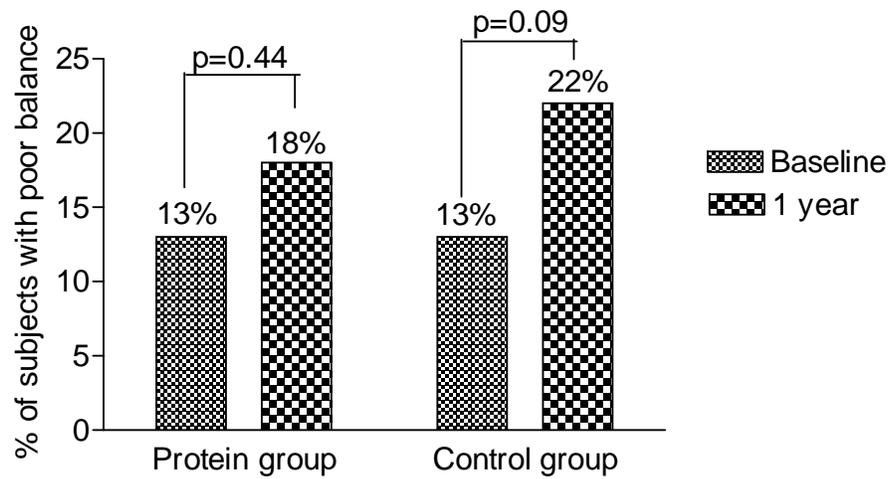
There were no differences in mobility between the trial and control drink groups at baseline and after one year using the GLM (general linear model) repeated measures test. The model showed that there was no significant drink effect ($p=0.64$), time effect ($p=0.12$), or interaction of treatment and time ($p=0.91$) for the 'Timed Up and Go' test. The absolute and percentage change for the 'Timed Up and Go' test at one year are shown in Table 4.15.

Balance ('Romberg eyes-open' and 'eyes-closed' tests)

There were no significant differences in the Romberg eyes-open ($\chi^2=8.9$, $p=0.54$) or eyes-closed ($\chi^2=29.9$, $p=0.27$) tests at one year between the two drink groups using the ordinal regression. The drink groups were used as factors and baseline values were used as covariates in the model (details are shown in Appendix 23).

Further analysis was conducted by defining poor standing balance as being unable to maintain a tandem stance for 10 seconds with the eyes-open (Guralnik, Ferrucci et al. 1995; Austin, Devine et al. 2007). The overall prevalence of poor balance was significantly increased at one year (odds ratio=4.33, $p=0.001$, 95% confidence interval 1.76 to 10.70) using logistic regression (drink group and baseline poor balance were entered in the model as covariates). When the two drink groups were analyzed separately using chi-square test, both drink groups showed an increase in the prevalence of poor balance from baseline to one year, but only the control group increased significantly as shown in Figure 4.14. There was no significant difference in the prevalence of poor balance between the protein group (18%) and the control group (22%) at one year (odds ratio: 0.74, 95% confidence interval: 0.36 to 1.53) using the logistic regression.

Figure 4.14 The percentage of subjects with poor standing balance at baseline and one year in the two drink groups.



(Poor standing balance was defined as being unable to maintain a tandem stance for 10 seconds with eyes-open. The within group changes were tested by chi-square test.)

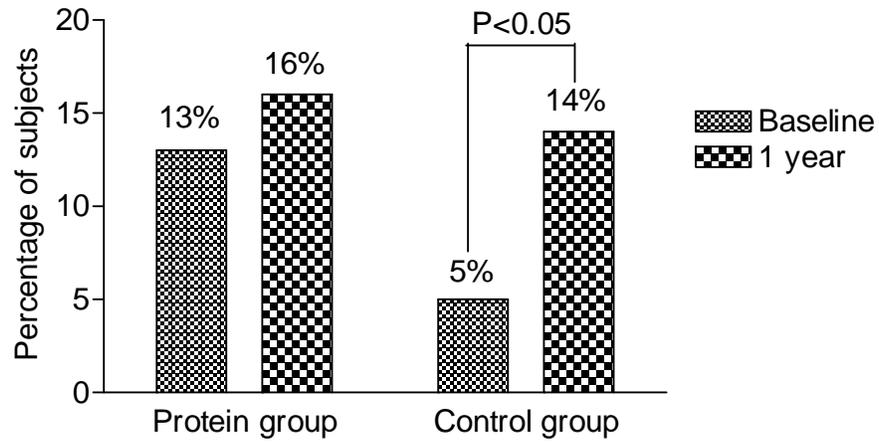
4.2.5 Changes in the prevalence of falls at one year

The prevalence of falls was assessed using the question of ‘How many times had you fallen in the last three months?’ which was added to the demographic questionnaire. The participants were grouped as ‘fallers’ and ‘non-fallers’. The fallers were those who had at least one fall in the three months prior to the clinic visit.

The prevalence of falls in the trial (protein drink) group was 13% at baseline and 16% at one year, and the control group were 5% at baseline and 14% at one year (details shown in Appendix 24).

There was no significant difference in the prevalence of falls between the protein group (16%) and the control group (14%) at one year (odds ratio: 1.07, 95% confidence interval: 0.47-2.41) using the logistic regression (drink group and baseline fall risk were as covariates in the model). However, there was a significant time effect on falls prevalence at one year (odds ratio: 3.21, $p = 0.036$, 95% confidence interval: 1.08-9.55), which means that overall there were more participants who had fallen at least once at one year compared with the baseline. When the two drink groups were analyzed separately using chi-square test, the control group showed a significant increase in falls rate at one year ($\chi^2 = 3.93$, $p = 0.047$), but the protein group remained at the similar level as at baseline compared with one year ($\chi^2 = 0.30$, $p = 0.59$) (Figure 4.15).

Figure 4.15 The percentage of subjects who had fallen at least once in the past three months before the clinic visit comparing baseline and one year in the two drink groups.



The within group changes between baseline and one year were tested by chi-square test. The protein group $n = 98$ at baseline and $n = 99$ at one year. The control group $n = 94$ at baseline and one year.

4.2.6 Serum IGF-1 analysis

4.2.6.1 Serum IGF-1 and baseline characteristics

There was no significant difference in Serum IGF-1 at baseline between the trial and control groups as shown in Table 4.16. The mean (\pm SD) IGF-1 concentration in all participants was 108.6 ± 39.0 ng/ml at baseline ($n = 184$). Baseline serum IGF-1 concentration was significantly correlated with whole body lean mass ($r = 0.17$, $p=0.02$ in spearman correlation test). There were no significant correlations of IGF-1 with other baseline characteristics, such as age, weight, muscle strength or protein intake.

4.2.6.2 Changes in Serum IGF-1 at one year

There were 184 participants (protein group $n = 93$ and control group $n = 91$) who had blood samples taken at baseline and at one year. The mean (\pm SD) IGF-1 in all participants was 110.9 ± 10.1 ng/ml at one year. Eight participants did not have a serum sample at baseline or at one year. Another two participants had either baseline or one year blood sample, but not both. Where blood samples were not available it was due to difficulty in obtaining a blood sample and these were treated as missing values and excluded from IGF-1 analysis.

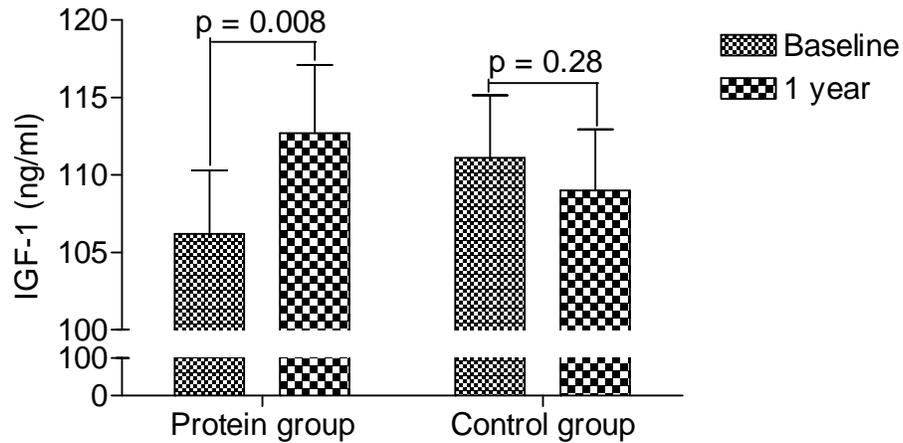
There was a significant increase in the serum IGF-1 at one year in the protein group compared with the control group ($F = 7.024$, $p = 0.009$) using ANCOVA analysis (baseline IGF-1 as covariate and drink code as fixed factor in the GLM model). The tests of between-subjects effects showed that both time and drink effects were significant (all $p < 0.01$). These indicate that the serum IGF-1 level was not only significantly different between two drink groups, but also significantly different at one year within the same drink group. The two drink groups were separately tested for a time effect using the paired sample t-test. The protein group showed a significant increase in serum IGF-1 at one year ($+6.5$ ng/ml, $p = 0.009$) while the control group decreased in serum IGF-1 at one year (-2.1 ng/ml, $p=0.28$) as shown in Figure 4.16.

The absolute and percentage changes of serum IGF-1 at one year between two drink groups were also tested by the independent sample t-test. There were significant

differences in the absolute and percentage changes in serum IGF-1 at one year between two drink groups as shown in Table 4.17 and Figure 4.17.

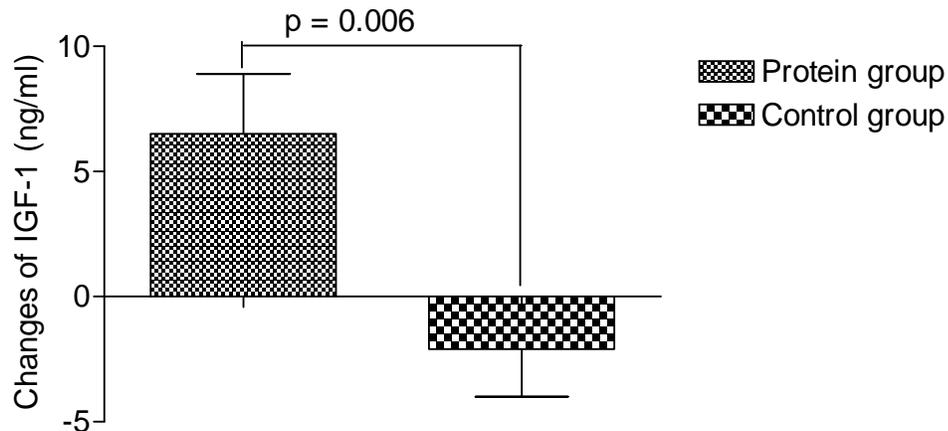
As there was a significant protein treatment effect on serum IGF-1 level, ANCOVA analysis was performed (drink group was entered as the fixed factor, baseline age and IGF-1 level were entered as covariates). After adjustment for baseline serum IGF-1 level and age, the protein group remained with a significantly higher IGF-1 at one year than the control group ($F = 7.068$, $p = 0.009$).

Figure 4.16 Serum IGF-1 concentrations comparing baseline and one year in the two drink groups.



Values are mean and SD. The within group changes between baseline and one year (protein group n = 93, control group n = 91) were tested by paired sample t-test.

Figure 4.17 The absolute changes of serum IGF-1 concentration comparing the protein group with the control group after one year intervention.



Values are mean and standard error. The difference in changes at one year between two drink groups was tested by the independent sample t-test (protein group n = 93, control group n = 91).

Table 4.16 Baseline and one year serum IGF-1 concentration in the two drink groups.

	Baseline IGF-1 (ng/ml)	1 year IGF-1 (ng/ml)
Protein group	106.1 ± 39.2 (n = 94)	112.7 ± 42.5 (n = 93)
Control group	111.1 ± 38.6 (n = 91)	108.9 ± 37.6 (n = 92)
p value of t-test of difference between drink groups	0.38	0.52

Values are mean ± SD.

Table 4.17 The absolute and percentage changes of serum IGF-1 concentration comparing the protein group with the control group after one year intervention.

	Absolute change (ng/ml)	% change
Protein group (n = 93)	6.5 (1.7, 11.3)	7.6 (2.6, 12.7)
Control group (n = 91)	-2.1 (-5.8, 1.7)	-1.0 (-4.2, 2.2)
p value of t-test of difference between drink groups	0.006	0.005

Values are mean and 95% confidence interval.

4.2.7 Changes in dietary intake at one year

A random sample of 40 participants had 3-day food records at one year (protein group n = 20, control group n = 20).

There were significant differences in the daily carbohydrate intakes at one year between the two drink groups ($p = 0.03$ for the drink effect in ANCOVA analysis, baseline value entered in the model as covariate and drink groups as the fixed factor). When the two drink groups were analysed separately using a paired sample t-test (Table 4.18), the control group showed a significant increase in daily carbohydrate intake at one year compared to at baseline ($p = 0.03$). The one year carbohydrate intake in the protein group was similar to the baseline values.

At one year, the protein group increased their total energy intake by 5% (120 kJ), protein intake 23% (7.6 g), and fat intake 10% (3.6 g). There was no change in carbohydrate intake at one year. The control group increased total energy intakes by 13% (648 kJ), protein intake 5% (2.2 g), fat intake 21% (4.8 g), and 17% (26.8g) at one year.

There was no significant difference in daily total energy intake ($p = 0.40$), protein intake ($p = 0.11$) or fat intake ($p = 0.81$) between two drink groups at one year using ANCOVA analysis to adjust for their baseline values as the covariate.

Table 4.18 Dietary intake of baseline and one year.

	Protein (n = 20)			Control (n = 20)		
	Baseline	1 year	p value	Baseline	1 year	p value
Energy (kJ/d)	7166 ± 1730	7287 ± 1344	0.76	6970 ± 1664	7618 ± 1602	0.16
Protein (g/d)	81 ± 31	89 ± 18	0.35	75 ± 20	77 ± 24	0.66
Fat (g/d)	62 ± 17	65 ± 20	0.39	62 ± 24	67 ± 19	0.46
Carbohydrate (g/d)	193 ± 64	187 ± 38	0.59	186 ± 48	212 ± 53	0.03

Values are mean ± SD. The dietary intake was assessed by 3-day food record. The within group differences between baseline and one year were tested by the paired sample t-test.

4.3 Summary of the key findings of the study

In summary, at one year, both the protein group and the control group significantly increased whole body lean mass, appendicular lean mass and body weight. Only the control group had a significant increase in total body fat mass. However, there were no significant differences in these variables between the two groups at baseline or at one year. Both drink groups significantly decreased hand grip strength, but increased ankle dorsiflexion strength, and total knee strength at one year. However, no significant differences were observed between the two groups in these variables at baseline or at one year. The prevalence of 'poor standing balance' and falls, at one year, were significantly increased in the control group, but remained at the same levels as at baseline in the protein group. Serum IGF-1 was significantly increased in the protein group and significantly decreased in the control group at one year, and the changes were significantly different between the two groups. The 24-hour urinary nitrogen was significantly increased in the protein group at one year, but remained at the same level as at baseline in the control group. These results will be discussed in the next chapter.

Chapter 5 Discussion

5.1 Baseline cross-sectional study

Dietary protein and energy intake at baseline

The mean protein intake at baseline was 76 g/day (or 1.14 g/kg/day) which was higher than the current recommendation of 0.94 g/kg/day level for Australian women age over 70 year (National Health and Medical Research Council 2006). The proportion of energy derived from protein intake in the current study was $19 \pm 4\%$, and carbohydrate and fat intake provided $46 \pm 7\%$ and $33 \pm 6\%$ energy intake respectively, which was consistent with the Acceptable Macronutrient Distribution Range recommended for chronic disease prevention (National Health and Medical Research Council 2006). The total energy intake in this study was 7140 kJ/day slightly higher than the Australia National Nutrition Survey of 1995-1996 for age group of 70-79, which was 6200 kJ/day (Australian Bureau of Statistics 1998).

Underreporting of dietary intake

The prevalence of underreporting in the current study was 11% as measured by the Goldberg EI:BMR 'cut-off' method (details are in section 2.14.6.3 and 4.1.5). The prevalence of underreporting ranged from 18-54% in large nutritional surveys and it can be as high as 70% in some subgroups (Macdiarmid and Blundell 1998). Similar to the previous study, the under-reporters identified in the current study also had higher BMI and body fat mass than the acceptable reporters and over-reporters. In general, women are more likely to under-report than men, and people with a high BMI are more likely to under-report than people with a low BMI (Macdiarmid and Blundell 1998). A study of 484 men and women aged 40-69 years found that 7% of women underreported both energy and protein intake using a 24 hours recall and 23% by food-frequency questionnaire (Subar, Kipnis et al. 2003). The use of doubly-labelled water would be the best way to obtain energy expenditure that was not related to subject compliance.

24 hour urinary nitrogen has been widely used as an external biomarker of dietary protein intake. The 24-hour urine nitrogen accounted for $90 \pm 2\%$ (mean \pm SE) of protein intake in the current study. Bingham's review of previous studies found that the average 24-hour urinary nitrogen constantly accounted for $81 \pm 2\%$ of nitrogen intake (Bingham 2003). The consistency of the 24-hour urinary nitrogen and protein

intake assessed by 3-day food record in the under-reporters in the current study indicated that the process of recording dietary intake influenced the eating habits in this subgroup during the recording period.

Correlation of baseline protein intake and body composition

Baseline whole body lean mass was significantly positively correlated with baseline protein intake, and baseline percentage of fat mass was significantly negatively correlated with protein intake assessed by the 3-day food record (Spearman rank correlation test). However, the beneficial effect of protein on lean body mass disappeared when adjusted for height and total energy intake in the model. This result is true even if the under-reporters were excluded from the analysis. This result is different from our previous cross-sectional cohort study in 862 elderly women, which found a strong correlation between the habitual high protein intake, as assessed by a food frequency questionnaire, and higher body lean mass (Meng, Zhu et al. 2009). However it is impossible to determine the direction of the relationships and the exact nature of risk factor in a cross sectional study. The relatively small sample size of the current study and the exclusion of the high protein intake volunteers at baseline may also help explain the difference between the two studies.

Prevalence of sarcopenia

At baseline, the prevalence of sarcopenia in this study population was 9% (20 subjects out of 219) when using the conventional definition of $ALM/height^2 \leq 5.454$ kg/m². As discussed in the literature review, the reported prevalence of sarcopenia varies depending on the definition used. Based on the same definition used in this study, the prevalence of sarcopenia for elderly women in most of studies was around 25% (Iannuzzi-Sucich, Prestwood et al. 2002; Estrada, Kleppinger et al. 2007), but also can be as high as 50% in 216 women aged 74 ± 5 years outpatient women in Italy (Coin, Perissinotto et al. 2008).

The prevalence of sarcopenia in our study population is low compared to the previous studies. The sources of study populations between studies may contribute to these differences. The participants in this current study were relatively healthy, community-dwelling ambulant elderly women. For example, women who had been diagnosed as osteoporosis and were taking bone active agents were excluded from

the study at the beginning. These women would be more likely to have sarcopenia. The physical activity level assessed at baseline showed that the study participants were overall fairly active and functional. The majority of the study participants had good performance in the TUAG and Romberg tests at baseline.

The current study used cut-points of 5.454 kg/m² for ALM/height² to define sarcopenia. This cut-point was derived from a random sample of Hispanic and white women and men from New Mexico (Baumgartner, Koehler et al. 1998). Therefore it may not be appropriate to apply this cut-point to a population of Australian elderly women, particularly since the previous study used a Lunar DXA and the current study used Hologic DXA. The mean (SD) of appendicular lean mass and ALM/height² in their study were 14.5 (2.2) and 5.9 (0.7) respectively in persons older 70 years. Participants in the current study had much higher values (mean±SD) of ALM and ALM/height² (16.5±2.4 and 6.4±0.8 respectively) compared to the Baumgartner study (Baumgartner, Koehler et al. 1998).

The prevalence of sarcopenia in the current study is close to the level found in a cross-sectional study conducted in Denmark which investigated the body composition in 754 healthy women aged 18 to 85 years (Tanko, Movsesyan et al. 2002). The body composition was measured by DXA QDR 4500A which is the same as the current study. The mean (SD) of ALM and ALM/height² for women aged >70 years were 15.7 (2.4) and 6.2 (0.8) respectively. These values are slightly lower than our current study, which were 16.5 (2.4) and 6.4 (0.8) respectively. The reason for small difference may be because this subgroups in their study (n=67) were slightly older than the current study population (70-80). In their study, the young-normal means were determined from 216 women aged 18 to 39 years in their study. Sarcopenia was defined as ALM/height² < young-normal mean 2 SD, which was 5.4 kg/m². In our lab, we have DXA body composition data measured on 71 young healthy females aged 24-36 years. The surprising finding is that the mean (SD) of ALM/height² of this young normal population was only 5.597 (0.681) kg/m². Therefore, if the value of <2SD is used from the young normals to define sarcopenia, the cut-point would be 4.235, which is even lower than 5.454. A larger normal young healthy female population sample may be needed in order to obtain a mean of ALM/height² which can represent this population.

The main difference in the study population between the current study and previous protein supplemented studies (Lauque, Arnaud-Battandier et al. 2000; Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003) was that the participants in the current study were generally healthy. They were recruited and selected at random from the electoral roll which includes all women aged between 70 and 80 years in Western Australian. Women such as those who were diagnosed as osteoporotic and on bone active agents, diabetic, obesity degree II or over ($BMI \geq 30 \text{kg/m}^2$) were excluded from study. The majority of previous studies of protein and energy supplementation were conducted in apparently unwell populations; especially those who had some degree of muscle wasting, such as occurs in bedridden hip fracture patients. In a meta-analysis, protein supplementation was found to have fewer unfavourable outcomes' compared to non-supplement hip fracture patients, but no significant effect of protein on mortality rate (Avenell and Handoll 2006).

Sarcopenia is defined as the progressive loss of muscle mass and strength with aging. However, there has been no agreed method to assess sarcopenia (Rolland and Vellas 2009), especially in the muscle strength or physical functions aspects, which varied widely between studies. Appendicular lean mass assessed by DXA, adjusted for body size or body fat mass, are the widely used methods to quantified muscle mass (Baumgartner, Koehler et al. 1998; Delmonico, Harris et al. 2007). For muscle strength or physical function, at an individual level, the muscle mass is not necessarily linearly correlated with muscle strength; and the muscle strength is not necessarily linearly correlated with physical function. All must be evaluated separately.

In a recent cross-sectional study of 1308 community-dwelling elderly women aged ≥ 75 year in France, researchers found that sarcopenia was not associated with difficulty in physical function in the nonobese but had a negative effect in the obese (Rolland, Lauwers-Cances et al. 2009). The physical functions were assessed by the self-reported difficulty in walking, climbing stairs, going down the stairs, rising from a chair or a bed, picking up and object from the floor, lifting heavy objects or reaching an object, and moving difficulties.

Correlation of body lean mass with muscle strength, mobility and balance

Most cross-sectional studies demonstrate that lean body mass is related to muscle strength in the elderly (Pedersen, Ovesen et al. 2002; Bunout, Barrera et al. 2004). The baseline data in the current study also indicated that whole body lean mass and appendicular lean mass were positively correlated with upper and lower limb muscle strength (hand grip, ankle dorsiflexion, knee and hip strengths).

Appendicular lean mass measured by DXA showed stronger correlation with hand grip strength ($r=0.38$, $p<0.01$) than the correlation between the corrected upper arm muscle area (CUAMA) and hand grip strength ($r=0.18$, $p<0.01$). CUAMA was derived from mid upper arm girth and skin fold measurements. A study of 874 free-living, apparently healthy Irish-born elderly people aged over 65 years, found that the arm muscle area was associated with handgrip strength (Corish and Kennedy 2003).

CUMAM tends to overestimate the arm muscle area in overweight or obese people because of the failure to account for the muscle fat infiltration by anthropometric skinfold techniques which measure only subcutaneous adipose tissue (Forbes, Brown et al. 1988). A study of 244 non-obese American men and women aged 40 ± 14 (SD) years found that of all the limb circumferences, including corrected thigh girth and corrected calf girth, the skinfold corrected upper arm girth had the highest correlation with total-body skeletal muscle mass assessed by magnetic resonance imaging (MRI) (Lee, Wang et al. 2000). The inconsistency between the previous studies may be due to the differences in age, gender, race, and health status in their study populations (Lee, Wang et al. 2000). Women have larger skinfolds than men and elderly have thinner skinfolds than young adults when assume they have similar percentage of body fat mass (Bellisari and Roche 2005). This indicates that fat tissue within and between muscles may be larger in women and in the elderly (Bellisari and Roche 2005). Therefore, using skinfold corrected upper arm muscle area to estimate the muscle size may not be reliable or accurate for this elderly female population.

Muscle quality may play a more important role in mobility and balance functions than muscle mass or size in elderly women. Muscle quality is generally defined as the relationship between muscle strength and muscle mass (Newman, Haggerty et al.

2003), such as maximal force production per unit of muscle mass (Ivey, Tracy et al. 2000). In a relatively healthy population, elderly woman may be able to function well despite having the smaller muscle mass. A study of 55 elderly men and women aged 70 ± 6 years in Switzerland found that muscle quality is the most important predictor of lower limb physical function (Misic, Rosengren et al. 2007). Their study assessed leg muscle quality by leg strength normalized for leg mineral free lean mass, and assessed lower limb functions using computed dynamic posturography and stair ascent/descent, a time up-and-go task and a 7-meter walk with and without obstacle. Another study of 2623 elderly men and women aged 70-79 in America found that body fat had a significant inverse association with muscle quality (defined by taking the ratio of strength to muscle mass for both upper and lower extremities) (Newman, Haggerty et al. 2003). Other factors, such as deterioration in neurological functions may also play a role in the decline in mobility and balance functions in the elderly.

The baseline data showed that 'Romberg eyes-closed test' had no correlation with any lower limb muscle strength. The 'Romberg eyes-open test' was only positively correlated with ankle dorsiflexion strength. The lack of correlation between Romberg test and muscle mass or strength could be due to two reasons. One is the larger inter and intra variation in study participants and observers as discussed before. Another is that the use of four categories for the test would attenuate the statistical power especially in the intervention studies which generally have much smaller sample sizes than epidemiological studies.

A cross-sectional validation study of the FICSIF (Frailty and Injuries: Cooperative Studies of Intervention Techniques) common database found that the parallel stance is an easy task and is only useful in populations with moderate to severe mobility problems such as nursing home residents (Rossiter-Fornoff, Wolf et al. 1995). Tandem stance has acceptable reliability and validity (Heitmann, Gossman et al. 1989). The current study found that to define participants who were unable to maintain a tandem stance for 10 seconds with eyes open as 'poor balance' is a better method for studying the correlation between standing balance performance and fall risk. This method was previously described by Guralnik (Guralnik, Ferrucci et al. 1995).

Physical activity level was not correlated with age or muscle mass or strength in the current study. An eight year longitudinal study also found no changes in physical activity level in twelve elderly men and women (Frontera, Reid et al. 2008). Previous studies also suggested that the loss of muscle with age could not be explained by declining physical activity levels (Rutherford and Jones 1992).

5.2 The effect of one year of protein supplementation

Introduction

This study examined the effect of adding a protein supplement drink or a placebo drink to the subject's usual diet for one year. The effect on muscle mass and strength and physical function was studied in 219 women aged 70-80 years. The randomization of the participants into the two groups was successful and there were no significant differences in the baseline main characteristics between the trial and the control groups. Inevitably some participants drop out of any study and in this case there were no significant differences between those who withdrew and those who completed the one year study.

The overall compliance of all participants both in the protein supplemented group and the control group with their allocated supplements was 47% in the current study. This is similar to a 18-month nutritional supplementation and resistance training study conducted in US in 149 healthy people aged ≥ 70 years, which the compliance was 48% (Bunout, Barrera et al. 2001), but slightly lower than a 9 month study of 57 men and women aged over 72 years living in a retirement home, which had a 54% compliance (Bonneyoy, Cornu et al. 2003). The compliance in the Bonneyoy study was assessed by monthly telephone interviews to ask the participants the number of days in the week and the number of times a day that they were consuming the product. In our study compliance was assessed by counting the empty bottles that the participants returned. This method is more likely to underestimate compliance when compared to other methods. Despite the modest compliance, consumption of the protein drink did result in an increase in the 24-hour urinary nitrogen in the protein supplemented group which confirmed that the participants were compliant with their allocated drinks.

Key findings

The key findings of the study were that both the protein supplemented and the control group experienced similar, and significant ($p<0.05$) increases in the whole body lean mass, appendicular lean mass, weight, ankle dorsiflexion strength and knee strengths (knee flexion and extension) at one year. Whole body lean mass increased by 1.6% in the protein supplemented and 2.3% in the control group. Appendicular lean mass, which is mainly muscle, increased by 1.3% in the protein supplemented group and 1.8% in the control group. Ankle dorsiflexion strength increased 27% in the protein supplemented and 31% in the control group. Total knee strength increased 20% in the trial (protein) group and 23% in the control group. The trial (protein) group did not differ from the control group in any of these variables as a result of the one year intervention.

There was, however, a significant increase in the serum IGF-1 level by 7.5% (+6.5 ng/ml) in the protein supplemented compared to a decrease by 1.0% (-2.1 ng/ml) in the control group at one year, and the changes were significantly different between the two drink groups ($p=0.006$). Self-reported falls were significantly increased in the control group (+9%) but not in the protein supplemented (+3%) at one year. Moreover, the total fat mass increased from baseline only in the control group (protein supplemented: +0.7%, $p=0.19$; control group: +1.5%, $p<0.05$).

Body composition

After one year of the intervention, whole body lean mass increased by 1.6% ($p<0.001$) in the protein supplemented and 2.3% ($p<0.001$) in the control group. Appendicular lean mass, which is mainly muscle, increased by 1.3% ($p=0.001$) in the protein supplemented and 1.8% ($p<0.001$) in the control group. Both groups significantly increased their body weight (trial group: +0.8% or 600g, $p<0.05$; control group: +1.5% or 1000g, $p<0.05$) and BMI (protein supplemented: +1.0%, $p<0.05$; control group: 1.6%, $p<0.05$). There were no significant differences in changes in these variables between the trial (protein) and the control group.

The initial explanation for no differences in changes in these variables was that the extra energy intake from the supplements may play a role in increasing whole body

and appendicular lean mass, body weight and BMI, regardless of the differences in the macronutrient composition of the supplements. Previous studies have suggested that without any intervention, elderly women would in general, experience a decrease in total body mass and body lean mass, and an increase in body fat mass with advancing age (Baumgartner, Waters et al. 1999; Guo, Zeller et al. 1999; Bunout, de la Maza et al. 2007; Fantin, Di Francesco et al. 2007; Chen, Lin et al. 2008). This phenomenon is more significant in white women aged over 75 years (Obisesan, Aliyu et al. 2005). In a population based study conducted in America, body composition was assessed in 2040 well-functioning black and white men and women aged 70-79 years using DXA (Visser, Pahor et al. 2003). In the women, total body mass, fat-free mass, and fat mass decreased by 0.3%, 0.1%, and 0.5% respectively after one year. A cross-sectional study in 1307 older (age \geq 70 years) and 576 young adults showed that women lost a mean of 221 ± 399 (SD) g of lean body mass per year (Bunout, de la Maza et al. 2007). Given no changes in the physical activity at one year in either the trial or the control group, the increased body mass in the current study subjects after one year intervention may due to the effect of the supplements. As there was no difference in changes between the trial and control groups in these variables (whole body and appendicular lean mass, body weight and BMI), the extra energy from the supplemented drink, regardless of macronutrient content, may contribute to these changes.

However, the analysis of the one year dietary intake showed that the energy intake (assessed by 3-day food record) did not increase, within the range of error inherent in this methodology, in either the trial or control group at one year (Table 4.18). The drinks consumed by the trial and control groups provided an energy supplement for the participants in both groups. After examining their 24-hour urinary nitrogen excretion, however, it appeared that underreporting of food intake may have contributed to the failure in detecting any changes in energy intake. The evidence of underreporting dietary intake was that the protein supplemented significantly increased their 24-hour urinary nitrogen excretion at one year (Table 4.12), but the reported protein intake derived from the 3-day food record did not show this increase in the trial (protein supplemented) group (Table 4.18). These data indicate that at least the trial (protein supplemented) group under reported their protein intake at one year. It was only possible for reasons of time to process and analyse 40 subjects' one

year 3-day food records. The results from the 40 records showed an increased trend in energy intake at one year in both the trial and control groups, although the changes were not statistically significant. The small sample size of this analysis may lack the power to detect the magnitude of the change expected. Therefore, it cannot be excluded that participants did increase their energy intake at one year, and the extra energy intake obtained from the supplements may have contributed to the increase in whole body and appendicular lean mass, weight and BMI. In order to exam whether there was a change in dietary energy or macronutrient intake at one year it will be necessary to analyse all of the participants' one year 3-day food records in the future.

If it is assumed that the energy intake did not change over one year, the other explanation for the increase in body weight and BMI would be a decrease in energy expenditure. Daily energy expenditure can be divided into three main components: basal metabolic rate, diet induced thermogenesis and physical activity, and the portion of each component varies between individuals (Butte 2006).

The current study did not detect any significant change in physical activity at one year in either the trial or control groups. There are two possible explanations: a) the study participants did not change their physical activity over one year; b) the change was smaller than can be detected by the International Physical Activity Questionnaire (IPAQ). The IPAQ was developed in the late 1990s to obtain internationally comparable data on health-related physical activity (Craig, Marshall et al. 2003). The short version, which is the one the current study used, is suitable for national and regional surveillance systems at a population level (International Physical Activity Questionnaire). Its reliability in detecting the potential changes over a short period in a small population is unknown, particularly in this age group. Validation studies have compared the IPAQ with the use of motion detectors such as accelerometer measurements, and these showed low correlations in the range of 0.26-0.33 (Johnson-Kozlow, Sallis et al. 2006; Wolin, Heil et al. 2008). As the sensitivity of detecting the changes in physical activity in the current study population using IPQA is unknown, it is not possible to exclude decreased physical activity as the reason for the increase in weight and BMI.

If it is assumed that the physical activity and energy intake did not change over one year, a decline in basal metabolic rate may account for the weight gain in the current study population. Basal metabolic rate represents about 70% of total daily energy expenditure in most people, and is the primary component of total caloric expenditure (Butte 2006). The main determinant of basal metabolic rate is body weight and body composition, and skeletal muscle consumes about 20% of resting energy expenditure. (Hill, Catenacci et al. 2006). In a sedentary person, the main determinants of energy expenditure is lean body mass which starts declining from the third decade of life (Evans and Cyr-Campbell 1997). Therefore, the reduction in body lean mass with aging is responsible for the decrease in basal metabolic rate found in the elderly (Tzankoff and Norris 1977; Ritz 2001). A four year study of 2163 (47% women) healthy elderly aged 70-79 showed that both weight loss and weight gain occurred (Newman, Lee et al. 2005). Their study showed that there were significantly more subjects with a loss of lean mass with weight loss than with weight gain, and more fat mass gain with weight gain than with weight loss. Small amounts of lean mass loss and fat mass gain were observed in weight stable subjects in their study. In contrast, participants in the trial (protein supplemented) group in the current study, significantly increased their body lean mass and their body fat mass did not change significantly, which implies that the protein supplement may play a positive role in preserving lean body mass and restricting body fat mass gain with weight gain. Therefore it seems less likely that a decrease in basal metabolic rate would account for the weight gain observed between the two groups.

Dietary intake appears to decline gradually with aging (Clarkston, Pantano et al. 1997; Morley 1997). It has been proposed that the low energy intake is the major reason for potential malnutrition in the elderly (Nes, Sem et al. 1992), which contributes to the occurrence of sarcopenia. The current study design provided an extra 30 g protein per day in the trial (protein supplemented) group when an energy controlled placebo was used in the control group. In contrast, there are few intervention studies which have tested the effectiveness of a supplement in this age group (Bunout, Barrera et al. 2001; Bonnefoy, Cornu et al. 2003; Bunout, Barrera et al. 2004) or in even older populations (Fiatarone, O'Neill et al. 1994; Payette, Boutier et al. 2002; Wouters-Wesseling, Van Hooijdonk et al. 2003). None of those studies used an isocaloric supplement without protein as a control drink. Therefore, any

effect have been detected from those studies could be due to the extra energy intake, and not specifically the high protein effect.

Bunout and colleagues conducted a nutritional supplementation study in 149 healthy free-living men and women aged 70 years or older recruited from three public outpatient clinics (Bunout, Barrera et al. 2001). The subjects were randomly allocated into four groups: supplement group (Total energy 873 kJ, protein 13 g), training group, supplement + training group, no intervention group. After the 18 months intervention, whole body lean mass and weight did not change in either group, and all subjects in the study gained ~1.9kg fat. The authors proposed that the reason for the lack of change in weight and whole body lean mass in their study may be because their study participants were healthier and had better baseline conditions than other studies which targeted frail elders and reported increases in body weight. Although their study reported a compliance of 49%, the lack of information on the changes in the total energy intake over the study period made it hard to confirm the reasons for their results. It is impossible to know if these dietary supplements resulted in increasing participants' total energy intake or just substituted for part of their habitual food intake. Consequently, the lack of significant changes in body composition, muscle strength or physical function found in the protein-energy supplemented group in their studies, may be because the supplements were substituted for their food intake resulting in no change in overall energy or macronutrient intake. Although the current study did not find a significant change in the participants' food intake over one year either, the evidence from the significant changes in 24-hour urinary nitrogen excretion at one year in the protein supplemented suggests the existence of underreporting food intake in the current study population or the dietary measurement method was not sensitive enough to detect the changes.

The results from the current study suggest that the difference in the source of energy made no difference to the weight change. Buchholze and Schoeller reviewed weight loss studies and found that neither macronutrient composition differences of the diet nor changes in energy expenditure explained the difference in weight loss but total energy intake did (Buchholz and Schoeller 2004). Evidence from previous short term (Noakes, Keogh et al. 2005) and long term (Brinkworth, Noakes et al. 2009)

weight loss studies in obese persons showed that energy restricted diets had similar effect on the weight loss and body composition changes independent of the composition of the diets. However, whether the high satiety of protein supplements contribute to the decreased total energy intake in an ad libitum diet needs to be investigated further. Whey protein is high in branched chain amino acids, especially leucine, and was found to be able to stimulate ‘satiety’ hormones, such as cholecystokinin (Veldhorst, Smeets et al. 2008). Findings from previous studies are inconsistent on whether the high satiation property of whey protein would impact subsequent energy intake (Lam, Moughan et al. 2009; Veldhorst, Nieuwenhuizen et al. 2009). Although the changes in weight were not statistically significant between the trial and control groups, there was a trend towards a smaller increase in weight in the trial (protein) group (+0.8% or 600g) when compared to the control group (+1.5% or 1000g) at one year.

Previous studies have found that high quality proteins, such as whey protein which is rich in essential amino acids, shows larger increases in energy expenditure than do lower-quality protein (Westerterp-Plantenga, Nieuwenhuizen et al. 2009). This is important for elderly people to manage weight gain when taking protein supplements. However in the current study no effect of the whey protein could be demonstrated between the trial and control groups.

The control group had a greater increase in the total energy intake (+13%) than the trial protein supplemented group (+5%), though the differences were not significant. The control group had a significant increase in carbohydrate intake, but there was no change in carbohydrate intake in the trial group. The differences in the level of changes in the total energy intake and carbohydrate intake between the two drink groups may explain the significant increase in body fat mass in the control group but not in the protein supplemented at one year. One of the study limitations is that the one year dietary intake was calculated based on 40 participants’ food records.

Muscle mass and muscle strength

In previous studies, protein-energy supplements alone showed the effects on weight gain (Gray-Donald, Payette et al. 1995; Payette, Boutier et al. 2002) or an increase in fat mass (Bunout, Barrera et al. 2001; Bunout, Barrera et al. 2004) . These studies did

not show a significant effect on increasing muscle mass, muscle strength or physical functional improvement in the elderly. The differences in protein content and protein type in the supplements between these studies and the current study may partially explain the differences in the results regarding the changes in muscle mass and muscle strength.

In these previous studies (Bunout, Barrera et al. 2001; Bunout, Barrera et al. 2004), the supplement used provided 13-15 g protein (~15% of energy of supplement), although the total energy of the supplements were similar or even higher than the current study. These protein levels were much lower than the current study in which the protein supplements contained 30 g protein (60% of the energy content of the supplement), and the protein sources were not reported. There were two other studies which had a similar protein content (protein 30g/day) in their supplements and much high total energy content compared to the current study (Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003). Both studies used a commercial drink (Ensure or Ensure Plus) in which the proteins are mainly casein and soy protein isolate. The protein used in the current study was whey protein which contains the highest concentration in essential branched-chain amino acids among all protein sources and thought to have the highest bioavailability (Walzem, Dillard et al. 2002). They are easily digested and utilized (Dangin, Boirie et al. 2002; Hall, Millward et al. 2003; Layman 2004; Bowen, Noakes et al. 2006) as whey protein is emptied rapidly from the stomach into the duodenum and thus amino acids appear in the blood stream faster (Boirie, Dangin et al. 1997). The branched chain amino acid are unique because they are metabolized by muscle not by liver (Walzem, Dillard et al. 2002). By contrast with whey protein, casein is a slow acting protein as it forms a gel or clot in the stomach, and the clot is able to slow the release of amino acid into the blood stream (Boirie, Dangin et al. 1997). Casein has been shown to induce a lower but more prolonged hyperaminoacidaemia and higher postprandial leucine deposition than whey protein in young men (Dangin, Boirie et al. 2001). Whey protein was digested faster than casein protein in both young (24 ± 1 years) and older men (71 ± 1 years), and protein synthesis was higher with whey protein than casein protein, especially in the elderly group (Dangin, Guillet et al. 2003). The use of whey protein in the trial group in the current study did not show any benefit

compared to the carbohydrate control supplements in improving muscle mass and strength aspects.

The adequate energy and protein intake in the current study population at baseline may also have contributed to the failure in detecting a protein supplement effect on improving muscle mass and strength. The average protein intake of this study population at baseline was already 76 ± 19 g/day or 1.14 ± 0.33 g/kg/day. This level is higher than the current recommended protein intake for Australian or New Zealand female aged over 70 years, which is 0.94 g/kg/day. At baseline, total energy intake was 7140 ± 1518 kJ/day, which is slightly higher than the Australian National Nutrition Survey of 1995-1996 for the age group of 70-79, which was 6200 kJ/day (Australian Bureau of Statistics 1998). This indicates that the participants in the current study already had adequate protein intake and sufficient energy intake. As the protein intake from their habitual diet already met their recommended requirements for protein, the extra protein from the supplement may not be of benefit on increasing muscle mass or strength compared to the extra carbohydrate intake.

Two population-based long-term studies, have demonstrated a positive relationship between high protein intake (habitual diet) and higher muscle mass or a slow rate of loss of muscle mass with aging (Houston, Nicklas et al. 2008; Meng, Zhu et al. 2009). The relationship between baseline protein intake and lean and bone mineral content five years later was examined in a cohort of 862 community-dwelling women aged 75 ± 3 years (Meng, Zhu et al. 2009). The study found women with a higher protein intake (>87 g/d) had 5.4-6.0% higher whole body and appendicular lean mass. The effects remained after adjustment for potential confounders (height, physical activity and energy intake).

The current study found no differences in change in muscle mass and strength between the control and trial groups at one year, suggesting that the effect of a high protein diet on muscle mass and strength may need a longer time to achieve results. This may explain why most of the long term cross-sectional or longitudinal studies have found a positive correlation between habitual high protein intake and high muscle mass and/or strength in the elderly (Hughes, Frontera et al. 2002; Meng, Zhu et al. 2009). However, most of the protein-energy intervention studies failed to show

this correlation (McMahon and Bistran 1990; Fiatarone, O'Neill et al. 1994; Bunout, Barrera et al. 2001; Payette, Boutier et al. 2002). Elderly people with a habitual high protein diet may also benefit from a lower risk in developing non-communicable diseases, such as diabetes and heart disease (Hu, Stampfer et al. 1999; Farnsworth, Luscombe et al. 2003). The presence of chronic disease causes deterioration in the overall health status in the long term, including muscle mass and strength and physical functions.

There was no significant fat mass gain in the trial (protein) group in the current study suggesting that the protein supplement may slow the expected gain in fat mass in these elderly women, despite the extra energy intake. One possible underlying mechanism is that protein can produce a larger diet-induced thermogenesis than carbohydrate and fat, resulting in an increase in energy expenditure (Raben, Agerholm-Larsen et al. 2003). Moreover, it has also been observed that with the same energy density (2500 kilojoules for women), postprandial fat oxidation was unchanged after protein meals (31.8% of energy), but was suppressed after carbohydrate meals (65.4% of energy) (Raben, Agerholm-Larsen et al. 2003). None of the previous protein-energy supplement intervention studies in this age group (Fiatarone, O'Neill et al. 1994; Bunout, Barrera et al. 2001; Bonnefoy, Cornu et al. 2003; Bunout, Barrera et al. 2004) compared the differences in fat mass gain between isocaloric protein and carbohydrate supplements. The total body fat mass increased in the control group (+1.5%, $p=0.04$) but not in the trial (protein) group (+0.7%, $p=0.19$) at one year in the current study. However the changes were not significantly different between control and trial groups. Caution is needed when interpreting this change in the percentage body fat as there was no significant difference between groups. The mean percentage of the total body fat mass of all participants in the control group in this study was $39.4 \pm 5.7\%$ at baseline and was similar to the level found in other studies.

The percentage body fat mass for 267 Italian women aged 70-80 years living in the Mediterranean area was $36.6 \pm 5.5\%$, (Coin, Sergi et al. 2008). They used the same brand of DXA machine as was used in the current study to measure the body composition. A high protein diet has been found to be associated with more favorable results for weight loss compared with high carbohydrate or low fat diets in

overweight or obese individuals (Farnsworth, Luscombe et al. 2003; Labayen, Diez et al. 2003; Due, Toubro et al. 2005). The effects on regulating body composition seem to be due to protein high satiety (Paddon-Jones, Westman et al. 2008) and diet-induced thermogenesis compared with fat or carbohydrate (Due, Toubro et al. 2005; Bowen, Noakes et al. 2006).

The current study showed that both protein and control groups significantly increased ankle dorsiflexion (protein supplemented +27%, control group +31%) and knee strengths (protein supplemented +20%, control group +23%) as a result of the intervention with no difference in changes between the trial and control groups. A 12-year follow-up study of eleven women aged over 73 years showed that ankle dorsiflexion strength declined 0.3% per year (Winegard, Hicks et al. 1996). The increase in the lower limb strength in the current study implies that the dietary supplements may play a role in preserving muscle strength. However, this result differs from previous randomized control trials of energy-protein supplementation with or without exercise (Fiatarone, O'Neill et al. 1994; Payette, Boutier et al. 2002; Bonnefoy, Cornu et al. 2003; Wouters-Wesseling, Van Hooijdonk et al. 2003). Most of the previous trials did not find that a dietary supplementation alone had any beneficial effect on increasing muscle strength in older people. Many studies however, found that exercise or resistance training alone or combined with dietary supplements had positive effect in the preserving of muscle strength (Fiatarone, O'Neill et al. 1994; Bunout, Barrera et al. 2001; Bunout, Barrera et al. 2004; Binder, Yarasheski et al. 2005; Aagaard, Magnusson et al. 2007; Levinger, Goodman et al. 2007) and balance (Orr, Raymond et al. 2008) in the elderly. LaStayo and co-authors have suggested that for studies which have combined resistance training with supplementation the 'learning effect' need to be considered in the analysis (LaStayo, Ewy et al. 2003). In the current study, hip strength increased at one year in the trial and control groups but the increases were not statistically significant in either group and there were no differences in changes between the drink groups.

The results of the increase in muscle strength in the trial and control groups, with no change between the groups are different from previous dietary intervention studies. Some previous studies (Fiatarone, O'Neill et al. 1994; Payette, Boutier et al. 2002; Wouters-Wesseling, Van Hooijdonk et al. 2003) which provided even higher energy

content supplement to their participants compared to the current study, failed to detect any changes in muscle strength following the intervention. Therefore, the extra energy intake from the supplements may not have an additional benefit for increasing muscle strength.

In a 16-week study of 83 elderly men and women aged 80 ± 7 years, participants were provided a nutrient-dense protein-energy liquid supplements (total energy 710 kcal/day, 30g protein) (Payette, Boutier et al. 2002). Their study found that although the total energy intake and weight gain were significantly higher in the supplemented group compared to the control group after 16 weeks, no differences in upper and lower limb muscle strength were detected. However, their elderly study participants were frail and under-nourished. The definition of frail and undernourished either an involuntary weight loss $>5\%$ body weight in the past month, 7.5% in the past 3 months, or $>10\%$ in the past 6 months and $BMI < 27$ or a $BMI < 24$. The current study participants, by comparison, were well-nourished (average BMI was 26.8 at baseline). Moreover, the current study duration is longer than their 16-week study.

Another study investigated the effect of dietary supplement and resistance training in 100 healthy, free-living men and women aged 70 years or older (Bunout, Barrera et al. 2004). Compared to the current study, their study had the same study duration (one year), a similar level of compliance in taking supplements (48% versus current study 47%) higher energy content (1600 kJ versus current study 810 kJ) but lower protein content (15 g versus current study 30 g) in the supplements. After the one year intervention, the protein-energy supplemented group did not change their lean body mass or muscle strength. However, no assessment of dietary intake was made to observe if total energy intake increased or not following the intervention.

Therefore, it is hard to evaluate whether the lack of changes in muscle strength following the protein-energy intervention was due to no effect of the supplements or due to compensation of normal food intake by the supplements.

Hand grip strength showed a different pattern from lower limb strength at one year in the current study. The protein supplements did not have a protective effect on preserving hand grip strength in the current study. Both the trial and control groups decreased hand grip strength at one year with a significant level in the trial (protein)

group but not in the control group. In general, hand grip strength peaks at 35 years of age and is positively correlated with forearm girth and length, hand size and body mass (Gunther, Burger et al. 2008). The CVs of hand grip strength varied largely between studies, and effort level was suggested to contribute to the high CV values over test occasions (Fairfax, Balnave et al. 1995). A CV above 7.5% indicates submaximal effort (Fairfax, Balnave et al. 1995). Submaximal effort was found to produce larger CVs, and females were also found to have greater strength variability (Fairfax, Balnave et al. 1995). The CV of hand grip strength in this study was 6.7%, which was within the acceptable range. The reasons for differences in response to the supplements between hand grip strength and lower limb strength are not understood and need further study.

Muscle size

Muscle size was evaluated by calf muscle cross-sectional area at 38% length of tibia and did not change over one year study period in the trial or control groups. Calf muscle cross-sectional area demonstrated a strong correlation with lower limb muscle mass and strengths at baseline. No correlation was found between the changes in calf muscle cross-sectional area and the increased in lower limb muscle strength after one year intervention. The calf muscle cross-sectional area as measured in this study had an acceptable inter and intra subjects and observers variation (detailed in 3.3.2.4). However, whether the muscle mass increased or decreased due to the intervention, the change of muscle size at this site was too small to detect at the one year intervention. The site chosen to assess the muscle cross-sectional area in this current study also could affect the ability to detect any change, as calf muscle cross-sectional at 38% length of tibia is small, and is not the site of maximum calf muscle area. The reliability of using this site to detect change over one year period has not been tested previously. Conventionally, muscle cross-sectional area at 66% tibia site were obtained using peripheral quantitative computed tomography (pQCT) (Stevenson, Moyer-Mileur et al. 2005; Popp, Hughes et al. 2009). As discussed in the methodology section, the manual tracing method was the only method available to our study to detect muscle density and intermuscular fat. If intermuscular fat increased, this method may not be sensitive enough to detect small changes in muscle area.

Following an extensive literature search, there have been few dietary protein intervention studies that have assessed muscle cross-sectional area in this age group of females. The one study that did, did not find any change in muscle cross-sectional area following the nutritional intervention (Fiatarone, O'Neill et al. 1994). Fiatarone and colleagues conducted a nutrition and exercise intervention trial in 100 men and women aged 72-98 years living in nursing homes (Fiatarone, O'Neill et al. 1994). The subjects were randomly allocated into four groups: supplement group (total energy 360 kcal, 17% protein which is about 15 g), resistance training group, supplement + training, no intervention group. After a 10 week study period, the mid-thigh muscle cross-sectional area was assessed by the computed tomography, and did not change in the supplemented group.

Data from previous longitudinal observation studies, however, showed an annual decline of thigh muscle cross-sectional area when measured by computed tomography in the range of 0.6-1.5% (Frontera, Reid et al. 2008; Delmonico, Harris et al. 2009). Previous studies demonstrated a strong correlation between muscle strength and cross-sectional area measured by quadriceps cross-sectional area from mid-thigh using X-ray computed tomography (CT) (Maughan, Watson et al. 1983; Visser, Kritchevsky et al. 2002; Visser, Goodpaster et al. 2005). A longitudinal study followed up twelve older volunteers (5 men) aged 71 ± 5 years for 8.9 years and found the knee extensor strength declined after eight years accompanied by a significant reduction in cross-sectional area of thigh muscle (-5.7% over 8.9 years which is about 0.6% per year) measured by computed tomography (Frontera, Reid et al. 2008). The variation of muscle fibre composition between individuals may contribute to the large variation in the ratio of strength to muscle cross-sectional area assessed by CT mid-thigh method (Maughan, Watson et al. 1983).

Fat infiltration into muscle may partially account for differences in muscle strength not attributed to muscle quantity (Goodpaster, Carlson et al. 2001). Evidence from magnetic resonance imaging also suggests that excess fat infiltration in leg skeletal muscle is associated with low calf muscle strength and impaired physical function (Hilton, Tuttle et al. 2008). It is worth remembering that although muscle mass changes influence the magnitude of the strength changes over time, strength declines in spite of muscle mass maintenance or even muscle mass gain in some subjects

(Hughes, Frontera et al. 2001). A combination of other factors such as cellular, neural or metabolic mediators may be involved in changes in muscle strength with aging (Hughes, Frontera et al. 2001).

A five year longitudinal study of 1678 ambulant men and women aged 70-79 showed that adjusted annualized (mean \pm SE) decrease in muscle torque (knee extensor) was 2-5 times greater than the loss of thigh muscle cross-sectional area measured by computed tomography (CT) in those who lost weight (thigh muscle area: $-1.5\pm 0.1\%$ cm², knee extensor torque: $-3.6\pm 0.3\%$) and in those who remained weight-stable (thigh muscle area: $-0.3\pm 0.1\%$, knee extensor torque: $-1.9\pm 0.3\%$) (Delmonico, Harris et al. 2009). In those whose weight increased, there was a small increase in thigh cross-sectional muscle area, but this increase did not prevent the loss of muscle torque (thigh muscle area: $0.4\pm 0.1\%$, knee extensor torque: $-1.9\pm 0.3\%$).

Mobility, balance function and falls risk

After the one year intervention, mobility tested by the 'Time Up and Go' test (TUAG) did not show any difference in either in the trial or control group in the current study. A study of 68 men and women aged 82 ± 7 years living in the nursing home also showed that there was no difference in changes in TUAG over a 6 months study period between the protein-energy supplemented group (1050 kJ/day, contained 14% protein) and the control group (Wouters-Wesseling, Van Hooijdonk et al. 2003). The baseline mean of the test in their study was 18.9 seconds. This is much higher than the baseline value of the current study, which was only 8.0 seconds. The much older study participants in their study may partially explain the difference of average of TUAG performance between the two studies.

In the protein supplemented, there was no change in the prevalence of poor balance and falls at one year. In comparison, the prevalence of poor balance was significantly increased in the control group (+9%), and the fall rate also significantly increased in the control group (+9%). These results are similar to a study of fifty community-living under-nourished men and women aged 78 ± 7 years, which showed that the supplemented group had significantly fewer falls after a twelve week study period (Gray-Donald, Payette et al. 1995). The study subjects were randomly assigned to the supplement and placebo groups. The supplemented group received

235mls of a commercial liquid (Ensure, Enrich with fibre, and Ensure Plus) twice daily which contained 1045-1480 kJ in total, and 14-14.7% of protein (about 8-13g). The proteins in these commercial liquid products are milk and soy protein concentrate. After 12 weeks intervention, the number of falls was significantly lower in the supplemented group (baseline: 25, 12-weeks: 0) than the control group (baseline: 4, 12-weeks: 21). It was suggested that malnutrition may play a role in the deterioration of muscle capacity and the loss of central and peripheral neurological function involved in gait and balance (Kinney 2004), however the underlying mechanism is not clear. The difference between the current study and Gray-Donald's study is that, the control group in the current study received an isocaloric carbohydrate drink but in Gray-Donald's study, the control group did not receive any treatment. As both studies observed an increase in fall rate in the control group, but no change or a decrease in fall rate in the protein supplemented, it is reasonable to consider that the protein supplement may have contributed to the preservation of balance function and may protect against falls in elderly women.

A common assumption made in intervention studies in the elderly is that the intervention designed to improve muscle mass or strength should lead to a greater physical function (Dutta 2000). However, most dietary or exercise intervention studies have failed to show this correlation (Bonney, Cornu et al. 2003; Kongsgaard, Backer et al. 2004; Orr, Raymond et al. 2008). Similarly, in the current study, lean mass gain was not correlated to strength gain in all muscle groups. For example, hip strength did not change significantly at one year although lower limb lean mass increased in this current study. A three year longitudinal study of 1880 older adults in America also showed that maintaining or gaining muscle mass does not prevent aging-associated decline in muscle strength which suggested a decline in muscle quality with aging (Goodpaster, Park et al. 2006). The larger inter- and intra-variation of lower limb muscle strength may also contribute to the lack of relationship between changes of lower limb muscle strength and changes of muscle mass or size. The coefficient of variation (CV) of the lower limb muscle strength (flexion, extension, abduction and adduction) of the current study ranged from 8.6% to 12.1%. These are similar to a study that examined the test-retest reliability of leg muscle strength in twelve men and women age 50-84 years (Pua, Wrigley et al.

2008). Their study found that the CV of leg muscle strength (flexors, abductors and extensors) ranged from 8% to 15.7%.

In the current study, the improvement in lower limb muscle strength was not correlated with physical functional performance. At baseline, ankle dorsiflexion, total knee and total hip strength were significantly negatively correlated with the 'Time Up and Go' test results ($r=-0.21$, $p<0.01$). However, although ankle dorsiflexion and knee strength increased at one year in both the trial and control groups, no changes were detected in TUAG performance in either group.

There are three possible explanations for the inconsistent results between the physical functional performance and lower limb muscle strength. One is referred to as a 'ceiling effect' (Stucki, Stucki et al. 1995; Colman 2006). That is, the TUAG test may not be challenging enough for this study population to detect a decline in mobility. Another possibility is that the study power was not great enough to detect the difference in TUAG in this population. The mean (SD) of TUAG in the current study was 8.0 (1.4) seconds at baseline. This was similar to the mean for 60 to 69 year olds in a meta-analysis which was 8.1 (7.1–9.0) seconds (Bohannon 2006). Even participants classed as sarcopenic might easily achieve the highest scores in this test. Therefore no difference could be detected between the groups or between study periods. Another reason is the large inter and intra variations of test subjects and observers, which leads to lack of precision of the tests, especially for the Romberg tests as discussed in the literature review (2.12.2). The third possibility is that muscle mass may not be highly correlated with physical function in this population. The possible non-linear age-related decline in balance and muscle strength reported by previous studies suggested the existence of threshold effects between these measurements (El Haber, Erbas et al. 2008). The threshold effects may also be a reason for the failure to detect the changes in TUAG and Romberg test in the current study.

The prevalence of falls at baseline in all participants was 10% and increased to 15% at one year. The finding of the decline in balance function with age in this study was consistent with the previous study which also suggested that postural balance deteriorates markedly after age 75 years old (Era, Heikkinen et al. 2002). Fall

prevalence can vary, largely depending on the study population and the definition of falls that is used. The one year fall prevalence of the current study was found to be similar to that reported by the Centers for Disease Control and Prevention (CDC), which used the same definition (self-reported falling during the preceding three months) to identify falls (Stevens, Mack et al. 2008). It was 15.9% in persons aged 65 years or over (14% in those aged 70-74; 15.7% in those aged 75-59; 20.8% in those aged ≥ 80). The CDC used data from Behavioural Risk Factor Surveillance system survey in 2006 which interviewed 92,808 elderly persons to assess fall rates.

The fact that there was no difference in changes in muscle mass or strength, but the differences in changes in standing balance between the trial and control groups over one year in the current study, indicated that the protective effect of protein on the deterioration of balance function with aging may be due to other mechanisms rather than simply an increase in lower limb strength. A study compared an inadequate protein intake (0.5 g/kg/d) to a control adequate diet (protein intake 1.2 g/kg/d) in twenty-one men and women aged 55-80 years by muscle biopsy. The study found that short-term inadequate protein intake changed the skeletal muscle transcript levels in these elderly participants and may resulted in muscle wasting, adverse metabolic and functional events (Thalacker-Mercer, Fleet et al. 2007) which means low protein intake could affect muscle quality.

The results reported in the literature from previous dietary intervention studies (Table 2.5) on the effect of a higher protein intake on muscle mass and function are inconsistent. Many of the reported dietary intervention studies in relatively healthy elderly people used protocols that combined supplements with resistance training.. The discrepancy in the results between studies may be due to the heterogeneity of the study cohort (sample size and target population which varied from chronically ill or fragile subjects to those who are relatively healthy), study duration, tests used, outcomes measured, the composition and amount of the supplements given, and the level of compliance with the trial regime.

The current study sample was population based. The participants were recruited randomly using the Electoral Roll, which included all women of this age range who have registered to vote. The participants were relatively healthy when they entered

in the current study. Ensuring that the trial (protein) supplement and the control supplement provided the same amount of energy and this enabled the current study to compare the effect of high protein supplement versus low protein supplement on preventing sarcopenia.

Serum IGF-1

Serum IGF-1 was significantly increased at one year in the protein supplemented group (+6.5 ng/ml) but decreased in the control group (-2.1 ng/ml). The difference of the changes between the two drink groups was highly significantly ($p = 0.009$ in t-test) at one year. A 3-month randomized controlled trial of high or moderate protein diet combined with resistance and cardiovascular training was conducted in 24 overweight or obese adults aged 31-59 years (Arciero, Gentile et al. 2008). The study found that all groups lost significant amount of body weight and total body fat after 3 months, and the serum IGF-1 was significantly increased in the subjects in the high protein diet with or without training groups. The increase in serum IGF-1 may explain the preservation of lean body mass despite having an overall loss of body weight.

It was hypothesized that participants in the trial (protein supplemented) group would have more lean mass gain than the control group over a one year period, and the increase would be associated an increase in the serum IGF-1 level. Although it was found that both the control and trial groups had similar increases in body lean mass after one year, there were significant differences in serum IGF-1 levels. The high protein diet of the trial group has a beneficial effect through increasing serum IGF-1 in the elderly women after one year. Given the fact that serum IGF-1 decreases with age (Corpas, Harman et al. 1993), the current study showed the beneficial effect of a high protein diet in increasing serum IGF-1.

As expected the overall serum IGF-1 concentration at baseline was significantly correlated with whole body lean mass in the current study. IGF-1 regulates muscle metabolism through complex biochemical pathways (Booth 2006). A decline in IGF-1 is correlated with a decrease in muscle mass and muscle strength (Baumgartner, Waters et al. 1999; Payette, Boutier et al. 2002; Roubenoff, Parise et

al. 2003), and poor lower limb function (Cappola, Bandeen-Roche et al. 2001) in elderly people.

Previous studies have shown that IGF-1 has positive effects on muscle and bone systems (Baumgartner, Waters et al. 1999; Roubenoff, Parise et al. 2003). Previous animal and human studies have also provided evidence of an inverse correlation of IGF-1 with some diseases, including diabetes (Bergerot, Fabien et al. 1995; Capoluongo, Pitocco et al. 2006; Chu, Moreland et al. 2008) and heart disease (Juil, Scheike et al. 2002). In the animal studies, IGF-1 was reported to protect non-obese mice from developing diabetes (Bergerot, Fabien et al. 1995) and reversed hypoalgesia and improved mobility in the mouse model of diabetic peripheral neuropathy (Chu, Moreland et al. 2008). A cross-sectional study in 170 subjects with metabolic syndrome, with or without diabetes, found that IGF-1 was significantly inversely correlated with C-reactive protein (CRP), a well-known marker of inflammation (Efstratiadis, Tsiaousis et al. 2006). A study of 246 type 1 diabetes patients in France found that serum IGF-1 concentration was significantly lower in patients with the diabetic complications, or a family history of diabetes (Capoluongo, Pitocco et al. 2006). A population-based case-control study in the US found that low serum IGF-1 was associated with increased risk of ischemic heart disease (Juil, Scheike et al. 2002).

The significant increase in serum IGF-1 in the trial (protein supplemented) group may play a role in preserving the balance functions in the protein supplemented group. All body tissues contain IGF-1 (Clemmons 2007), and IGF-1 levels decline with aging, and this phenomenon exists in community-dwelling elderly with no malnutrition and no inflammation (Raynaud-Simon 2003). The prevalence of poor balance and falling in the trial (protein) group did not change significantly at one year compared to the baseline, but the prevalence of poor balance and the reported falls were significantly increased in the control group at one year. As both the trial and the control groups increased in muscle mass at one year with no difference between the groups, the increased in muscle mass alone cannot explain the differences in changes in the balance functions between the drink groups. The increased IGF-1 may contribute to an improvement in the quality of muscle (Wang, Messi et al. 2002) and/or neurological functions (Gonzalez, Messi et al. 2003).

Muscle quality includes fibre-type composition, contractile properties, innervations, capillarity and metabolic capacity (Dutta 2000). Muscle quality in humans can be assessed by muscle testing and muscle biopsy to assess the number of muscle fibre or the cross-sectional area of muscle type I and II fibres (Brooks, Layne et al. 2007). One-repetition maximum (1 RM) is used by many studies to assess muscle quality (Kostek, Delmonico et al. 2005; Tanton, Cappaert et al. 2009), and most of these studies assessed the effect of resistance training on muscle quality. Other methods, such as muscle function per unit of muscle mass (Ivey, Tracy et al. 2000) or muscle function per unit of muscle cross-sectional area (Delmonico, Harris et al. 2009) have also been used. Muscle function can be defined as 1 RM, muscle peak power, or torque (Dutta 2000). A study was undertaken to compare the influence of age and gender on muscle quality response to strength training in 42 young and elderly men and women (Ivey, Tracy et al. 2000). The study calculated muscle quality by dividing quadriceps 1RM strength by quadriceps muscle volume determined by magnetic resonance imaging. Evidence supporting the existence of a positive correlation between IGF-1 and muscle quality are mainly from animal studies (Saatman, Contreras et al. 1997; Messi and Delbono 2003; Payne, Zheng et al. 2006).

A study in mice showed that an increased level of IGF-1 reduced age-related losses of skeletal muscle motor neurons and type IIB muscle fibres (Messi and Delbono 2003). IGF-1 administration improved neuromotor function in brain injured rats (Saatman, Contreras et al. 1997). A study that used a tetanus toxin fragment-C fusion protein to target IGF-1 to motor neurones in mice was used to study age-related declines in muscle function. It was shown in mice to be associated with motor neurone loss, (Payne, Zheng et al. 2006). After three monthly injections of IGF-1, muscle weight or muscle fibre size did not change but the single fibre specific force increased significantly. Their study demonstrates that IGF-1 administration prevents muscle fibre specific force decline. Although both animal and human studies have demonstrated the positive correlation between IGF-1 and muscle quality (as discussed above), and the beneficial effect of growth hormone treatment on improving muscle mass and strength (Barton-Davis, Shoturma et al. 1998; Attanasio, Bates et al. 2002), whether these improvements translate into improved physical function, such as balance performance, in elderly remains largely unknown.

The mean IGF-1 level varies considerably between studies. The mean \pm SD of IGF-1 concentration was 108.6 ± 39.0 ng/ml at baseline and 110 ± 40.1 ng/ml at one year in all participants in the current study. These levels are similar to previous studies (Barber, Braid et al. 2003; Dantas, Pires et al. 2008). A study of 98 elderly women aged 63-78 years found that the mean \pm SD of IGF-1 in women engaged in weight training for at least six months was 107.5 ± 38.6 ng/ml, and the control group was 60.1 ± 6.4 ng/ml (Dantas, Pires et al. 2008). The median (interquartile range) IGF-1 level at baseline (when admitted to the rehabilitation unit) of 32 patients (12 females) with median age of 79 years who had surgical fixation for proximal femoral fracture was 120.0 (92.0-129.5) ng/ml (Barber, Braid et al. 2003). The differences between studies could be due to the differences in analytical methods (Brabant and Wallaschofski 2007), and the study populations, including differences in age, gender and health status. The results from previous dietary intervention studies are inconsistent regarding the effect of protein-energy supplement on IGF-1.

A study of 62 postmenopausal women aged 55 ± 5 showed that IGF-1 increased in both the soy protein supplemented group (25 g soy protein) (+12.8%) and the control diet group (+26.3%) after one year of study (Arjmandi, Lucas et al. 2005). The reason for a significantly lower increase in IGF-1 in the soy protein supplemented group compared to the control group is unknown. The authors did not mention the composition of the control diet in their paper, and therefore it is hard to assess whether the control diet was an appropriate control. Moreover, compared to the current study, the study subjects are much younger. A study investigated the effect of combined exercise and dietary supplements on IGF-1 in 51 young adult men and women aged 18-25 years (Lee, Pivarnik et al. 1996). After 6 months of intervention, the increase in plasma IGF-1 was greater in the protein supplemented group (42 g protein, 24 g carbohydrate, 2 g fat) compared to the carbohydrate controlled group (70 g carbohydrate). Their paper did not report the exact values of the changes, but from the graph in their paper, IGF-1 was slightly decreased in the carbohydrate controlled group (about 2 ng/ml), and IGF-1 was increased about 3 ng/ml in the trial group, and the difference between two groups was significant ($p = 0.01$). Although the effect of protein supplements in their study is similar to the current study which is that the protein supplements significantly increased IGF-1 level, caution is needed

when making the comparison, as their study population is much younger than the current study population.

Previous observation studies have shown that deprivation of nutrients is associated with decreased essential amino acids and circulating regulatory hormones and either of these factors could affect the formation of IGF-1 in the liver (Phillips and Young 1976; Phillips and Young 1976; Phillips, Harp et al. 1990). Both animal (Phillips, Orawski et al. 1978) and human (Clemmons, Klibanski et al. 1981) studies revealed that protein is particularly important in sustaining levels of circulating IGF-1 as well as circulating IGF bioactivity. An in vitro study in rat hepatocytes found that deprivation of the essential amino acids lysine or tryptophan from culture medium for 3-5 days led to 63% and 76% declines in IGF-1 release respectively, but no significant change occurred when the non-essential amino acids, cysteine, was deleted (Harp, Goldstein et al. 1991).

Muscle and IGF-I levels may respond to extra protein or energy intake differently. Muscle mass or strength may be more sensitive to energy intake regardless of the nutrient component. IGF-1 may be more related to specific dietary components, such as protein. Muscle mass and strength alone cannot provide a full range of information on muscle quality.

The current study has shown that one year of dietary protein supplementation significantly increased serum IGF-1 level compared with the control drink. The mechanisms that link an increase in IGF-1 levels and changes in muscle mass and strength, body composition and balance function need further investigation. Because of the strong correlation between IGF-1 and health of musculoskeletal system, the significant increase in IGF-1 in the trial group (protein supplemented) group provides support for increasing the recommended protein intake for the elderly, a non-pharmaceutical strategy to prevent sarcopenia.

Summary of this section

The results of the current study suggests that the extra energy intake from the supplements (control and trial drinks) may have a role in increasing muscle mass and improving lower limb muscle strength in these elderly women regardless of the

macronutrient composition of the supplement. However, data that shows that energy intake actually increased in the groups at one year, it is difficult to draw a final conclusion. A longer study period is needed and to complete the analysis together with more data on energy intake. Ideally this would be done through the use of doubly-labeled water.

The protein supplement showed a tendency to preserve balance function with aging and had a beneficial effect on preventing increased fall risk with aging. The isocaloric carbohydrate drink did not show these beneficial effects. However, the trial drink (protein supplement) was similar to the control (carbohydrate) drink in building lean body mass and muscle strength in these elderly women during twelve months period. As nutritional intake and muscle mass decrease with age, a small increase in energy intake irrespective of its nutritional composition, may reverse the reduction in muscle mass and strength. Moreover, the increase energy intake obtained from protein has been shown in this study to have a beneficial effect on the preservation standing balance functions. However, caution is needed before recommending increasing energy intake for people who are overweight or obese. The observation of a significant increase in serum IGF-1 concentration and improved standing balance and decreased prevalence of falls in the trial (protein) group indicates that protein supplement may have a role in improving muscle quality. A longer follow-up period is needed to confirm the positive correlation of the improved IGF-1 level with balance performance and rate of falls. More studies are needed to investigate the underlying mechanisms involved.

5.3 Limitations of the study

There are a few limitations that need to be considered when interpreting the result so this study and some of these are related to the measurement methods. The first is that the para-amino benzoic acid test (PABA) was not used to verify the completeness of 24-hour urine collection. The conclusion drawn in this report was based on the assumption that participants collected their 24-hour urine completely. The second limitation is that using IPAQ for testing physical activity may not be appropriate for this one year study. IPAQ was validated for large population based studies, but its reliability in detecting the potential changes in physical activity over a

short period in a small population is unknown. The third limitation was the issue of measurement error for the anthropometric data. The same anthropometrist was not used throughout the study, increasing the possibility of measurement error between the baseline values and the one year testing. Ideally, all anthropometric measurements should be done in duplicate or triplicate but time did not always permit this to occur. However the measurements were conducted by experienced anthropometrists following a strict protocol. Therefore, while it is unlikely, measurement error cannot be ruled out as a cause of the changes in these variables. The fourth limitation is that due to the use of bone parameters in performing pQCT scan for leg, calf muscle cross-sectional area was analysed by the manual tracing method, therefore the information about muscle density is not available. Moreover, measuring of calf muscle cross-sectional area at 38% length of tibia may not be able to detect the change of muscle as this muscle at this site is relatively small.

The compliance (overall 47%) rate was comparable to similar studies at overseas centres, but is still of some concern. The slight imbalance in compliance between the two drink groups (higher compliance in the protein supplemented group) may affect the outcomes. However this could also be due to the subjects in the trial group (the protein supplemented group) may have perceived a positive benefit, even though every possible effort was made to maintain a strictly double blind study. Although we conducted a subgroup analysis which only included those whose compliance >70% this showed no significant difference from the analyses using whole study population, the limit number of this subgroup (n = 38) would affect the power to generalize the conclusion.

Moreover, the baseline data indicates that there may have been a limited expected effect. As shown in Appendix 19, at baseline there was no difference in any measures of mobility and balance or in muscle strength between subjects in the lowest, middle and highest tertile of protein intake. The difference between the highest and lowest tertile was 11.1 g of protein/day assessed by food-record but as high as 39.4 g estimated using 24 hour urinary excretion. With a supplement of just 30 g protein and a 52 % compliance in the protein supplemented group, the 'average' supplement achieved would be only about 15g/day. The changes of protein intake assessed by 24-hour urinary nitrogen excretion showed that the protein supplemented

group only increase average of 8 g/day of protein intake at one year. Given the baseline data, 30 g/day protein supplement is not likely to be effective for achieving the changes in mobility, balance or muscle strength.

Although it is best practice to use an 'intention to treat' analysis, it would also be beneficial to know if there was any tendency for an effect on any of the outcomes if only the high compliers were assessed. This analysis approach was also attempted but did not find any significant finding. Higher levels of supplementation could be tested in future studies.

The extra energy intake had a beneficial effect on preventing muscle wasting with aging. However, accompanied with the increased muscle mass and strength, the weight also increased in both drink groups. A longer period of follow-up is needed to verify that if the weight gain would continue over a longer period.

For the assessment of dietary energy intake it would be preferable to use the doubly-labeled water method as this is relatively independent of compliance and is now regarded as the gold standard for assessing energy expenditure and the extent of underreporting. Unfortunately it remains prohibitively expensive for similar levels of funding available to this study.

Due to the small number of falling reports, the correlations between protein supplement, serum IGF-1 and improved balance and fall rates in the protein supplemented group need to be interpreted with caution. A longer follow-up period is needed to confirm this finding.

The use of the cut-point of 5.454 kg/m² to define sarcopenia may not be appropriate for this study population as this cut-point was derived from a random sample of Hispanic and white women and men from New Mexico. A study investigating body composition using the same DXA machine in normal young healthy female Australian population is needed for obtaining a mean value which can represent this population. This may then to be used to calculate more appropriate population cut-points for the elderly female Australia to identify the prevalence of sarcopenia.

Chapter 6 Summary and Conclusions

1) Underreporting of food intake at baseline, prevalence of sarcopenia at baseline, and the correlation of baseline dietary protein intake and body composition:

The mean protein intake was 76 g/day (1.14 g/kg/day) at baseline. By using the Goldberg 'cut-off' method, 24 subjects (11% of study population) were defined as under-reporters. Baseline protein intake was not correlated with baseline whole body lean mass or appendicular lean mass after adjusting for height in the analysis. The prevalence of sarcopenia was 9%, which is low compared to previous studies. Using a cut-point of 5.454 kg/m² of ALM/height² to define sarcopenia may not be appropriate for the current study population. Upper and lower limb muscle strength were significantly positively correlated with DXA measured whole body lean mass and appendicular lean mass, and the correlation remained significant after adjusted age and BMI.

2) The effect of one year protein supplementation on muscle mass, muscle size, and body composition measured by dual energy x-ray absorptiometry and peripheral computed tomography (objective 1, 2, &6):

Whole body lean mass (protein supplemented group +1.6%; control group +2.3%) and appendicular lean mass (supplemented group +1.3%; control group +1.8%) measured by DXA were increased significantly both in the protein supplemented group and the control groups after a one year intervention. There were no significant differences in changes of these parameters between the two groups. Body weight also increased significantly in both the protein supplemented group (+0.8%) and the control group (+1.5%). Total body fat mass only significantly increased in the control group (+1.5%, $p < 0.05$) but not in the protein supplemented group (+0.7%, $p = 0.19$). There was no significant difference in changes in total body fat mass between the two groups. Calf muscle cross-sectional area measured by the peripheral computed tomography did not change in either the protein supplemented group or the control group after the one year intervention. No changes in any anthropometry measurement were detected in either the protein supplement group or the control group after the one year intervention.

3) The effect of one year protein supplementation on muscle strength, mobility, balance, falling risk, and serum IGF-1 level (objective 3, 4, & 5):

Lower limb strength, including ankle dorsiflexion strength (protein supplemented group +27%; control group +31%) and knee strength (protein supplemented group +20%; control group +23%), increased significantly both in the protein and the control groups after the one year intervention. There were no significant differences in changes in lower limb muscle strength after one year intervention between the two drink groups. The prevalence of poor balance (baseline 13%, one year 22%, $p < 0.05$) and falls (baseline 5%, one year 14%, $p < 0.05$) were significantly increased only in the control group. In contrast, after the one year intervention, the protein supplemented group maintained similar levels of poor balance rate (baseline 13%, one year 18%, $p = 0.44$) and self-reported falling rate (baseline 13%, one year 16%, $p = 0.59$) as at baseline. The serum IGF-1 level was significantly increased in the protein supplemented group (+7.6%, $p = 0.008$) but slightly decreased in the control group (-1.0%, $p = 0.28$), and the difference was significant between the two groups ($p = 0.006$).

The protein supplement has shown to be superior to the isocaloric control drink in the current study in three areas. Firstly, the standing balance at one year remained at the similar level as at baseline in the protein supplemented, but became worse in the control group. These differences were consistent with the changes in the prevalence of falls at one year, the protein supplemented stayed at the similar level as at baseline but there was an increase in the control group. Longer follow-up is needed to confirm these findings. Secondly, the protein supplements limited body fat gain compared to the control supplements. Fat mass increased only in the control group at one year. Thirdly, protein supplements resulted in a significant increase in serum IGF-1 level at one year compared with the control group in which the IGF-1 level slightly decreased. The significantly increased serum IGF-1 level in the protein supplemented group may relate to the stable balance function and fall rate in this group at one year. Whereas, the slight decrease in serum IGF-1 level in the control group at one year may contribute to the decrease in balance function and the increase in fall rate in this group. However, given the small changes in serum IGF-1 although significantly different between the groups, caution is needed when interpreting these

results. A longer follow-up is needed to confirm these correlations and further studies are needed to investigate the mechanisms involved in the relationship between IGF-1 and muscle quality and balance function.

These data are consistent with the concept that in this age group increased energy intake regardless of the macronutrient composition of the supplements improves muscle mass and function. It is possible that achieving this through increased protein rather than carbohydrate intake may prevent the increase in fat mass noted with the carbohydrate supplement. The metabolic significance of this remains to be explored.

Appendices

Appendix 1 Ethics approval letters

memorandum

To	Dr Deborah Kerr, Public Health
From	Professor Charles Watson, Chairperson, Human Research Ethics Committee
Subject	Protocol Approval HR 23/2007
Date	5 April 2007
Copy	Professor Colin Binns, Public Health, Xingqiong Meng (Rm1 Flat D2W, Guild House, 63 Jackson Rd Karawara WA 6152) Graduate Studies Officer, Division of Health Sciences

Thank you for your application submitted to the Human Research Ethics Committee (HREC) for the project titled "A Randomised Controlled Trial of Twelve Months Protein Supplementation on the Muscle Mass and Strength in Elderly Women". Your application has been reviewed by the HREC and is **approved**.

- You are authorised to commence your research as stated in your proposal.
- The approval number for your project is **HR 23/2007**. Please quote this number in any future correspondence
- Approval of this project is for a period of twelve months **03-04-2007 to 03-04-2008**. To renew this approval a completed Form B (attached) must be submitted before the expiry date **03-04-2008**.
- If you are a Higher Degree by Research student, data collection must not begin before your Application for Candidacy is approved by your Divisional Graduate Studies Committee.
- The following standard statement **must be** included in the information sheet to participants:

This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number HR 23/2007). If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784 or by emailing hrec@curtin.edu.au.

Applicants should note the following:

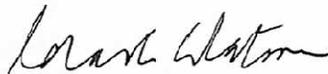
It is the policy of the HREC to conduct random audits on a percentage of approved projects. These audits may be conducted at any time after the project starts. In cases where the HREC considers that there may be a risk of adverse events, or where participants may be especially vulnerable, the HREC may request the chief investigator to provide an outcomes report, including information on follow-up of participants.

The attached **FORM B** should to be completed and returned to the Secretary, HREC, C/- Office of Research & Development:

When the project has finished, or

- If at any time during the twelve months changes/amendments occur, or
- If a serious or unexpected adverse event occurs, or
- 14 days prior to the expiry date if renewal is required.
- An application for renewal may be made with a Form B three years running, after which a new application form (Form A), providing comprehensive details, must be submitted.

Regards,



Professor Charles Watson
Chairperson
Human Research Ethics Committee

Wednesday, 17 January 2007

A/Prof Richard Prince
Bone and Mineral Research Group
Sir Charles Gairdner Hospital
Verdun Street
Nedlands WA 6009

Dear A/Prof Prince

HUMAN RESEARCH ETHICS COMMITTEE TRIAL 2006-181 Dietary protein effects in elderly women: musculoskeletal, renal, cardiovascular and body composition endpoints

Thank you for responding to the queries of the Medical and Allied Health Committee raised at it's meeting on the 21st December, 2006.

Outcome/Action required:

The Proposal is approved. The study will be tabled at the next Human Research Ethics Committee meeting to be held on the 6th February, 2007.

Please quote trial number: 2006-181 on all correspondence.

Yours sincerely

MR M CAIN
CHAIRMAN
MEDICAL AND ALLIED HEALTH COMMITTEE

Appendix 2 Recruitment letter



6th March 2007

<Given Name> <Surname>
<Address>
<Suburb> <State> <Postcode>

Dear Mrs <Surname>,

We would like ask you to become a research participant in the Protein Intake and Metabolic Effects Study

Researchers from the University of Western Australia, Sir Charles Gairdner Hospital, Curtin University and Edith Cowan University are looking at ways to prevent bone and muscle weakness through increased protein intake in the diet. Bones weakness is called osteoporosis, a condition in which bones become fragile and brittle, leading to a high risk of fractures. Muscle weakness may lead to increased risk of falling. To try and stop these problems occurring we have been given money by the National Health and Medical Research Council (NHMRC) to study the effects of a daily high protein drink or an identical low protein drink on your bones and muscles. The study is for 2 years during which time you would have take one of the two drinks every day and come to Sir Charles Gairdner Hospital seven times. If you are eligible you will receive (at no charge):

*** bone density scans * blood tests * diet assessment * ongoing study care**

You are not eligible for this study if you are on:

*** bone active treatment such as Fosamax or Actonel * lactose intolerant**

I appreciate that you did not ask to receive this letter. However, many of you have been prepared to help in similar studies in the past. So I thank you for considering this request. Your assistance is very much appreciated as it might be of assistance to yourself and others. The study has been approved by the Human Ethics Research Committee of Sir Charles Gairdner Hospital as well as the NHMRC.

Yours sincerely,

A/Prof Richard Prince, Chief Investigator
Bone and Mineral Research Group, Sir Charles Gairdner Hospital

If you are interested in finding out more please fill in the form below and

- a) return in the reply paid envelope enclosed OR
- b) fax it to us on 9346 1317 OR c) contact us by telephone 9346 1747

✂-----

I would like one of your staff to contact me about the "Protein intake and Metabolic Effects Study".

My name is _____ My date of birth is _____

My address is _____

_____ Postcode _____

My contact phone number is _____ Other comments _____

Signed _____

Appendix 3 PIMES study telephone recruitment prompter

PIMES Study Telephone Recruitment Prompter

Hello, could I please speak to Mrs/Miss/Ms _____.
Good morning/afternoon Mrs/Miss/Ms _____, my name is _____.
I am calling from the Bone and Mineral Research Group at the University of Western Australia. You recently responded to a letter in regards to a protein study. Is it possible to talk to you for the next 5 minutes, regarding the selection criteria utilised in this particular study? There are a few important questions that I need to clarify with you.

Yes / No

If no, Time: _____ **Day:** _____

If yes

Would you mind proceeding with the following screening questionnaire, so we can determine if you will be eligible for the present study?

Ineligible

Mrs/Miss (name) thank you very much for your time and assistance. Unfortunately the selection criteria make you ineligible to participate in the present study.

Eligible

With all the questions I asked you so far, you are eligible to come in for clinic screening visits. There will be two screening visits, in which we will further determine whether this study suit you. Before I book you in for the clinic visit, I just briefly explain to you what the study will involve.

The purpose of this study is to determine whether high protein intake will prevent bone loss and osteoporotic fracture in elderly people. Should you choose continue, this study will run for 2 years and would require you to consume 250ml of a milk like fluid every day. We would require you to come to Sir Charles Gairdner Hospital in Nedlands a total of 7 times over this 2 year period. The visits can take up to four hours and will include a variety of tests and questionnaires. You may be required to attend the hospital on three separate occasions at the baseline or beginning of the study, at 1 and 2 years you may need to come back to the hospital on 2 separate occasions. A physical general examination, blood and urine tests, anthropometry, bone density, muscle function, and food intake will be incorporated into your baseline visit.

Should you be eligible and choose to be part of the (PIMES) study, on your second visit you will be randomized into one of the two groups. Both groups will be asked to drink 250ml of the milk like fluid (in chocolate, caramel or coffee flavors) per day. The first group is a treatment group with the milk product containing 600mg calcium and 30g of extra protein per day for 2 years. The second is a placebo or dummy milk fluid, which looks and tastes the same but doesn't contain protein, however still contains the 600mg of calcium. You will be randomly assigned to one of the two groups and both you and I/staff won't know which milk product you have been assigned until the end of the study, unless there is a medical emergency. We call this a double blinded study.

During the study, you will also be asked to complete of a health diary, that will assist us in making informed decisions about any health or dietary problems that may develop. Your contribution to the study will help to determine whether we can prevent or delay disease through the use of extra protein.

What is your local GP or Doctors name? Find/ look up list OR type in details.

GP Name: _____

GP Address: _____

GP Telephone _____

We run our clinics on Tuesday and Thursday, so you can choose the day that suits you. The clinic starts at 8.30 am and goes for 4 hours until 12.30pm. When is the soonest convenient day for you to come into the study clinic.

Make appointment Day/Date: _____

We will send you the information in the mail, the soonest I can get it to you is in the next day or two. I will now explain the contents of the letter that I am going to send to you. You do not need to write anything down as it is explained in a covering letter to you.

There are green sheets which explain the study and the protein milk drink, have a read and there is a consent form that you need to bring with you and sign when you get here. As we will be taking blood and urine samples, it is essential that you fast prior to your appointment. There is a sheet of paper asking you to fast from the night before until you get here and just drink water. We will give you toast and tea or coffee once you have had your blood taken. ie. no breakfast the morning of your appointment.

There is a stapled blue questionnaire asking you lots of questions about your lifestyle. It will be great if you could complete as much of it as you possibly can. Anything you're unsure about or uncomfortable with, just leave blank until you get here and you can discuss it with us then. The last page of this questionnaire is asking about your medications and illnesses. If you can complete this and bring in your medications with you to the clinic that would be great (**only say this section if we are going to mail the demographic questionnaire**).

We will send you directions and a map on where to come, and a car parking voucher. The clinic will take four hours, so if you want to bring some knitting, a crossword or a book with you to pass the time that's okay. There are about eight other ladies in the clinic and they usually say they are really happy they came. They were a little bit anxious about coming but had a lovely morning once they got here. I will put the information to be sent to you in the outside/university mail today and you will get it within the next few days. Our phone number is on the sheets of paper we send to you. So if you have any queries, or want to change your appointment or cancel it for whatever reason, please phone us.

Thank you for your time on the phone now and I look forward to seeing you on the _____.

Appendix 4 Telephone screening and visit 1 questionnaire

Screening ID:

Protein Intake Metabolic Effects Study (PIMES) – Study Entry Criteria

Grey section – telephone screening White section – screening visit section

Full name:		Date of Birth:
Contact phone	Home	Mobile
Mailing address:		Post Code:
Telephone screening:		
<input type="checkbox"/> Eligible Date booked for first clinic screening visit: □□ / □□ / □□ ask participants to bring the medications they are using and their GP 's details		
<input type="checkbox"/> Exclude Reason for exclusion:		
Date of telephone screening:		Name of person completing this form:

Inclusion Criteria – IF NO EXCLUDE	Yes	No
Aged 70-80 years		
Have transport to get to SCGH?		
Exclusion Criteria – IF YES EXCLUDE	Yes	No
1. Lactose intolerant or do not like milk products		
2. Currently or within last year taking any of the following medications for osteoporosis (Bisphosphonates, Fosamax, Actonel, Zoledronate by drip, Evista, Teriparatide injection, Protos (strontium ranelate), HRT, Premarin or any other oestrogens) Name of medication _____		
3. Taking steroid tablets (eg Cortisone prednisone) in the past 3 months or have taken more than 7 g in total in life (daily dose _____ mg, start date _____, stop date _____)		
4. Metabolic bone disease eg Paget's disease		
5. Clinical diagnosis of diabetes		
6. Any fracture at wrist, hip or spine in the last 10 years falling from standing height or less than 1 meter If yes write details of when/what _____ The patient should be informed that they may be eligible for osteoporosis treatment covered by the Pharmaceutical benefits scheme (PBS). If patient is interested in receiving treatment, they should investigate this further before committing to PIMES.		
7. Participation in another clinical trial in the past 3 months. If yes write details of when/what _____		
8. Have one of the following: Liver failure Kidney failure on dialysis		

Bowl Surgery or difficulty in absorbing foods		
Coeliac disease		
9. BMI > 35 kg/m ² Height: _____m Weight: _____kg BMI: _____kg/m ²		
10. Clinical hepatic insufficiency		
Screening visit inclusion exclusion criteria		
Inclusion Criteria – IF NO EXCLUDE	Yes	No
1. A signed and dated written informed consent obtained prior to participation and given to patient		
2. Able to comply with the requirements of the protocol, including consumption of the drink		
Exclusion Criteria – IF YES EXCLUDE	Yes	No
3. A Mini Mental State Score of less than 24		
4. Total hip bone density more than 2 SD below the mean for their age		
5. High protein intake as assessed by Food Frequency Questionnaire (equivalent to protein intake more than 1.5 g/kg body weight per day)		
6. Renal insufficiency - creatinine more than twofold the upper limit of normal		
7. Participants who, in the opinion of the investigator are not likely to complete the study for any reason		
8. Participants who, in the opinion of the investigator are not likely to complete the study due to serious physical or mental disease		
9. Participants who, in the opinion of the investigator, abuse alcohol or drugs		
10. Participants with any clinically significant abnormality following review of screening laboratory data		
11. Contraindicated medications for osteoporosis, oestrogen or corticosteroids		
Clinic screening:		
<input type="checkbox"/> Eligible for randomization (has fulfilled all inclusion/exclusion criteria)		
<input type="checkbox"/> Eligible to act as community control (has high protein intake but fulfilled all the other inclusion / exclusion criteria)		
<input type="checkbox"/> Excluded		
Reason for exclusion:		

To be reviewed and completed at screening visit two

The participant has fulfilled all inclusion/exclusion criteria: Yes No

Study Coordinator's Signature: _____ Date: _____

Appendix 5 Screening visit appointment letter



THE UNIVERSITY OF
WESTERN AUSTRALIA

FACULTY OF
Medicine and Dentistry



Sir Charles Gardiner Hospital
Department of Diabetes and Endocrinology
1st Floor, C Block
NEDLANDS WA 6009
Telephone: 61 8 9346 1747

Dear Mrs,

05/09/2009

Thank you for agreeing to come in to see us. This visit is to determine if you are eligible for the study. The following information is important for your visit on:

Date: Tuesday 11 September 2007

Time: Start at 9.30am and finish at approximately 1.30PM

At: Department of Endocrinology & Diabetes, 1st Floor C Block, Sir Charles Gairdner Hospital, Hospital Ave, Nedlands 6009 (please find map enclosed)

Before your appointment:

Please fast from 10pm the night before your appointment and follow the enclosed instructions carefully (Yellow)

Please read the enclosed Participant Information Sheet and Consent Form (Green and white)

Please bring the following to your appointment:

ALL your medications (prescription AND non-prescription)

Your local GP or Doctors details (Name, address, telephone)

Reading Glasses, and Interpreter/Assistant if required

Please do not wear pantyhose and wear enclosed comfortable shoes

Parking voucher (enclosed). Place this on your dashboard so you don't need to pay for parking

The Participant Information and Consent (Green and white)

At your visit you will:

Go through the Participant Information Sheet and Consent Form

Have FASTING blood and urine samples collected

Have height, weight and body measurements

Undergo DXA and pQCT bone density scans

Complete several questionnaires

If you are unable to attend the appointment that has been made to you, please contact us on **9346 1747 or 9346 4970**. We look forward to seeing you at your appointment.

Yours sincerely,

Rosie Meng
PIMES study coordinator

Appendix 6 Demographic questionnaire

3. Which **one** of the following best describes your **normal place of residence**?

(please tick one)

- House/Flat/Unit/Villa
- Granny flat/Self-care unit/Retirement village
- Boarding house/Rooming house
- Hostel/Hostel type
- Caravan

4. Do you (or your Husband or partner) own your own home?

- Yes
- No

5. Which **one** of the following best describes your **living arrangements**?

(please tick one)

- Live alone
- Lives with Husband/partner ONLY
- Lives with relative(s)
- Has resident housekeeper
- Other _____

6. Which **one** of the following best describes your **main source of income**? *(please tick one)*

- Government pension or benefit
- Superannuation (including annuities, interest and dividends)
- Wage or salary from an employer
- Private business or rental property(ies)
- Other _____

7. Have you ever undertaken paid employment for more than one year?
(please tick one)

- Yes
- No
- Don't know

If yes, which **one** of the following best describes your **main paid occupation during your working life?** (please tick one)

- Professional
- Teaching/Nursing
- Clerical
- Domestic Duties
- Factory/Agriculture
- None of the above

8. At what age did you achieve your highest level of education?

--	--

 yrs

What is the highest level of education that you have completed?
(please tick one)

- Primary School
- Some High School
- Year 12 High School
- Trade or technical qualifications
- Degree from a University, College of Advanced Education or other tertiary institution

9. How many children have you had?
(Do not count miscarriages)

--

10. Did you breastfeed any children?

(3 or more times a day for the first month of their life.)

For each child tick "Yes" or "No". If yes, please tell us how long you breastfed that child.

- (a) First child Yes If yes, how many months _____
 No
- (b) Second child Yes If yes, how many months _____
 No
- (c) Third child Yes If yes, how many months _____
 No
- (b) Fourth child Yes If yes, how many months _____
 No
- (e) More than four children
 Yes If yes, how many months _____
 No

11. How old were you when you had your **last** menstrual period?

--	--

 yrs

12. Have you had a hysterectomy? (please tick one)

- Yes
 No

(a) If yes, at what age was this?

--	--

 yrs

(b) If you had a hysterectomy:

How many ovaries did you have removed? (please tick one)

- None
 One
 Two
 Don't Know

Did you have hot flushes? (please tick one)

- Yes
 No

If yes, how old were you when they started?

--	--

 yrs

13. (a) How old was your mother when she died?

--	--

 yrs
(if still alive leave blank)
- (b) How old was your father when he died?

--	--

 yrs
(if still alive leave blank)

14. How often do you go outside into the street or garden per week?
(please circle one)

Never	2-3 days	4-5 days	Most days
-------	----------	----------	-----------

15. Do you avoid direct sunshine? *(please circle one)*

Never	Usually	Always
-------	---------	--------

16. Have you had a suntan in the past 12 months? *(please circle one)*

No	Slight	Obvious
----	--------	---------

17. Do you use a walking aid? *(please tick one)*

- Yes
 No

If yes, what aid(s) do you use inside the house?

If yes, what aid(s) do you use outside the house?

18. How many times have you fallen in the last 3 months?

--	--	--

(If no falls write "0")

19. Are you afraid of falling? *(please tick one)*

- Yes
 No

20. Do you limit any household activities because you are frightened you may fall? *(please tick one)*

- Yes
 No

21. Do you limit any outdoor activities because you are frightened you may fall?

(please tick one)

Yes

No

22. Please tick the category that best describes the number of times you experience pain in **each** of the following parts of your body:

	Never	Less than once a month	Once a week to once a month	Once a day to once a week	Once a day or more
Hip joints					
Knee joints					
Feet joints					
Lower Back					
Upper Back					

23. Have you ever smoked at least one cigarette per day for as long as three months?

(please tick one)

Yes

No

(a) If yes, what year did you start?

--	--	--	--

(b) What year did you stop?

(Leave blank if currently smoking)

--	--	--	--

(c) On average, how many cigarettes do/did you smoke per day?

--	--

24. Have you had any serious illnesses or operations? YES/NO

If YES, could you please give details:

a		e	
b		f	
c		g	
d		h	

25. Are you taking/using any prescribed or over the counter medications? YES/NO

If YES, could you please give details:

Name of drug (generic or trade name)	For condition	Regimen eg: once a day	Dose Eg: 5 mg	Comments / checked MIMS (study staff use only)
Example: Tritace	Hypertension	Once daily	10 mg	
1				
2				
3				
4				
5				
6				
7				
8				

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

PLEASE REMEMBER TO BRING THE COMPLETED QUESTIONNAIRE ALONG WITH YOU TO YOUR APPOINTMENT AT SIR CHARLES GAIRDNER HOSPITAL

**Appendix 7 International physical activity questionnaire – short last 7 days self
– administered format**

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

No vigorous physical activities → *Skip to question 3*

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

No moderate physical activities → *Skip to question 5*

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

No walking → **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ hours per day

_____ minutes per day

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix 8 SF-36 questionnaire

ENGLISH (AUSTRALIA)

SF-36

IQOLA SF-36 English (Australia) Version 1.0

SF-36 HEALTH SURVERY

INSTRUCTIONS: This questionnaire asks for your views about your health, how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:		(circle one)
Excellent		1
Very good		2
Good		3
Fair		4
Poor		5

2. <u>Compared to one year ago</u> , how would you rate your health in general <u>now</u> ?		(circle one)
Much better now than one year ago		1
Somewhat better now than one year ago		2
About the same		3
Somewhat worse now than one year ago		4
Much worse now than one year ago		5

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(circle one number on each line)

ACTIVITIES	Yes, Limited A Lot	Yes, Limited A Little	No, Not limited At All
a. Vigorous activities , such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. Moderate activities , such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing several flights of stairs	1	2	3
e. Climbing one flight of stairs	1	2	3
f. Bending, kneeling, or stooping	1	2	3
g. Walking more than a mile	1	2	3
h. Walking several blocks	1	2	3
i. Walking one block	1	2	
j. Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? (circle one number on each line)

	Yes	No
a. Cut down the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Were limited in the kind of work or other activities	1	2
d. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(circle one number on each line)

	Yes	No
a. Cut down the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Didn't do work or other activities as carefully as usual	1	2

6. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

(circle one)

- Not at all1
- Slightly2
- Moderately3
- Quite a bit4
- Extremely5

7. How much bodily pain have you had during the past 4 weeks?

(circle one)

- None1
- Very mild2
- Mild3
- Moderate4
- Severe5
- Very severe6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

- Not at all 1
- A little bit 2
- Moderately 3
- Quite a bit 4
- Extremely 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks - (circle one number on each line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
a. Did you feel full of life?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt down?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

- All of the time1
Most of the time2
Some of the time3
A little of the time4
None of the time5

11. How TRUE or FALSE is each of the following statements for you.

(circle one number on each line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get sick a little easier than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5

Appendix 9 Food frequency questionnaire

Affix Participant's Label Here _____

Study ID _____

Date _____

FOOD FREQUENCY QUESTIONNAIRE

This questionnaire has been developed to provide information on your normal eating patterns, over the last 12 months. On the following pages please write in **number of times each week OR each day** that you have a serving of each food. Do not use a X or a √. If you eat a food less than once a week, DO NOT include it. If you eat a food every day, write the number of servings in the “every day” column. If you eat a food weekly, then write the number of servings in the “every week” column.

For example:

You drink 1 middle-size glass of milk every day and

You eat 3 doughnuts every week.

Food Type	Serving size	I Eat This Food Every Week (Write Number of Servings)	I Eat This Food Every Day (Write Number of Servings)
Milk	1 Small Cup (100 ml)		
	1 Middle-size glass (200 ml)		1
	1 Big glass, bowl (250 ml)		
Doughnuts (cream sweets)	110 g (1 doughnut)	3	

Food Type	Serving size	I Eat This Food Every Week (Write Number of servings)	I Eat This Food Every Day (Write Number of servings)
Milk (any type)	1 Small Cup (100 ml)		
	1 Middle-size glass (150 ml)		
	1 Big glass, bowl (250 ml)		
Yoghurt (any type)	150-180 g carton		
Doughnuts (cream sweets)	110 g (1 doughnut)		
Ice cream	2 large scoops		
White cheese (ricotta, cottage)	150 g		
Swiss/cheddar cheese	1 slice		
Melted cheese	30 g		
Grated cheese	30 g		

Food Type	Serving size	I Eat This Food Every Week (Write Number of servings)	I Eat This Food Every Day (Write Number of servings)
Soft cheese (Camembert)	Small (1/8 or 30g)		
	Middle (1/4 or 60 g)		
Red meat (65-100 g = 1/2C mince, 2 chops, 2 slices roast meat)	Small (50g)		
	Middle-size (80-100 g)		
	Large (150-200g)		
Poultry (50 g - 1 thick slice, 1/4 chicken -155g, 1 large drumstick-100g)	Small (50g)		
	Middle-size (80-100 g)		
	Large (150-200g)		
Fish (1 fillet = 100 g)	Small (50g)		
	Middle-size (80-100 g)		
	Large (150-200g)		
Processed meat (e.g. ham, polony)	Small (1 slice)		
	Middle-size (2 slices)		
	Large (1 Cup)		
Eggs	1 Egg (55g)		
Potato	Small (100g)		
	Middle-size (140g)		
	Large (200g)		
Pasta, rice or polenta	Small (1/2 Cup)		
	Middle-size (3/4 Cup)		
	Large (1 Cup)		
Peas, beans, lentils	Small (3 Tb)		
	Middle-size (5 Tb)		
	Large (10 Tb)		
Vegetables, cooked	Small (75g or ½ Cup)		
	Middle-size (1 Cup)		
	Large (1 ½ Cup)		
Bread (any type)	1 slice (Thick toast cut)		
	1 slice (Sandwich cut)		
Pizza (any type)	400 g per serve		
Cheese cake	1 moderate slice		
Sandwich, pannino, foccacia	1 Serving		

Appendix 10 Mini mental state exam

Affix Participant Label Here

Screening ID _____

Date _____

MMSE

1. Ask the patient for the following information. Score 1 for each correct answer.

Today's date _____ Season _____ day _____ month _____ year _____ /5

2. Ask the patient for the following information in regards to where they are. Score 1 for each correct answer.

State _____ Country _____ Town/City _____ Hospital _____ Floor _____ /5

3. Name three objects (apple, penny, table) taking one second to say each. Repeat the answers until the patient learns all three. Then ask the patient to tell you the three. Score 1 point for each correct answer. /3

4. Ask the patient to begin with 100 and count backwards by 7. Stop after 5 subtractions (93, 86, 79, 72, 65). Score the total number of correct answers. If the patient cannot or will not perform the task, ask him or her to spell the word 'world' backwards. The score is the number of letters in correct order, eg, dlrow 5, dlwrw 3. /5

5. Ask for the names of the three objects learned in #3. Score 1 point for each item recalled correctly (in any order) /3

6. Point to a pencil and a watch. Have the patient name them as you point.. Score 1 point for each item correctly identified /2

7. Have the patient repeat 'no if's, ands or but's'. If patient does not pronounce 's' score 0. /1

8. Have the patient follow this three-stage command, "take the paper in your right hand. fold the paper in half. put the paper on the floor." Score one point for each part correctly executed. /3

9. Have the patient read and obey the following "close your eyes". (Write in large letters). Score one point only if patient closes their eyes. /1

10. Have the patient write a sentence of his or her own choice. Do not dictate a sentence. The sentence must contain a subject and a verb. Score 1 point. /1

11. Have the patient copy the design attached (overlapping pentagons). /1

Education:

Primary of less

Secondary

Tertiary

Total /30

RA Name _____

Appendix 11 Screening visit one checklist

**Protein Intake Metabolic Effects Study
Checklist – Screening Visit 1**

Name and address label here

Date of screening visit 1: / / Study ID:

1. Consent form signed
(and copy given to patient) YES NO Initials:

2. Inclusion/exclusion form completed YES NO Initials:

3. Mini Mental State passed
(tick yes or no on exclusion criteria 11) YES NO Initials:

4. Questionnaires Initials:

◆ Demographic YES NO

◆ SF-36 YES NO

◆ International Physical Activity YES NO

◆ FFQ YES NO

5. Blood and urine samples

Blood sample YES NO
If yes, Sample ID _____ Time of blood _____ Initials

Urine sample YES NO
If yes, Sample ID _____ Time of urine _____ Initials

6. Weight: . kg Height: . cm

7. Waist circumference . cm

Hip circumference . cm

8. Triceps skin fold: . mm

Upper arm girth: . cm

Calf girth: . cm

9. Blood pressure: 1. mm Hg / mm Hg
 2. mm Hg / mm Hg
 3. mm Hg / mm Hg
 Mean mm Hg / mm Hg

11. Protein intake _____ g/day
 Protein intake / Weight = _____ g/kg body per day (tick yes or no or exclusion criteria 13)

12. DXA

Scan site	Scan speed	Scan No.	Comment
Hip L/R			Z score < -2 <input type="checkbox"/> YES <input type="checkbox"/> NO (tick yes or no on exclusion criteria 12)
Total body			
VFA			

13. pQCT

Scan site	Pixel Resolution	Scan No.	Comment
Forearm			
Tibia			

14. Final check list

- ◆ 3-day food record and scale given to participant YES
- ◆ Urine bottles given to participant YES
- ◆ 3-day test drink given to participant YES
- ◆ Clinic visit booked YES

Appendix 12 Screening visit 2 checklist

**Protein Intake Metabolic Effects Study
Checklist – Screening Visit 2**

Name and address label here

Date of screening visit 2: / / Study ID:

1. 24-hour urine bottles collection Initials: _____

Total weight (bottle and urine) _____ gram

Bottle weight Urine Volume _____ ml

Collection start date / / Time _____ hrs

Collection finish date / / Time _____ hrs

2. 3-day food record returned and checked YES Initials: _____

3. Timed Up and Go . seconds

4. Grip strength . kg/N

5. Lower limb muscle strength

Ankle dorsiflexion		Hip extension	
Knee flexion		Hip abduction	
Knee extension		Hip flexion	
		Hip adduction	

6. Romberg

Position	Open	Closed
Unable to perform	0	0
Side-by-side	1	1
Semi Tandem	2	2
Tandem (0-9 secs)	3	3
Tandem (>9 secs)	4	4

7. Drink acceptability

YES

NO

8. Check eligibility for randomization from telephone screening questionnaire

YES

NO

9. Completed all screening requirements

YES – go to 9

NO – go to 10

10. 3-month supply of test drink given to participant

YES NO

11. Preferred way of getting future test drink supply:

Pick up from the Sir Charles Gairdner Hospital

Pick up from the Curtin University of Technology

Deliver to home

Every 3 months 6 months

12. Date of appointment for QCT visit:

/ /

13. Eligible to act a community control

YES Inform participant that we will contact her after one year to make an appointment for clinic visit

NO

Appendix 13 3-day food record log book

Affix participant label here

PIMES STUDY

FOOD RECORD

The food record should include two weekdays and one weekend day (i.e. Thursday, Friday and Saturday or Sunday, Monday and Tuesday).

Started Diary on ____/____/____ *dd/mm/yy*

Day of week _____

Finished on ____/____/____ *dd/mm/yy*

Day of week _____

Name _____

Contact telephone number _____

Date of Birth _____

Height _____

Weight _____

Email _____

Please don't hesitate to contact the PIMES study team on 9346 1747 for further assistance.

Guidelines for keeping your 3-day Food Diary

PLEASE RECORD EVERYTHING THAT YOU EAT AND DRINK FOR THREE CONSECUTIVE DAYS.

- Start a new page for each day
- Record the date, the day and the time
- Record each item as close to the time of eating/drinking as possible
- Try to carry the diary with you always and record food as you eat it, so that nothing is forgotten

1. GIVE A FULL DESCRIPTION OF YOUR FOOD

- Record the *type* and *brand* of each item, which cut of meat is used and whether the fat has been trimmed, which brand of spread is used and which type of bread
- How the food is prepared and which cooking method has been used (for example; boiling, frying, roasting or baking). If fat has been added, please state the brand and the amount
- When 'take-aways' have been bought please say where they have been bought from

2. DESCRIBE THE AMOUNT OF FOOD YOU EAT & RECORD

INDIVIDUAL ITEMS

- **Where possible weigh the food** with the scales provided (this is the most accurate) and provide the quantity consumed in grams
- Record all fluids even water and where possible measure the volume with the measure cup provided and provide the volume consumed in mls. If you provide the volume consumed in cups, please indicate the size of the cup (eg a medium glass, a paper cup, coffee mug)
- If you are unable to weigh the food or drink record the amount in household measures (for example: cups, spoons, volume or teaspoons)
- Estimate the ingredients of a mixed dish separately (eg a salad with 1 lettuce leaf, ½ tomato and 3 slices of cucumber lightly dressed with French dressing)
- Don't forget accompaniments such as; butter, gravies, salt, butter added to vegetables, milk and sugar in coffee
- Record all recipes: there is a 'notes' section at the end of each page for this. Record the recipe and then tell us how much of the total is your portion

3. EATING AWAY FROM HOME

When eating away from home, it will not be possible to include recipes but we would like you to describe the food and the amount as much as possible.

4. PLEASE REMEMBER:

- Record what you eat and drink as close as possible to the time that you consume it
- At the end of the day – think back – you may have forgotten to record a biscuit or a piece of cake eaten with a cup of tea: please write it all down

EXAMPLE OF HOW TO RECORD WHAT YOU EAT AND DRINK

Time	Food and Drink Eaten	
	Description eg. Weetbix	Quantity eg. 50 grams
7.30am	Cornflakes – Kellogs Milk – Brownes Calcium Plus Sugar - white Toast, white bread – Helgas Margarine – Flora Light Tea – Lipton Milk – Brownes Skim Sugar - white	45g 250 ml 2 teaspoons 2 large thick slices 3 teaspoons 1 cup 30ml 3 teaspoons
10.30am	Granita – Arnotts Black Coffee Green Apple – Granny Smith	1 biscuit 1 cup 1 medium
1 pm	Bought from deli: 1 x cheese & salad sandwich White bread – brand unknown Butter – brand unknown Cheese grated - brand unknown Lettuce Tomato Beetroot, canned Apple – Red Delicious	2 standard slices Thickly spread ½ a cup 2 small leaves 3 medium slices 3 slices 1 large
3.30pm	Water – Mount Franklin Chocolate slice from deli	1 bottle 750ml 1 medium slice
7.30pm	Emu Export Spaghetti, white – Maggi Bolognaise Sauce – lean mince Parmesan Cheese – Kraft Red wine, Shiraz Salad carrots tomatoes lettuce French dressing – Kraft Fat Free Ice cream (vanilla) – Blue Ribbon	1 x 375ml can 320g 1.5 cups (half the recipe) 3 teaspoons 2 glasses 3 slices 1 whole 4 small leaves 1 tablespoon 3 scoops
9.00pm	<p>Notes: Bolognaise Sauce: 500g lean mince, 1 x 420g can tinned whole tomatoes, 2 tablespoons Leggo tomato paste, 1 tablespoon canola oil, ½ cup dry white wine, oregano, thyme, basil, salt and pepper</p>	

Day 1

Date: _____ Day of week: _____

Time	Food and Drink Consumed	
	Description eg. Weetbix	Quantity eg. 50 grams
	_____ _____ _____ _____ _____	_____ _____ _____ _____
	_____ _____ _____ _____	_____ _____ _____ _____
	_____ _____ _____ _____ _____	_____ _____ _____ _____ _____

Was it a usual day? Yes No

If not, please comment:

Notes:

Day 2

Date: _____ Day of week: _____

Time	Food and Drink Consumed	
	Description eg. Weetbix	Quantity eg. 50 grams
	_____ _____ _____ _____ _____	_____ _____ _____ _____
	_____ _____ _____ _____	_____ _____ _____ -
	_____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____

Was it a usual day? Yes No

If not, please comment:

Notes:

Day 3

Date: _____ Day of week: _____

Time	Food and Drink Consumed	
	Description eg. Weetbix	Quantity eg. 50 grams
	_____ _____ _____ _____ _____	_____ _____ _____ _____
	_____ _____ _____ _____	_____ _____ _____
	_____ _____ _____ _____ _____	_____ _____ _____ _____

Was it a usual day? Yes No

If not, please comment:

Notes:

Appendix 14 Checklist for recording 3 days food record and 24 hour urine

Checklist for after your 1st appointment

1. 3-day Diet Record

1. Record everything you eat and drink for 3 days starting on _____.
2. Try not to alter what you eat or drink during this time and record as accurately as you can (but don't worry we will clarify any minor details).
3. Follow the guidelines for keeping the 3-day food diary.

2. 24-hour Urine Collection

This involves collecting your urine for a period of 24 hours. Please begin and finish as close to the same time of the day as possible e.g. 7:00am one day to 7:00am the next. To collect your 24-hour specimen, please follow these instructions.

- * Begin your 24 hour collection in the morning of: _____.
- * To begin, empty your bladder in the toilet as usual.

Record the time. **Time:** _____ **(am)**

- * Every time you go to the toilet in the next 24 hours, collect your urine with the urinary hat provided and pour it into the plastic collection bottle provided.
- * Keep your urine collection bottle in a cool place (no need to refrigerate).
- * Collect your last specimen in the morning of: _____
(As close to the same time as you began the morning before.)

Record the time. **Time:** _____ **(am)**

Your collection is now complete.

If you are unsure about anything, please do not hesitate contact us on:
9346 1747 or 9346 4970.

Please bring **your urine collection along with this sheet, your 3-day food diary and the food scale** to your next appointment on: _____ at Bone Mineral Research Group, Sir Charles Gairdner Hospital (see over for map).

At your next appointment, you will have measurements of balance, mobility and muscle strength, so please wear enclosed comfortable shoes. You do not need to be fasting.

Appendix 15 Baseline characteristics by quartile of ALM/height² (n = 219).

	1 st quartile < 5.88 kg/m ² (n = 53)	2 nd quartile 5.88–6.34kg/m ² (n = 55)	3 rd quartile 6.34–6.86kg/m ² (n = 55)	4 th quartile > 6.86 kg/m ² (n = 56)	P value of ANOVA test
Age (year)	74.6 ± 2.7	74.1 ± 2.7	74.3 ± 2.8	74.0 ± 2.5	0.60
Height (cm)	159.2 ± 6.4	159.1 ± 5.1	160.8 ± 6.9	160.3 ± 5.5	0.39
Weight (kg)	59.3 ± 8.7 ^{bcd}	65.4 ± 6.6 ^{ad}	69.3 ± 9.6 ^{ad}	79.6 ± 9.1 ^{abc}	<0.001
BMI (kg/m ²)	23.3 ± 2.7 ^{bcd}	25.8 ± 2.5 ^{ad}	26.8 ± 2.8 ^{ad}	31.0 ± 2.8 ^{abc}	<0.001
SF-36 standard physical health (0-100)	47 ± 10	46 ± 9	48 ± 8	43 ± 10	0.06
SF-36 standard mental health (0-100)	53 ± 9	56 ± 8	53 ± 9	55 ± 8	0.44
Physical activity (log(Mets/week))	3.27 ± 0.50	3.49 ± 0.48 ^{cd}	3.25 ± 0.37 ^b	3.27 ± 0.45 ^b	0.015
Body composition †					
Whole body lean mass (kg)	33.3 ± 3.5 ^{bcd}	35.9 ± 2.5 ^{acd}	38.2 ± 3.7 ^{abd}	42.3 ± 3.7 ^{abc}	<0.001
Whole body fat mass (kg)	21.4 ± 6.3 ^{bcd}	24.6 ± 5.5 ^{ad}	26.1 ± 6.9 ^{ad}	32.2 ± 6.9 ^{abc}	<0.001
Percentage of whole body fat mass (%)	37.4 ± 6.2 ^d	39.3 ± 5.3	39.0 ± 5.7 ^d	41.9 ± 5.0 ^{ac}	0.001
ALM/height ² (kg/height ²)	5.49 ± 0.29 ^{bcd}	6.16 ± 0.13 ^{acd}	6.58 ± 0.15 ^{abd}	7.43 ± 0.44 ^{abc}	<0.001
Calf muscle cross-sectional area (cm ²)	26.9 ± 3.4 ^{bcd}	30.5 ± 4.7 ^{ad}	31.9 ± 4.5 ^{ad}	35.3 ± 5.8 ^{abc}	<0.001
Corrected upper arm muscle area (cm ²)	28.7 ± 9.0 ^{cd}	32.0 ± 8.3 ^{cd}	36.8 ± 8.6 ^{ab}	39.2 ± 11.7 ^{ab}	<0.001
Protein intake assessed by 24-hour urinary nitrogen					
Protein intake (g/day)	58.3 ± 18.2 ^{cd}	66.8 ± 15.3	68.8 ± 20.6 ^a	70.4 ± 19.1 ^a	0.004
Dietary intake assessed by 3-day food record					
Protein intake (g/day)	73.3 ± 17.6	73.2 ± 18.7	77.8 ± 24.5	81.3 ± 14.8	0.08
Protein intake (g/kg/day)	1.25 ± 0.32 ^d	1.13 ± 0.33	1.14 ± 0.37	1.04 ± 0.24 ^a	0.007
Total fat intake (g/day)	62.3 ± 18.9	59.8 ± 18.3	59.8 ± 17.5	66.6 ± 20.6	0.20
Carbohydrate intake (g/day)	184.7 ± 40.1	179.4 ± 43.7 ^d	188.6 ± 43.8	203.5 ± 56.7 ^b	0.045

	1 st quartile < 5.88 kg/m ² (n = 53)	2 nd quartile 5.88–6.34kg/m ² (n = 55)	3 rd quartile 6.34–6.86kg/m ² (n = 55)	4 th quartile > 6.86 kg/m ² (n = 56)	P value of ANOVA test
Energy intake (kJ/day)	7004 ± 1367	6877 ± 1514	7055 ± 1578	7608 ± 1535	0.05
% of energy from protein (%)	18.3 ± 3.1	18.6 ± 3.5	19.0 ± 3.6	19.0 ± 3.8	0.69
% of energy from fat (%)	33.3 ± 6.2	32.5 ± 5.1	31.7 ± 5.2	32.9 ± 6.4	0.51
% of energy from carbohydrate (%)	45.9 ± 6.1	45.4 ± 7.8	46.6 ± 5.6	45.8 ± 7.0	0.83
Mobility and balance					
Timed Up and Go (second)	7.9 ± 1.3	7.9 ± 1.3	8.1 ± 1.5	8.0 ± 1.6	0.81
Romberg eye-open (0-4)	3.8 ± 0.4	4.0 ± 0.2	3.8 ± 0.5	3.9 ± 0.3	0.047
Romberg eye-close (0-4)	3.1 ± 0.6	3.3 ± 0.7	3.1 ± 0.7	3.1 ± 0.7	0.13
Muscle strength					
Hand grip strength (kg)	19.6 ± 5.4 ^{cd}	21.0 ± 4.8	22.5 ± 6.0 ^a	23.4 ± 4.8 ^a	0.001
Ankle dorsiflexion (kg)	10.0 ± 4.5	10.3 ± 4.2	10.7 ± 5.8	11.2 ± 3.9	0.57
Knee flexion (kg)	7.7 ± 3.4 ^{cd}	9.1 ± 3.5	9.9 ± 4.2 ^a	10.5 ± 3.8 ^a	0.001
Knee extension (kg)	13.6 ± 4.7 ^d	15.7 ± 5.7	15.7 ± 6.1	17.4 ± 7.4 ^a	0.02
Hip extension (kg)	14.7 ± 5.1 ^d	16.3 ± 6.0	16.5 ± 6.4	18.3 ± 7.6 ^a	0.03
Hip abduction (kg)	10.2 ± 3.4 ^d	10.6 ± 3.9	10.9 ± 4.2	12.4 ± 6.0 ^a	0.05
Hip flexion (kg)	11.0 ± 4.8	11.6 ± 4.0	12.0 ± 4.6	12.8 ± 4.4	0.18
Hip adduction (kg)	10.3 ± 5.0	12.4 ± 5.9	12.1 ± 5.4	12.4 ± 5.8	0.16
Total knee strength (kg)	21.3 ± 6.6 ^{cd}	24.7 ± 7.6	25.5 ± 9.0 ^a	27.9 ± 9.0 ^a	0.001
Total hip strength (kg)	46.2 ± 16.4 ^d	50.8 ± 16.9	51.4 ± 17.4	55.9 ± 20.0 ^a	0.042

Results are mean ± SD.

† Whole body fat mass and percentage of fat mass were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.
^a significantly different from the 1st quartile, p < 0.05, ^b significantly different from the 2nd quartile, p < 0.05, ^c significantly different from 3rd quartile, ^d significantly different from the 4th quartile (ANOVA with Tukey test).

Appendix 16 Baseline characteristics by quartile of regression residuals* (n = 219).

	1 st quartile Residual <-1136 (n = 55)	2 nd quartile Residual (-1136) – (-162) (n = 54)	3 rd quartile Residual (-162) – (1047) (n = 54)	4 quartile Residual >1047 (n = 56)	P value of ANOVA test
Age (year)	74.4 ± 2.6	74.4 ± 3.0	74.1 ± 2.6	74.1 ± 2.5	0.83
Height (cm)	160.4 ± 6.2	158.7 ± 4.8	160.4 ± 6.4	160.0 ± 6.5	0.40
Weight (kg)	66.1 ± 10.5 ^d	66.9 ± 10.1 ^d	67.9 ± 11.4	73.2 ± 12.0 ^{ab}	0.003
BMI (kg/m ²)	25.6 ± 3.3 ^d	26.5 ± 3.4 ^d	26.4 ± 4.0 ^d	28.6 ± 4.1 ^{abc}	<0.001
SF-36 standard physical health (0-100)	46 ± 10	45 ± 9	45 ± 10	47 ± 9	0.40
SF-36 standard mental health (0-100)	53 ± 10	55 ± 7	56 ± 7	52 ± 10	0.05
Physical activity (log(Mets/week))	2.09 ± 0.75	2.26 ± 0.62	2.35 ± 0.65	2.16 ± 0.71	0.15
Body composition †					
Whole body lean mass (kg)	34.8 ± 4.1 ^{cd}	36.0 ± 3.6 ^d	37.8 ± 4.0 ^{ad}	41.2 ± 4.5 ^{abc}	<0.001
Whole body fat mass (kg)	26.4 ± 7.0	25.9 ± 7.0	25.1 ± 7.8	27.0 ± 8.3	0.59
Percentage of whole body fat mass (%)	41.5 ± 5.0 ^{cd}	40.2 ± 5.3	38.1 ± 5.9 ^a	37.9 ± 6.0 ^a	0.001
ALM/height ² (kg/kg/height ²)	5.67 ± 0.44 ^{bcd}	6.20 ± 0.42 ^{acd}	6.54 ± 0.45 ^{abd}	7.28 ± 0.58 ^{abc}	<0.001
Calf muscle cross-sectional area (cm ²)	27.5 ± 3.8 ^{cd}	29.9 ± 4.6 ^d	32.1 ± 4.7 ^{ad}	35.4 ± 5.7 ^{abc}	<0.001
Corrected upper arm muscle area (cm ²)	30.8 ± 10.0 ^d	34.5 ± 9.8	33.7 ± 9.9	38.0 ± 10.4 ^a	0.003
Protein intake assessed by 24-hour urinary nitrogen					
Protein intake (g/day)	60.4 ± 17.1 ^d	66.2 ± 18.6	67.9 ± 16.7	70.1 ± 21.6 ^a	0.04
Dietary intake assessed by 3-day food record					
Protein intake (g/day)	70.7 ± 17.6	76.5 ± 23.0	79.6 ± 20.0	79.1 ± 15.3	0.06
Protein intake (g/kg/day)	1.09 ± 0.31	1.16 ± 0.36	1.21 ± 0.37	1.11 ± 0.25	0.23
Total fat intake (g/day)	59.2 ± 19.8	60.2 ± 15.7	63.5 ± 18.4	65.7 ± 21.3	0.26

	1 st quartile Residual <-1136 (n = 55)	2 nd quartile Residual (-1136) – (-162) (n = 54)	3 rd quartile Residual (-162) – (1047) (n = 54)	4 quartile Residual >1047 (n = 56)	P value of ANOVA test
Carbohydrate intake (g/day)	176.1 ± 38.6	193.0 ± 47.8	187.9 ± 40.2	199.7 ± 57.6	0.06
Energy intake (kJ/day)	6729 ± 1393	7114 ± 1484	7246 ± 1421	7473 ± 1697	0.07
% of energy intake from protein (%)	18.4 ± 3.4	18.7 ± 3.4	19.2 ± 3.8	18.8 ± 3.5	0.73
% of energy intake from fat (%)	32.8 ± 6.7	31.8 ± 4.5	32.7 ± 5.4	32.9 ± 6.1	0.76
% of energy intake from carbohydrate (%)	45.8 ± 7.1	46.8 ± 6.0	45.1 ± 7.1	45.9 ± 6.4	0.61
Mobility and balance					
Timed Up and Go (second)	7.8 ± 1.2	8.1 ± 1.4	8.3 ± 1.5	7.8 ± 1.4	0.14
Romberg eye-open (0-4)	3.9 ± 0.3	3.9 ± 0.4	3.8 ± 0.5	3.9 ± 0.3	0.29
Romberg eye-close (0-4)	3.1 ± 0.7	3.2 ± 0.6	3.0 ± 0.8	3.2 ± 0.7	0.58
Muscle strength					
Hand grip strength (kg)	20.7 ± 5.4 ^d	20.5 ± 5.4 ^d	21.8 ± 4.9	23.6 ± 5.6 ^{ab}	0.008
Ankle dorsiflexion (kg)	10.1 ± 4.4	10.7 ± 5.6	10.2 ± 3.8	11.1 ± 4.6	0.65
Knee flexion (kg)	8.4 ± 3.3 ^d	9.5 ± 4.4	8.7 ± 3.5	10.6 ± 3.8 ^a	0.01
Knee extension (kg)	15.2 ± 5.6	15.0 ± 5.6 ^d	14.0 ± 5.1 ^d	18.1 ± 7.6 ^{bc}	0.004
Hip extension (kg)	15.5 ± 5.4 ^d	15.5 ± 5.6 ^d	15.6 ± 5.6 ^d	19.2 ± 8.2 ^{abc}	0.003
Hip abduction (kg)	10.5 ± 4.0	10.7 ± 3.6	10.3 ± 3.9 ^d	12.5 ± 6.0 ^c	0.03
Hip flexion (kg)	11.6 ± 4.7	12.2 ± 4.3	11.1 ± 4.4	12.4 ± 4.4	0.40
Hip adduction (kg)	10.8 ± 5.0	12.6 ± 6.1	11.0 ± 5.5	12.9 ± 5.4	0.10
Total knee strength (kg)	23.6 ± 7.3 ^d	24.5 ± 8.6 ^d	22.8 ± 7.1 ^d	28.7 ± 9.4 ^{abc}	0.001
Total hip strength (kg)	48.3 ± 16.8	51.1 ± 17.1	48.0 ± 15.9 ^d	57.0 ± 20.5 ^c	0.03

* The residuals were obtained from linear regression of appendicular lean mass (kg) adjusted for height (m) and total body fat mass (kg). Values are mean ± SD.

† Whole body fat mass and percentage of fat mass were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.
^a significantly different from the 1st quartile, p < 0.05, ^b significantly different from the 2nd quartile, p < 0.05, ^c significantly different from 3rd quartile, ^d significantly different from the 4th quartile (ANOVA with Tukey test).

Appendix 17 Baseline characteristics by protein intake tertile assessed by 3-day food record (n = 218).

	1 st tertile Protein intake < 69 g/day (n = 74)	2 nd tertile Protein intake 69 - 83 g/day (n = 72)	3 rd tertile Protein intake > 83 g/day (n = 72)	P value of ANOVA test
Age (year)	74.5 ± 2.7	74.6 ± 2.7	73.6 ± 2.6	0.05
Height (cm)	158.3 ± 6.4 ^b	161.2 ± 5.3 ^a	160.0 ± 5.7	0.01
Weight (kg)	67.1 ± 11.7	69.7 ± 11.8	68.7 ± 10.4	0.37
BMI (kg/m ²)	26.7 ± 3.6	26.8 ± 4.1	26.9 ± 3.9	0.94
SF-36 standard physical health (0-100)	46 ± 9	46 ± 9	45 ± 10	0.62
SF-36 standard mental health (0-100)	55 ± 9	54 ± 8	54 ± 9	0.86
Physical activity (log(Mets/week))	3.33 ± 0.49	3.35 ± 0.46	3.27 ± 0.44	0.50
Protein intake assessed by 24-hour urinary nitrogen				
Protein intake (g/day)	58.3 ± 14.8 ^{bc}	68.1 ± 20.3 ^a	72.2 ± 18.7 ^a	<0.001
Dietary intake assessed by 3-day food record				
Protein intake (g/day)	57.7 ± 9.2 ^{bc}	75.1 ± 4.0 ^{ac}	97.1 ± 15.6 ^{ab}	<0.001
Protein intake (g/kg/day)	0.88 ± 0.20 ^{bc}	1.11 ± 0.22 ^{ac}	1.44 ± 0.28 ^{ab}	<0.001
Fat intake (g/day)	51.6 ± 18.0 ^{bc}	61.6 ± 14.9 ^{ac}	73.6 ± 17.1 ^{ab}	<0.001
Carbohydrate intake (g/day)	160.9 ± 32.3 ^{bc}	192.0 ± 42.6 ^{ac}	215.4 ± 48.7 ^{ab}	<0.001
Energy intake (kJ/day)	5910 ± 1123 ^{bc}	7140 ± 1144 ^{ac}	8403 ± 1114 ^{ab}	<0.001
% of energy from protein	17.3 ± 3.2 ^{bc}	18.8 ± 3.5 ^{ac}	20.2 ± 3.2 ^{ab}	<0.001
% of energy from fat	32.3 ± 6.5	32.4 ± 4.6	33.0 ± 6.0	0.73
% of energy from carbohydrate	47.5 ± 7.3 ^c	46.2 ± 5.6	44.1 ± 6.5 ^a	0.007
Calcium intake (mg/day)	768 ± 411 ^c	891 ± 305 ^c	1056 ± 371 ^{ab}	<0.001
Body composition				
Whole body lean mass (kg)	36.0 ± 4.6 ^{bc}	38.3 ± 4.6 ^a	38.1 ± 4.6 ^a	0.004
Appendicular lean mass (kg)	15.7 ± 2.4 ^{bc}	16.9 ± 2.3 ^a	16.7 ± 2.3 ^a	0.002
Whole body fat mass (kg)	26.2 ± 7.7	26.5 ± 8.1	25.6 ± 6.8	0.78
% of whole body fat mass	40 ± 5	39 ± 6	39 ± 5	0.16
ALM/height ² (kg/m ²)	6.23 ± 0.69 ^c	6.50 ± 0.76	6.54 ± 0.79 ^a	0.03
Residuals	-532 ± 1469 ^{bc}	158 ± 1700 ^a	356 ± 1624 ^a	0.003
Calf cross-sectional muscle area (cm ²)	29.9 ± 4.6 ^c	31.6 ± 5.9	32.1 ± 5.9 ^a	0.04
Correct upper arm muscle area (cm ²)	34.4 ± 10.6	34.2 ± 11.0	34.0 ± 9.4	0.97
Mobility and balance				
Timed Up and Go (second)	8.0 ± 1.4	8.1 ± 1.3	7.9 ± 1.5	0.80
Romberg eye-open (0-4)	3.8 ± 0.5	3.8 ± 0.3	3.9 ± 0.3	0.60
Romberg eye-close (0-4)	3.1 ± 0.8	3.1 ± 0.8	3.2 ± 0.6	0.50

	1 st tertile Protein intake < 69 g/day (n = 74)	2 nd tertile Protein intake 69 - 83 g/day (n = 72)	3 rd tertile Protein intake > 83 g/day (n = 72)	P value of ANOVA test
Muscle strength				
Hand grip strength (kg)	21.4 ± 5.0	21.4 ± 5.9	22.1 ± 5.3	0.63
Ankle dorsiflexion (kg)	10.0 ± 4.3	11.3 ± 5.4	10.2 ± 4.0	0.21
Knee flexion (kg)	8.8 ± 2.6	9.7 ± 4.0	9.3 ± 4.0	0.36
Knee extension (kg)	15.3 ± 5.6	15.7 ± 6.6	15.8 ± 6.5	0.85
Hip extension (kg)	16.7 ± 6.1	16.0 ± 6.3	16.6 ± 7.0	0.77
Hip abduction (kg)	11.3 ± 4.8	10.6 ± 3.8	11.1 ± 5.0	0.64
Hip flexion (kg)	12.2 ± 4.4	11.4 ± 4.2	12.0 ± 4.8	0.50
Hip adduction (kg)	11.9 ± 5.3	11.6 ± 5.7	12.0 ± 5.8	0.88
Total knee strength (kg)	24.1 ± 7.4	25.4 ± 9.1	25.1 ± 8.8	0.60
Total hip strength (kg)	52.1 ± 17.7	49.5 ± 16.9	51.8 ± 19.5	0.65
Total leg strength (kg)	86.4 ± 24.7	86.0 ± 25.3	87.1 ± 27.1	0.97

Results are mean ± SD.

† Whole body and percentage of whole body lean and fat mass and appendicular lean mass (ALM) were derived from DXA; Residuals was the regression residual of ALM adjusted for height² and fat mass; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.

^a significantly different from the 1st tertile, ^b significantly different from the 2nd tertile, ^c significantly different from 3rd tertile (ANOVA with Tukey's test, p < 0.05).

Appendix 18 Estimated marginal means (SE) of baseline muscle mass measurements by protein intake tertile assessed by 3-day food record adjusted for height (n = 218).

	1 st tertile Protein intake < 69 g/day (n = 74)	2 nd tertile Protein intake 69 - 83 g/day (n = 72)	3 rd tertile Protein intake > 83 g/day (n = 72)	P value of ANCOVA test
Whole body lean mass (kg)	36.7 ± 0.4	37.6 ± 0.4	38.0 ± 0.4	0.11
Appendicular lean mass (kg)	16.0 ± 0.2	16.6 ± 0.2	16.7 ± 0.2	0.07
Whole body fat mass (kg)	26.2 ± 0.7	26.5 ± 0.8	25.6 ± 0.7	0.56
% of whole body fat mass	40 ± 0.5	39 ± 0.6	39 ± 0.5	0.14
Calf cross-sectional muscle area (cm ²)	30.2 ± 0.6	31.4 ± 0.6	32.1 ± 0.6	0.10
Correct upper arm muscle area (cm ²)	34.6 ± 0.1	34.1 ± 0.1	34.0 ± 0.1	0.93

Results are mean ± SD. † Whole body and percentage of whole body lean and fat mass and appendicular lean mass (ALM) were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.

Appendix 19 Baseline characteristics by protein intake tertile assessed by 24-hour urinary nitrogen (n = 218).

	1 st tertile Protein intake < 56.7 g/day (n = 72)	2 nd tertile Protein intake 56.7 - 73 g/day (n = 73)	3 rd tertile Protein intake > 73 g/day (n = 73)	P value of ANOVA test
Age (year)	74.3 ± 2.7	74.6 ± 2.6	73.8 ± 2.6	0.22
Height (cm)	159.3 ± 6.4	159.7 ± 6.3	160.6 ± 5.3	0.40
Weight (kg)	66.7 ± 11.9	69.3 ± 11.3	69.6 ± 10.8	0.24
BMI (kg/m ²)	26.2 ± 4.0	27.1 ± 3.8	27.0 ± 3.8	0.31
SF-36 standard physical health (0-100)	47 ± 9	45 ± 9	46 ± 10	0.48
SF-36 standard mental health (0-100)	54 ± 9	54 ± 8	54 ± 9	0.97
Physical activity (log(Mets/week))	3.34 ± 0.45	3.25 ± 0.43	3.37 ± 0.49	0.28

Protein intake assessed by 24-hour urinary nitrogen

Protein intake (g/day)	47.4 ± 6.7 ^{bc}	64.0 ± 4.3 ^{ac}	86.8 ± 14.8 ^{ab}	<0.001
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Dietary intake assessed by 3-day food record

Protein intake (g/day)	70.9 ± 18.3 ^c	76.5 ± 20.7	82.0 ± 17.7 ^a	0.002
Protein intake (g/kg/day)	1.09 ± 0.34	1.13 ± 0.33	1.20 ± 0.31	0.13
Fat intake (g/day)	59.6 ± 20.7	60.4 ± 17.5	66.3 ± 18.2	0.07
Carbohydrate intake (g/day)	182.9 ± 50.4	190.7 ± 48.3	192.5 ± 41.6	0.43
Energy intake (kJ/day)	5910 ± 1123	7140 ± 1144	8403 ± 1114	0.10
% of energy from protein	18.0 ± 3.2	18.9 ± 3.8	19.4 ± 3.4	0.06
% of energy from fat	32.4 ± 6.8	31.8 ± 5.5	33.5 ± 4.8	0.23
% of energy from carbohydrate	46.3 ± 8.0	46.2 ± 6.1	45.1 ± 5.5	0.45
Calcium intake (mg/day)	818 ± 348	919 ± 388	969 ± 400	0.05

Body composition

Whole body lean mass (kg)	36.4 ± 4.9 ^c	37.9 ± 4.7	38.2 ± 4.4 ^a	0.04
Appendicular lean mass (kg)	15.9 ± 2.5	16.7 ± 2.4	16.8 ± 2.1	0.03
Whole body fat mass (kg)	25.5 ± 7.8	26.5 ± 7.5	26.4 ± 7.3	0.68
% of whole body fat mass	39 ± 6	40 ± 5	39 ± 6	0.91
ALM/height ² (kg/m ²)	6.23 ± 0.81 ^b	6.54 ± 0.72 ^a	6.49 ± 0.71	0.03
Residuals	-424 ± 1782 ^b	246 ± 1560 ^a	129 ± 1502	0.03
Calf cross-sectional muscle area (cm ²)	3002 ± 649	3186 ± 466	3171 ± 525	0.08
Correct upper arm muscle area (cm ²)	34.4 ± 10.8	35.3 ± 10.8	33.1 ± 9.4	0.41

Mobility and balance

Timed Up and Go (second)	7.8 ± 1.4	8.2 ± 1.5	7.9 ± 1.3	0.12
Romberg eye-open (0-4)	3.9 ± 0.3	3.9 ± 0.3	3.8 ± 0.5	0.63
Romberg eye-close (0-4)	3.1 ± 0.7	3.2 ± 0.6	3.1 ± 0.8	0.94

	1 st tertile Protein intake < 56.7 g/day (n = 72)	2 nd tertile Protein intake 56.7 - 73 g/day (n = 73)	3 rd tertile Protein intake > 73 g/day (n = 73)	P value of ANOVA test
Muscle strength				
Hand grip strength (kg)	21.0 ± 5.4	21.8 ± 5.4	22.1 ± 5.5	0.45
Ankle dorsiflexion (kg)	10.2 ± 4.5	10.4 ± 5.1	11.0 ± 4.3	0.60
Knee flexion (kg)	9.6 ± 3.9	9.1 ± 4.0	9.3 ± 3.7	0.76
Knee extension (kg)	15.8 ± 7.1	15.0 ± 6.0	16.0 ± 5.5	0.52
Hip extension (kg)	16.9 ± 6.6	15.6 ± 5.7	16.9 ± 7.0	0.35
Hip abduction (kg)	11.2 ± 4.6	11.0 ± 4.0	10.9 ± 5.1	0.93
Hip flexion (kg)	11.9 ± 5.0	11.8 ± 3.9	11.9 ± 4.5	0.99
Hip adduction (kg)	12.4 ± 5.6	11.6 ± 5.2	11.4 ± 6.0	0.57
Total knee strength (kg)	25.4 ± 8.9	24.0 ± 8.7	25.3 ± 7.7	0.55
Total hip strength (kg)	52.4 ± 19.0	49.9 ± 15.2	51.1 ± 19.6	0.72
Total leg strength (kg)	88.0 ± 27.2	84.6 ± 23.3	87.2 ± 26.5	0.71

Results are mean ± SD. Whole body and percentage of whole body lean and fat mass and appendicular lean mass were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; Corrected upper arm muscle area (cm²) = ((Arm girth in cm) – π(triceps skin fold in cm))² / 4π – 6.5.

* p < 0.05 of ANOVA F-ratio.

^a significantly different from the 1st tertile, p < 0.05, ^b significantly different from the 2nd tertile, p < 0.05, ^c significantly different from 3rd tertile (ANOVA with Tukey's test).

Appendix 20 Romberg tests and percentage of subjects who reported falling at least once during the preceding three month at baseline.

Balance test	Number of subjects (%)		
	Overall n = 219	Protein group n = 109	Control group n = 110
Romberg eyes-open			
1	1 (1%)	0	1 (1%)
2	0	0	0
3	28 (13%)	14 (13%)	14 (13%)
4	190 (86%)	95 (87%)	95 (86%)
Romberg eyes-closed			
1	10 (5%)	1 (1%)	9 (8%)
2	12 (5%)	8 (7%)	4 (4%)
3	136 (62%)	65 (60%)	71 (64%)
4	62 (28%)	35 (32%)	26 (24%)
Self-reported falling	Overall n = 216	Protein group n = 107	Control group n = 109
Ever had a fall	21 (10%)	16 (15%)	5 (5%)
Never had a fall	195 (90%)	91 (85%)	104 (95%)

0 = not be able to perform side by side stance; 1 = side by side stance; 2 = semi tandem stance; 3 = tandem stance (1-9 seconds); 4 = tandem stance (>9 seconds).

Appendix 21 Differences in baseline characteristics between two drink groups in the participants who completed the one year study.

	Protein group mean \pm SD (n = 100)	Control group mean \pm SD (n = 95)	p value of t-test
Age (year)	74 \pm 3	74 \pm 3	0.75
Height (m ²)	159.8 \pm 6.3	159.8 \pm 5.7	0.96
Weight (kg)	66.8 \pm 11.1	69.6 \pm 11.3	0.08
BMI (kg/m ²)	26.1 \pm 3.8	27.2 \pm 4.0	0.05
Waist circumference (cm)	87.1 \pm 9.4	90.3 \pm 9.6	0.02
Hip circumference (cm)	103.6 \pm 8.6	105.4 \pm 9.5	0.15
Triceps skinfold (mm)	28.0 \pm 7.8	29.2 \pm 8.5	0.30
Arm girth (cm)	31.0 \pm 3.2	31.8 \pm 3.5	0.09
Calf girth (cm)	35.3 \pm 2.8	36.0 \pm 3.0	0.08
Physical activity (log(Mets/week))	3.35 \pm 0.52	3.32 \pm 0.51	0.66
Dietary intakes assessed by 3-day food record			
Energy intake (kJ/day)	7140 \pm 1633	7147 \pm 1422	0.97
Protein intake (g/day)	77.6 \pm 22.2	76.9 \pm 16.9	0.81
Fat intake (g/day)	63.0 \pm 18.6	61.7 \pm 19.7	0.64
Carbohydrate intake (g/day)	187.5 \pm 49.9	190.7 \pm 44.1	0.63
% energy intake from protein (%)	19.0 \pm 3.8	18.8 \pm 3.4	0.72
Body composition †			
Whole body lean mass (kg)	37.0 \pm 4.7	37.7 \pm 4.7	0.29
Appendicular lean mass (kg)	16.2 \pm 2.4	16.6 \pm 2.4	0.27
Whole body fat mass (kg)	24.8 \pm 7.2	27.0 \pm 7.6	0.04
% of whole body fat mass (%)	38.6 \pm 5.5	40.0 \pm 6.1	0.08
Calf cross-sectional muscle area (cm ²)	30.6 \pm 5.3	31.8 \pm 6.2	0.16
CUAMA (cm ²)	33.3 \pm 10.0	34.9 \pm 10.2	0.30
Mobility and balance tests			
Timed Up and Go test (second)	7.9 \pm 1.3	8.0 \pm 1.5	0.61
Romberg eye-open (0-4) *	4 (4 - 4)	4 (4 - 4)	0.47
Romberg eye-close (0-4) *	3 (3 - 4)	3 (3 - 4)	0.05
Muscle strength measurements			
Hand grip strength (kg)	21.7 \pm 5.2	21.6 \pm 5.6	0.92
Ankle dorsiflexion (kg)	10.4 \pm 4.5	10.2 \pm 4.2	0.72
Total knee strength (kg) ^a	24.6 \pm 7.5	25.8 \pm 9.1	0.31
Total hip strength (kg) ^b	52.1 \pm 18.1	51.0 \pm 18.6	0.67

* Results are median (interquartile range) with p value of Chi-square test.

† Whole body and percentage of whole body lean and fat mass and appendicular lean mass were derived from DXA; Calf muscle cross-sectional area was obtained from pQCT measurements at 38% length of tibia; CUAMA: Corrected upper arm muscle area (cm²) = ((Arm girth in cm) - π (triceps skin fold in cm))² / 4 π - 6.5.

^a Total knee strength = knee flexion + knee extension

^b Total hip strength = hip extension + hip abduction + hip flexion + hip adduction

Appendix 22 Differences in baseline characteristics between those who withdrew and those who completed the one year study.

	Completed n = 195	Withdrawals n = 23	p value t-test
Age (year)	74.2 ± 2.7	75.0 ± 2.5	0.17
Height (m ²)	159.8 ± 6.0	160.5 ± 6.1	0.61
Weight (kg)	68.1 ± 11.2	71.8 ± 11.6	0.14
BMI (kg/m ²)	26.7 ± 3.9	27.8 ± 3.3	0.19
SF-36 standard physical health (0-100)	46.4 ± 9.2	40.9 ± 11.0	0.007
SF-36 standard mental health (0-100)	54.4 ± 8.3	52.9 ± 10.2	0.41
Physical activity (log(Mets/week))	3.34 ± 0.46	3.16 ± 0.44	0.08
24 hour urinary nitrogen (g/d)	8.77 ± 3.06	7.07 ± 2.09	0.009
Dietary intake assessed by 3-day food record			
Energy intake (kJ/day)	7143 ± 1530	7111 ± 1452	0.92
Protein intake (g/day)	77.2 ± 19.7	70.3 ± 15.4	0.10
Fat intake (g/day)	62.4 ± 19.0	60.4 ± 18.1	0.64
Carbohydrate intake (g/day)	189.1 ± 47.0	189.7 ± 49.4	0.95
Mobility and balance			
Timed Up and Go test (second)	7.9 ± 1.4	8.4 ± 1.3	0.14
Romberg (eye-open) (0-4)	3.9 ± 0.4	3.8 ± 0.4	0.33
Romberg (eye-close) (0-4)	3.2 ± 0.7	3.0 ± 0.6	0.37
Muscle strength			
Hand grip strength (kg)	21.6 ± 5.4	21.8 ± 5.8	0.92
Ankle dorsiflexion (kg)	10.3 ± 4.3	12.1 ± 6.5	0.08
Total knee strength (kg)	25.2 ± 8.3	22.9 ± 9.2	0.21
Total hip strength (kg)	51.5 ± 18.3	48.0 ± 14.8	0.36
Total leg strength (kg)	87.1 ± 25.7	82.9 ± 25.1	0.46

Values are mean ± SD.

Appendix 23 Romberg tests by drink groups at baseline and at one year in the participants who completed one year visit.

	Protein group		Control group	
	Baseline n = 100	1 year n = 99	Baseline n = 95	1 year n = 94
Romberg eyes-open				
1	0	1 (1%)	1 (1%)	1 (1%)
2	0	1 (1%)	0	0
3	13 (13%)	16 (16%)	11 (12%)	20 (21%)
4	87 (87%)	81 (82%)	83 (87%)	73 (78%)
Romberg eyes-close				
0	0	0	0	2 (2%)
1	1 (1%)	1 (1%)	8 (9%)	1 (1%)
2	7 (7%)	6 (6%)	4 (4%)	11 (12%)
3	58 (58%)	73 (74%)	59 (62%)	58 (62%)
4	34 (34%)	19 (19%)	24 (25%)	22 (23%)

0 = not be able to perform side by side stance; 1 = side by side stance; 2 = semi tandem stance; 3 = tandem stance (1-9 seconds); 4 = tandem stance (>9 seconds).

Appendix 24 Percentage of subjects who ever had a fall in the past three months before the clinic visit at baseline and one year by drink groups in participants who completed one year visit.

	Protein group		Control group	
	Baseline n = 98	1 year n = 99	Baseline n = 94	1 year n = 94
Ever had a fall	13 (13%)	16 (16%)	5 (5%)	13 (14%)
Never had a fall	85 (87%)	84 (84%)	89 (95%)	81 (86%)

Appendix 25 Statement of the role of the candidate in this clinical trial.

The current study is part of a two year randomized controlled trial funded by the NHMRC. The large study title is: ‘Dietary protein effects in elderly women: musculoskeletal, renal, cardiovascular and body composition endpoints’. The investigators include: A Prof Richard Prince, Dr Kathy Zhu, Dr Deborah Kerr, Dr Amanda Devine, A Prof Vicky Solah and Prof Colin Binns.

The NHMRC study has two arms, outcomes related to bone health and muscle health. I was responsible for baseline and one year muscle outcome measurements. A detailed list has been made as below to state what my work involved, and an acknowledgement to others who contributed to this project.

My role in this one year study reported in the thesis:

1. Preparation for the equipment for the trial: ordering laboratorial materials, such as blood and urine sample tubes, urine bottles, and electronic food scales; participants’ document files preparation; drafted the standardized operating procedure, such as 24-hour urinary nitrogen collection instruction for participants and the instruction of manipulation of the samples for the research team; prepare the instruction for operating DXA and peripheral quantitative computerized tomography (pQCT); and the instruction for muscle strength, mobility and balance test.
2. Involvement in the recruitment (e.g., letter printing, sending and telephone screening).
3. Conducted all pQCT scans and some DXA scans and anthropometry measures when needed.
4. Conducted the majority of the baseline and one year muscle strength, mobility, and balance tests.
5. Conducted the majority of the baseline clinical preparation, clinical data entry, and Realbeach data entry (medications and medical conditions).
6. Analyzed all pQCT scan for calf muscle cross-sectional area for baseline and one year.
7. Involved in some DXA scan imagine analyses and re-analyses when cleaning the baseline and one year data.

8. Conducted all the CVs tests and the CVs analyses for pQCT calf muscle cross-sectional area, muscle strength and mobility test.
9. Cleaned all the baseline data and part of the one year data (DXA, pQCT muscle area, dietary intake and 24-hour urinary nitrogen).
10. Performed all the baseline and one year data analyses presented in this thesis.
11. Responsible for all the data analyses interpretation presented in this thesis.

Acknowledgement to those who involved in this one year study:

1. PIMES research team:
 - 1) Prof Richard Prince, the main investigator of this study has used any possible resource to ensure this trial followed the protocol and finished on time.
 - 2) Other investigators for this NHMRC funded projects are Dr Kathy Zhu, Associate Professor Deborah Kerr, Dr Amanda Devine, Associate Professor Vicky Solah, and Professor Colin Binns. I was part of the management team for the project and attended regular management meetings.
 - 3) The clinical research team, especially Monika Trapanovski, Cynthia Adikara, Emily Greenwood and Maria Pollock recruited most of the participants and telephone screened them.
 - 4) Kathy Zhu, Deborah Kerr and Amanda Devine conducted most of the DXA scan and analyzed the baseline scan images and Emily Greenwood interviewed most of the baseline participants and checked most of their questionnaires and medications.
 - 5) Maria Pollard, study coordinator collected most of the baseline and one year blood samples, ensuring a smooth communication between the research team and study participants and their GPs, and also prepared all the one year visit invitation letters.
 - 6) Felicia Novana assisted with the one year clinic visit preparation, checked the participants' one year questionnaires and medications, recorded the number of the returned consumed drink containers, and also involved the one year data entry and cleaning.
 - 7) Emily Greenwood checked the baseline 3-day food records and Felicia Novana checked the one year 3-day food records.

- 8) Senior students studying nutrition in Curtin University entered most of the baseline 3-day food records in the Foodworks database.
- 9) Deborah Kerr and Felicia Novana entered forty of the one year 3-day food records in the Foodworks database.
2. The Laboratory staff of the School of Public Health, Curtin University analyzed the baseline and one year 24-hour urinary nitrogen.
3. Royal Perth Hospital analyzed the baseline and one year serum IGF-1 concentration.

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